UNIVERSITEIT GENT

Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen

Academiejaar 2002-2003

# ISOLATION AND STRUCTURAL ELUCIDATION OF NATURAL PRODUCTS FROM *PENTAS BUSSEI* K. Krause, *PENTAS LANCEOLATA* (Forsk.) Deflers AND *PENTAS PARVIFOLIA* Hiern (RUBIACEAE)

# ISOLATIE EN STRUCTUURBEPALING VAN NATUURPRODUCTEN UIT *PENTAS BUSSEI* K. Krause, *PENTAS LANCEOLATA* (Forsk.) Deflers EN *PENTAS PARVIFOLIA* Hiern (RUBIACEAE)

door

**JACQUES BUKURU**

Thesis submitted in fulfilment of the requirements for the degree of Doctor (Ph.D.) in Applied Biological Sciences: Chemistry

Proefschrift voorgedragen tot het bekomen van de graad van doctor in de Toegepaste Biologische Wetenschappen: Scheikunde

Op gezag van
Rector: **Prof. Dr. A. DE LEENHEER**

Decaan:
**Prof. Dr. ir. H. VAN LANGENHOVE**

Promotoren:
**Prof. Dr. ir. N. DE KIMPE**
**Dr. L. VAN PUYVELDE**

# COPYRIGHT



June 2003,
Ghent, Belgium

De auteur:

Jacques BUKURU

De promotor:

Prof. Dr. ir. N. De KIMPE

To my brothers T. Ngarukiyinka,
J. Murengerantwari and A. Butoyi.

Dedicated to all the Burundians for their hunger
for peace, science and development.

## Examination committee

Promoters

- **Prof. Dr. ir. N. De Kimpe**

Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium

- **Dr. L. Van Puyvelde**

Project Leader-TIBOTEC
Natural Products Drug Discovery, Cau Glay – Hanoi – Vietnam

Chairman

- **Prof. Dr. ir. J. Viaene**

Department of Agricultural Economy, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium

Members

- **Prof. Dr. L. Pieters**

Laboratory of Pharmacognosy and Phytochemistry, Department of Pharmaceutical Sciences, University of Antwerp, Antwerp, Belgium

- **Prof. Dr. ir. P. Van Damme**

Department of Plant Production, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium

- **Prof. Dr. T. van Beek**

Laboratory of Organic Chemistry, Phytochemical Section, Wageningen University, Wageningen, The Nederlands

- **Prof. Dr. ir. C. Stevens**

Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium

- **Prof. Dr. ir. W. Steurbaut**

Department of Crop Protection, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium

- **Dr. P. Wigerinck**

TIBOTEC, Mechelen, Belgium

# Preface

It may be considered as a common thing, which is not more than a habit, to express gratitude at the end of a PhD research work like mine. However, let me wish all the individual persons and all the institutions mentioned here to find, through the following lines, a sincere and deep expression of what I have and I feel in my heart about them, upon the termination of my PhD thesis.

First of all, my deepest appreciation is expressed to my promoters, Prof. Dr. ir. N. De Kimpe and Dr. L. Van Puyvelde, for their guidance and continuous help throughout the course of my doctororate studies. The supervision and criticism of Prof. Dr. ir. N. De Kimpe have been very crucial. Under his guidance, I have learnt science and I have made my real first steps in widely sharing knowledge, as attested by the high quality publications we have made together. I am grateful to him for having shared with me his high level scientific skill. The long experience of Dr. L. Van Puyvelde in the isolation of natural products, together with his long stay in Africa (Rwanda) have been beneficial to me from both the technical and the human point of vue. I am very thankful to him.

Nothing could have been done without the financial support of my country throughout these years of hard work. I am highly grateful to The Republic of Burundi for this financial support. May the University of Ghent (Commission of Scientific Research of the Faculty of Agricultural and Applied Biological Sciences) also find here my gratitude for a two-months financial support at the very end of my studies. This support has been of a great rescue for me and my whole family.

With all respect and gratitude, I refer to Prof. Dr. N. Schamp, the former head of the Department of Organic Chemistry, for having played a key role which lead to my admission at Ghent University. Through the collaboration I had with the late Prof. Dr. H. L. De Pooter since 1991, I knew Prof. Dr. N. Schamp only indirectly. After my Master Degree in Pharmaceutical Sciences at the Catholic University of Louvain in 1998, he welcomed me and introduced me to Prof. Dr. ir. N. De Kimpe.

My special thanks also go to Prof. Dr. ir. H. Van Langenhove, the dean of the Faculty of Agricultural and Applied Biological Sciences, for his concern about me.

I am very much grateful to Prof. Dr. ir. R. Verhé, the head of the Department of Organic Chemistry for his kindness and encouragement.

I would like to thank the members of the reading committee: Prof. Dr. ir. C. Stevens, Prof. Dr. L. Pieters, Prof. Dr. T. van Beek, Dr. L. Van Puyvelde and Prof. Dr. ir. N. De Kimpe for their critical evaluation of my thesis. The contribution of the examination committee including Prof. Dr. ir. J. Viaene, Prof. Dr. ir. P. Van Damme, Prof. Dr. ir. W. Steurbaut and Dr. P. Wigerinck is also highly appreciated.

I also appreciate the key contribution of Mr. Simon G. Mathenge and Mr. Francis P. Mudida (Botany Department, University of Nairobi, P. O. Box 30197, Nairobi, Kenya, and TRAMEDA, P. O. Box 66514, Nairobi, Kenya) for providing all the plant materials used for this research.

I am thankful to Dr. Tuyen Van Nguyen from Vietman for his crucial contribution to my research work, particulary for all the time we spent together discussing my results, even Saturday. I will always remember positively his contribution to the articles I wrote.

I would not forget to thank all the people of the Department of Organic Chemistry, Secretaries, Scientific staff, PhD students and students from Belgium and elsewhere, for their kind help and friendly cooperation.

My heartfelt thanks go to my dear wife Evelyne. Her understanding, tolerance and love during so many years when most of the time I was away from home are deeply appreciated for the rest of my life. Had I known before the heavy sacrifices she was going to suffer of and endure, I am afraid that I would have hesitated to start the PhD studies! I wish her to consider that this research work is also hers!

Last not the least, I would like to thank my daughters Rachel and Aurore, although not yet old enough to read and understand what I am writing at this moment. They often knocked on the door of my home office at the moments I needed silence around me, bringing to me, however, the nice and funny drawings they had been making all the day long and saying: “Open the door! We have our own works to show you!” Probably unaware of that, they provided me with a psychological refreshment, and I have learnt from them what is responsibility. I wish them to do more than I have done and to succeed where I failed!

Should there be anyone I failed to acknowledge, my sincere regret, my apology and my thanks go to him/her too.

Jacques BUKURU
Ghent, June 2003

## TABLE OF CONTENT

**Chapter I.**

# GENERAL INTRODUCTION

# Chapter I. GENERAL INTRODUCTION

A large variety of structurally complex compounds has been isolated from plants. This complexity does not allow a simple organisation of an overview of plant-derived chemicals. For a clear presentation of this general introduction, the chapter is divided into four parts.

The first part is devoted to a survey of important plant-derived drugs and agrochemicals.

In the second part will be given a brief presentation of the Rubiaceae plant family and some of its biologically active compounds. Besides, a special emphasis will be made on the genus *Pentas* (from which are the plants under investigation in the present study), wich will includes an overview of the traditional medicine usages (in Africa) of its species, together with a presentation of the results of phytochemical investigations already made on *Pentas longiflora* Oliver and *Pentas zanzibarica* (Klotsch) Vatke.

In the third part, the purpose and the goal of the present research work will be presented, whereas the fourth part will be devoted to the materials and methods used.

## I.1. Nature: An Invaluable Source of Useful Chemical Agents

Nature has been a source of medicinal treatments for thousands of years. Nowadays, the search for new chemotherapeutic agents has been expanded to the whole biodiversity: plants, microorganisms, as well as marine organisms.

From 1960 to 1982, more than 180,000 microbial-derived, some 16,000 marine organism-derived, and more than 114,000 plant-derived extracts were screened for antitumor activity, and a number of clinically effective drugs were developed. Microorganisms are a profilic source of structurally diverse bioactive metabolites, and have yielded some of the most important products of the drug industry, including penicillins, aminoglycosides, tetracyclines, cephalosporines, and other classes of antibiotics that have revolutionized modern medicine (1a). The most prominent of the marine-derived chemotherapeutic agents is bryostatin 1, isolated from the bryozoan *Bugula neritina* (2), and which is in phase II clinical trials (USA) as anticancer agent (1a).

Of the newly approved drugs reported between 1983 and 1994, drugs of natural origin predominated (78%) in the area of antibacterials, while 61% of the 31 anticancer drugs approved in the same period were either natural products, nature-derived products or compounds modeled on natural product parents or "leads". In addition, 50% of the best selling pharmaceuticals in 1991 were either natural products or their derivatives (3).

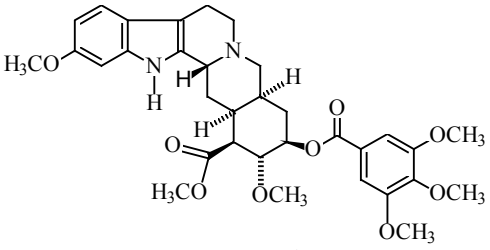
Plant-based systems continue to play an essential role in the primary health care of 80% of the world's population (4). An increasing number of chemotherapeutic agents are discovered as a result of chemical studies directed towards the isolation of the active substances from plants used in traditional medicine (1a). Tropical forest plant species have served as a source of medicines for people of the tropics for millennia. Many medical practitioners are well aware of the number of modern therapeutic agents that have been derived from tropical forest species. In fact, over one-hundred and twenty pharmaceutical products currently in use are plant-derived, and some 75% of these were discovered by examining the use of these plants in traditional medicine. Of these, a large portion has come from tropical forest species (1b). Given the arising of new diseases, particularly in the area of infectious diseases, and the occurrence of a variety of drug resistance such as in the AIDS and tropical malaria treatments, it is expected that this interest in nature, and rather in the plant kingdom, as a source of potential drug agents will continue to grow.

It has to be also noted that plants, as living organisms, possess biological self-defense mechanisms which served as the basis for the discovery of a number of agrochemicals such as insecticides, fungicides, and herbicides.

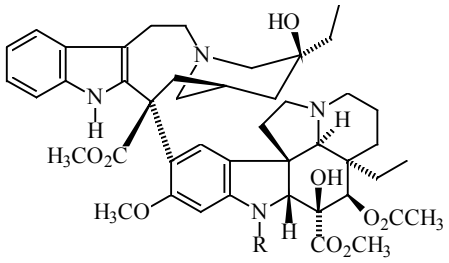
### I.1.1. Survey of Plant-derived Drugs

An increasing number of modern drugs have been isolated and purified from plant extracts. Well-known examples of plant-derived medicinal agents include the antimalarial drug quinine, isolated from the bark of *Cinchona officinalis*, the analgesics codeine and morphine from *Papaver somniferum*, the cardiac glycoside digitoxin from *Digitalis purpurea*, the antihypertensive reserpine **1** from *Rauwolfia serpentina* (5), and the anticancer drugs vinblastine **2** and vincristine **3**, isolated from the Madagascar periwinkle, *Catharanthus*

*roseus* (6), to name a few. Of the established plant-derived anticancer drugs, vinblastine **2** and vincristine **3** are still produced by their direct isolation from the plant material (1a).



Reserpine **1**



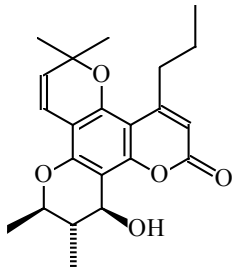
Vinblastine **2** R = $CH_3$
Vincristine **3** R = CHO

*I.1.1.1. Infectious Diseases Area*

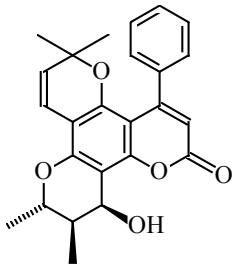
The occurrence of new bacterial and viral pathogens and the increasing clinical importance of drug-resistant variants of well-known pathogens have given additional urgency to antiinfectious diseases research in the last decades. Many effective antibacterial, antimicrobial, antiviral or antifungal agents have been isolated from various plant species. (+)-Calanolide A **4** (phase I clinical trials) is a reverse-transcriptase inhibitor discovered from the Malaysian rainforest tree *Calophyllum lanigerum* (Clusiaceae) by the U. S. National Cancer Institute (7,8). In vitro studies of this compound demonstrated its effectiveness against the Human Immunodeficiency Virus Type 1 (HIV-1), including strains resistant to AZT and other non-nucleoside reverse-transcriptase inhibitors (9,10). It also exhibited synergistic anti-HIV activity in combination with nucleoside reverse-transcriptase inhibitors, including AZT, ddI, and ddC (11,12).

In an effort aiming at the discovery of more potent anti-HIV pyranocoumarins of the (+)-calanolide A (**4**) type from plants of the genus *Calophyllum*, 31 species have been analyzed and their anti-HIV activity tested (13). In addition to isomers of (+)-calanolide A (**4**), more than 10 other pyranocoumarins were isolated, among which soulattrolide **5** (from *C. teysmannii*), which was found to be also a potent inhibitor of HIV-1 reverse transcriptase ($IC_{50}$

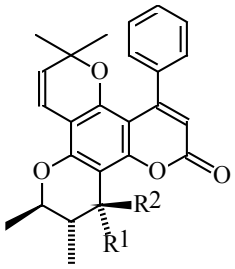
of 0.34 μM) (13,14). Two isomers of this last compound, inophyllum B (**6**) and inophyllum P (**7**) were isolated from *C. inophyllum*, and inhibited HIV reverse transcriptase with $IC_{50}$ values of 38 and 130 nM, respectively (15).



(+)-Calanolide A **4**



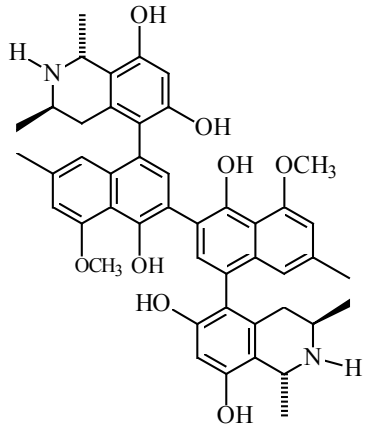
Soulattrolide **5**



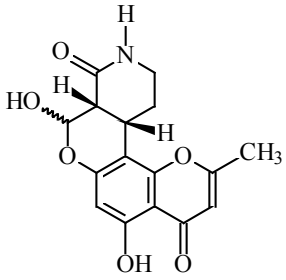
Inophyllum B **6** $R^1$ = H $R^2$ = OH
Inophyllum P **7** $R^1$ = OH $R^2$ = H

Another class of anti-HIV natural products, the atropisomeric naphthylisoquinoline alkaloids dimers, michellamines A, B, and C, were isolated from *Ancistrocladus korupensis* (Ancistrocladaceae), an extremely rare tropical rainforest plant of Cameroon (16). The most potent and abundant member of the series, michellamine B (**8**), inhibited the enzymatic activities of reverse transcriptases from both HIV-1 and HIV-2. Enzymatic assays showed michellamine B (**8**) to inhibit drug-sensitive and drug-resistant HIV-1 and HIV-2 reverse transcriptases, including the AZT-resistant strain G910-6 (16,17).

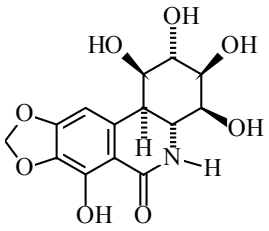
From the root bark of *Schumanniophyton magnificum* was isolated the chromone secondary amine, schumannificine **9**, which displayed activity against HIV, whereas potent anti-Herpes simplex (anti-HSV) activity was also observed for a number of its derivatives (18,19). Pancratistatin **10** is an Amaryllidaceae isoquinoline alkaloid isolated from the bulbs of the Hawaian *Pancratium littorale* Jacq. (20), and which exhibited consistent *in vitro* activity against flaviviruses (Japanese encephalitis, yellow fever, and dengue viruses) and the bunyaviruses (Punta Toro and Rift Valley fever virus) (21).



Michellamine B **8**

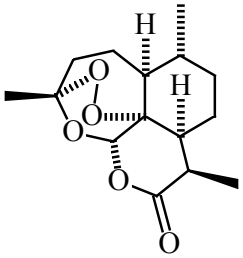


Schumannificine **9**

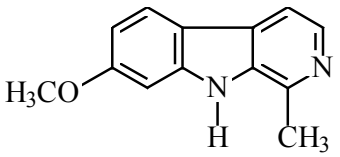


Pancratistatin **10**

In the early 1970's, the antimalarial artemisinin **11** was isolated (22-23) from *Artemisia annua* L. (Asteraceae plant family), a plant used in traditional Chinese medicine for the treatment of febrile diseases. The drug is effective in treating chloroquine-resistant malaria and other severe cases without major toxicity. Its synthetic and fat-soluble analogue, artemether, which is a methyl ether of the corresponding lactol, is as effective as quinine in the treatment of severe malaria. The *in vitro* concentration at which artemisinin can inhibit 50% of the growth of *Plasmodium falciparum* ranges from 3 to 30 μg/L, while artemether is approximately twice as active. Artemisinin **11** and its derivatives have found their way into clinical use in many areas where malaria is endemic (8,24).



Artemisinin (qinhaosu) **11**

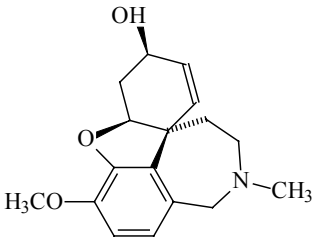


Harmine **12**

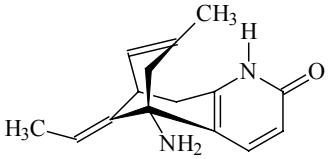
Various tropical species from the Malpighiaceae plant family, which are used regularly by tropical tribe groups in South America, contain *β*-carboline alkaloids (25-27). In an *in vitro* test against epimastigotes of *Trypanosoma cruzi* (Costa Rica strain), one of these alkaloids, harmine **12**, was effective in reducing growth for more than 90% at a concentration of 50 μg/mL, and showed significant activity at 5 μg/mL as anti-leishmanial compound (28).

*I.1.1.2. Neurological Diseases Area*

Natural products research afforded some interesting compounds which are effective medicines for neurological diseases. Galanthamine **13**, a natural product originally isolated from Caucasian snowdrop *Galanthus woronowi* (29a), and the common snowdrop *Galanthus nivalis* (29b) in the 1950s, is a long-acting, centrally active competitive cholinesterase inhibitor. It has also been found in several South African *Amaryllidaceae* (29c). *Galanthus* species have been used for hundreds of years in traditional medicine to treat painful neurological conditions such as facial neuralgia, with such treatment being essentially topical (8). Galanthamine **13** has been used extensively as a curare reversal agent in anaesthetic practice in Eastern bloc countries (30). In the 1990s, preliminary clinical trials have claimed that Galanthamine **13** has some beneficial effects in restoring cholinergic transmission in patients with Alzheimer's disease (31a-c). Phase II clinical trials carried out in patients with senile dementia of Alzheimer's type have shown a statistically significant improvement in cognitive performance (32).




Galanthamine **13**



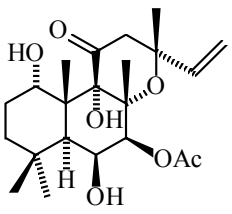

Huperzine A **14**

Another example of a plant derived candidate for the treatment of Alzheimer's disease is huperzine A **14**. For several centuries, elderly people in some parts of mainland China have brewed *Huperzia serata* (Lycopodiaceae) for improvement of their memory. In the early 1980's, huperzine A **14** was isolated from this traditional medicinal plant (33a-c) as a potent, reversible, and selective inhibitor of acetylcholinesterase (34). In a prospective, multicenter, double-blind trial with 103 patients, huperzine A **14** induced improvement in memory cognition and behaviour in about 58% of patients with Alzheimer's disease (35). Because of the fact that the compound is produced at very low levels in nature, its total synthesis has been developed (36a-b).

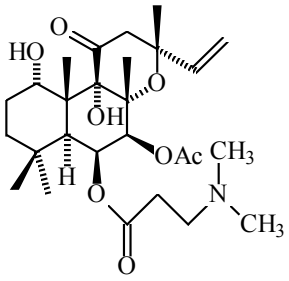
*I.1.1.3. Cardiovascular and Metabolic Diseases Area*

Recently, research efforts have been focussed on the discovery of oral antihyperglycemic agents for the treatment of Type II diabetes primarily from the screening of medicinal plants in animal models.

A number of orally active natural products were shown to reduce blood glucose in these *in vivo* models (37). An example of an active cardiovascular and metabolic plant-derived compound is forskolin (colforsin) **15**, which is a labdane-type diterpene isolated from the Indian *Coleus forskohlii* (Lamiaceae) (38a-b). The compound was first found to have blood pressure lowering and cardioactive properties (8,39). Due to its powerful and direct action on adenylate cyclase, therapeutic benefit in various diseases, such as congestive heart failure, hypertension, and asthma, has been expected. However, because of its low water solubility (0.0026%), the usage of forskolin **15** as a drug has been limited. To overcome this problem, semisynthetic efforts generated a water-soluble forskolin derivative, colforsin daproate (NHK-477) **16** which exhibited reversible effects on the respiratory, circulatory, and autonomic nervous systems. Its preliminary clinical trials demonstrated beneficial hemodynamic effects in heart failure patients. The compound was then brought into phase II trials in Japan for treatment of asthma and phase III clinical trials for treatment of cardiac insufficiency (8,40).




Forskolin **15**

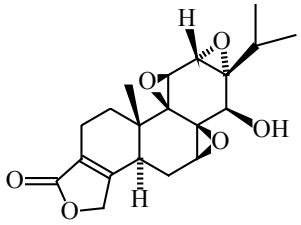



Colforsin daproate **16**

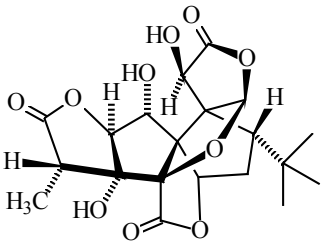
*I.1.1.4. Immunological, Inflammatory, and Related Diseases Area*

Interesting compounds with effective activity against immunological and inflammatory diseases have been isolated from some Chinese medicinal plants. Triptolide **17** is a major active component isolated from *Tripterygium wilfordii* (Celastraceae), a plant traditionally used in China for the treatment of rheumatoid arthritis. A variety of formulations of the plant extract were developed in mainland China and were shown to be effective in the treatment of

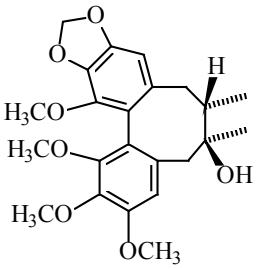
patients with inflammatory and autoimmune diseases. The compound was demonstrated to significantly inhibit arthritis in animal models. The immunosuppressive effect is mediated by inhibition of IL-2 signal transduction (8).



Triptolide **17**



Ginkgolide B **18**



Gomisin A **19**

*Ginkgo biloba* (Ginkgoaceae) is a Chinese tree which has been used therapeutically for thousands of years. Ginkgolides, a class of unique diterpene cage-like compounds, are among the natural products isolated from the leaves. In the late 1980's, it was demonstrated that the ginkgolides were highly active as selective platelet-activating factor (PAF) receptor antagonists. One of them, ginkgolide B (BN-52021) **18**, has been advanced to phase III clinical trials for the treatment of septic shock in patients with severe sepsis caused by Gram-negative bacterial infections. Good results were also found in inflammatory and autoimmune disorders (41a-b).

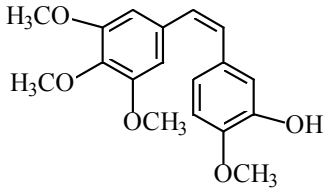
Another plant-derived drug of immunological activity is gomisin A (**19**), a lignan derivative isolated from the dry fruits of *Schisandra chinensis*, a plant used in Chinese traditional medicine for the treatment of liver intoxication. Gomisin A (**19**) was found to protect against hepatocarcinogenesis and liver damage in various animal models. Its mechanism of action includes induction of hepatic metabolizing enzyme systems, increase in the proliferation of the endoplasmic reticulum of liver cells, and antioxidant activity (42).

*I.1.1.5. Oncological Diseases Area*

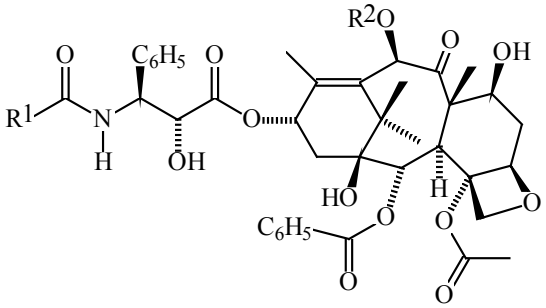
Plants have a long history of use in the treatment of cancer (43), although many of the claims for the efficacy of such treatment should be viewed with some skepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine (1a).

Of the plant-derived anticancer drugs in clinical use, the best known are the so-called *Vinca* alkaloids, vinblastine **2** and vincristine **3**, isolated from the Madagascar periwinkle, *Catharanthus roseus* (6) in the early 1960's. In the 1970's, combretastatin A-4 (**20**) was isolated from *Combretum caffrum* (Combretaceae), an African willow tree that has a history of being used by the Zulu for various purposes. The compound **20** has proved to be a powerful inhibitor of tubulin assembly and a variety of human cancer cell lines (44).

The toxicity of European yew trees has been known for thousands of years (45) and the use of various parts of some of the species (e.g., *Taxus brevifolia*, *T. canadensis*, *T. baccata*) by several native American tribes for the treatment of some non cancerous conditions has been reported (43). However, it was only in the 1960's, that laboratory tests demonstrated that the extracts of the Western Pacific Yew, *Taxus brevifolia* (Taxaceae), could kill cancer cells (46). The active compound, the diterpenoid paclitaxel (Taxol®) **21**, was initially isolated from a species collected in USA, Washington State (47). In test systems using human tumor cell lines, paclitaxel **21** showed distinct activity against several types of cancer, while phase I clinical studies have shown that this compound has significant clinical benefit for the treatment of ovarian and breast cancer (46). As paclitaxel **21** occurs in low yield in the bark of the Western Pacific yew, its production as a clinical drug is problematic. A related diterpenoid, baccatin III, and other key precursors (the baccatins) occur in higher yields in the needles (which represent a renewable source for the tree) of several species of *Taxus*. Semisynthetic modification of baccatin III readily led to the paclitaxel analogue docetaxel (Taxotere®) **22**, which proved to be a serious rival of paclitaxel **21** (45,48).



Combretastatin A-4 **20**



| | $R^1$ | $R^2$ | |
|---|---|---|---|
| **21** | $C_6H_5$ | $CH_3CO$ | Paclitaxel (Taxol®) |
| **22** | $(CH_3)_3CO$ | H | Docetaxel |

## I.1.2. Agrochemicals of Plant Origin

### *I.1.2.1. General Overview*

Enhancement of health quality by fighting against endemic and novel diseases is certainly one of the major challenges for the whole world today. However, pest management is also an important and vital component in actual production of food, which is needed in increasing quantities to meet the demands of the world's burgeoning population.

The main important strategy of this management is the use of chemicals to control insect (but also bacteria, fungi and viruses) pests, which not only attack plants and plant products at various stages of production, but also destroy structures and transmit human and livestock diseases. There are approximately one million insect species in the world, representing ~ 70% of all species in the animal kingdom. Of these, around 10,000 have been recognized as harmful. It has been estimated that 14% of crop losses worldwide are caused by insect pests. Thus, control of harmful insects is a must for mankind (49).

But there is an enormous scope for the discovery of safer, more potent, and environmentally non-polluting insecticides. Because biologically derived chemicals are perceived by consumers as having less environmental toxicity and lower mammalian toxicity, chemical companies currently tend to have a greater desire to discover and develop natural product-based plant pesticides (50). At present, the commercial success is below the results expected.
Of the various strategies used for developing new biologically active compounds for specific applications, probing nature's bounty of so-called secondary metabolites has proved quite

effective in the past, and promises to remain so in the future. These secondary metabolites are now thought to mediate plant defense mechanisms by producing chemical barriers against animal and microbial predators. Plants must also compete with other plants (allelopathic interactions), often of the same species, for sunlight, water and nutrients. This chemical warfare between plants and their pathogens consistently provides new natural product leads (49,50).

Plant-derived substances have been used as botanical pesticides since ancient times, as attested by the so-called "Persian dust" or "Persian insect powder" (*Pyrethrum*) (51). However, from early Roman times to the mid-20$^{th}$ century, only pyrethrins, rotenone, nicotine, sabadilla and quassin were widely used as insect repellents and toxicants in the Western hemisphere (52).

Over 2000 plant species belonging to some sixty families (Table 1.1) are now known to possess insecticidal properties. Of these, the following families have provided plants of commercial value, both present and past: Asteraceae (Compositae), Flacourtiaceae, Fabaceae (Leguminosae), Liliaceae and Solanaceae (49).

Table 1.1. Plant Families with Insecticidal Species (49)

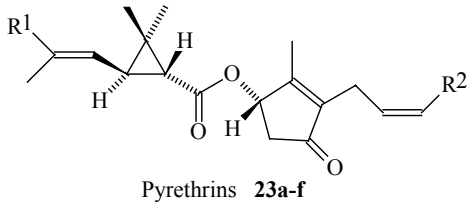
| N° Family | N° Family | N° Family | N° Family |
|---|---|---|---|
| 1. Acanthaceae | 17. Asteraceae (Compositeae) | 33. Lauraceae | 49. Ranunculaceae |
| 2. Agavaceae | 18. Convolvulaceae | 34. Fabaceae (Legumunosae) | 50. Rosaceae |
| 3. Annonaceae | 19. Cucurbutaceae | 35. Liliaceae | 51. Rubiaceae |
| 4. Apocyanaceae | 20. Dioscoreaceae | 36. Loganiceae | 52. Rutaceae |
| 5. Araceae | 21. Ebenaceae | 37. Lycopodiaceae | 53. Sapindaceae |
| 6. Aristolochiaceae | 22. Ericaceae | 38. Magnoliaceae | 54. Sapotaceae |
| 7. Asclepidiaceae | 23. Euphorbiaceae | 39. Malvaceae | 55. Simaroubaceae |
| 8. Balanitaceae | 24. Flacourtiaceae | 40. Meliaceae | 56. Solanaceae |
| 9. Berberidaceae | 25. Guttiferae | 41. Menispermaceae | 57. Stemonaceae |
| 10. Boraginaceae | 26. Helleboraceae | 42.Myristicaceae | 58. Taxaceae |
| 11. Brassicaceae | 27. Hippocastanaceae | 43. Myrtaceae | 59. Theaceae |
| 12. Burseraceae | 28. Hypericaceae | 44. Papaveraceae | 60. Apiaceae (Umbelliferaceae) |
| 13. Capparaceae | 29. Illiciaceae | 45. Piperaceae | 61. Verbenaceae |
| 14. Capparidaceae | 30. Juglandaceae | 46. Poaceae | |
| 15. Celastraceae | 31. Lamiaceae | 47. Polygonaceae | |
| 16. Chenopodiaceae | 32. Lamiaceae | 48. Polypodiaceae | |

*I.1.2.2. Few Examples of Plant-derived Insecticides*

Currently and commercially, the most important insecticidal plant widely used is pyrethrum (53), which consists of the fried flower-heads of any of the two species of the genus *Chrysanthemum* (Family: Asteraceae) now revised to the genus *Tanacetum* (54): the Persian insect flower (red flower), *T. coccineum* Willd and the Dalmatian insect flower (white flower) *T. cinerariaefolium Vis.* Of the two species, *T. cinerariaefolium*, a native to former Yugoslavia, is commercially the most important and is cultivated on a large scale in Kenya, Tanzania, Ecuador, Brazil, Russia, Japan and India.

The insecticidal principles of pyrethrum have been fully characterized (53,55) and consist of six esters, namely pyrethrins **23a-f** belonging to the two series I, II. Esters of series I are derivatives of (+)-*trans*-chrysanthemic acid and have excellent insect killing properties, while

series II, derived from pyrethric acid, has high knock-down activity. Products based on pyrethrum are essentially used for indoor applications.



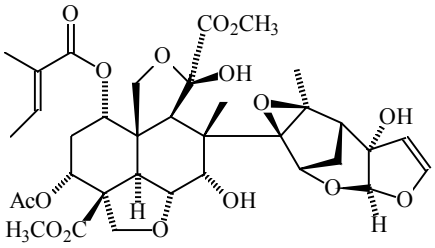

Pyrethrins **23a-f**

| | | $R^1$ | $R^2$ | |
|---|---|---|---|---|
| Pyrethrin | I | $CH_3$ | $CH{=}CH_2$ | **a** |
| Jasmolin | I | $CH_3$ | $CH_2CH_3$ | **b** |
| Cinerin | I | $CH_3$ | $CH_3$ | **c** |
| Pyrethrin | II | $CO_2CH_3$ | $CH{=}CH_2$ | **d** |
| Jasmolin | II | $CO_2CH_3$ | $CH_2CH_3$ | **e** |
| Cinerin | II | $CO_2CH_3$ | $CH_3$ | **f** |

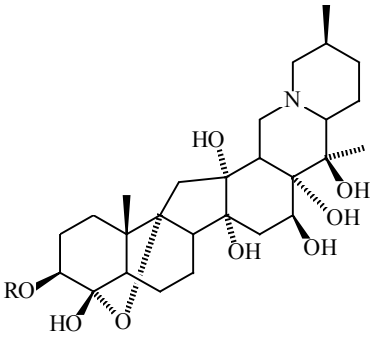
Pyrethrins are quite photolabile and their half-life in open environment is such that their use on agricultural crops is excluded essentially on economic grounds. On the other hand, they have a high safety margin for mammals ($LD_{50}$ for pyrethrin I 23a: 260-400 mg/kg, rat, oral), chiefly because of their rapid metabolic disposal. Thus, there has been a distinct need to develop pyrethrin analogues, which would be photostable, safe and environmentally non-persistent (49,51). Intense efforts aiming at the latter goal led, in between the late 1940's and the lates 1960's, to the synthesis of several very effective household insecticides, namely the so-called pyrethroids (56a-c), opening a major area of industrial and academic research. The development of pyrethroids has been one of the major success stories in the use of natural products as a source of leads for novel compounds possessing useful insecticidal activity. Their synthesis strategies lie in the modulation of the natural pyrethrins, both in the carboxylic acid as well as in the alcohol moieties.

The neem tree or margosa, *Azadirachta indica*, A. Juss (Meliaceae) is widespread in many Asian and African countries. Centuries before synthethic insecticides became available, farmers in India protected crops with natural repellents found in neem fruits and leaves (57). The active principles or “bitters” have been identified as limonoids, a group of stereochemically homogenous tetranortriterpenoids. The most potent and chemically the most important of them is azadirachtin **24**, although some 64 triterpinoids have been reported from the seeds, wood, bark, leaves, and fruit of the neem tree (58a-b). Neem derivatives have

diverse behaviour and physiological effects ranging from repellency to feeding deterrence, growth disruption, sterilizing effects, mating disruption, oviposition inhibition, etc. (59a-b).



Azadirachtin **24**

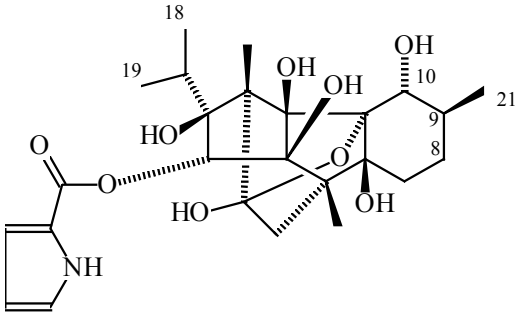


| | R | |
|---|---|---|
| Veratridine | 3,4-(MeO)$_2$PhCO (= veratronyl) | **25** |
| Cevadine | [3-(*Z*)-2-methylbut-2-enoyl] (= angenoyl) | **26** |
| Veracevine | H | **27** |

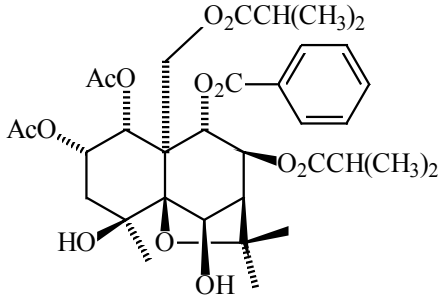
Two other phytoinsecticides (60), sabadilla (61,62) and ryania (61,63) may be mentioned. Sabadilla is the dried powdered barley-like ripe seeds of the South and Central American plant *Schoenocaulony officinale* Gray (Liliaceae), also known as *Sabadilla officinalis* Brant, and *Veratrum sabadilla* Retz (61b), which the local people had been using for a long time to control insect-infestation. The major insecticidal components of Sabadilla are veratridine **25**, the 3-veratronyl [3-(3,4-dimethoxybenzoyl)], and cevadine **26**, the 3-angenoyl [3-(*Z*)-2-methylbut-2-enoyl] derivative of veracevine **27**. These alkaloids are highly poisonous (e. g. $LD_{50}$ for veratridine **25**: 1.35 mg/kg, mouse, intraperitoneal) (49,59b). Veratridine **25** is one of several alkaloids identified as neurotoxins that affect sodium ion channels in excitable membranes (62). An extensive study on the effect of varying the nature of the acyl group, attached to the 3-position (sixty-five 3-acyl derivatives tested) of veracevine **27**, on insecticidal activity and toxicity to mice has been reported. Of all the derivatives, the naturally occurring veratridine **25** and cevadine **26** were near optimal in potency (62).

Ryania consists of the powdered roots and stems of the South American plant *Ryania speciosa* Vahl (Flacourtiaceae) and has been used as a contact and stomach poison to insects (49,59b). The active principle is the diterpene ryanodine **28** which is present in the roots with a yield of 0.1-0.25%. It is less photolabile than pyrethrum and its mammalian toxicity is also low ($LD_{50}$: 750 mg/kg, rat, oral) (49). Dehydroryanodine **29** was also isolated from Ryania.

The two compounds were found essentially equipotent and accounted for almost all of the biological activity of Ryania insecticide (61c).




Ryanodine **28**
Dehydroryanodine (9-21: $C=CH_2$) **29**

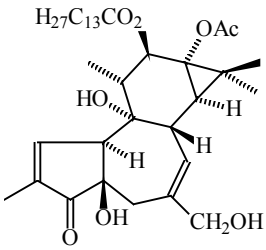



Angulatin A **30**

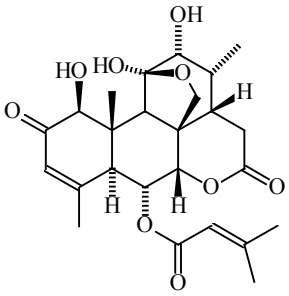
The sesquiterpene polyol ester angulatin A (**30**) is claimed to be strongly antifeedant (and insecticidal) against a variety of insects (64). Angulatin A (**30**) and close antifeedant analogues (65) are derived from the root bark of *Celastrus angulatus* Max. (Celastraceae), which is used, in China, to protect plants from insects (59b).

From the seeds of *Croton tiglium* L.(Euphorbiaceae) was isolated the tigliane diterpene ester **31** which showed both growth inhibitory and insecticidal activities in diet against newly hatched larvae of *Pectinophora gossypiella* (100% kill at 20 ppm) (66). It also gave 100% kill of *Culex pipiens* larvae at 0.6 ppm but was ineffective against *Oncopeltus fasciatus* and *Tribolium confusum*. Esters of this type are also well-known for their vesicant and tumor-promoting properties (61b,66).

Several quassinoid drugs have been used for a long time for their tonic and insecticidal properties (27). A series of antileukemic and cytotoxic quassinoids, isolated from *Simaba multiflora* Juss. and *Soulamea soulameoides* (Gray) Nooteboom (Simaroubaceae), were evaluated in diets for their growth-inhibitory and insecticidal activities against the tobacco budworm *Heliothis virescens* and for their antifeedant activity against *H. virescens* and the fall armyworm *Spodoptera frugiperda* (67). The most effective quassinoid was 6α-senecioyloxychaparrinone **32** which was equivalent to azadirachtin **24** as a growth inhibitor of newly hatched *H. virescens* but was, at an $LD_{50}$ of 7 ppm, 2.5-3.5 times less toxic (59b).




"Tigliane diterpene ester" **31**




6α-Senecioyloxychaparrinone **32**

## I.2. Rubiaceous Plants in African Traditional Medicine

The Rubiaceae plant family is one of the six largest angiosperm families (Table 1.2) in terms of number of genera and species. Its essentially tropical and its species are mainly woody. Less than 20% of the genera are herbaceous and twenty nine of the thirty eight currently accepted tribes are predominantly woody (68). In East Africa, there are 100 genera and about 600 species (69).

Various natural products occur in rubiaceous plants. Extensive phytochemical investigation has been realized regarding the natural occurrence of iridoids (70), anthraquinones (71), and indole alkaloids (72) in the plant family.

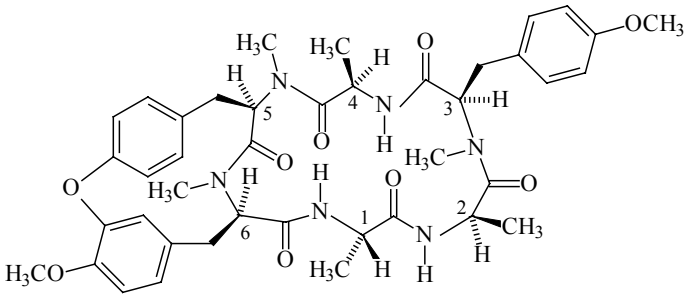
Table 1.2. The six largest angiosperm families, with their number of genera and species (68)

| | genera | Species |
|---|---|---|
| Asteraceae | 1 317 | 21 000 |
| Orchidaceae | 795 | 17 500 |
| Fabaceae | 657 | 16 400 |
| **Rubiaceae** | **637** | **10 700** |
| Poaceae | 737 | 7 950 |
| Euphorbiaceae | 326 | 7 750 |

### I.2.1. Biologically Active Species

Several rubiaceous plant species are widely used in African traditional medicines. *Rubia cordifolia* is widespread in tropical and southern Africa. In the Cape Province (South Africa), a decoction of the leaf and root of the plant is used as a remedy for pleurisy and other inflammatory conditions of the chest. The southern Sotho use a decoction of the root to relieve colic, sore throat and chest complaints, and to wash the teeth (73). The dried roots and rhizomes of the plant are listed officially as a herbal medicine in the Chinese Pharmacopeia for the treatment of arthritis, dismenorrhea, hematorrhea and hemostasis, as a tonic, and for wound healing (74). In India, it has also been used for menstrual pain, rheumatism, and urinary disorders (75).

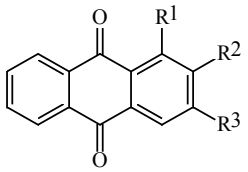
Several quinones (76,77) and anthracenes (78) have been isolated from the plant. In addition, the complex molecule RA-VII **33** is a typical representative of antitumor bicyclic hexapeptides (RAs series) (79) isolated from the dried roots of *Rubia cordifolia*. (80).



RA-VII **33**

*Morinda lucida* Benth is a commonly used medicinal plant in Africa. Its stem bark decoction is used for the treatment of asthma and bronchitis, whereas an infusion of the leaf is used as antiseptic (81). In Central Africa, the decoction of leaves is used as diuretic, and the decoction of the roots as purgative. In West Africa, the roots are used as remedy for fever and malaria, and the leaf as an astringent and as an application to ulcers (73,82). Bark extracts of the plant showed strong but short-lasting hypotensive effects, along with sedative effect (83). However, the alcoholic stem wood extract exhibited a remarkably strong hypertensive effect (84). The plant also inhibited significantly, in a dose-dependent manner, the growth of the malarial parasite *Plasmodium falciparum*, *in vitro* (85). The antimalarial (86,87) and the antileishmanial (87) activities of the stem bark and the roots of *M. lucida* have been related to their anthraquinone **34-36** contents. The most potent anthraquinones were found to be the 2-carboxyaldehyde substituted ones (87).

Another widely used medicinal plant is *Pentanisia prunelloides* Walp. In Southern Africa, the plant has been used for a long time for the treatment of rheumatism, fever, venereal diseases, and as anthelminthic in infants. It is also used for relieving pain in the chest (73). In the Zulu traditional medicine (South Africa), it is believed that the plant is effective in relieving inflammation, bacterial and viral infections and also stimulating uterine contraction.



| | $R^1$ | $R^2$ | $R^3$ | |
|---|---|---|---|---|
| **34** | H | CHO | OH | |
| **35** | $OCH_3$ | CHO | OH | Damnacanthal |
| **36** | $OCH_3$ | $CH_3$ | OH | Rubiadin-1-methyl ether |

aqueous, the ethanolic, and ethyl acetate extracts of leaves and roots showed cyclooxygenase-1 inhibition in an anti-inflammatory assay, together with inhibition of viral replication of Influenza A virus (88). A crude decoction of the plant also exhibited direct stomach muscle activity on rat uterus and ileum preparation (89).

## I.2.2. Studies on Plant Species of the Genus *Pentas*

The genus *Pentas* comprises about 40 species, widely distributed throughout tropical Africa from West Africa and Somali Republic to Angola and Natal (South Africa), also in tropical Arabia, Madagascar and Comoro Islands (69,90).

Many species of this genus are widely used by local people as medicinal plants. In Kenya, a decoction of the roots of *Pentas bussei* is taken as a remedy for gonorrhoea, syphilis and dysentery. Roots of *Pentas decora* are used as a cure for pimples (the roots are normally pounded, mixed with some ghee and rubbed on the pimples). *Pentas hindsioides* is used as a remedy for scabies (pounded roots and leaves are soaked in warm water for bathing), while *Pentas micrantha* is used as a cough remedy (fresh roots chewed or boiled or pounded and soaked in water and the infusion drunk). The juice of the pounded leaves of *Pentas zanzibarica*, mixed with a little water, is drunk as a drastic purgative, whereas a decoction of the roots is taken as a remedy for gonorrhoea and syphilis or given to children as a tonic (91).

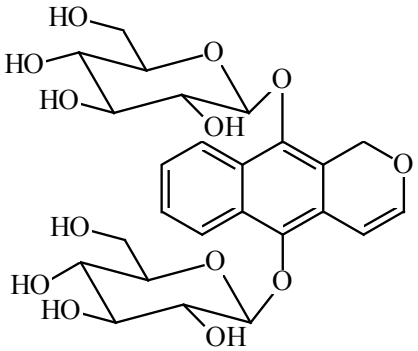
Roots of *Pentas longiflora* are used as a cure both for tapeworm, itchy rashes and pimples; a decoction of the roots is mixed with milk and taken as a cure for malaria. In Rwanda, the powder of the roots of this plant mixed with butter is used as an ointment to treat skin diseases, e.g. scabies and pityriasis versicolor (91,92a). A decoction of the roots of *Pentas purpurea*, mixed with sugar cane, is used by Tanzanian women to initiate the menstruation,

whereas the juice of the plant is taken as a remedy for headache, fever and rheumatic pains. The plant is reported in Kenya to be toxic to sheep. The juice of the leaves of another *Pentas sp.*, mixed with a small amount of water, is taken as a drastic purgative in East Africa (73).

*I.2.2.1. Phytochemical Investigation of Pentas longiflora* Oliver.

*Pentas longiflora* Oliver has been the first species from the genus *Pentas* to be investigated. Two major constituents, mollugin **38** and pentalongin **39** have been isolated from the root bark of a plant sample collected in Burundi (93). Mollugin **38** was already isolated from two other rubiaceous plant species, *Galium molluga* (94) and *Rubia cordifolia* (95).

An alcoholic root extract of *P. longiflora* (in Rwanda) is used as an ointment to cure *Pityrias versicolor* and the bioassay guided-fractionation led to the isolation of pentalongin **39** as active principle (92). Pentalongin **39** also exhibited an antifungal activity against *Pityrosporum ovale*, an algicidal activity against freshwater green algae *Chemydomonas sphagmophilla* var. *dysosmos* and *Chlorella vulgaris*, and marine algae *Phaeodactylum tricornutum* and *Porphyridium purpureum*. The compound also showed antibacterial activity against freshwater blue-green bacterium *Anabaena cylindrica* (96). A pentalongin hydroquinone diglycoside, harounoside **37** (97), has been isolated from *Mitracarpus scaber* Zucc (Rubiaceae), a plant used in African traditional medicine (Niger) for antifungal and antiparasital activity (98).




**37** Harounoside

Pentalongin **39** has been synthesised by a photochemical [2+2] addition of 2-chloro-1,4-naphthoquinone and acrolein dimethyl acetal and subsequent treatment of the resulting 1-

dimethoxymethyl-1,2-dihydrocyclobuta[*b*]naphthalene-3,8-dione with *p*-toluenesulfonic acid (99). The total synthesis of the compound was also successfully performed by various strategies (100). The photochemical reactions of pentalongin **39** under sunlight conditions (in various organic solvents) met with difficulties in duplication in several hands. Therefore, efforts have been performed to elaborate suitable syntheses.

A detailed phytochemical study of *P. longiflora*, which was aiming at the identification of minor constituents and new physiologically active compounds, led to the isolation of several compounds belonging to different chemical families (Figure 1.1): naphthoquinones, anthraquinones, coumarins, and steroids (96).

In addition to mollugin **38** and pentalongin **39**, six other known naphthoquinones were isolated from the plant (96): psychorubrin **40** (100,101), 3-hydroxymollugin **41** (76a), *trans*-3,4-dihydroxy-3,4-dihydromollugin **42** (76a), 3-methoxymollugin **43** (76a), and the prenylated naphthoquinones, methyl 2,3-epoxy-3-prenyl-1,4-naphthoquinone-2-carboxylate **44** (76a,102) and methyl 3-prenyl-1,4-naphthoquinone-2-carboxylate **45** (103). The plant also yielded three novel naphthoquinones, the dimeric derivative of pentalongin **39**, i.e. (3α,3′α,4β,4′β)-3,3′- dimethoxy-*cis*-[4,4′-*bis*-(3,4,5,10-tetrahydro-1*H*-naphtho[2,3-*c*]pyran)]-5,5′,10,10′-tetraone **46** (104), the tetracyclic isagarin **47** (105,106), and *cis*-3,4-dihydroxy-3,4-dihydromollugin **48** (104). Isagarin **47** has been successfully synthesised (106) as shown in Scheme 1.1.

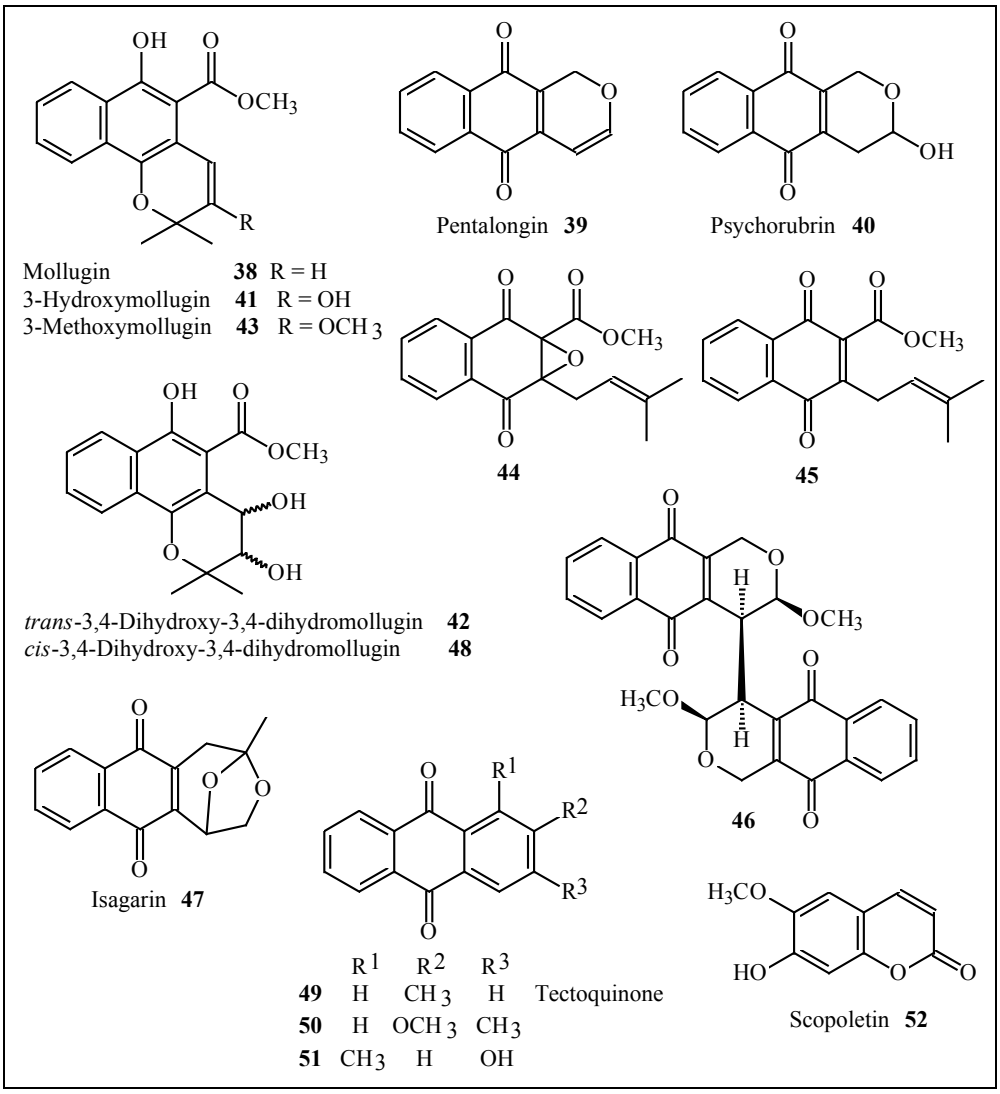
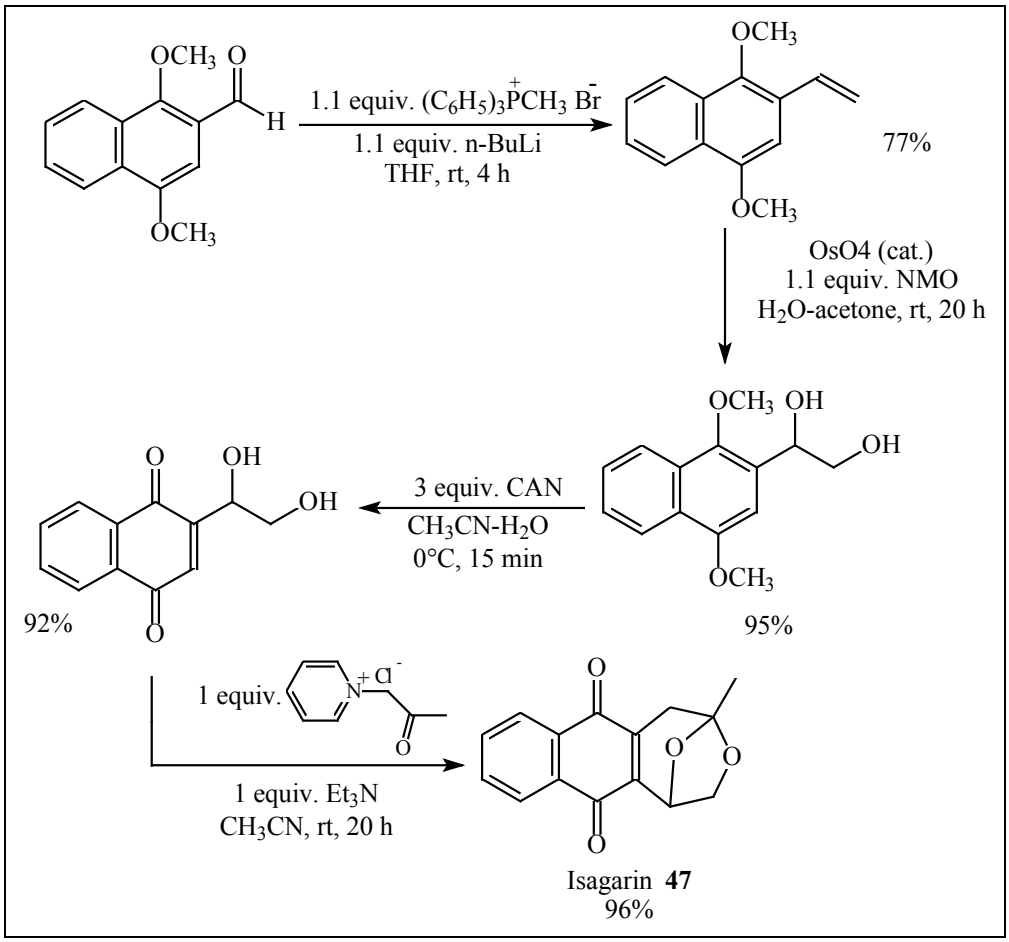



Figure 1.1. Natural Products Isolated from the Roots of *Pentas longiflora*

Three anthraquinones were isolated from the plants during the same study (96), the known tectoquinone **49** (102) and 2-methoxy-3-methyl-9,10-anthraquinone **50** (107), together with the new 3-hydroxy-1-methyl-9,10-anthraquinone **51**. However, literature studies (108,109) revealed a possible erroneous substituent positioning for the latter anthraquinone, and therefore its structure elucidation needs to be reinvestigated.

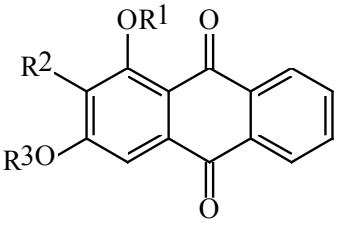
The coumarin scopoletin **52** (110), the two steroids *ß*-stigmasterol (111) and *ß*-sitosterol (111), together with stearic acid were also isolated from *Pentas longiflora* (96).

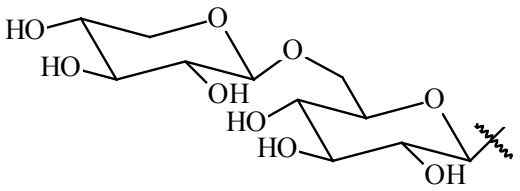



**Scheme 1.1**: Synthesis of Isagarin **47** (ref. 106)

*I.2.2.2. Phytochemical Investigation of Pentas zanzibarica* (Klotsch) Vatke

The methanol extract of stems of *P. zanzibarica* (from Congo) has been studied (112) and yielded the anthraquinones rubiadin **53**, damnacanthal **35** and rubiadin-1-methyl ether **36**, together with the anthraquinone glycosides rubiadin-3-*O*-*β*-primeveroside **54**, rubiadin-1-methyl ether 3-*O*-*β*-primeveroside **55**, lucidin-3-*O*-*β*-primeveroside **56** and damnacanthol-3-*O*-*β*-primeveroside **57**.




Prim (= primeverose)

| | $R^1$ | $R^2$ | $R^3$ | |
|---|---|---|---|---|
| **35** | H | CHO | H | Damnacanthal |
| **36** | $CH_3$ | $CH_3$ | H | Rubiadin-1-methyl ether |
| **53** | H | $CH_3$ | H | Rubiadin |
| **54** | H | $CH_3$ | Prim | Rubiadin-3-*O*-*β*-primeveroside |
| **55** | $CH_3$ | $CH_3$ | Prim | Rubiadin-1-methylether-3-*O*-*β*-primeveroside |
| **56** | H | $CH_2OH$ | Prim | Lucidin-3-*O*-*β*-primeveroside |
| **57** | $CH_3$ | $CH_2OH$ | Prim | Damnacanthol-3-*O*-*β*-primeveroside |

Figure 1.2. Anthraquinones and Anthraquinone Glycosides Isolated from the Stems of *Pentas zanzibarica*

## I.3. Purpose and Goal of the Present Research

The motivations of the present study lie in some important facts which can briefly be described as follow:

The phytochemistry of the important *Pentas* species is only scarcely investigated. From the about 40 known species, only *P. longiflora* (96) and *P. zanzibarica* (112) have been studied.

These species display a broad range of uses in traditional medicine, particularly in Africa (73,91,92). These medicinal uses constitute an important indication of the presence of various constituents responsible of the biological effects.

The two *Pentas* species already investigated revealed a predominant presence of quinones, compounds known to have a broad spectrum of biological activities. Particular quinone derivatives, which contain a pyran unit in their structures, were isolated from *P. longiflora* (93,96)

One of the latter compounds was pentalongin **39**, which has served as a "lead" compound for the synthesis of new compounds having antibiotic activity (100).

Based on the above considerations, the present research was undertaken to achieve the double purpose of 1) investigation of unstudied *Pentas* species and 2) search for novel naturally occurring compounds which may be used as "lead" for the synthesis of more potentially active compounds. Three *Pentas* species have been chosen for the study: *P. bussei* K. Krause, *P. lanceolata* (Forsk.) Deflers and *P. parvifolia* Hiern.

In addition, in order to confirm their structural assignment, the synthesis of two new natural products isolated from *P. longiflora* (96), i.e. *cis*-3,4-dihydroxy-3,4-dihydromollugin **48** and 3-hydroxy-1-methylanthraquinone **51**, will be performed.

## I.4. Materials and Methods

### I.4.1. Plant Materials

The roots of *Pentas bussei* K. Krause were collected at Shimba Hills, Coast Province (Kenya) at an altitude of 1-450 m on May 15, 1999. The roots of *Pentas lanceolata* (Forsk.) Deflers were collected at Chiromo (Nairobi, Kenya) at an altitude of 1520-3000 m on May 5, 1999. The two plants were identified by S. G. Mathenge (Botany Department, University of Nairobi, P. O. Box 30197, Nairobi, Kenya). The roots of *Pentas parvifolia* Hiern were collected in the Carnivore plains (Nairobi, Kenya) at an altitude of 650-2400 m on April 25, 1999. The plant was identified by F. P. Mudida (TRAMEDA, P. O. Box 66514, Nairobi, Kenya). The voucher herbarium specimen of the three plants (LVP-PB, LVP-PL and LVP-PP) were deposited at the herbarium of the Department of Botany, Faculty of Sciences, Ghent University. The roots were dried in a ventilated oven at 40 °C during 5 days and powdered mechanically.

### I.4.2. Instrumentation

Medium Pressure Liquid Chromatography (MPLC) was executed with a Büchi system consisting of a 688 chromatography pump (maximum pressure: 40 bars), a 687 gradient former, borosilicate glass columns (various sizes), and a 684 fraction collector (Büchi, Switzerland), together with a SEDEX 55 evaporative light scattering detector (LSD) (S.E.D.E.R.E, France). Silica gel 60 (0.015-0.040 mm for column chromatography; Merck) was used as normal phase, whereas LiChroprep RP-18 ( LiChroprep RP-18 for liquid chromatography, 40-63 µm particle size; Merck) was used as reversed phase column material. Preparative Centrifugal Partition Chromatography (CPC) was carried out on a CCC-1000 High Speed Countercurrent Chromatograph (Pharma-Tech Research Corp., Baltimore, Maryland, USA) equipped with SSI 300 pump, a Pharmacia LKB Uvicord S II detector (254 and 280 nm) and a Retriever II fraction Collector.

Analytical High Pressure Liquid Chromatography (HPLC) was executed on a Kontron instrument equipped with a single 422 pump (maximum pressure: 350 bars), and a 430 UV detector (Kontron, Germany). Preparative HPLC was carried out on a Kontron instrument

equipped with two 422 pumps (maximum pressure: 200 bars), a 430 UV detector (Kontron, Germany), and a Retriever II fraction collector.

Silica gel plates 60 $F_{254+366}$, 20 x 20 cm (Merck) were used for analytical TLC, whereas preparative TLC was performed on Silica gel PLC plates 60 $F_{254+366}$, 20 x 20 cm, 2 mm (Merck). Analytical reversed phase (RP-18 $F_{254}$) plates, 20 x 20 cm (Merck), were also used for TLC monitoring of highly polar fractions.

Büchi Rotavapor R-124 systems, connected to diaphragm vacuum pumps, type MZ 2C, were used for evaporation of the solvents. For powdering the plant material, a Retsch GmbH, type SK1, 1100 watt, 2840 u/min, DR 80B/2Q, was used. Solvents for large scale extraction and chromatographic purification were from Merck (Germany) or Rathburn Chemicals Ltd (Welkerburn, Scotland). Extraction was executed by sonication with a 8210E-DTH ultrasonic bath (Branson Ultrasonic Corporation, CT 06813, USA).

NMR spectra were recorded on a JEOL-JNM-EX 270 MHz FT NMR spectrometer (270 MHz for $^{1}H$ NMR, 67.5 MHz for $^{13}C$ NMR) (JEOL, Japan). The HMBC NMR data were obtained with a Bruker Avance DRX-500 spectrometer (500 MHz for $^{1}H$ NMR, 125 MHz for $^{13}C$ NMR) or a JEOL-DELTA-ECP400 NMR spectrometer (JEOL, Japan). Deuterated NMR solvents were obtained from Aldrich or Acros.

Direct inlet mass spectroscopy (for the EI-MS) was carried out on a Varian MAT 112 mass spectrometer (70 eV). ESIMS was performed using the LC-MS technique with mass spectra recorded on a Waters ZMD spectrometer coupled to an LC system using a Waters Alliance 2690 separation module with 996 PDA detector, a Waters Xterra MS $C_{18}$ (50 x 4.6 mm, i.d., 2.5 μm) column with a gradient of 10 mM $HCOONH_4$ (0.1% HCOOH)/$CH_3CN$ (0.1% HCOOH) from 85:15 to 100:0 (%) for 5 min, and a flow rate of 1.2 mL $min^{-1}$, maximum pressure: 300 bars. Another system alternately used for ESIMS was composed of an Agilent 1100 Series HPLC system coupled with an Agilent LC-MS mass detector (VL type). The HPLC was executed on a Phenomenex Luna $C_{18}$(2) (250 x 4.6 mm, i. d., 5μm) column and elution performed under a gradient mode with 0.5% (w/v) $NH_4OAc$ (in $H_2O$, w/v)/$CH_3CN$, stepwise: 100% of 0.5% $NH_4OAc$ (in $H_2O$) for 2 min, a gradient of 0.5% $NH_4OAc$ (in $H_2O$,

w/v)/$CH_3CN$ from 100:0 to 0:100 (%) for 15 min, and 100% of $CH_3CN$ for 5 min; flow rate, 1.5 mL $min^{-1}$; maximum pressure 300 bars.

The IR spectra were obtained using a Perkin-Elmer Spectrum One™ instrument using Spectrum™ software and the UV spectra was recorded on a Varian Cary 50 probe spectrophotometer. The optical rotation was obtained on an AA-10 automatic polarimeter (l = 1 dm). The melting point measurement was carried out with a Büchi melting point apparatus.

I.4.3. Partial Hydrolysis of Anthraquinone Glycosides on TLC

Anthraquinone glycosides were applied on silica gel TLC plates (HPTLC plates, 10 x 10 cm, silica gel 60 $F_{254}$) and left in a HCl atmosphere at room temperature for 3 hours. HCl vapours were eliminated under hood ventilation (30 minutes), and authentic sugars were applied to the plates. The TLC plates were then developed with $CHCl_3$/MeOH/$H_2O$ (8:5:1) and spots detected by spraying with aniline/diphenylamine/$H_3PO_4$/MeOH (1:1:5:48), followed by heating in an oven at 110 °C for 30 minutes.

**Chapter II.**

# STUDY OF THE ROOTS OF *PENTAS BUSSEI* K. Krause (Rubiaceae)

# Chapter II. STUDY OF THE ROOTS OF *PENTAS BUSSEI*

## II.1. Introduction

*Pentas bussei* K. Krause is a woody herb or shrub, about 0.5-4 m high, sometimes scrambling, leaves ovate, base cuneate, apex acute or acuminate, 3.5-15 by 1.5-5 cm, stems with sparse to dense white or brown hairs above, usually drying ferruginous or yellowish, white-velvety beneath. Flowers red, in axillary and terminal many-flowered cymes; corolla tube 7-20 mm, lobes 3-10 mm long. Fruiting inflorescences with individual branches; fruit oblong, 3-6 mm long. The plant was collected in Kenya where it is mainly located in coastal bushland, forest margins and thickets (69,113).

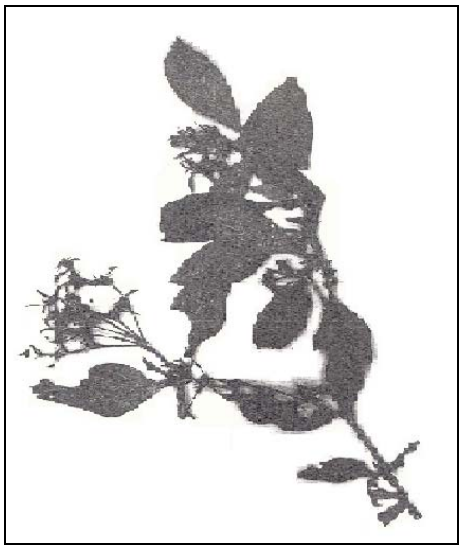


Figure 2.1. *Pentas bussei* K. Krause

In this area, where it is called "Mdobe" or "Mudobe" in a local dialect ("Digo"), a decoction of the roots of the plant is taken as a remedy against gonorrhoea, syphilis and dysentery (91). In a screening of antitumor cyclic hexapeptides in some rubiaceous plant, aerial parts and roots of *P. bussei* revealed no presence of these compounds (114). No other phytochemical study of this species is reported in the literature.

## II.2. Experimental

### II.2.1. Extraction and Purification

#### *II.2.1.1. Extraction*

The ground dried roots of the plant (613.80 g) were extracted exhaustively (x 3) with *n*-hexane, dichloromethane ($CH_2Cl_2$), ethyl acetate (EtOAc) and methanol (MeOH), respectively. A volume of 0.75 litres of each solvent was used for each extraction. The solvent was removed and the sample dried before the extraction with a new solvent started. Extraction was executed under sonication at room temperature. The extracts were filtered and concentrated by evaporation under reduced pressure on a rotavapor at 40 °C to afford a dark green *n*-hexane residue (7.52 g, 1.23 % yield), a dark $CH_2Cl_2$ residue (16.65 g, 2.71 % yield), a dark EtOAc residue (1.74 g, 0.28 % yield), and a dark brown MeOH residue (31.27 g, 5.09 % yield).

#### *II.2.1.2. Study of the n-Hexane Extract of the Roots of Pentas bussei*

The *n*-hexane extract (7,52 g, 1.23 % yield) was chromatoghraphed over a silica gel column in a MPLC system (Büchi) with elution using a *n*-hexane/$CH_2Cl_2$/MeOH gradient (Table 2.1).

Table 2.1. Programme for separation of the *n*-hexane extract of the roots of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| *n*-Hexane/$CH_2Cl_2$ | 90:10 | 90:10 | ---- | 5 |
| *n*-Hexane/$CH_2Cl_2$ | 70:30 | 70:30 | ---- | 10 |
| *n*-Hexane/$CH_2Cl_2$ | 60:40 | 0:100 | 10% | 200 |
| $CH_2Cl_2$/MeOH | 95:5 | 80:20 | 5% | 220 |
| $CH_2Cl_2$/MeOH | 70:30 | 70:30 | ---- | 50 |
| MeOH (clean up) | 100 | 100 | ---- | 50 |

The MPLC conditions were: column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 53.4 g of silica gel and packed in a 230 x 36 mm i.d. sample column; flow rate 75 ml/min at a back pressure < 40 bar; detection, LSD, 42 °C, 2 bar $N_2$ flow; collection, 65 seconds per fraction. A total of 26 different fractions (Table 2.2) were obtained after monitoring by TLC.

Table 2.2. MPLC fractionation of the *n*-hexane extract of the roots of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 1:1 | 1-11 | 91.9 |
| 1:2 | 12-21 | 33.3 |
| 1:3 | 22-38 | 65.8 |
| 1:4 | 39-75 | 74.5 |
| 1:5 | 76-84 | 46.5 |
| 1:6 | 85-92 | 87.7 |
| 1:7 | 93-101 | 132.3 |
| 1:8 | 102-105 | 79.5 |
| 1:9 | 106-127 | 894.2 |
| 1:10 | 128-133 | 181.7 |
| 1:11 | 134-143 | 174.4 |
| 1:12 | 144-192 | 286.6 |
| 1:13 | 193-224 | 165.4 |
| 1:14 | 225-264 | 101.3 |
| 1:15 | 265-283 | 128.1 |
| 1:16 | 284-299 | 208.1 |
| 1:17 | 300-311 | 89.5 |
| 1:18 | 312-323 | 91.5 |
| 1:19 | 324-333 | 27.7 |
| 1:20 | 334-361 | 832.5 |
| 1:21 | 362-375 | 745.4 |
| 1:22 | 376-383 | 85.6 |
| 1:23 | 384-413 | 339.1 |
| 1:24 | 414-448 | 1234.3 |
| 1:25 | 449-475 | 748.6 |
| 1:26 | 476-494 | 458.2 |

II.2.1.2.1. Isolation of Methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate **58**

Fractions 1:8 to 1:11 (1.33 g, 0.23 % yield) were combined together and rechromatographed over a normal phase MPLC (Table 2.3) to afford, after monitoring by TLC, 9 fractions (Table 2.4). MPLC conditions were: column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 8.94 g of silica gel and packed in a 230 x 26 mm i.d. sample column; flow rate 70 ml/min at a back pressure < 40 bar; detection, LSD; collection 48 seconds per fraction.

Table 2.3. Programme for separation of fractions 1:8-1:11 of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| *n*-Hexane/EtOAc | 100:0 | 100:0 | ---- | 50 |
| *n*-Hexane/EtOAc | 98:2 | 98:2 | ---- | 50 |
| *n*-Hexane/EtOAc | 95:5 | 95:5 | ---- | 50 |
| EtOAc/MeOH (clean up) | 90:10 | 90:10 | ---- | 50 |

Table 2.4. MPLC fractionation of fractions 1:8-1:11 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 2:1 | 1-6 | 38.5 |
| 2:2 | 7-46 | 27.2 |
| 2:3 | 47-85 | 485.6 |
| 2:4 | 86-98 | 95.0 |
| 2:5 | 99-127 | 311.8 |
| 2:6 | 128-158 | 247.6 |
| 2:7 | 159-191 | 30.8 |
| 2:8 | 192-215 | 22.9 |
| 2:9 | 216-250 | 35.6 |

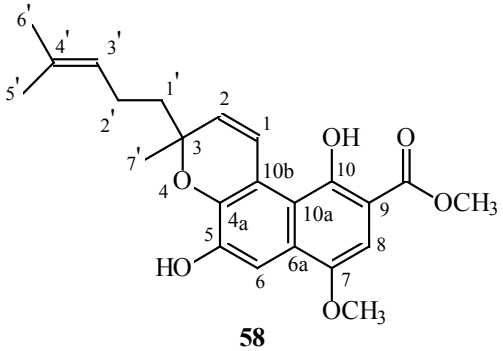
Fractions 2:3 to 2:6 (1.14 g, 0.19 % yield) were combined together and submitted to MPLC using *n*-hexane/EtOAc (98:2) in isocratic mode to yield 3 fractions (Table 2.5). The MPLC conditions were: column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 7.66 g of silica gel and packed in a 230 x 26 mm i.d. sample column; flow rate 60 ml/min at a back pressure < 40 bar; detection, LSD; elution for 75 min; collection 45 seconds per fraction.

Table 2.5. MPLC fractionation of fractions 2:3-2:6 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 3:1 | 1-19 | 251.4 |
| 3:2 | 20-34 | 127.5 |
| 3:3 | 35-60 | 680.1 |

Fraction 3:3 (680.1 mg, 0.11 % yield) was purified by preparative TLC with *n*-hexane/acetone (9:1) as eluent. A band with a $R_f$ 0.32 was colored in yellow and blue under UV light at 254 and 365 nm, respectively. The band was scraped off and the compound removed from the adsorbent by extraction with acetone. Further purification by preparative HPLC on a RP18 column yielded a pure product (350.6 mg, 0.057 % yield). The analytical HPLC conditions were: column, a Kromasil C18, 5µm, 10 x 0.4 cm i.d., Teknokroma (Barcelona); elution, gradient mode with $H_2O/CH_3CN$ (30:70 to 0:100); flow rate, 1 ml/min; detection, UV 254 nm. The preparative HPLC conditions were: column, a Kromasil C18, 5 µm, 25 x 2 cm i.d., Teknokroma (Barcelona); elution, isocratic mode with $H_2O/CH_3CN$ (12.5:87.5); flow rate, 15 ml/min corresponding to a back pressure ≈ 176 bar; detection, UV 254 nm; collection 36 seconds per fraction. The sample was dissolved in 15 ml of $H_2O/CH_3CN$ (5:95) and filtered through 0.45 µm filter (Acrodisc 13 CR PTFE, 13 mm, Gelman) prior to injection. A sample volume of 3 ml was injected. The running time (elution) was 45 min and 5 injections were executed for all the samples. The eluates from 17.2-20.0 min were mixed together and dried under reduced pressure to afford the new compound **58** as a yellow solid.

Based on the analysis of the spectral and physical data, the compound was identified as methyl-5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate **58**.




**58**

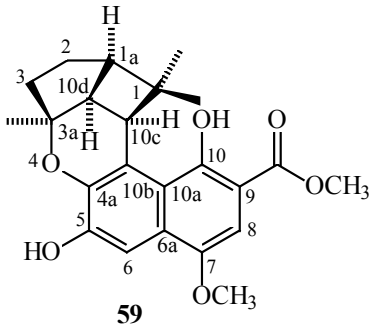
**Methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate 58**: Yellow solid (from hexane); mp 89.9-92.1 °C; $[\alpha]^{21}_{D}$ +34.8° (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 252.0 (4.63), 255.0 (4.57), 272.0 (4.62), 283.0 (4.62), 328.0 (4.28) nm; IR $\nu_{max}$ (KBr) 3455 (OH), 2960, 2919, 2848, 1727, 1632, 1593, 1455, 1359, 1273 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 270 MHz) δ (ppm) 12.26 (1H, s, OH-10), 8.02 (1H, d, *J* = 10.4 Hz, H-1), 7.60 (1H, s, H-6), 6.92 (1H, s, H-8), 6.05 (1H, s, OH-5), 5.66 (1H, d, *J* = 10.4 Hz, H-2), 5.04-5.14 (1H, br t, *J* = 7.1 Hz, H-3'), 3.97 (3H, s, COOC$\underline{H}_3$-9), 3.91 (3H, s, OC$\underline{H}_3$-7), 2.08-2.22 (2H, m, $H_2$-2'), 1.76-1.86 (2H, m, $H_2$-1'), 1.66 (3H, s, $H_3$-6'), 1.57 (3H, s, $H_3$-5'), 1.47 (3H, s, $H_3$-7'); $^{13}$C NMR ($CDCl_3$, 67.5 MHz) δ (ppm) 171.98 ($\underline{C}OOCH_3$-9), 157.60 (C-10), 147.30 (C-5), 146.96 (C-7), 140.97 (C-4a), 131.96 (C-4'), 127.81 (C-2), 127.18 (C-8), 123.82 (C-3'), 123.31 (C-1), 117.35 (C-10b), 115.98 (C-10a), 105.33 (C-6), 103.53 (C-9), 99.59 (C-8), 78.95 (C-3), 55.78 (O$\underline{C}H_3$-7), 52.15 (COO$\underline{C}H_3$-9), 40.33 (C-1'), 25.61 (C-7'), 25.56 (C-6'), 22.78 (C-2'), 17.62 (C-5'); ESIMS *m*/*z* 399 $[M+H]^+$ (73), 367 (100), 355 (12), 315 (74), 283 (13), 279 (19), 257 (12); HREIMS *m*/*z* 399.1735 (calcd for $C_{23}H_{26}O_6$ + H, 399.1808).

II.2.1.2.2. Isolation of Methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a,10c,10d-hexahydro-1*H*-4-oxacyclobuta[3,4]indeno[5,6-*a*]naphthalene-9-carboxylate **59**

Fraction 3:2 (127.5 mg, 0.021 % yield) was purified by preparative TLC with *n*-hexane/ acetone (9:1) as eluent. A blue (under UV light at 254 and 365 nm) band with a $R_f$ 0.41 was scraped off and the compound removed from the adsorbent by extraction with acetone. Further purification by HPLC on a RP18 column yielded a pure product (22.4 mg, 0.0036 % yield). The analytical HPLC conditions were: column, a Kromasil C18, 5µm, 10 x 0.4 cm i.d., Teknokroma (Barcelona); elution, gradient mode with $H_2O/CH_3CN$ (30:70 to 10:90); flow rate, 1 ml/min; detection, UV 254 nm. The preparative HPLC conditions were: column, a Kromasil C18, 5 µm, 25 x 2 cm i.d., Teknokroma (Barcelona); elution, isocratic mode with $H_2O/CH_3CN$ (15:85); flow rate, 12 ml/min corresponding to a back pressure ≈ 170 bar; detection, UV 254 nm; collection 40 seconds per fraction; running time, 60 min. The sample was dissolved in 3 ml of $H_2O/CH_3CN$ (5:95) and filtered with 0.45 µm filter (Acrodisc 13 CR PTFE, 13 mm, Gelman) prior to injection. The eluates from 26.0-29.3 min were mixed together and dried under vacuum to obtain pure compound **59**.

Based on its spectral and physical data, the compound was identified as methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a,10c,10d-hexahydro-1*H*-4-oxacyclobuta[3,

4]indeno[5,6-*a*]naphthalene-9-carboxylate **59**. The compound is a new naphthohydroquinone of the cyclol-type.




**59**

**Methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a, 10c,10d-hexahydro-1*H*-4-oxacyclobuta[3,4]indeno[5,6-*a*]naphthalene-9-carboxylate 59**: Yellow crystals (from hexane), mp 158.0-159.4 °C; $[\alpha]^{20}_{D}$ = +120.0° (*c* 1.15, $CHCl_3$); ); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 274.0 (4.53), 283.0 (4.56), 302.0 (3.86), 315.0 (3.91), 327.0 (3.76), 370.0 (3.97), 503.0 (2.37), 505.0 (2.37), 515.0 (2.32) nm; IR $\nu_{max}$ (KBr) 3409 (OH), 2953, 2860, 1660, 1626, 1517, 1449, 1365, 1272, 1238, 1153, 1027, 800 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 270 MHz) δ (ppm) 12.21 (1H, s, OH-10), 7.60 (1H, s, H-6), 6.93 (1H, s, H-8), 6.42 (1H, s, OH-5), 4.57 (1H, d, *J* = 9.6 Hz, H-10c), 3.96 (3H, s, $COOC\underline{H}_3$-9), 3.92 (3H, s, $OC\underline{H}_3$-7), 2.70 (1H, dd, $J_1$ = 9.6 Hz, $J_2$ = 9.2 Hz, H-10d), 2.49 (1H, ddd, $J_1$ = 9.2 Hz, $J_2$ = 7.9 Hz, $J_3$ = 4.4 Hz, H-1a), 2.00-2.07 (1H, m, $H_2$-3(1)), 1.64-1.80 (3H, m, $H_2$-2 and $H_2$-3(2)), 1.49 (3H, s, $H_3$-1α), 1.36 (3H, s, $H_3$-3a), 0.54 (3H, s, $H_3$-1β); $^{13}$C NMR ($CDCl_3$, 67.5 MHz) δ (ppm) 172.22 ($\underline{C}OOCH_3$-9), 157.90 (C-10), 148.91 (C-5), 146.79 (C-7), 141.51 (C-4a), 127.81 (C-6a), 123.47 (C-10b), 119.08 (C-10a), 102.68 (C-6), 102.32 (C-9), 99.53 (C-8), 85.41 (C-3a), 55.78 ($O\underline{C}H_3$-7), 52.09 ($COO\underline{C}H_3$-9), 46.68 (C-1a), 41.83 (C-1), 41.28 (C-10d), 40.59 (C-3), 38.98 (C-10c), 33.95 ($CH_3$-1α), 25.55 ($CH_3$-3a), 25.28 (C-2), 19.17 ($CH_3$-1β); ESIMS *m/z* 399 $[M+H]^+$ (100), 367 (60), 315 (42), 289 (19), 277 (16).

II.2.1.2.3. Isolation of Methyl 8-hydroxy-1,4,6,7-tetramethoxy-2-naphthoate **60**

Fractions 1:20 to 1:26 were mixed together (4443.70 mg, 0.72 % yield), split into two equal parts (2221.85 mg, each) and each part submitted to a reversed phase separation with MPLC conditions (Table 2.6) to give 3 fractions (Table 2.7) monitored by TLC (RP18 plates). MPLC conditions were: column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample (each part) adsorbed on 12 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate 60 ml/min at a back pressure < 40 bar; detection, LSD; collection 50 seconds per fraction).

Table 2.6. Programme for separation of fractions 1:20-1:26 of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| Water/MeOH | 50:50 | 0:100 | 10% | 160 |

Table 2.7. MPLC fractionation of fractions 1:20-1:26 of *P. bussei*.

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 4:1 | 38-101 | 998.6 |
| 4:2 | 102-168 | 938.4 |
| 4:3 | 169-192 | 2217.0 |

Fraction 4:1 (998.6 mg, 0.16 % yield) was subjected to CPC using *n*-hexane/EtOAc/MeOH/ $H_2O$ (3:7:5:5) as biphasic solvent system and under the following conditions: column 120 ml x 3; elution mode, head to tail, the upper phase as the stationary phase and the lower phase as mobile phase; flow rate 1 ml/min with a pressure ~ 90 psi; revolution speed, 1014 rpm; equilibrium volume, 97 ml; sample loop, 14 ml; detection, UV 254 nm and 280 nm; sample collection, 5 min per fraction; push out at 320 min with a flow rate of 4 ml/min collected at 16 ml per tube; the sample was divided into 3 equal parts and chromatographed separately. The separation gave rise to 8 fractions listed in Table 2.8.

Table 2.8. CPC fractionation of fraction 4:1 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 5:1 | 10-22 | 604.8 |
| 5:2 | 23-33 | 126.1 |
| 5:3 | 34-52 | 27.6 |
| 5:4 | 53-65 | 21.4 |
| 5:5 | 66-69 | 28.2 |
| 5:6 | 70-75 | 46.2 |
| 5:7 | 76-79 | 92.6 |
| 5:8 | 80-91 | 26.0 |

Fractions 5:6 and 5:7 (138.80 mg, 0.023 % yield) were mixed together and rechromatographed over a reversed phase MPLC (Table 2.9) to afford 4 fractions (Table 2.10). MPLC conditions used were: columns 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 1 g of RP18 and packed in a 230 x 26 mm i.d. sample column; flow rate 24 ml/min at back pressure < 40 bar; detection LSD; collection 25 seconds per fraction.

Table 2.9. Programme for separation of fractions 5:6-5:7 of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 30:70 | 0:100 | 10% | 90 |

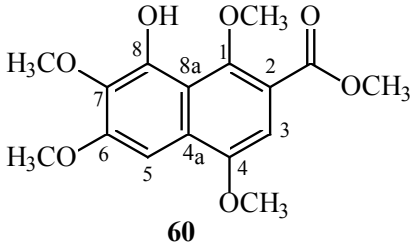
Table 2.10. MPLC fractionation of fractions 5:6–5:7 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 6:1 | 1-22 | 10.3 |
| 6:2 | 23-47 | 54.3 |
| 6:3 | 48-96 | 57.0 |
| 6:4 | 97-216 | 15.8 |

Fraction 6:2 (54.3 mg, 0.0089 % yield) was subjected to preparative TLC using *n*-hexane/ EtOAc (4:1) as eluent. Two bands were observed. The first band (blue under UV 254 and 365 nm) with *Rf* 0.59 was scraped off and the compound removed from the adsorbent by extraction with EtOAc. Solvent removal by evaporation at reduced pressure yielded a white solid (19.7 mg, 0.00321% yield). The compound was additionally purified by HPLC on a RP18 column to afford a pure product (7.4 mg, 0.00120% yield). The analytical HPLC

conditions were the following: column, a Kromasil C18, 5µm, 10 x 0.4 cm i.d., Teknokroma (Barcelona); elution, gradient mode with $H_2O$/MeOH (50:50 to 10:90); flow rate, 1 ml/min; detection, UV 254 nm. The preparative HPLC conditions were: column, a Kromasil C18, 5 µm, 25 x 2 cm i.d., Teknokroma (Barcelona); elution, isocratic mode with $H_2O$/MeOH (25:75); flow rate, 14 ml/min corresponding to a back pressure ≈ 185 bar; detection, UV 254 nm; collection 45 seconds per fraction; running time, 120 min. The sample was dissolved in 3 ml $H_2O/CH_3CN$ (5:95) and filtered with 0.45 µm filter (Acrodisc 13 CR PTFE, 13 mm, Gelman) prior to injection. The eluates from 52.3-56.7 min were mixed and dried under vacuum to afford compound **60** as a reddish solid.

Based on its spectral data and physical properties, the compound was identified as methyl 8-hydroxy-1,4,6,7-tetramethoxy-2-naphthoate **60**, which is a new compound.

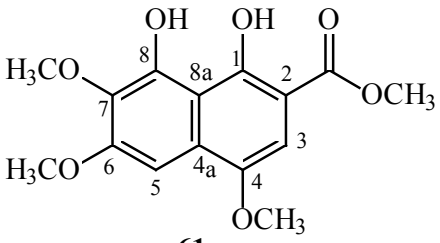



**60**

**Methyl 8-hydroxy-1,4,6,7-tetramethoxy-2-naphthoate 60**: Amorphous reddish solid; mp 167.9-169.4 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 231.4 (4.19), 260.2 (4.67), 295.5 (3.68), 308.7 (3.45), 323.2 (3.52), 379.7 (3.88), 501.8 (2.94), 514.4 (2.79) nm; IR (KBr) $\nu_{max}$ 3318 (OH), 3020, 2930, 2855, 1715 (C=O), 1630 (C=C), 1607(C=C), 1514, 1472, 1429, 1369, 1218, 1137, 1109, 1058, 762 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) δ (ppm) 9.85 (1H, s, OH-8), 7.14 (1H, s, H-5), 7.11 (1H, s, H-3), 4.02 (3H, s, OC$\underline{H}_3$-1), 4.00 (3H, s, OC$\underline{H}_3$-6), 3.98 (3H, s, COOC$\underline{H}_3$-2), 3.97 (3H, s, OC$\underline{H}_3$-7), 3.96 (3H, s, OC$\underline{H}_3$-4); $^{13}C$ NMR (67.5 MHz, $CDCl_3$) δ (ppm) 165.99 ($\underline{C}OOCH_3$-2), 155.22 (C-6), 152.36 (C-1), 150.62 (C-8), 147.99 (C-4), 135.05 (C-7), 126.56 (C-4a), 115.11 (C-8a), 113.52 (C-2), 103.59 (C-3), 93.40 (C-5), 64.56 (O$\underline{C}H_3$-1), 60.81 (O$\underline{C}H_3$-7), 55.92 (O$\underline{C}H_3$-6), 55.81 (O$\underline{C}H_3$-4), 52.34 (COO$\underline{C}H_3$-2); ESIMS *m/z* 323 $[M+H]^+$ (7), 291 (100).

II.2.1.2.4. Isolation of Methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61**

Fraction 6:3 (57.0 mg, 0.0093 %) was recrystallised in MeOH to yield red needles. Analysis of the spectral data and the physical properties of the compound allowed to identify it as being the new highly oxygenated naphthohydroquinone methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61**. The compound is a novel natural product.




**61**

**Methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate 61**: Reddish needles (from MeOH); mp 175.8-176.8 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 230.0 (4.25), 261.9 (4.56), 296.0 (3.64), 308.0 (3.66), 322.1 (3.60), 379.1 (3.97), 502.1 (2.81), 514.0 (2.80) nm; IR (KBr) $\nu_{max}$ 3341 (OH), 2946, 2837, 1655 (C=O), 1638 (C=C), 1620 (C=C), 1522, 1480, 1450, 1434, 1376, 1337, 1285, 1258, 1227, 1191, 1137, 1119, 1060, 1047, 1003, 794, 603 $cm^{-1}$; $^{1}H$ NMR (270 MHz, $CDCl_3$) δ (ppm) 12.95 (1H, s, OH-1), 9.67 (1H, s, OH-8), 7.06 (1H, s, H-5), 6.82 (1H, s, H-3), 3.98 (3H, s, OC$\underline{H}_3$-6), 3.97 (3H, s, COOC$\underline{H}_3$-2), 3.95 (3H, s, OC$\underline{H}_3$-7), 3.92 (3H, s, OC$\underline{H}_3$-4); $^{13}C$ NMR (67.5 MHz, $CDCl_3$) δ (ppm) 171.71 ($\underline{C}OOCH_3$-2), 156.04 (C-6), 155.79 (C-1), 149.45 (C-8) 147.13 (C-4) 135.25 (C-7), 127.81 (C-4a), 110.17 (C-8a), 101.79 (C-2), 99.67 (C-3), 93.74 (C-5), 60.75 (O$\underline{C}H_3$-7), 55.92 (O$\underline{C}H_3$-6), 55.61 (O$\underline{C}H_3$-4), 52.42 (COO$\underline{C}H_3$-2); ESIMS *m/z* 309 $[M+H]^+$ (8), 277 (100).

II.2.1.2.5. Isolation of 9-Methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol **62**

On the one hand, fraction 5:1 (604.8 mg, 0.099 % yield) (Table 2.8) was subjected to reversed phase MPLC (Table 2.11) yielding 4 fractions (Table 2.12) monitored by TLC (on RP18 plates). MPLC conditions were: column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 4.0 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate, 32 ml/min at back pressure < 40 bar; detection LSD; collection 45 seconds per fraction.

Table 2.11. Programme for separation of fraction 5:1 of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 50:50 | 50:50 | ---- | 10 |
| $H_2O$/MeOH | 40:60 | 0:100 | 10% | 100 |

Table 2.12. MPLC fractionation of fraction 5:1 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 7:1 | 1-33 | 34.4 |
| 7:2 | 34-40 | 15.6 |
| 7:3 | 41-92 | 458.6 |
| 7:4 | 93-147 | 82.0 |

On the other hand, fraction 4:2 (938.4 mg, 0.15 % yield) (Table 2.7) was submitted to CPC using *n*-hexane/EtOAc/MeOH/$H_2O$ (3:7:5:5) as biphasic solvent system to afford 4 fractions (Table 2.13) monitored by TLC (RP18 plates). The CPC conditions were: column 120 ml x 3; elution mode, head to tail, the upper phase as the stationary phase and the lower phase as mobile phase; flow rate 1 ml/min with a pressure ~ 85 psi; revolution speed, 1023 rpm; equilibrium volume, 106 ml; sample loop, 14 ml; detection, UV 254 nm and 280 nm; sample collection, 5 min per fraction; push out at 300 min with a flow rate of 4 ml/min collected at 16 ml per tube; the sample was divided into 3 equal parts which were chromatographed separately.

Table 2.13. CPC fractionation of fraction 4:2 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 8:1 | 13-19 | 139.5 |
| 8:2 | 20-39 | 342.9 |
| 8:3 | 47-54 | 19.7 |
| 8:4 | 59-82 | 410.1 |

Based on their identity (monitoring by TLC on RP18 plates), fractions 7:3, 7:4, 8:1 and 8:2 were mixed together (1023.0 mg, 0.17 % yield) and submitted to a reversed phase MPLC under isocratic elution mode utilizing $H_2O$/MeOH (3:7) as mobile phase to give rise to 3 fractions (Table 2.14) monitored by TLC (RP18 plates). MPLC conditions were: column 460

x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 7.2 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate, 28 ml/min at a back pressure ≈ 30 bar; detection LSD; collection 45 sec per fraction; duration, 90 min.

Table 2.14. MPLC fractionation of fraction 7:3-7:4 and 8:1-8:2 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 9:1 | 1-34 | 139.3 |
| 9:2 | 35-64 | 601.6 |
| 9:3 | 65-120 | 206.8 |

Fraction 9:2 (601.6 mg, 0.098 % yield) was rechromatographed on a normal phase MPLC eluting with EtOAc/MeOH under a gradient mode (Table 2.15) to afford 4 fractions (Table 2.16) monitored by TLC (RP18 plates; eluent: $H_2O$/MeOH (2:3)). MPLC conditions were: column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 4 g of silica gel and packed in a 230 x 36 mm i.d. sample column; flow rate 40 ml/min at a back pressure < 40 bar; detection LSD; collection, 15 seconds per fraction.

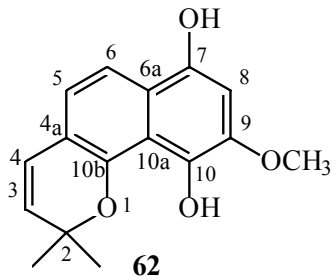
Table 2.15. Programme for separation of fraction 9:2 of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| EtOAc/MeOH | 100:0 | 70:30 | 10% | 60 |
| EtOAc/MeOH | 60:40 | 60:40 | ---- | 30 |

Table 2.16. MPLC fractionation of fraction 9:2 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 10:1 | 1-137 | 34.5 |
| 10:2 | 138-176 | 135.3 |
| 10:3 | 177-188 | 21.5 |
| 10:4 | 189-360 | 69.2 |

Based on its spectral data and its physical properties, fraction 10:2 (135.3 mg, 0.022 % yield), which eluted at $R_t$ = 45-65 min, was identified as 9-methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol **62**.




**62**

**9-Methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol 62**: White solid (from hexane/$(CH_3)_2CO$ (1:1)); mp 201.8-204.0 °C; UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 255.0 (4.12), 360.0 (3.63) nm; IR (KBr) $\nu_{max}$ 3430 (broad, OH), 2974, 1605 (broad), 1459, 1394, 1317, 1264, 1202, 1162, 1138, 1118, 1046, 967, 876, 800, 727 $cm^{-1}$; $^1H$ NMR (270 MHz, $CD_3OD$) δ (ppm) 8.07 (1H, d, *J* = 8.9 Hz, H-5), 7.62 (1H, d, *J* = 10.2 Hz, H-4), 7.36 (1H, s, H-8), 6.90 (1H, d, *J* = 8.9 Hz, H-6), 5.51 (1H, d, *J* = 10.2 Hz, H-3), 3.79 (3H, s, $OC\underline{H}_3$-9), 1.39 (6H, s, 2 x $CH_3$-2); $^{13}C$ NMR (67.5 MHz, $CD_3OD$) δ (ppm) 156.63 ($=C_q$-O), 156.11 (2 x $=C_q$-O), 150.92 (C-9), 129.11 (C-3), 128.12 (C-6a), 127.26 (C-5), 125.62 (C-4), 124.33 (C-10a), 119.67 (C-6), 116.96 (C-4a), 109.56 (C-8), 77.14 (C-2), 57.52 ($O\underline{C}H_3$-9), 28.89 (2 x $CH_3$-2); ESIMS *m*/*z* (rel. int.) 295 $[M + Na]^+$ (9), 255 (100), 141 (15).

II.2.1.2.6. Isolation 9-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]chromene-7,10-diol **63**

Fraction 9:3 (206.8 mg, 0.034 % yield) was submitted to normal phase MPLC using EtOAc/MeOH under a gradient mode (Table 2.17) and the following chromatographic conditions: column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample dissolved in 12 ml of EtOAc and injected into a loop of 15 ml as maximum volume; flow rate, 40 ml at a back pressure < 40 bar; detection LSD; collection 15 sec per fraction. The separation yielded 3 fractions (Table 2.18) monitored by TLC (RP18 plates; eluent: $H_2O$/MeOH (2:3)).

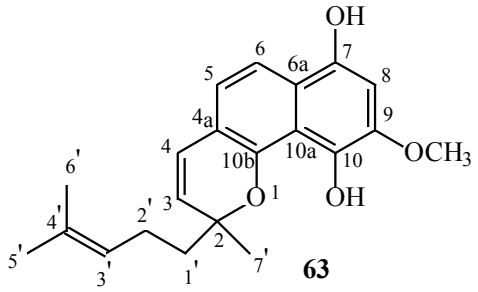
Table 2.17. Programme for separation of fraction 9:3 of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| EtOAc/MeOH | 100:0 | 70:30 | 10% | 60 |

Table 2.18. MPLC fractionation of fraction 9:3 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 11:1 | 1-81 | 45.9 |
| 11:2 | 82-144 | 73.7 |
| 11:3 | 145-240 | 22.0 |

Fraction 11:3 (73.7 mg, 0.012 % yield) eluted at $R_t$ = 38-54 min. Analysis of its spectral data and its physical properties allowed to identify it as 9-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]chromene-7,10-diol **63**.




**63**

**9-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]chromene-7,10-diol 63**: White solid (from hexane/$(CH_3)_2CO$ (1:1)); mp 174.5-176.4 °C; UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 254.1 (4.25), 369.0 (3.70) nm; IR (KBr) $\nu_{max}$ 3500 (broad, OH), 2855, 1595, 1563, 1453, 1428, 1392, 1313, 1259, 1137, 1083, 1046, 814 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 270 MHz) δ (ppm) 8.08 (1H, d, *J* = 8.9 Hz, H-5), 7.72 (1H, d, *J* = 10.4 Hz, H-4), 7.34 (1H, s, H-8), 6.92 (1H, d, *J* = 8.9 Hz, H-6), 5.51 (1H, d, *J* = 10.4 Hz, H-3), 5.09 (1H, broad t, *J* = 7.1 Hz, H-3'), 3.82 (3H, s, OC$\underline{H}_3$-9), 2.11-2.17 (2H, m, $H_2$-2'), 1.66-1.72 (2H, m, $H_2$-1'), 1.62 (3H, s, $H_3$-5'), 1.55 (3H, s, $H_3$-6'), 1.37 (3H, s, $H_3$-7'); $^{13}C$ NMR (67.5 MHz, $CD_3OD$) δ (ppm) 155.54 (=$C_q$-O), 155.07 (2 x =$C_q$-O), 149.74 (C-9), 132.36 (C-4'), 127.06 (C-6a), 126.88 (C-3), 126.05 (C-5), 125.41 (C-3'), 124.78 (C-4), 123.05 (C-10a), 118.36 (C-6), 115.56 (C-4a), 108.26 (C-8), 78.27 (C-2),

56.30 (O<u>C</u>H$_3$-9), 41.63 (C-1'), 26.04 (C-7'), 25.88 (C-5'), 23.84 (C-2'), 17.70 (C-6'), ESIMS *m*/*z* (rel. int.) 341 $[M + H]^+$ (7), 323 (19), 286 (100), 256 (10).

II.2.1.2.7. Isolation of (24*S*)-24-Ethylcholesta-5-(*E*)-22-dien-3-*β*-ol (*β*-Stigmasterol) **64**

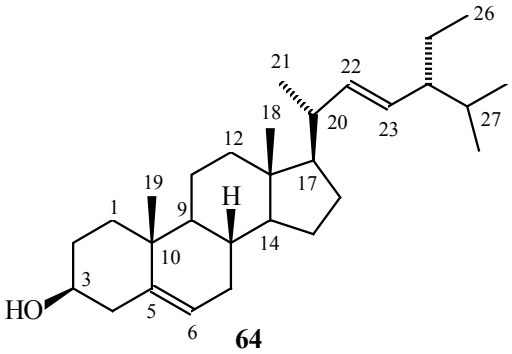
A normal phase MPLC was applied to fractions 1:16 to 1:19 (Table 2.2) mixed together (46.8 mg, 0.068 %), under an isocratic mode elution utilizing *n*-hexane/EtOAc (90:10) as solvent system. MPLC conditions were: column, 460 x 49 mm i.d.; sample adsorbed on 3 g of silica gel and packed in 230 x 36 mm i.d. sample column; flow rate 25 ml/min at a back pressure ≈ 30-35 bar; detection, LSD; collection 60 seconds per fraction; running time, 180 min. The separation yielded 5 fractions (Table 2.19) monitored by TLC.

Table 2.19. MPLC fractionation of fractions 1:16-1:19 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 12:1 | 7-23 | 51.3 |
| 12:2 | 24-35 | 40.7 |
| 12:3 | 36-51 | 125.4 |
| 12:4 | 52-66 | 39.8 |
| 12:5 | 67-180 | 118.6 |

Fraction 12:3 (125.4 mg, 0.020 % yield) was submitted to preparative TLC using *n*-hexane/ acetone (3:2) as eluent. The sample was dissolved in 5 ml of a mixture 1:1 of *n*-hexane and acetone, and spotted on 3 preparative TLC plates, in a line 3 mm wide and 18 cm length at 1.5 cm from the bottom edge of the plate. The compound, wich showed a $R_f$ 0.63, was scraped off and extracted from the silica gel material with $CH_2Cl_2$ followed by filtration. Evaporation under reduced pressure afforded a white solid material (55.2 mg, 0.0090 % yield).

According to its spectral data and physical properties, the compound was identified as (24*S*)-24-ethylcholesta-5-(*E*)-22-dien-3*β*-ol (*β*-stigmasterol) **64**.




**64**

**(24*S*)-24-Ethylcholesta-5-(*E*)-22-dien-3$\beta$-ol ($\beta$-Stigmasterol) 64**: White solid (from hexane); mp 164.0-165.9 °C, lit. mp 164.2-166.0 °C (115); IR $\nu_{max}$ (KBr) 3414 (OH), 2940, 2866, 1546, 1459, 1376, 1051, 712 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 270 MHz) $\delta$ (ppm) 5.35-5.38 (1H, m, =CH), 4.96-5.18 (AB qd, $\Delta\nu$ = 62.7 Hz, $^2J$ = 15.2 Hz, $^3J$ = 8.7 Hz and 8.4 Hz, CH=CH), 3.46-3.61 (1H, m, C<u>H</u>-OH), 2.31-2.35 (1H, m, CH), 2.26 (1H, m, CH), 1.85 (2H, m, $CH_2$), 1.81 (1H, m, CH), 1.04 (3H, d, $J$ = 6.8 Hz), 1.01 (3H, s, $CH_3$), 0.84 (3H, d, $J$ = 6.2 Hz, $CH_3$), 0.81 (3H, t, $J$ = 7.0 Hz, $CH_3$), 0.77 (3H, d, $J$ = 6.4 Hz, $CH_3$), 0.68 (3H, s, $CH_3$); $^{13}C$ NMR ($CDCl_3$, 67.5 MHz) $\delta$ (ppm) 140.75 (=$C_q$), 138.33 (=CH), 129.27 (=CH), 121.72 (=CH), 71.82 (<u>C</u>H-OH), 56.85 (CH), 55.94 (CH), 51.23 (CH), 50.15 (CH), 42.32 ($CH_2$), 42.21 ($C_q$), 40.50 (CH), 39.68 ($CH_2$), 37.25 ($CH_2$), 36.51 ($C_q$), 31.89 ($CH_2$), 31.89 (CH), 31.89 ($CH_2$), 31.66 ($CH_2$), 28.93 ($CH_2$), 25.41 ($CH_3$), 24.37 ($CH_2$), 21.22 ($CH_3$), 21.08 ($CH_3$), 21.08 ($CH_2$), 19.41($CH_3$), 19.03 (CH), 12.26 ($CH_3$), 12.04 ($CH_3$); EIMS *m/z* (rel. int.): 412 ($M^+$; 3%), 380 (2), 299 (3), 255 (52), 240 (20), 227 (21), 179 (46), 163 (36), 143 (88), 133 (65), 121 (77) 109 (53), 91 (76), 83 (98), 69 (75), 55 (100), 43(62).

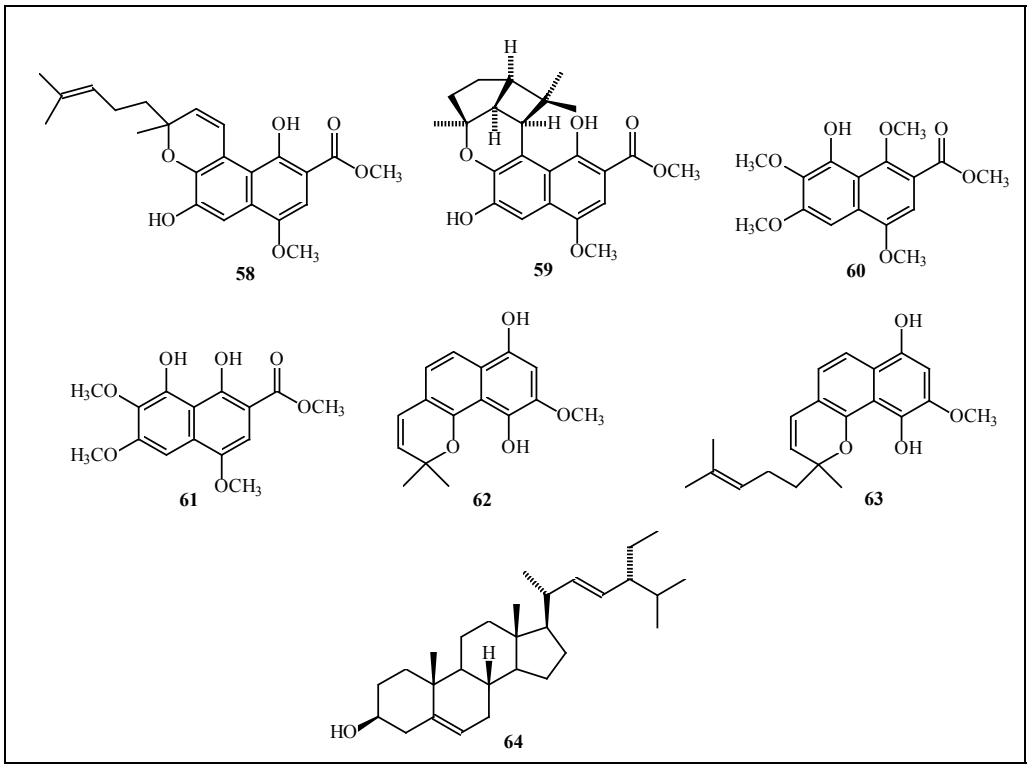



Figure 2.2. Natural Products Isolated from the *n*-Hexane Extract of the Roots of *Pentas bussei*

*II.2.1.3. Study of the Dichloromethane Extract of the Roots of Pentas bussei*

The $CH_2Cl_2$ extract of the roots of *Pentas bussei* (16.65 g, 2.71 % yield) was submitted to a reversed phase MPLC separation under a gradient mode (Table 2.20) to afford 7 different fractions (Table 2.21) monitored by TLC (on RP18 plates). MPLC conditions were: column 460 x 70 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 120 g of RP18, divided into 4 equal parts chromatographed separately and each part packed in a 230 x 36 mm i.d. sample column; flow rate 30 ml/min at a back pressure < 40 bar; detection LSD, collection 80 seconds per fraction.

Table 2.20. Programme for separation of the $CH_2Cl_2$ extract of the roots of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 40:60 | 0:100 | 10% | 175 |

Table 2.21. MPLC fractionation of the $CH_2Cl_2$ extract of the roots of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 13:1 | 7-23 | 662.7 |
| 13:2 | 24-35 | 775.4 |
| 13:3 | 36-51 | 424.1 |
| 13:4 | 52-66 | 3973.8 |
| 13:5 | 67-83 | 3306.2 |
| 13:6 | 84-109 | 1485.0 |
| 13:7 | 110-131 | 5309.9 |

II.2.1.3.1. Isolation 7-Hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromene-8-carboxylic acid **65**

Fractions 13:4 and 13:5 were mixed together (7.28 g, 1.19 % yield), divided into 4 parts (1.82 g per parts) and each part subjected to CPC with *n*-hexane/EtOAc/MeOH/$H_2O$ (1.5:1.5:1:1) as biphasic solvent system. The separation conditions were: column 120 ml x 3; elution mode, head to tail, the upper phase as the stationary phase; flow rate 1 ml/min with a pressure ~ 80 psi; revolution speed, 1060 rpm; equilibrium volume, 72 ml; sample loop, 14 ml; detection, UV 254 nm and 280 nm; sample collection, 4 min per fraction; push out at 245 min with a flow rate of 5 ml/min collected at 16 ml per tube. The separation yielded the three fractions listed in Table 2.22.

Table 2.22. CPC fractionation of fraction 13:4-13:5 of *P. bussei*

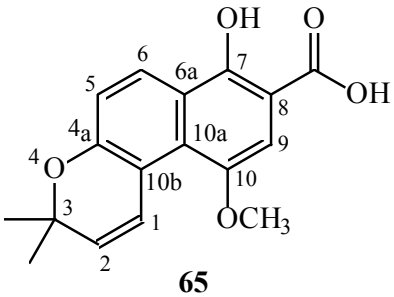
| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 14:1 | 13-23 | 2754.9 |
| 14:2 | 73-88 | 3465.2 |
| 14:3 | 91-105 | 56.3 |

Fraction 14:2 (3465.2 mg, 0.56 % yield) was rechromatographed with an isocratic reversed phase MPLC elution with $H_2O$/MeOH (15:85) to yield 4 fractions (Table 2.23) monitored by TLC (RP18 plates). MPLC conditions were: 2 coupled columns 460 x 49 mm i.d. and 230 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 20 g of RP18 and split into 2 equal parts, each one packed in a 230 x 36 mm i.d. sample column and eluted separately; flow rate 36 ml/min at a back pressure < 40 bar; detection, LSD; collection 65 seconds per fraction, run time 180 min.

Table 2.23. MPLC fractionation of fraction 14:2 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 15:1 | 1-27 | 262.1 |
| 15:2 | 28-72 | 643.8 |
| 15:3 | 73-104 | 950.4 |
| 15:4 | 105-167 | 720.6 |

After solvent evaporation, fraction 15:3 afforded fine yellow crystals of compound **65**. Based on its physical and spectral data, the compound was identified as 7-hydroxy-3,3-dimethyl-10-methoxy-*3H*-benzo[*f*]chromene-8-carboxylic acid **65**.




**65**

**7-Hydroxy-3,3-dimethyl-10-methoxy-*3H*-benzo[*f*]chromene-8-carboxylic acid 65**: Yellow fine crystals (from $CHCl_3$); mp 196.7-198.0 °C; UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 259.1 (4.57), 261.0 (4.57), 373.9 (4.17) nm; IR $\nu_{max}$ (KBr) 3440 (OH), 2974 (broad), 2597, 1648, 1620, 1575, 1509, 1445, 1396, 1301, 1274, 1224, 1201, 1136, 1119, 1041, 891, 869, 785, 737, 716 $cm^{-1}$; $^1H$ NMR (400 MHz, Acetone-$D_6$) δ (ppm) 8.22 (1H, d, $J$ = 8.9 Hz, H-6), 7.75 (1H, dd, $J_1$ = 10.6 Hz, $J_2$ = 0.7 Hz , H-1), 7.17 (1H, s, H-9), 7.11 (1H, dd, $J_1$ = 8.9 Hz, $J_2$ = 0.7 Hz, H-5), 5.70 (1H, d, $J$ = 10.6 Hz, H-2), 3.94 (3H, s, OC$\underline{H_3}$-10), 1.46 (6H, s, 2 x C$\underline{H_3}$-3); $^{13}C$ NMR

(100 MHz, Acetone-$D_6$) δ (ppm) 173.10 (COOH-8), 156.87 (C-7), 155.90 (C-4a), 150.06 (C-10), 128.66 (C-2), 127.69 (C-10a), 126.04 (C-6), 123.46 (C-1), 122.23 (C-6a), 119.31 (C-5), 115.76 (C-10b), 105.16 (C-9), 103.30 (C-8), 76.01 (C-3), 56.15 ($OCH_3$-10), 27.60 (2 x $CH_3$-3); ESIMS *m/z* 301 $[M+H]^+$(81), 271 (100), 257 (7), 199 (14), 146 (7).

In addition, fraction 14:3 afforded compound **61**, while the MPLC-RP18 fractionation of fraction 13:6 (Table 2.21) gave rise to compounds **58**, **62** and **63**. All these compounds were already isolated from the *n*-hexane extract of the roots of *P. bussei* (see description of these compound in Section II.2.1.2).

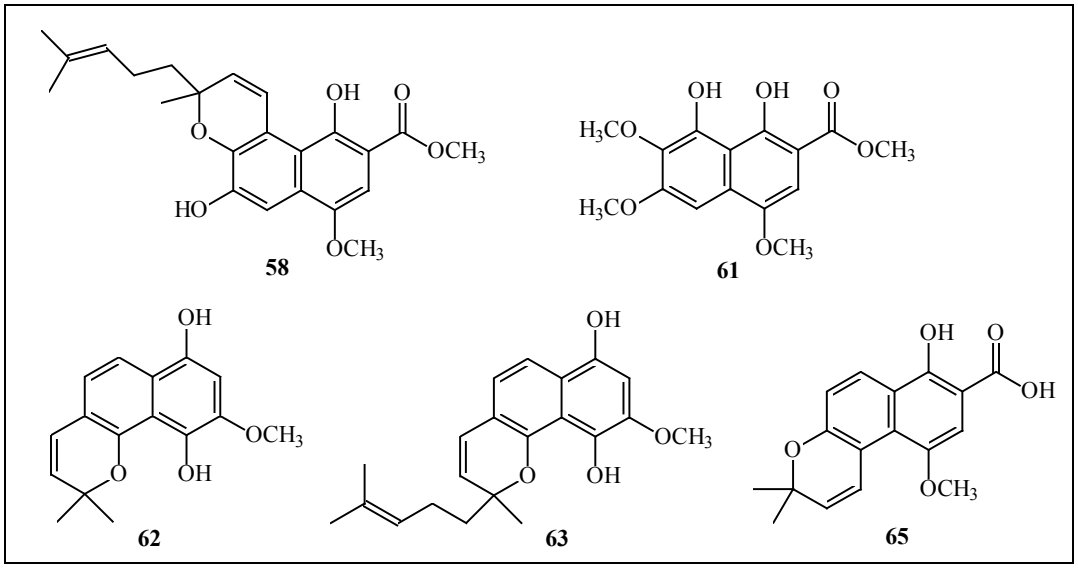



Figure 2.3. Natural Products Isolated from the Dichloromethane Extract of the Roots of *Pentas bussei*

*II.2.1.4. Study of the Ethyl Acetate Extract of the Roots of Pentas bussei*

A reversed phase (RP18) MPLC was applied to the ethyl acetate extract of the roots of *P. bussei* (1740 mg, 0.2834% yield) with elution under a gradient of acetonitrile/water (Table 2.24) and the following conditions: column 460 x 46 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 12 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate 48 ml/min at a back pressure < 40 bar; detection LSD, collection 45 seconds per fraction. The

eluates obtained were combined into 6 different fractions (Table 2.25) depending upon the monitoring on TLC (RP18 plates).

Table 2.24. Programme for separation of the ethyl acetate extract of the roots of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O/CH_3CN$ | 100:0 | 0:100 | 10% | 165 |

Table 2.25. MPLC fractionation of the ethyl acetate extract of the roots of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 16:1 | 36-51 | 197.3 |
| 16:2 | 52-80 | 154.7 |
| 16:3 | 81-130 | 285.6 |
| 16:4 | 131-154 | 183.9 |
| 16:5 | 155-180 | 150.5 |
| 16:6 | 229-243 | 60.5 |

II.2.1.4.1. Isolation of Rubiadin-1-methyl ether 3-*O*-*ß*-primeveroside **55**

Fractions 16:1 and 16:2 were combined together (352.0 mg, 0.057 % yield) and submitted to a reversed phase MPLC eluted with a gradient (Table 2.26) of acetonitrile/water under the following conditions: 2 coupled columns 460 x 26 mm i.d. and 230 x 26 mm i.d.; precolumn, 140 x 10 mm i.d.; sample dissolved in 12 ml of acetonitrile/water (1:9), filtered through a 0.45 µm filter (25 mm syringe filter, PVDF, Alltech Associates, Inc.) and introduced into a 15 ml sample loop; flow rate, 42 ml/min at a back pressure < 40 bar; detection LSD, collection 80 seconds per fraction. The eluates were monitored by TLC (RP18 plates) and combined into 5 different fractions (Table 2.27).

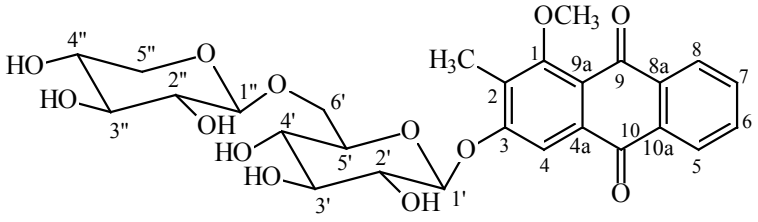
Table 2.26. Programme for separation of fractions 16:1-16:2 of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O/CH_3CN$ | 100:0 | 90:0 | 5% | 90 |

Table 2.27. MPLC fractionation of fractions 16:1-16:2 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 17:1 | 24-38 | 21.2 |
| 17:2 | 82-102 | 69.8 |
| 17:3 | 104-122 | 29.5 |
| 17:4 | 138-148 | 16.5 |
| 17:5 | 160-180 | 51.7 |

After solvent evaporation under reduced pressure, the fraction 17:3 (29.5 mg, 0.0049 % yield) afforded a yellowish solid. Analysis of its physical and spectral data, together with comparison with data available in the literature (112), allowed to elucidate the structure of the compound and to identify it as rubiadin-1-methyl ether 3-*O*-*β*-primeveroside **55**.

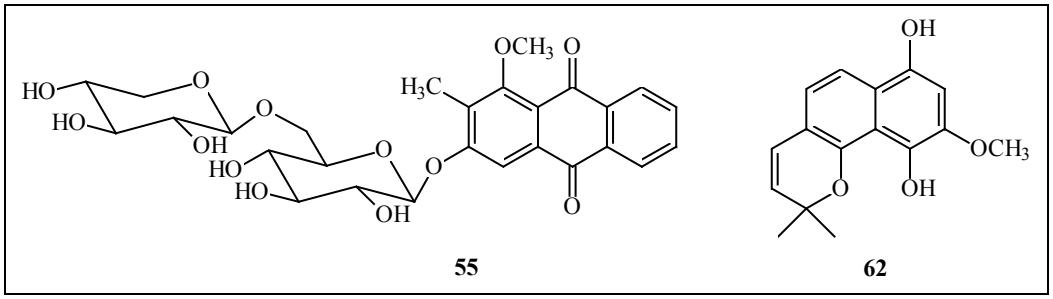



**55**

**Rubiadin-1-methyl ether 3-*O*-*β*-primeveroside 55**: mp 161.1 – 163.0 °C, lit. mp. 158-160 °C (116); $[\alpha]^{22}_D$ = -90 ° (*c* 0.1, $H_2O$); UV ($H_2O$) $\lambda_{max}$ (log $\varepsilon$) 271.0 (4.53), 315.0 (4.09), 349.0 (4.11), 370.0 (4.08), 374.1 (4.07), 422.9 (3.85), 442.0 (3.86) nm; IR $\nu_{max}$ 3342 (br, hydrogen bonded OH), 2929, 1672 (C=O, unchelated), 1582, 1331, 1080 (br, C-O), 779, 714, 602 $cm^{-1}$. $^1H$ NMR ($C_5D_5N$, 270 MHz) δ (ppm) 8.35 (1H, s, H-4), 8.30-8.32 (2H, m, H-5 and H-8), 7.56-7.70 (2H, m, H-6 and H-7), 5.84 (1H, d, *J* = 6.3 Hz, H-1'), 4.93 (1H, broad d, *J* = 6.3 Hz, H-1"), 3.63-3.89 (the rest of the sugar protons), 3.97 (3H, s, $OCH_3$-1), 2.36 (3H, s, $CH_3$-2); $^{13}C$ NMR (67.5 MHz, DMSO-$d_6$) δ (ppm) 182.19 (C-10), 180.52 (C-9), 160.00 (C-3), 159.78 (C-1), 134.53 (C-7), 134.19 (C-10a), 133.80 (C-8a), 133.55 (C-6), 132.00 (C-4a), 128.84 (C-

2), 126.59 (C-8), 126.16 (C-5), 120.05 (C-9a), 108.10 (C-4), 103.93 (C-1"), 100.18 (C-1'), 76.30 (C-3"), 76.01 (C-3'), 75.56 (C-5'), 73.21 (C-2"), 73.06 (C-2'), 69.38 (C-4"), 69.04 (C-4'), 67.92 (C-6'), 65.52 (C-5"), 60.77 ($OCH_3$-1), 9.22 ($CH_3$-2); ESIMS *m/z* 585$[M+Na]^+$ (57), 563 $[M+H]^+$ (18), 431 (31), 381 (5), 269 (100).

In addition, a series of reversed and normal phase MPLC separations was applied to fraction 16:5 (150.5 mg, 0.025 % yield) which resulted in the isolation of the benzochromene **62**, already isolated from the *n*-hexane and the dichloromethane extracts of the roots of *P. bussei*.




**55** **62**

Figure 2.4. Natural Products Isolated from the Ethyl Acetate Extract of the Roots of *P. bussei*

*II.2.1.5. Study of the Methanol Extract of the Roots of Pentas bussei*

A portion of 10.55 g was taken from the methanol extract of the roots of *P. bussei* (31.27 g, 5.095 % yield) and submitted to a CPC with EtOAc/*n*-BuOH/$H_2O$ (2:1:3) as biphasic solvent system under the following conditions: column 320 ml x 3; elution mode, head to tail, the upper phase as the stationary phase and the lower phase as mobile phase; flow rate 3 ml/min with a pressure ~ 80 psi; revolution speed 1065 rpm; equilibrium volume, 250 ml; sample loop, 14 ml; detection, UV 254 nm and 280 nm; sample collection, 2 min per fraction; push out at 360 min with a flow rate of 9 ml/min collected at 18 ml per tube; the sample was divided into 2 equal parts partitioned separately. Based on the TLC monitoring (on RP18 plates), the eluates were combined into 5 different fractions listed in Table 2.28.

Table 2.28. CPC fractionation of the methanol extract of the roots of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 18:1 | 38-45 | 8637.9 |
| 18:2 | 46-69 | 808.4 |
| 18:3 | 92-133 | 132.9 |
| 18:4 | 141-156 | 210.8 |
| 18:5 | 159-165 | 347.2 |

II.2.1.5.1. Isolation of Damnacanthol-3-*O*-*ß*-primeveroside **57**

Fraction 18:2 (808.4 mg, 0.13 % yield) was rechromatographed (MPLC on reversed phase) under a gradient mode (Table 2.29) to afford 4 fractions (Table 2.30) after TLC monitoring on RP18 and combination of identical eluates. MPLC conditions were: column 460 x 36 mm i.d.; sample adsorbed on 6 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate 36 ml/min at a back pressure < 40 bar; detection LSD; collection 40 seconds per fraction.

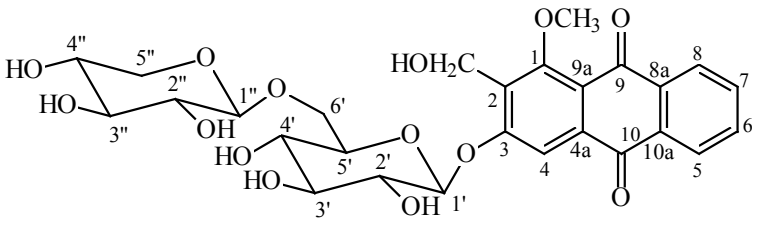
Table 2.29. Programme for separation of fractions 18:2 of *P. bussei* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 80:20 | 60:40 | 10% | 75 |
| $H_2O$/MeOH | 60:40 | 60:40 | ---- | 40 |

Table 2.30. MPLC fractionation of the fraction 18:2 of *P. bussei*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 19:1 | 11-25 | 149.3 |
| 19:2 | 26-47 | 99.8 |
| 19:3 | 84-109 | 181.7 |
| 19:4 | 117-175 | 159.9 |

The solvent of fraction 19:3 (181.7 mg, 0.030 % yield) was evaporated to dryness to give rise to a yellow solid. Its physical and spectral data allowed to identify it as damnacanthol-3-*O*-*ß*-primeveroside **57**. The compound is already described in the literature (112).

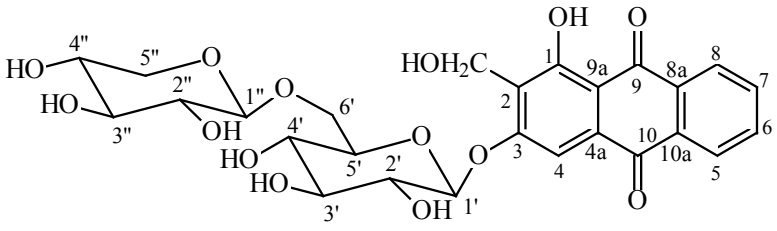


**57**

**Damnacanthol-3-*O*-*ß*-primeveroside 57**: mp 138.4-140.6 °C, lit. mp 140-142 °C (112); $[\alpha]^{16}_{D}$ = -130 ° (*c* 0.1, $H_2O$), lit. $[\alpha]^{20}_{D}$ = -94.8 ° (*c* 0.18, MeOH) (112); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 246.0 (3.50), 266.0 (3.64), 308.0. (2.98), 317.1 (2.99), 323.0 (2.99), 325.0 (3.00), 328.0 (2.99), 374.0 (2.86) nm; IR (KBr) $\nu_{max}$ 3391 (broad, OH groups), 2923, 1673 (unchelated C=O), 1579, 1454, 14091330, 1284, 1229, 1167, 1069 (br, C-O), 900, 715, 612 $cm^{-1}$; $^1H$ NMR (270 MHz, $C_5D_5N$) δ (ppm) 8.42 (1H, s, H-4), 8.26-8.34 (2H, m, H-5 and H-8), 7.22-7.67 (2H, m, H-6 and H-7), 5.77 (1H, d, *J* = 6.6 Hz, H-1'), 5.12-5.17 and 5.22-5.28 (2H, 2 x d, *J* = 11.2 Hz, AB system $CH_2OH$-2), 4.90-4.94 (1H, broad d, *J* = 6.3 Hz, H-1"), 4.17 (3H, s, $OCH_3$-1), 3.68-4.87 (the rest of sugar protons); $^{13}C$ NMR (67.5 MHz, DMSO-$d_6$) δ (ppm) 182.08 (C-10), 180.34 (C-9), 160.59 (C-3), 160.59 (C-1), 135.63 (C-10a), 134.64 (C-7), 134.21 (C-8a), 133.58 (C-6), 131.91 (C-4a), 131.46 (C-2), 126.63 (C-8), 126.22 (C-5), 120.37 (C-9a), 108.98 (C-4), 103.95 (C-1"), 100.77 (C-1'), 76.30 (C-5'), 75.76 (C-3'), 75.60 (C-3"), 73.22 (C-2"), 73.15 (C-2'), 69.42 (C-4'), 69.07 (C-4"), 67.92 (C-6'), 65.53 (C-5"), 62.66 ($O\underline{C}H_3$-1), 51.84 ($\underline{C}H_2OH$-2); ESIMS *m/z* (rel. int.) 285 (13), 283 [M – primeverosyl]$^+$ (7), 267 (12), 253 (100).

II.2.1.5.2. Isolation of Lucidin-3-*O*-*ß*-primeveroside **56**

The above separation (Tables 2.29 and 2.30) also afforded fraction 19:4 (159.9 mg, 0.026 % yield). After solvent evaporation under reduced pressure, an orange red solid was obtained and identified as lucidin-3-*O*-*ß*-primeveroside **56** based on its physical and spectral data, together with the comparison of the latter with data available in the literature (112).



**56**

**Lucidin-3-*O*-*ß*-primeveroside 56**: mp 206.5-208.3 °C, lit. mp 208-210 °C (116); $[\alpha]^{17}_{D}$ = -140 ° (*c* 0.05, $H_2O$); UV ($H_2O$) $\lambda_{max}$ (log $\varepsilon$) 247.0 (4.43), 265.0 (4.49) 362.0 (4.11) nm; IR $\nu_{max}$ 3399 (broad, OH groups), 2895, 1677 (unchelated C=O), 1635 (chelated C=O), 1593, 1526, 1485, 1416, 1372, 1334, 1295, 1229, 1164, 1079 (br, C-O), 1005, 903, 796, 763, 717, 611 $cm^{-1}$; $^1H$ NMR (270 MHz, $C_5D_5N$) δ (ppm) 8.20-8.34 (2H, m, H-5 and H-8), 8.16 (1H, s, H-4), 7.50-7.72 (2H, m, H-6 and H-7), 5.75 (1H, d, *J* = 6.6 Hz, H-1'), 5.17-5.25 and 5.34-5.41 (2H, 2 x d, *J* = 11.5 Hz, AB system $CH_2OH$-2), 4.90-4.94 (1H, broad d, *J* = 6.3 Hz, H-1''), 3.63-4.88 (the rest of sugar protons); $^{13}C$ NMR (67.5 MHz, DMSO-$d_6$) δ (ppm) 187.11 (C-9), 181.70 (C-10), 161.50 (C-3), 160.90 (C-1), 134.68 (C-7), 134.52 (C-6), 133.74 (C-10a), 133.00 (C-8a), 132.81 (C-4a), 126.81 (C-8), 126.45 (C-5), 123.68 (C-2), 111.23 (C-9a), 106.19 (C-4), 103.97 (C-1''), 100.66 (C-1'), 76.30 (C-5'), 75.81 (C-3'), 75.56 (C-3''), 73.19 (C-2'), 73.19 (C-2''), 69.40 (C-4'), 69.04 (C-4''), 67.92 (C-6'), 65.53 (C-5''), 51.36 ($\underline{C}H_2OH$-2); ESIMS *m*/*z* (rel. int.) 547 (22), 269 (100).

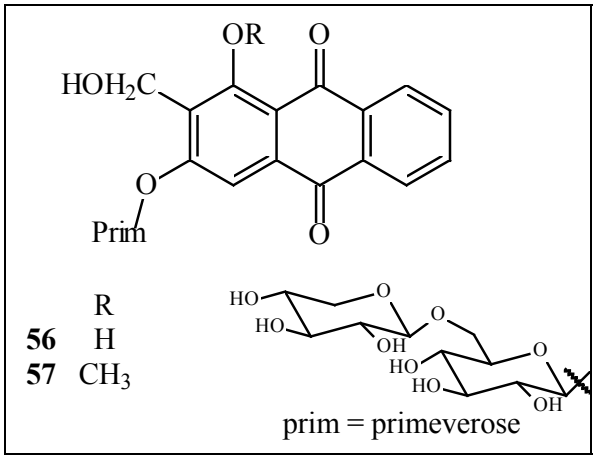
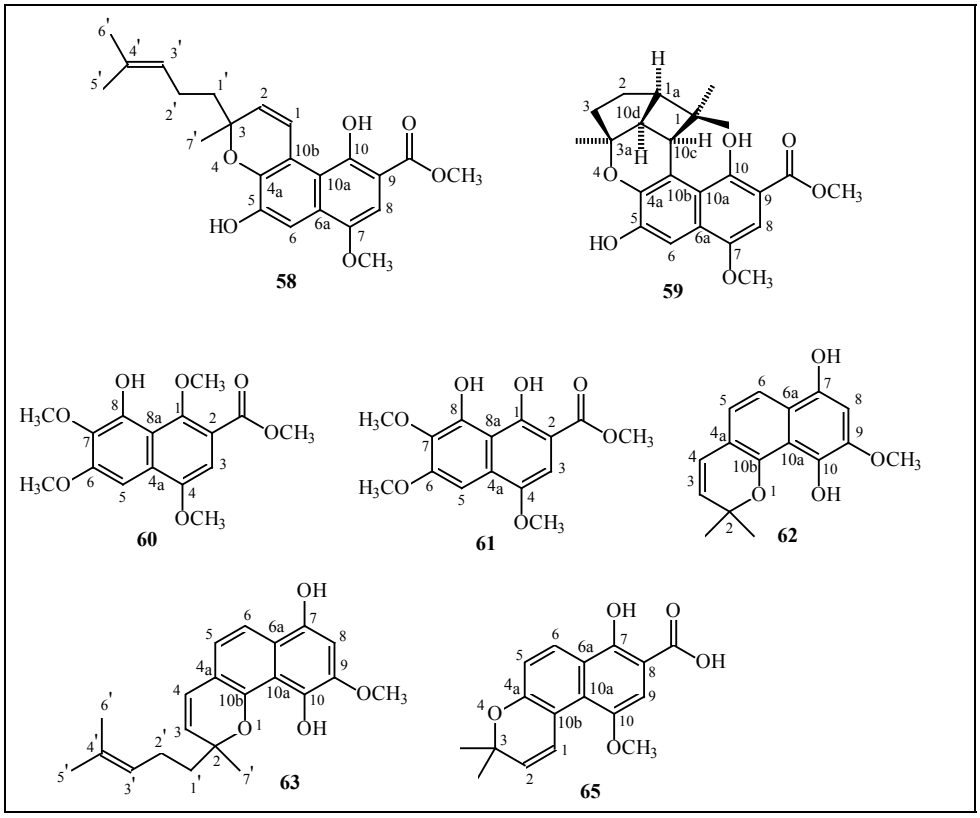



Figure 2.5. Natural Products Isolated from the Methanol Extract of the Roots of *Pentas bussei*

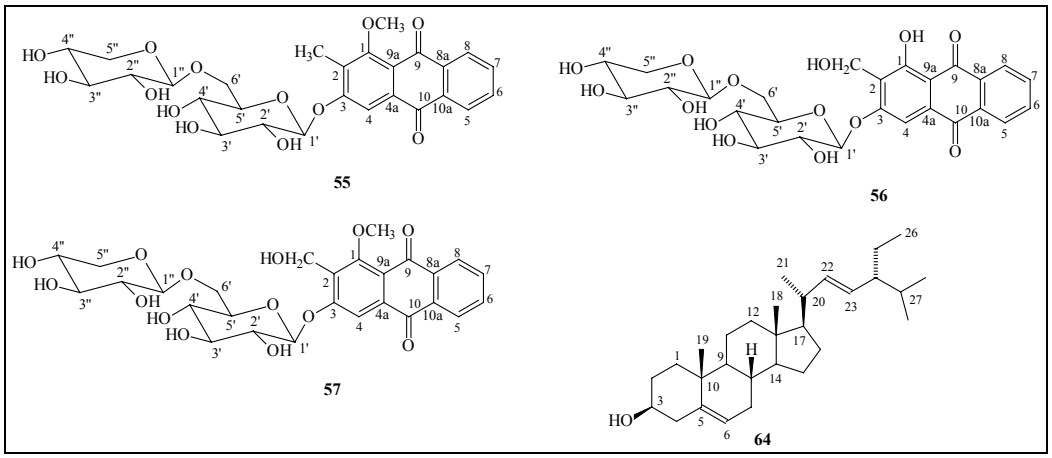
## II.3. Results and Discussion

II.3.1. Novel Natural Products from the Roots of *P. bussei*

The present study is the first investigation of *P. bussei*. Seven novel natural products (**58**-**63** and **65**) were isolated from the roots of the plants, together with four known compounds (**55**-**57** and **64**). Five of the new compounds were naphthohydroquinones of the benzochromene type (**58**-**59**, **62**-**63** and **65**), whereas the remaining two were highly oxygenated naphthohydroquinones (**60**-**61**).




The known compounds are the anthraquinone glycosides rubiadin-1-methyl ether-3-*O*-*ß*-primeveroside **55**, lucidin-3-*O*-*ß*-primeveroside **56** and damnacanthol-3-*O*-*ß*-primeveroside **57**, together with the ubiquitous *ß*-stigmasterol **64**. All the compounds were characterized by spectrometric methods (NMR, UV, IR and MS).

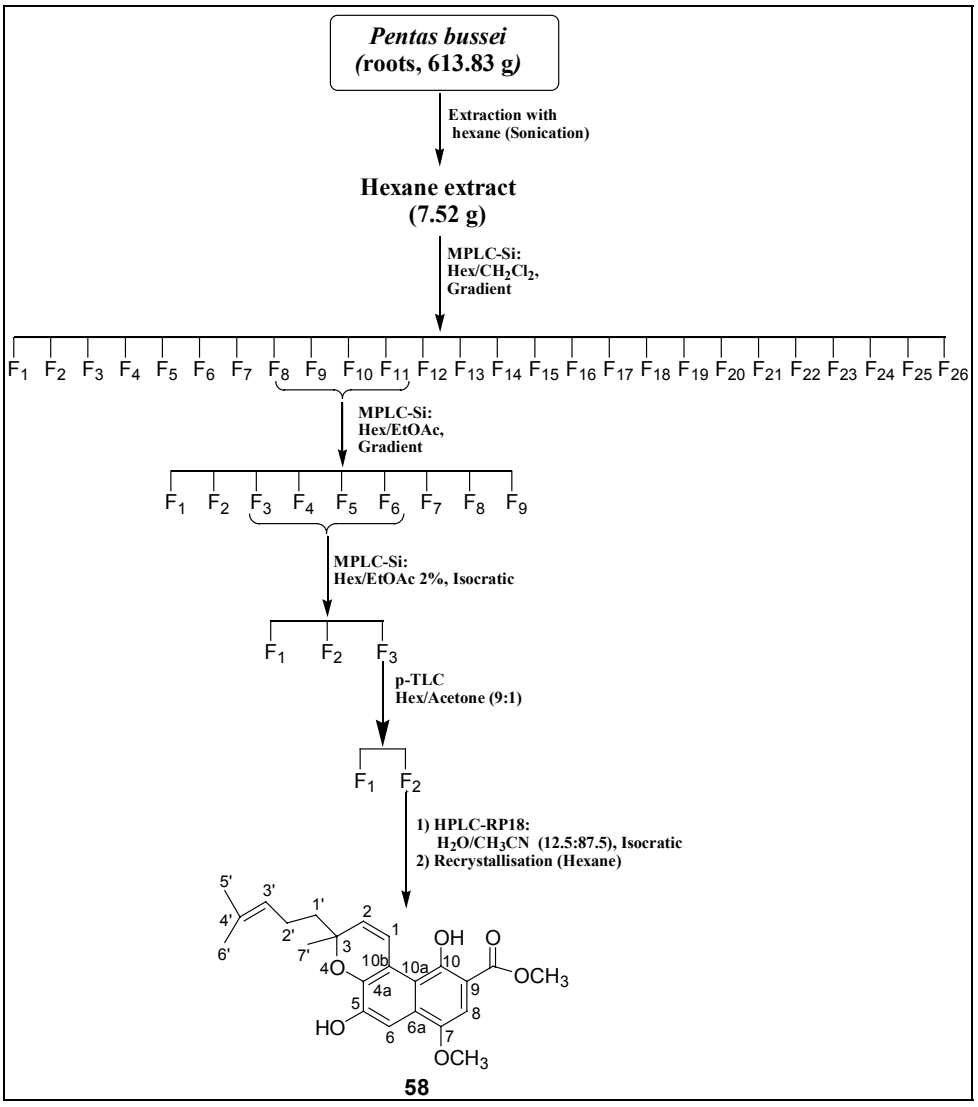



*II.3.1.1. Naphthohydroquinone Derivatives of the Benzochromene Type*

II.3.1.1.1. Methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate **58**

A series of MPLC-Si fractionation of mixed fractions 1:8 to 1:11 (Table 2.2) from the hexane extract of the roots of *P. bussei*, followed by p-TLC and HPLC purification (Scheme 2.1) led to this isolation of compound **58** which is a novel natural product.

**Methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate 58**, yellow solid (from hexane), mp 89.9-92.1 °C. HRMS provided the exact mass at *m/z* 399.1735 (calcd *m/z* 399.1808 $[M + H]^+$), suggesting $C_{23}H_{26}O_6$ as the molecular formula, with an unsaturation index of 11. The $^1H$ NMR, $^{13}C$ NMR and HMBC NMR spectra (Figures 2.6-2.7, Table 2.31) revealed the presence of a 4-methyl-3-pentenyl side chain from the observation of two methyl groups [$\delta_H$ 1.57 (3H, s), $\delta_C$ 17.62, C-5' and $\delta_H$ 1.66 (3H, s), $\delta_C$ 25.56, C-6'], two methylene groups [$\delta_H$ 1.76-1.86 (2H, m), $\delta_C$ 40.33, C-1' and $\delta_H$ 2.08-2.22 (2H, m), $\delta_C$ 22.78, C-2'], and one methine and a quaternary olefinic carbon forming a carbon-carbon double bond with the respective NMR spectral data $\delta_H$ 5.04-5.14 (1H, br t, $J$ = 7.1 Hz), $\delta_C$ 123.82, C-3' and $\delta_C$ 131.96, C-4'. The data were similar to those found in the literature for this alkenyl chain in other compounds (117-120).




**Scheme 2.1**: Isolation of Methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-Pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate **58** from the Roots of *P. bussei*.

Besides, an oxygenated quaternary carbon was shown from the $^{13}C$ NMR spectrum at $\delta_C$ 78.95 (C-3). A *cis* vicinally coupled two-proton olefinic system (as AX coupling system) was observed in the $^{1}H$ NMR spectrum and was confirmed by the $^{1}H$-$^{1}H$ COSY NMR. The two protons resonate at $\delta_{H\text{-}2}$ 5.66 and $\delta_{H\text{-}1}$ 8.02 (1H each, d, $J$ = 10.4 Hz), respectively. The $^{13}C$ NMR and HETCOR spectra showed that their corresponding carbons resonated at $\delta_C$ 123.37 (C-1) and $\delta_C$ 127.81 (C-2). A further methyl group [$\delta_H$ 1.47 (3H, s); $\delta_C$ 25.61, C-7'] and an ester carbonyl carbon ($\delta_C$ 171.98) were also identified.

The long-range HMBC spectrum (Figures 2.8-2.9) showed that H-7', H-1 and H-2, together with H-1' and H-2' all gave a strong coupling with the oxygenated quaternary carbon C-3. This suggested that the side chain must be located at C-3. This was also supported by the observation of a fragment at *m/z* 315 ($M^+$-83) in the ESI mass spectrum corresponding to [M - $(CH_2)_2CH=C(CH_3)_2]^+$, a loss of the side chain by an ether α-cleavage (Scheme 2.2). This type of cleavage has been observed in similar compounds (117). The HMBC correlations showed a strong coupling between H-2 and the aromatic quaternary carbon C-10b ($\delta_C$ 117.35) and between H-1 with the aromatic carbon C-4a ($\delta_C$ 140.97), which was assigned as the oxygenated aromatic carbon C-4a involved in the pyran ring.

In addition, a combination of $^1$H NMR, $^{13}$C NMR, DEPT and HETCOR spectra revealed the presence of two aromatic CH units, respectively [$\delta_H$ 6.92 (1H, s), $\delta_C$ 99.59, C-8 and $\delta_H$ 7.60 (1H, s), $\delta_C$ 105.33, C-6], along with two methoxy groups [$\delta_H$ 3.91 (3H, s), $\delta_C$ 55.78, O$\underline{CH_3}$-7 and $\delta_H$ 3.97 (3H, s), $\delta_C$ 52.15, COO$\underline{CH_3}$-9]. The two remaining signals were deduced as being two hydroxy protons substituted at aromatic rings [$\delta_H$ 6.05 (1H, s), OH-5 and $\delta_H$ 12.26 (1H, s), OH-10]. The presence of a methoxycarbonyl group (COO$CH_3$-9) was established first, from the $^{13}$C NMR spectrum which revealed an ester-like carbonyl carbon at $\delta_C$ 171.98 and, second, by the HMBC spectrum which showed a strong long-range coupling between the methyl protons (COOC$\underline{H_3}$-9) and the carbonyl carbon ($\underline{C}$OO$CH_3$-9).

The locations of this methoxycarbonyl, the two hydroxy groups as well as the methoxy group on the aromatic rings was established by HMBC. The significant deshielding of the OH-10 proton was taken as an indication that this hydroxy group is located in an *ortho* position with respect to the methoxycarbonyl group. Long-range coupling of this proton with the two quaternary aromatic carbons $\delta_C$ 103.53 (C-9) and $\delta_C$ 115.98 (C-10a) confirmed this proposed arrangement. The hydroxyl proton was strongly coupled to a carbon at $\delta_C$ 157.60 which is unequivocally carbon C-10 ($^2J$ long-range coupling) on which the hydroxy group is directly substituted. In addition, long-range coupling of H-8 ($\delta_H$ 6.92) with the carbonyl carbon, C-9, and C-7 gave further evidence for the substitution pattern of H-8. The location of OH-5 was confirmed by a strong coupling of its proton with C-6 and with the oxygenated quaternary carbon C-4a ($\delta_C$ 140.97). The location of C-6 was confirmed by long-range coupling of the proton H-6 with C-4a, C-5, C-7 and C-10a.

The UV spectrum of compound **58** showed particular intense absorption at 272.0 and 283.0 nm, in agreement with a naphthalene chromophore extended by lone-pair donating or by π-bonded systems (121); in addition, IR spectrum showed an OH band (3455 $cm^{-1}$), a band at 1727 $cm^{-1}$ typical to the α,β-unsaturated carbonyl of benzoate, together with bands belonging to conjugated C=C (1632 and 1593 $cm^{-1}$).

In conclusion, based on the analysis of all the spectroscopic data obtained, the structure of compound **58** was established as being the new natural product methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]-chromene-9-carboxylate **58** (122). This is the first time that a compound possessing this type of skeleton is isolated from a plant source.

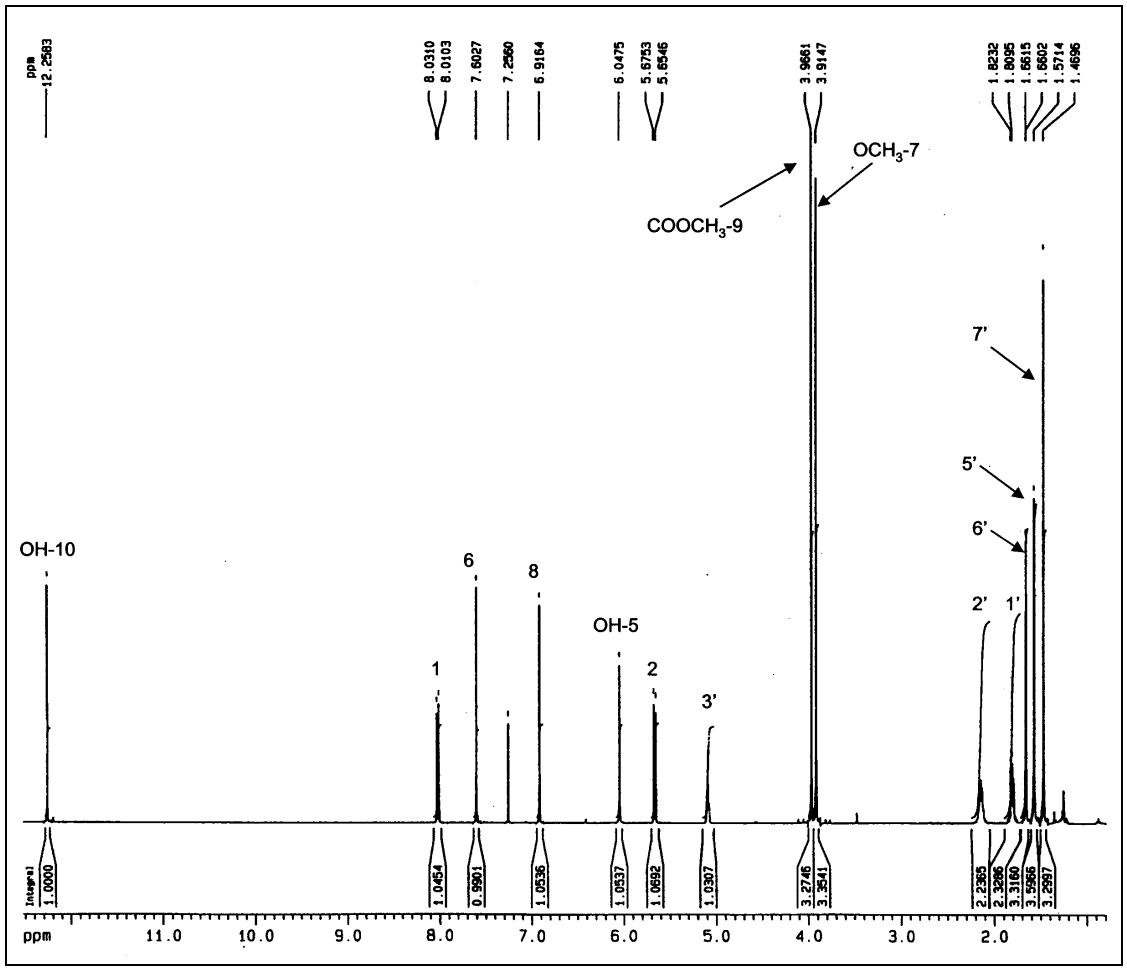
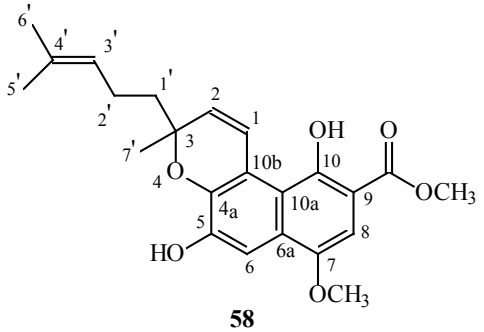



Figure 2.6. $^{1}$H NMR Spectrum of Compound **58** (500 MHz, $CDCl_3$),




**58**

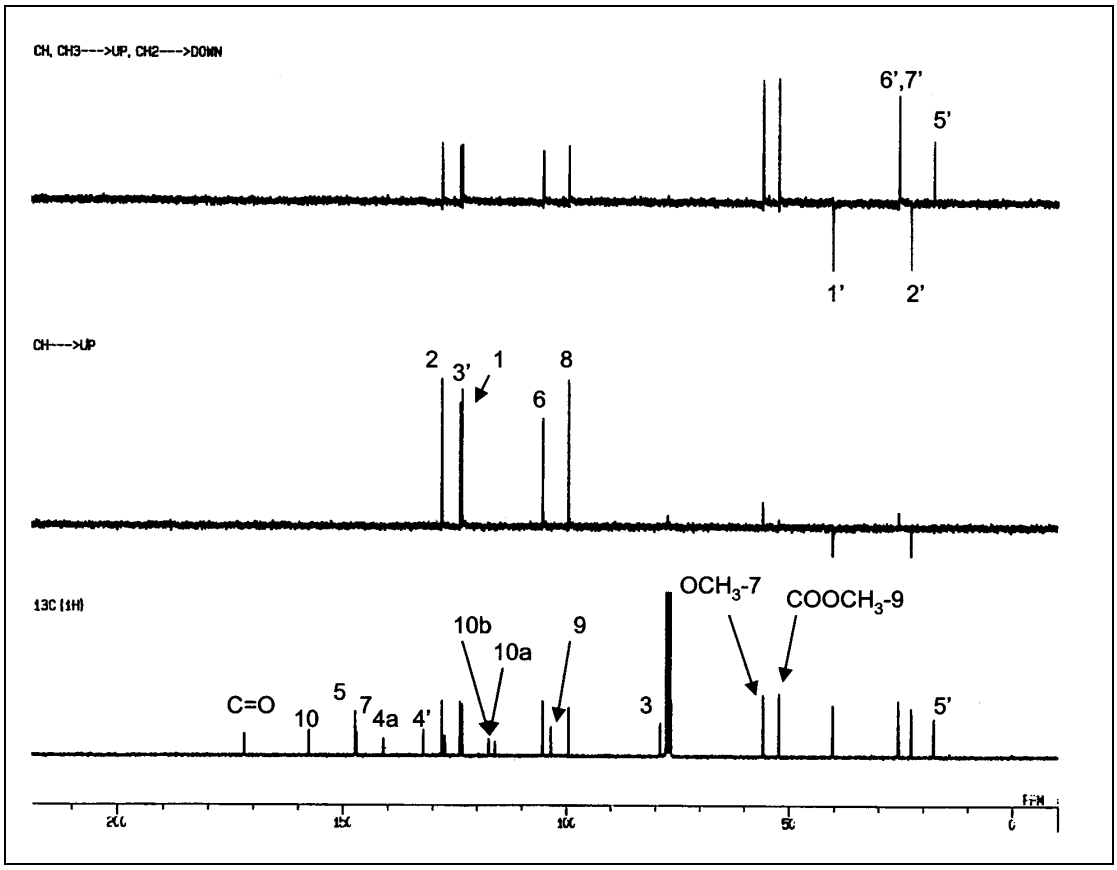
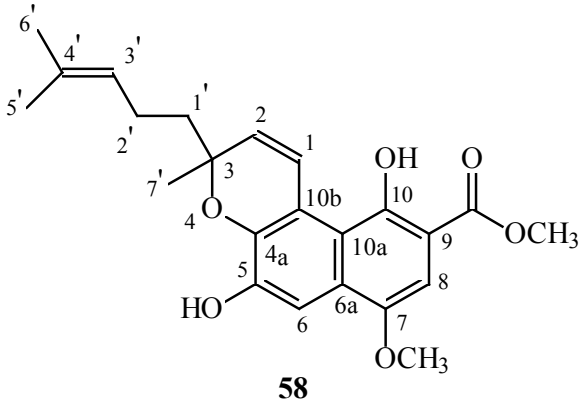



Figure 2.7. $^{13}C$ and DEPT NMR Spectra of Compound **58** (67.5 MHz, $CDCl_3$)




**58**

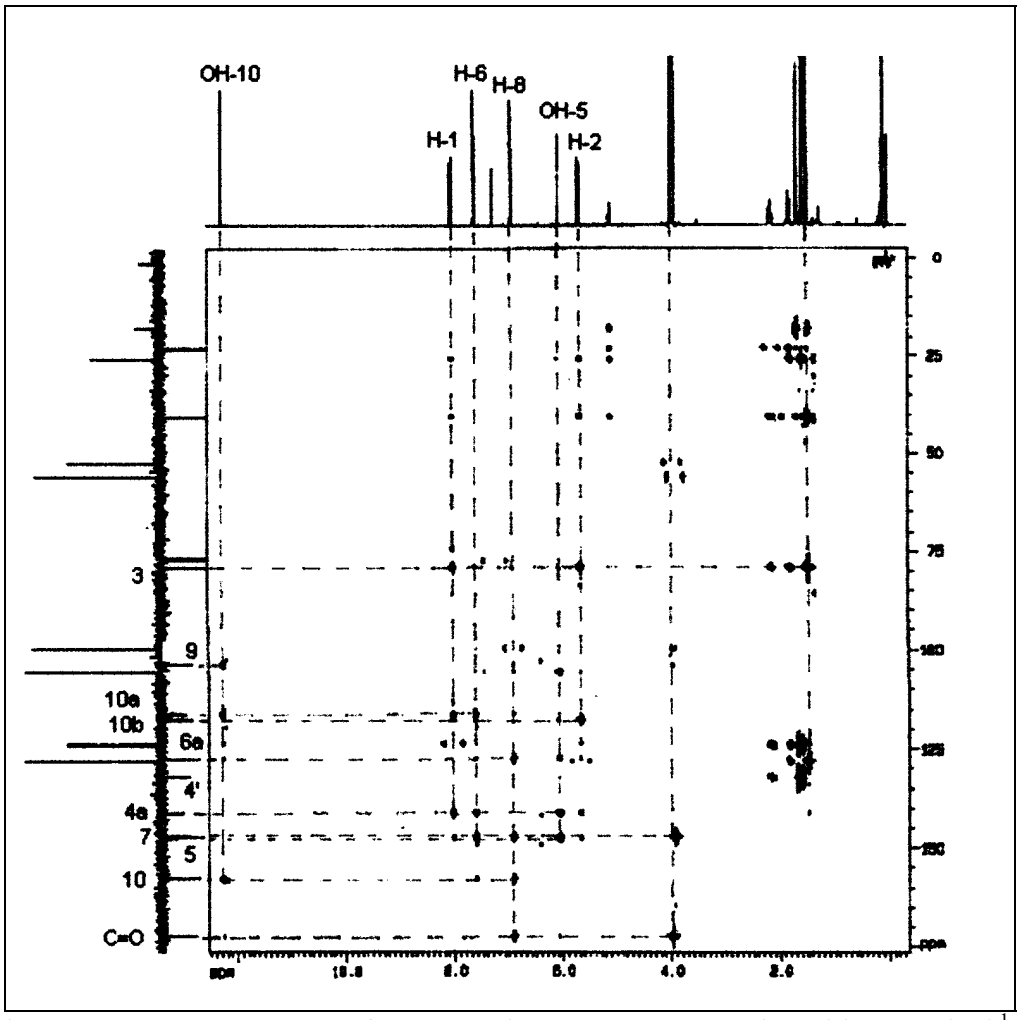



Figure 2.8. HMBC Spectrum of Compound **58** (500 MHz, $CDCl_3$), with a Standard $^{1}H$ Spectrum and a Spin-echo Modulated $^{13}C$ Spectrum

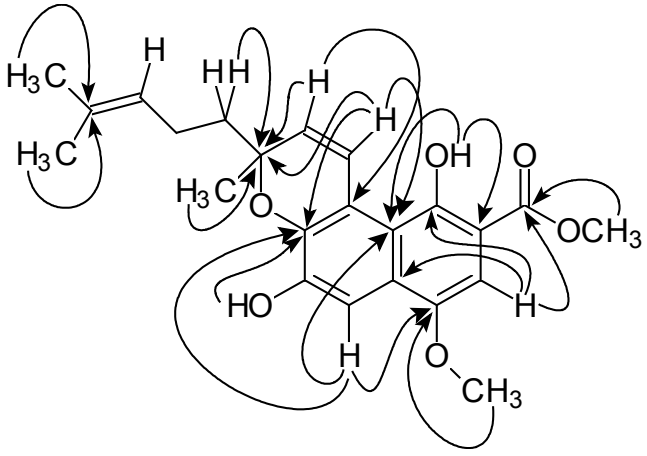
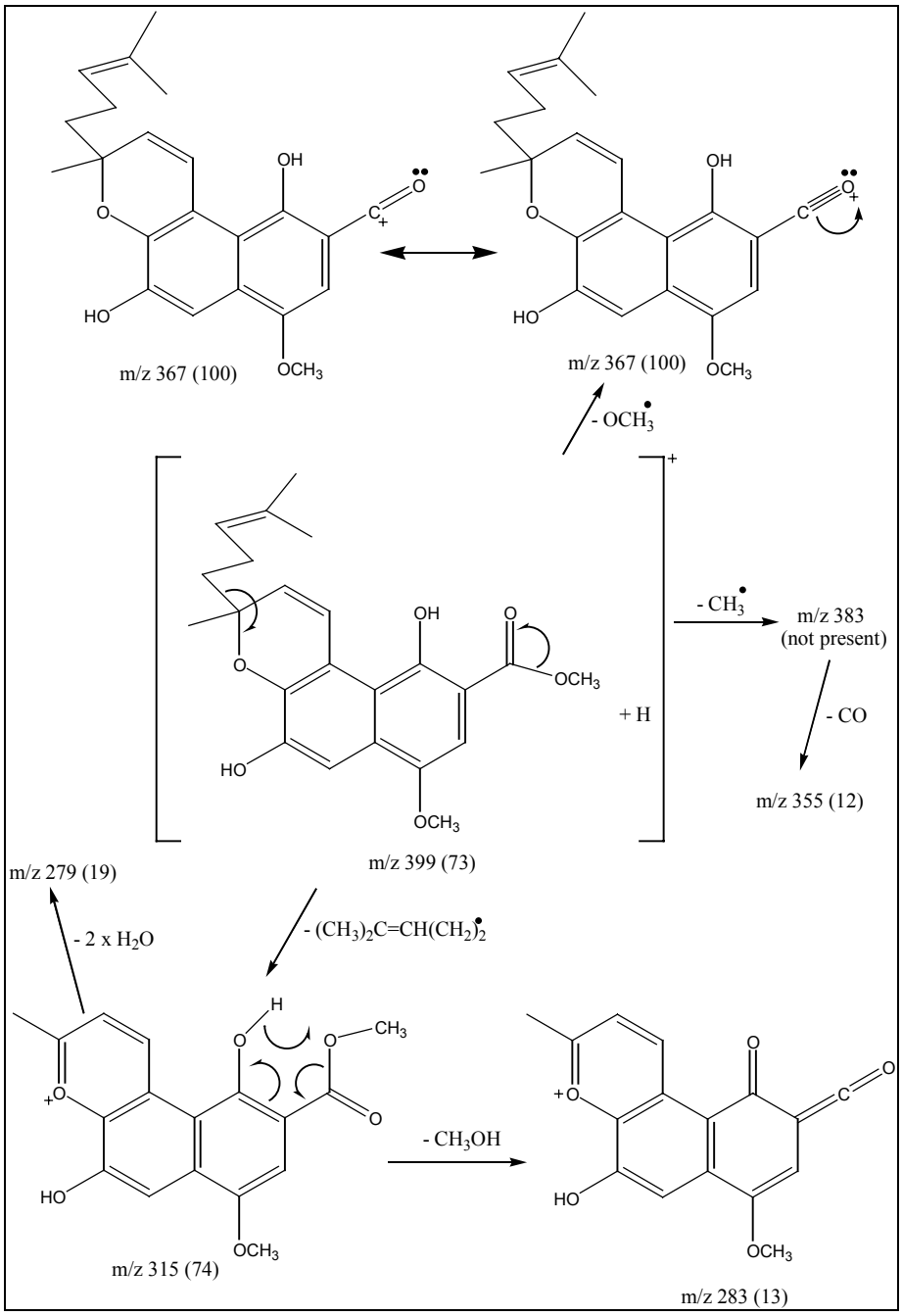



Figure 2.9. Selective HMBC Correlations for Compound **58**



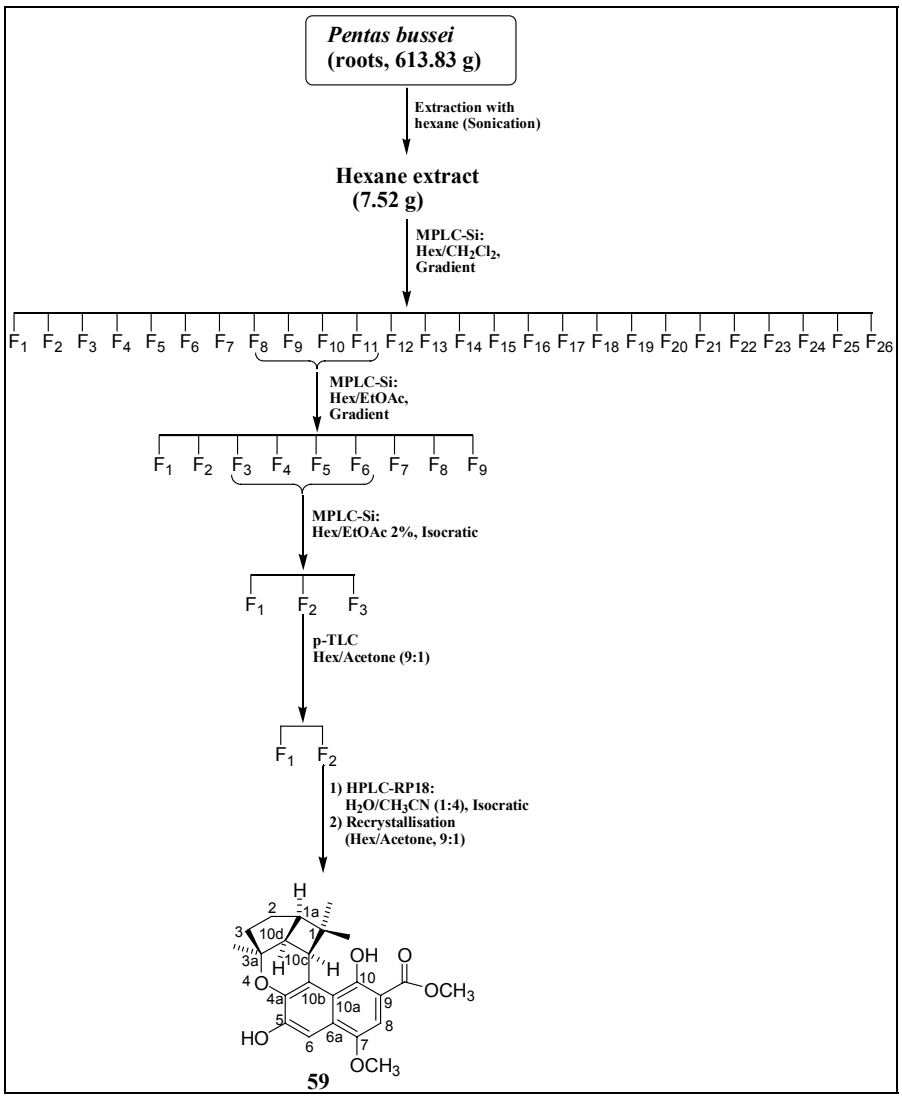

**Scheme 2.2**: Main Fragmentation Patterns in the Mass Spectrum of Compound **58**

Table 2.31. $^{13}C$ and $^{1}H$ NMR data (270 and 67.5 MHz, $CDCl_3$), and observed HMBC (500 MHz for $^{1}H$ and 125 MHz for $^{13}C$) correlations for compound **58** (δ in ppm, *J* in Hz).

| Position | $\delta_H$, multiplicity, (*J*) | $\delta_C$ | HMBC |
|---|---|---|---|
| 1 | 8.02 d (10.4) | 123.37 | H-2 |
| 2 | 5.66 d (10.4) | 127.81 | H-1', H-7' |
| 3 | - | 78.95 | H-1, H-2, H-1', H-2', H-7' |
| 4a | - | 140.97 | H-1, OH-5 , H-6 |
| 5 | - | 147.30 | OH-5, H-6 |
| OH-5 | 6.05 s | - | - |
| 6 | 7.60 s | 105.33 | OH-5 |
| 6a | - | 127.18 | H-8 |
| 7 | - | 146.96 | H-6, H-8, OC$\underline{H_3}$-7 |
| 8 | 6.92 s | 99.59 | - |
| 9 | - | 103.53 | H-8, OH-10 |
| 10 | - | 157.60 | H-8, OH-10 |
| OH-10 | 12.26 s | - | - |
| 10a | - | 115.98 | H-1, H-6, OH-10 |
| 10b | - | 117.35 | H-1, H-2 |
| 1' | 1.76-1.86 m | 40.33 | H-2, H-2', H-3', H-7' |
| 2' | 2.08-2.22 m | 22.78 | H-1', H-3' |
| 3' | 5.04-5.14 br t (7.1) | 123.82 | H-1', H-2', H-5', H-6' |
| 4' | - | 131.96 | H-2', H-5', H-6' |
| 5' | 1.57 s | 17.62 | H-3', H-6' |
| 6' | 1.66 s | 25.56 | H-3', H-5' |
| 7' | 1.47 s | 25.61 | H-1', H-2 |
| O$\underline{CH_3}$-7 | 3.91 s | 55.78 | - |
| COO$\underline{CH_3}$-9 | 3.97 s | 52.15 | - |
| $\underline{C}$OOCH$_3$-9 | - | 171.98 | H-8, COOC$\underline{H_3}$-9 |

II.3.1.1.2. Methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a,10c,10d-hexahydro-1*H*-4-oxacyclobuta[3,4]indeno[5,6-*a*]naphthalene-9-carboxylate **59**

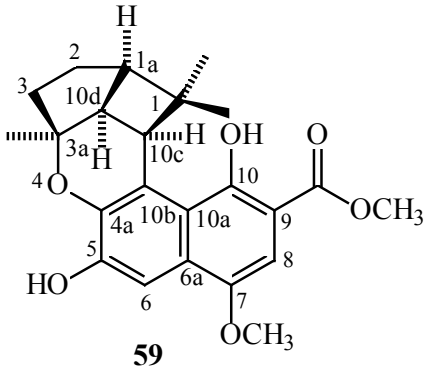
Preparative TLC of fraction 3:2 (Table 3.5) from the hexane extract of the roots of *P. bussei* followed by HPLC gave rise to the compound **59** (Scheme 2.3) in low yield (0.0036 % yield).




**Scheme 2.3**: Isolation of Methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a, 2, 3, 3a,10c,10d-hexahydro-1*H*-4-oxacyclobuta[3,4]indeno[5,6-*a*]naphthalene-9-carboxylate **59** from the Roots of *P. bussei*.

**Methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a, 2, 3, 3a, 10c,10d-hexahydro-1*H*-4-oxacyclobuta[3,4]indeno[5,6-*a*]naphthalene-9-carboxylate 59** was obtained as yellowish

dark crystals from hexane, mp 158.0-159.4 °C. ESIMS showed a base peak at *m/z* 399 corresponding to $[M+H]^+$. HRMS provided the exact mass at *m/z* 399.1736 (calcd *m/z* 399.1808 $[M+H]^+$), and therefore suggesting $C_{23}H_{26}O_6$ as molecular formula which is accounting for 11 degrees of unsaturation. The IR spectrum showed absorption bands at 3409 $cm^{-1}$ and 1741 $cm^{-1}$, assigned to the OH functions and the carbonyl group, respectively.




**59**

The $^1H$ NMR and $^{13}C$ NMR spectra (Figures 2.2.10-2.11, Table 2.32) of compound **59**, along with DEPT and HETCOR spectra (Figures 2.12-2.13) showed twenty-two proton and twelve carbon signals in the aliphatic region corresponding to three $CH_3$ [$\delta_H$ 0.54 (3H, s), $\delta_C$ 19.2, $CH_3$-1(ß); $\delta_H$ 1.36 (3H, s), $\delta_C$ 25.6, $CH_3$-3a; $\delta_H$ 1.49 (3H, s), $\delta_C$ 34.0, $CH_3$-1(a)], two $CH_2$ [$\delta_H$ 1.64-1.80 (2H, m), $\delta_C$ 25.3, C-2, and $\delta_H$ 1.64-1.80/2.00-2.07 (2H, m), $\delta_C$ 40.6, C-3], three CH [$\delta_H$ 2.49 (1H, ddd, $J_{H\text{-}2(1)/H\text{-}1a}$ = 4.40, $J_{H\text{-}1a/H\text{-}2(2)}$ = 7.91, $J_{H\text{-}1a/H\text{-}10d}$ = 9.24 Hz), $\delta_C$ 46.7, C-1a; $\delta_H$ 2.70 (1H, dd, $J_{H\text{-}10d/H\text{-}10c}$ = 9.57 Hz, $J_{H\text{-}10d/H\text{-}1a}$ = 9.24 Hz), $\delta_C$ 41.3, C-10d; and $\delta_H$ 4.57 (1H, d, $J_{H\text{-}10c/H\text{-}10d}$ = 9.57 Hz), $\delta_C$ 39.0, C-10c], two $OCH_3$ [$\delta_H$ 3.92 (3H, s), $\delta_C$ 55.8, O$\underline{CH_3}$-7; $\delta_H$ 3.96 (3H, s), $\delta_C$ 52.1, COO$\underline{CH_3}$], and two aliphatic $sp^3$ quaternary carbons at $\delta_C$ 41.8 and 85.4. The aromatic region showed two $sp^2$ CH and eight $sp^2$ quaternary carbons. In addition, an ester carbonyl carbon, and two OH groups, one of which is chelated ($\delta_H$ 12.21), were observed. $^1H$-$^1H$ COSY spectrum showed the different coupling systems observed in the molecule (Figure 2.14). HMBC analysis confirmed the proposed data attribution (Figure 2.15, Table 2.32). The relative stereochemistry in the alicyclic ring system was established by a DIFNOE and ROESY study which revealed NOE effects of 12% for $H_3$-1(β)/$H_3$-1(α), 9% for $H_3$-1(α)/H-1a, 5% for H-1a/H-10d, 11% for H-10d/H-10c, 4% for H-10c/$H_3$-1(α), and 8% for H-10d/ $H_3$-3a (Figure 2.16). These results suggested an all *cis* configuration for H-1a, $CH_3$-3a, H-10c, H-10d, and $CH_3$-1(α), and such a configuration is typical for this type of structure (123).

In addition, NMR data of the alicyclic system of compound **59** were fitting with those of eriobrucinol (123b) (Figure 2.17), although the effects of different aromatic rings may be accountable for the slight differences observed, particularly in the $^{13}C$ NMR chemical shifts. The coupling systems observed therein , i.e. $J_{H\text{-}10c/H\text{-}10d}$ = 9.57 Hz, $J_{H\text{-}10d/H\text{-}1a}$ = 9.24 Hz, were also typical for the cyclol ring (123,124a) (Table 2.33). Highly shielded $CH_3$ protons such as $H_3$-1 resonating at $\delta_H$ 0.54 have been related to a puckered conformation of the cyclobutane ring which induces an equatorial-oriented anisotropy (124). However, shielding contributions from aromatic rings have also to be taken into account (125). A Dreiding model of compound **59** showed that the $CH_3$-1(β) is almost lying within the shielding cone of the aromatic current, and therefore its protons resonating at high fields. This was also supported by ROESY effects observed between $H_3$-1(β)/$H_2$-3 and which are possible if the cyclobutane and cyclopentane are puckered in such a way that $CH_3$-1(ß) and $CH_2$-3 are brought closer each other, and therefore $CH_3$-1(ß) is positioned over the pyranonaphthoquinone ring. A part from the alicyclic system, the observed spectral data for the aromatic ring system and its substituents were similar to those observed for compound **58** from which it may derive through the cyclisation of the homoprenyl side chain. Based on all the previous data, the structure of compound **59** was established as methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a, 10c,10d-hexahydro-1*H*-4-oxacyclobuta[3,4]indeno[5,6-*a*]naphthalene-9-carboxylate. This type of compound is structurally attached to the class of meroterpenes. To the best of our knowledge, no natural quinonic cyclol is yet reported in the literature.

The biosynthetic pathways leading to the new naphthohydroquinone type compound could be the same as proposed in the case of natural meroterpenoid coumarins (126). Biogenetic condensation of terpenes with natural acetate-derived phenols leads to "meroterpene" metabolites. "Terpenylphenols", formed in the first instance, such as the geranylphenol **66**, are likely starting products for elaboration *in vivo*, giving rise to chromenes **67** and more structurally complex systems such as those known generically as "citrans" **68** and "cyclols" **69** (126) (Scheme 2.4). Compound **59** may derive, through a (2+2) concerted process, from the homoprenylated benzochromene **58** (122) by a light-induced mechanism (Scheme 2.5). However, the intramolecular cycloaddition required for the formation of cyclols from appropriate prenylated compounds has been induced both photochemically, but also thermally, and with acid catalysis (125a,127).

"Cyclol" units are characterised by a tricyclic terpenoid core involving a dihydropyran, a cyclopentane, and a cyclobutane ring. These units have already been found integrated into the structure of natural phenolic compound such us cannabinoids, i.e. cannabicyclol **70** (127a,128) isolated from *Cannabis sativa* L. (Cannabaceae), of terpenoid alkaloids isolated from the Rutaceae plant *Murraya koenigii* Spreng, e. g., bicyclomahanimbine **71** (125a,129), and more recently, of eriobrucinol **72** (and its angular regioisomers **73** and **74**) and hydroxyeriobrucinol **75**, a set of coumarins isolated from another rutaceous plant, *Eriostemon brucei* F. Muell (123,130) (Figure 2.13). The common spectral features of all these compounds are an all *cis* configuration for the three aliphatic methines arising from the cyclisation, together with highly shielded protons belonging to one of the two methyl groups substituted on the cyclobutane ring.

Table 2.32. $^{1}H$ and $^{13}C$ NMR data (270 and 67.5 MHz, $CDCl_3$), and observed HMBC (300 MHz for $^{1}H$ and 75 MHz for $^{13}C$) correlations for compound **59** (δ in ppm, *J* in Hz).

| Position | $\delta_H$, multiplicity, (*J*) | $\delta_C$ | HMBC |
|---|---|---|---|
| 1 | - | 41.83 | $H_3$-1(α), $H_3$-1(ß), H-10c, H-10d |
| 1a | 2.49 ddd (4.40; 7.91; 9.24) | 46.68 | $H_2$-3, H-10d, H-10c, $H_3$-1(α), $H_3$-1(ß) |
| 2 | 1.64–1.80 m | 25.28 | $H_2$-3, H-10d |
| 3 | 1.64–1.80 m, $H_2$-3(1) | 40.59 | H-10d, H-1a, $H_3$-3a |
| | 2.00–2.07 m, $H_2$-3(2) | | - |
| 3a | - | 85.41 | H-10c, $H_2$-3, $H_3$-3a |
| 4a | - | 141.51 | H-10c, OH-5, H-6 |
| 5 | - | 148.91 | OH-5, H-6 |
| OH-5 | 6.42 s | - | - |
| 6 | 7.60 s | 102.68 | OH-5 |
| 6a | - | 127.81 | H-8 |
| 7 | - | 146.79 | H-8, OC*H*$_3$-7, H-6 |
| 8 | 6.93 s | 99.53 | - |
| 9 | - | 102.32 | OH-10, H-8 |
| 10 | - | 157.90 | OH-10, H-8 |
| OH-10 | 12.21 s | - | - |
| 10a | - | 119.08 | OH-10, H-6, H-10c |
| 10b | - | 123.47 | H-10c |
| 10c | 4.57 d (9.57) | 38.98 | H-1a, H-10d, $H_3$-1(α), $H_3$-1(ß) |
| 10d | 2.70 dd (9.57; 9.24) | 41.28 | $H_2$-3, $H_2$-2, $H_3$-1(α), $H_3$-1(ß) |
| $CH_3$-1 (α) | 1.49 s | 33.95 | $H_3$-1(ß), H-10c |
| $CH_3$-1 (β) | 0.54 s | 19.17 | $H_3$-1(α), H-10c |
| $CH_3$-3a | 1.36 s | 25.55 | $H_2$-3, H-10d |
| O$\underline{C}H_3$-7 | 3.92 s | 55.78 | - |
| COO$\underline{C}H_3$-9 | 3.96 s | 52.09 | - |
| $\underline{C}OOCH_3$-9 | - | 172.22 | COOC$\underline{H}_3$-9, H-8 |

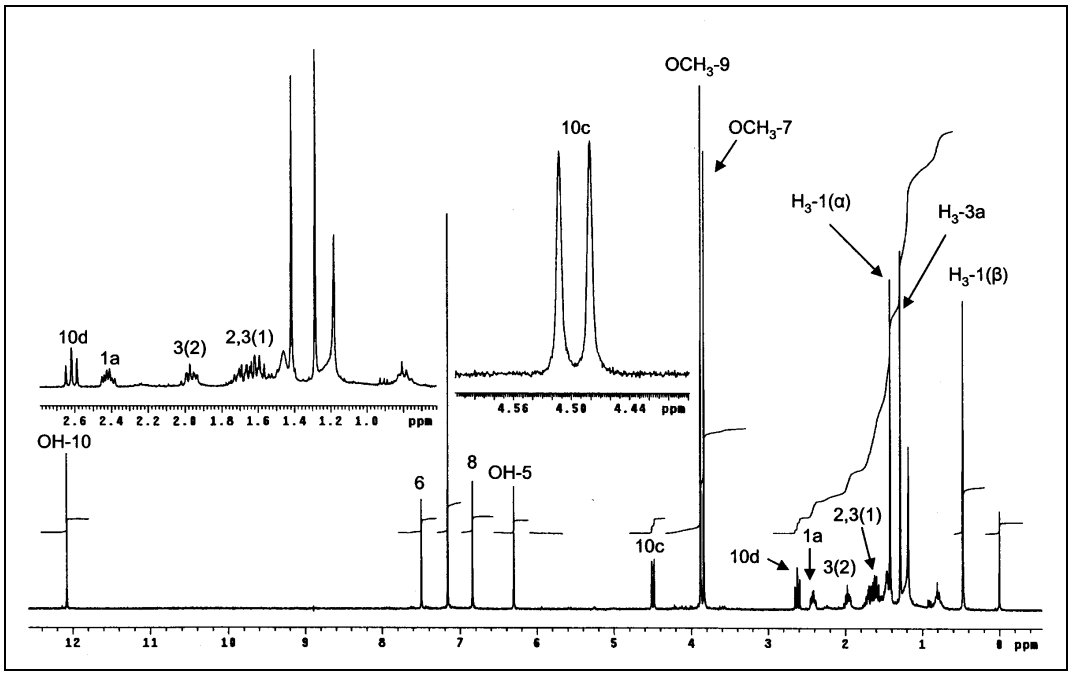



Figure 2.10. $^{1}$H NMR Spectrum of Compound **59** (300 MHz, $CDCl_3$)

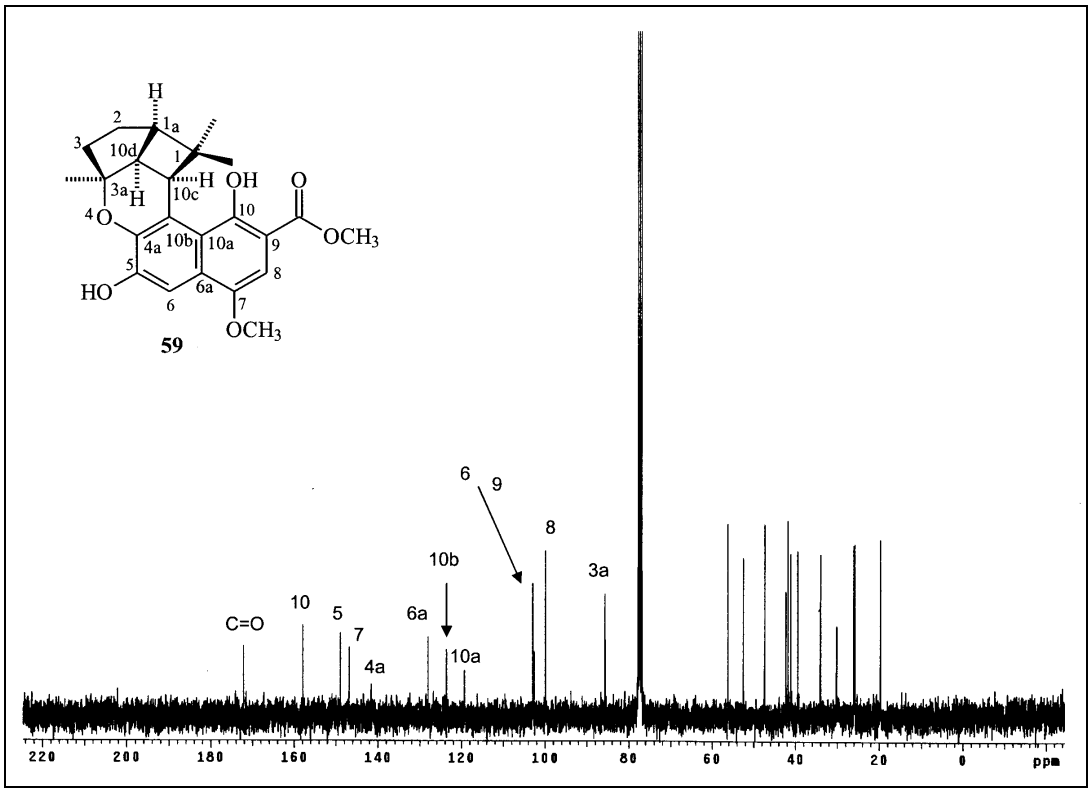



Figure 2.11. $^{13}$C NMR Spectrum of Compound **59** (75 MHz, $CDCl_3$)

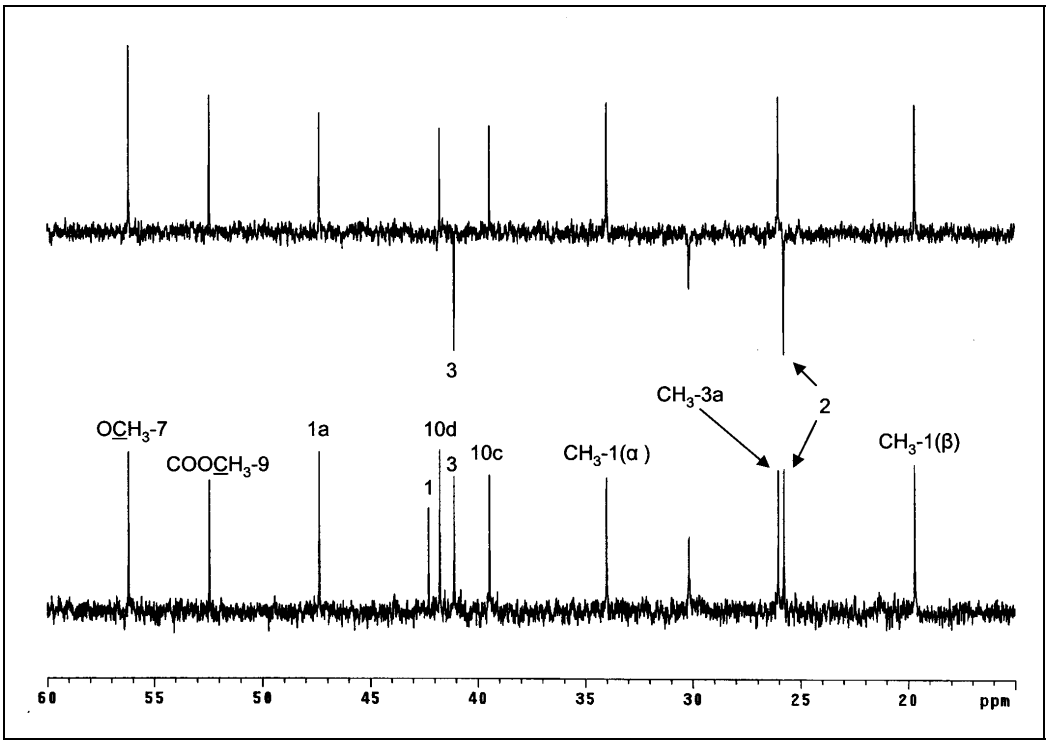



Figure 2.12. DEPT NMR Spectrum of Compound **59** (75 MHz, $CDCl_3$): aliphatic region

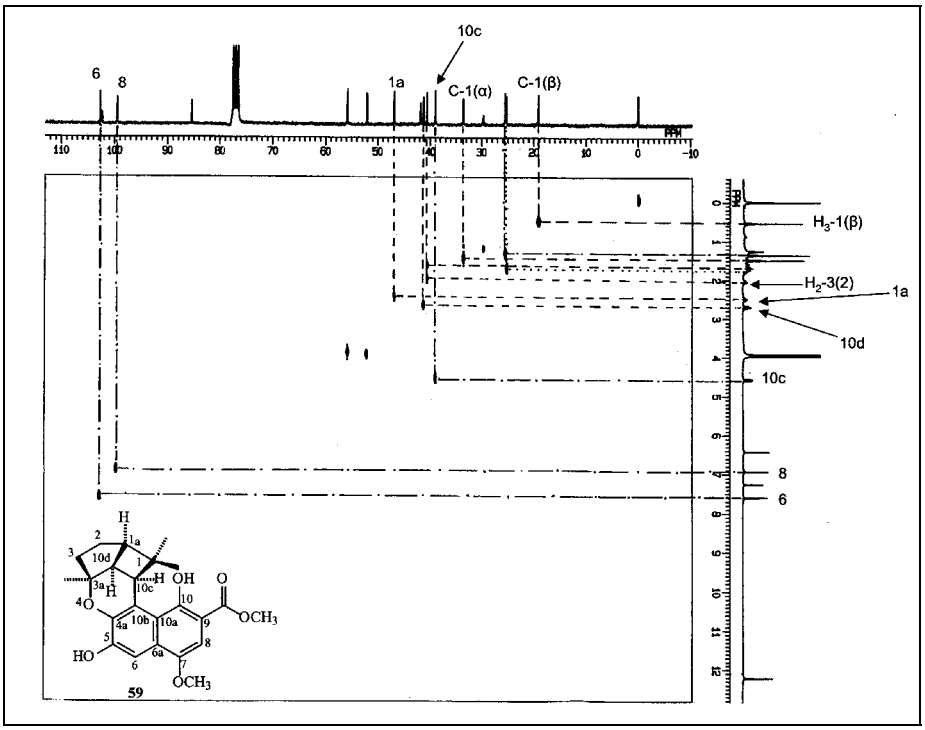



Figure 2.13. HETCOR Spectrum of Compound **59** (270 MHz, $CDCl_3$)

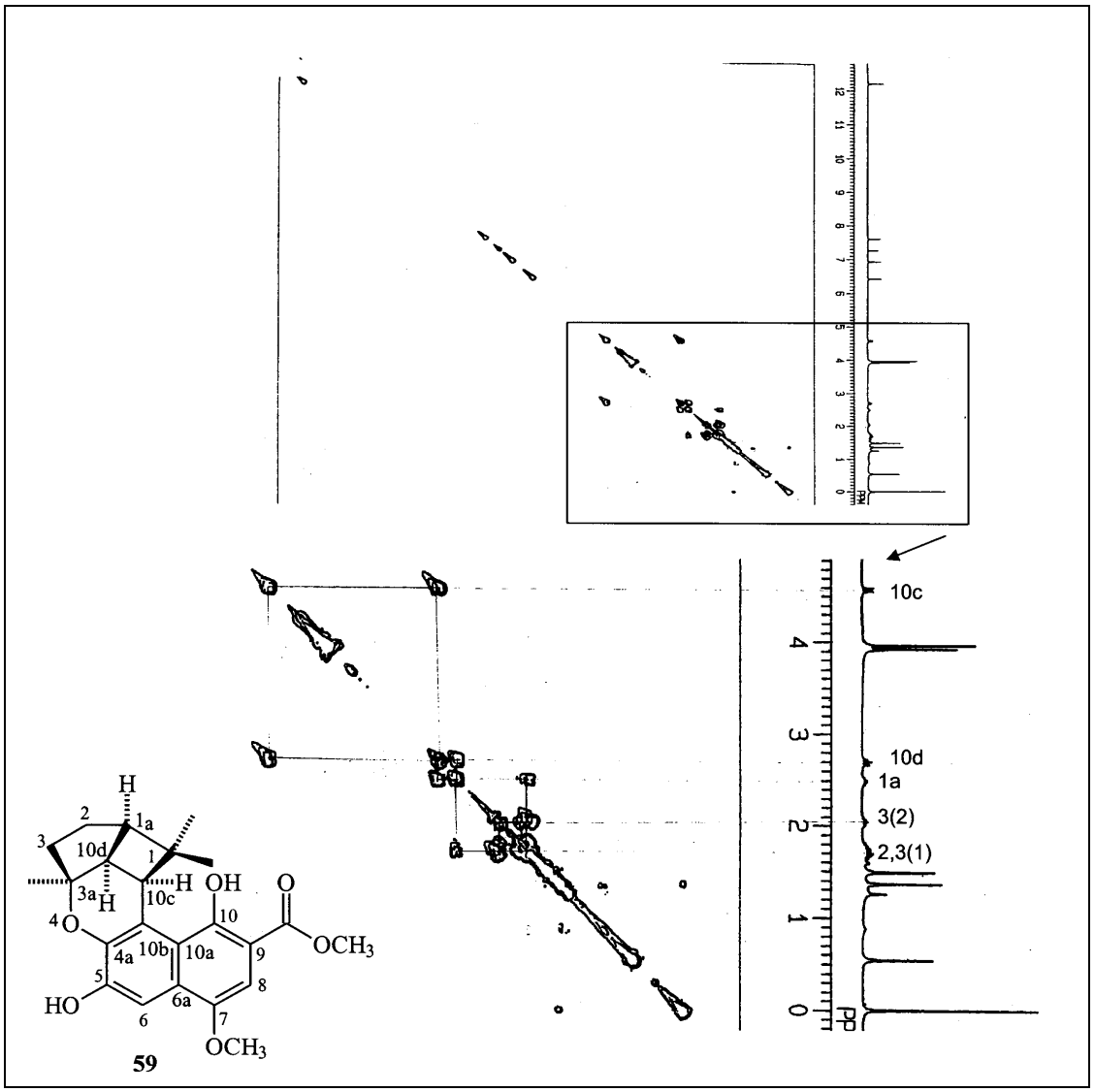



Figure 2.14. $^{1}H$-$^{1}H$ COSY Spectrum of Compound **59** (270 MHz, $CDCl_3$)

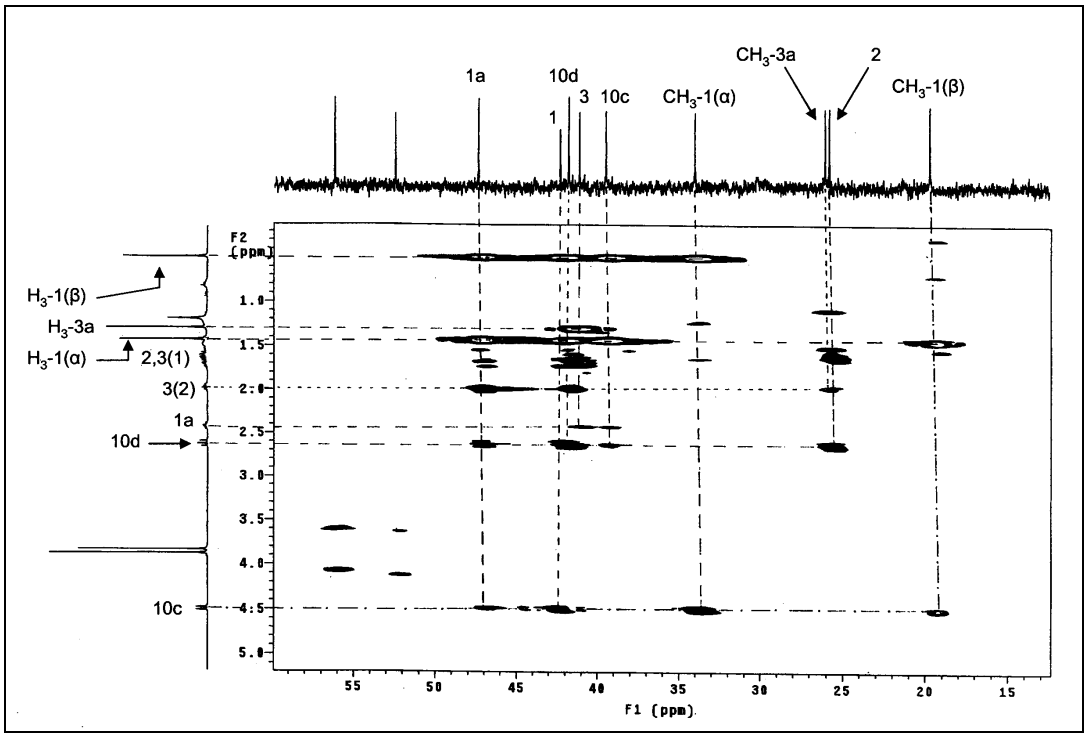



Figure 2.15. HMBC Spectrum of Compound **59** (300 MHz, $CDCl_3$)

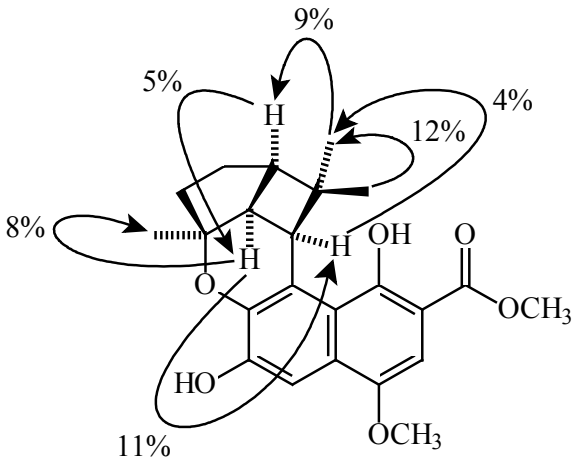
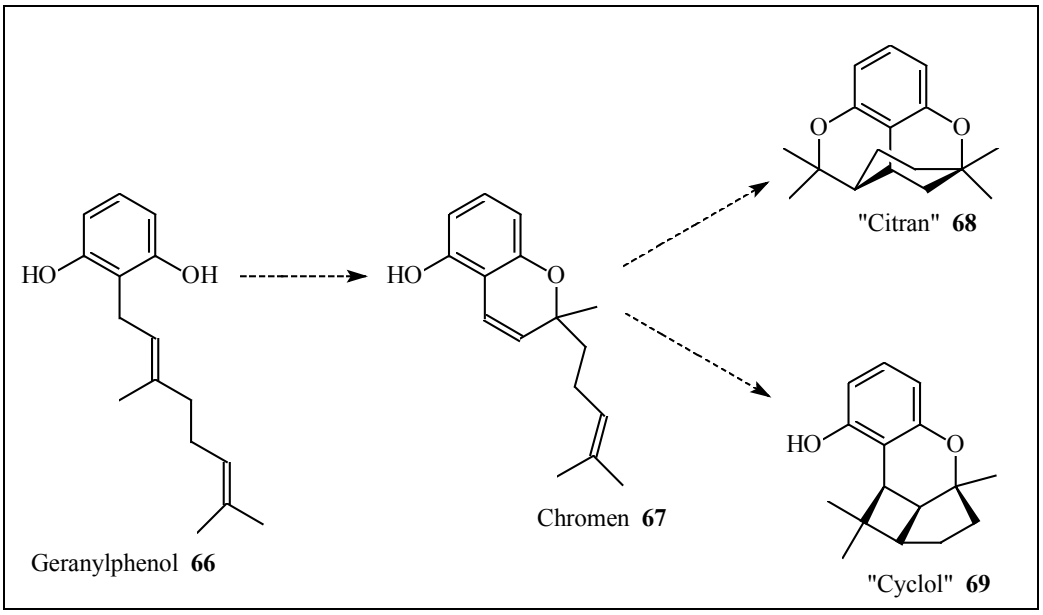
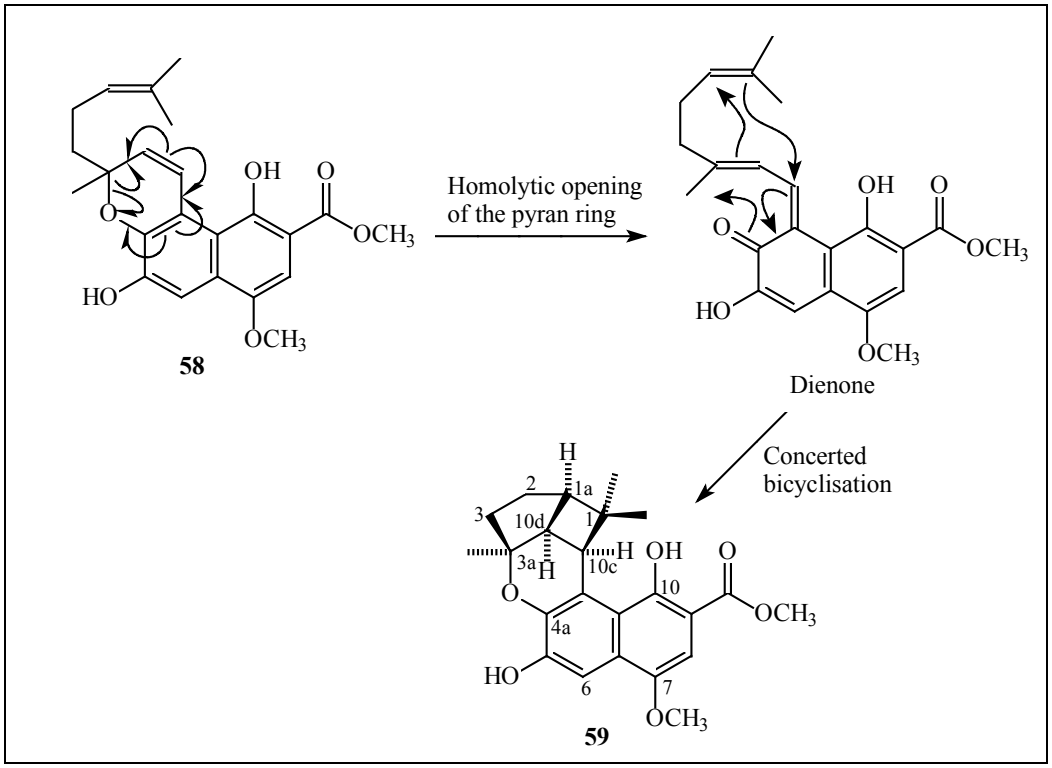



Figure 2.16. Major NOE Effects in the Alicyclic Moiety of Compound **59**




**Scheme 2.4**: Meroterpenes: From Geranylphenols to Complex Structures




**Scheme 2.5**: Possible Biogenetic Pathway Leading to Compound **59**

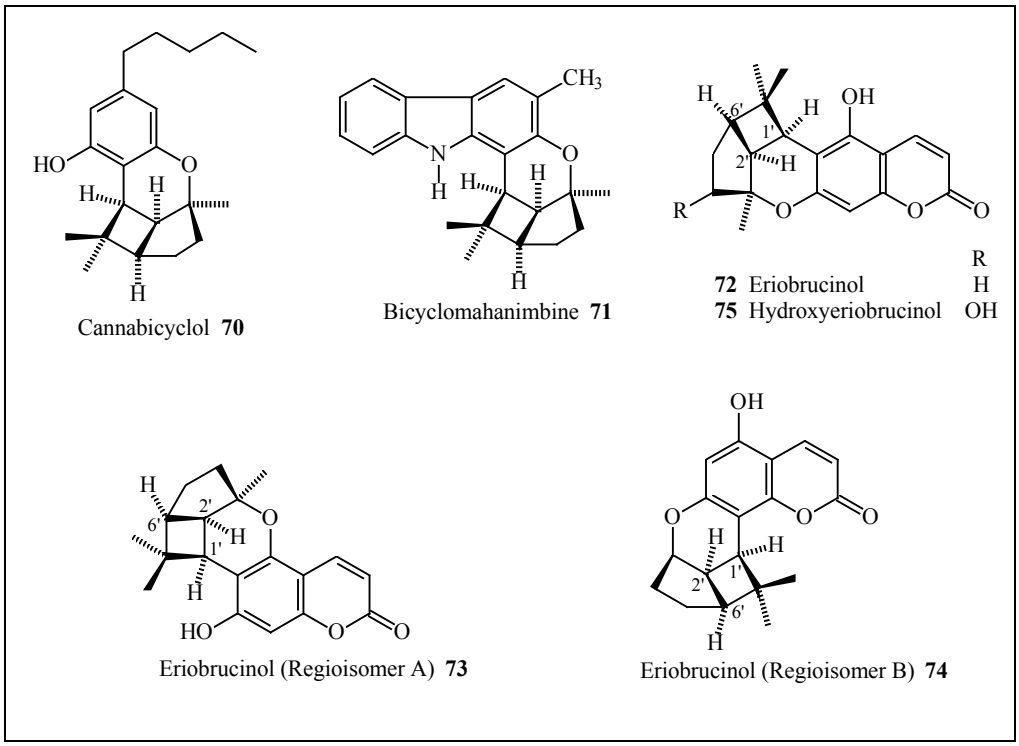



Figure 2.17. Some Examples of Natural Occurring "Cyclol" Derivatives

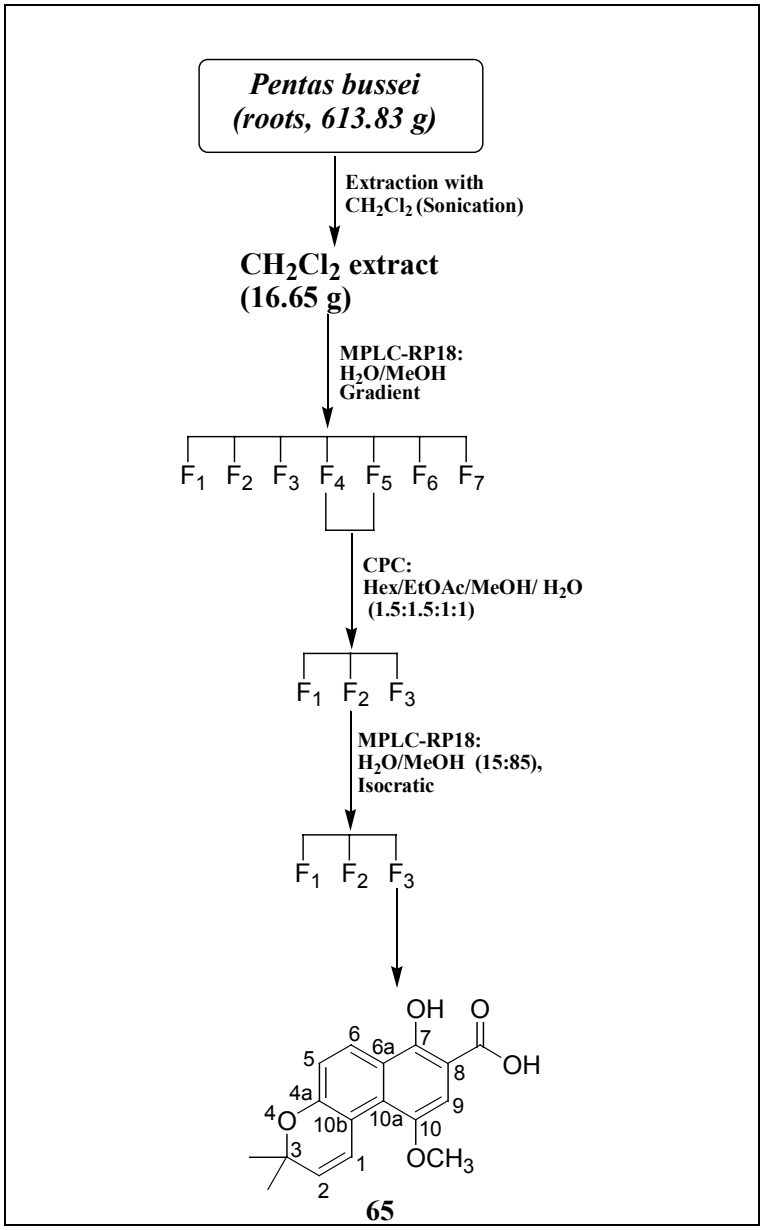
Table 2.33. Comparison of the NMR data ($^{1}H$ and $^{13}C$) of the alicyclic moiety (δ (ppm), multiplicity, *J* in Hz) of compound **59** and eriobrucinol isomers **73** and **74** (ref. 123b).

| Compound **59**[1] | | | Eriobrucinol **72**[2] (ref. 123b) | | | Eriobrucinol (Regioisomer A) **73**[3] (ref. 123b) | | Eriobrucinol (Regioisomer B) **74**[4] (ref. 123b) | |
|---|---|---|---|---|---|---|---|---|---|
| Position | $^{1}H$ | $^{13}C$ | Position | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ |
| 1 | - | 41.83 | 7’ | - | 39.1 | - | - | - | - |
| 1a | 2.49 ddd (4.4; 7.9; 9.2) | 46.68 | 6’ | 2.49 t (7.4) | 46.5 | 2.49 t (7.4) | - | 2.31 t (7.6) | - |
| 2 | 1.64-1.80 m | 25.28 | 5’ | 1.65 m and 1.92 dt (12.0; 6.8) | 25.7 | 2.12 m | - | 1.90 m | - |
| 3 | 1.64-1.80 and 2.00-2.07 m | 40.59 | 4’ | 1.71-1.74 m | 38.8 | 1.55 dt (11.8; 7.6) and 1.62 m | - | 1.50-1.75 m | - |
| 3a | - | 85.41 | 3’ | - | 84.7 | - | - | - | - |
| 10c | 4.57 d (9.6) | 38.98 | 1’ | 3.09 d (9.7) | 35.7 | 3.31 d (9.5) | - | 3.26 d (9.4) | - |
| 10d | 2.70 dd (9.6; 9.2) | 41.28 | 2’ | 2.67 dd (9.7; 7.4) | 37.4 | 2.56 dd (9.5, 7.6) | - | 2.60 dd (9.4; 7.4) | - |
| Me-1(α) | 1.49 s | 33.95 | Me-7’(α) | 1.46 s | 34.6 | 1.46 s | - | 1.52 s | - |
| Me-1(β) | 0.54 s | 19.17 | Me-7’(β) | 0.80 s | 18.3 | 0.96 s | - | 0.77 s | - |
| Me-3a | 1.36 s | 25.55 | Me-3’ | 1.42 s | 27.4 | 1.44 s | - | 1.42 s | - |

NMR run at: [1] 270 MHz in $CDCl_3$, [2] 400 MHz in $CDCl_3$, [3] 250 MHz in $C_5D_5N$, [4] 250 MHz in $CDCl_3$.

II.3.1.1.3. 7-Hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromene-8-carboxylic acid **65**

The fractionation of the mixture of fractions 13:4 and 13:5 (Table 2.21) from the $CH_2Cl_2$ extract of the roots of *P. bussei* with MPLC-RP18 and CPC (Scheme 2.6) led to the isolation of compound **65**. The compound is a new natural product isolated for the first time.




**Scheme 2.6**: Isolation of 7-Hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromene-8-carboxylic acid **65** from the Roots of *P. bussei*.

**7-Hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromene-8-carboxylic acid 65**, yellow crystals, mp 196.7-198.0 °C. The ESI mass spectrum of compound **65** displayed the pseudomolecular ion peak at 301 ($[M + H]^+$ (81%) consistent with the molecular formula $C_{17}H_{16}O_5$. The UV spectrum displayed two intense bands at 259.1 nm (log$\varepsilon$ 4.57) and 373.9 nm (log$\varepsilon$ 4.17). This pattern is typical for a naphthalene ring with the extension of the conjugation by substituents.

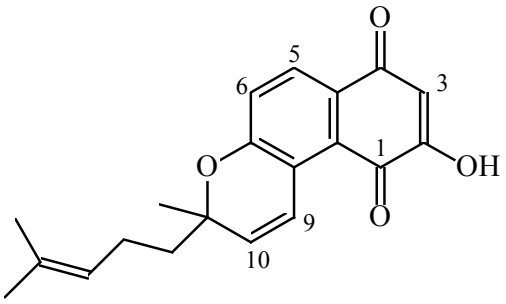
The $^1$H NMR spectrum (in acetone-$D_6$) of compound **65** displayed a relatively simple pattern (Figure 2.18a): two methyl groups [$\delta_H$ 1.46 (6H, s, 2 x $CH_3$-3)], one methoxy group [$\delta_H$ 3.94 (3H, s, $OCH_3$-10)], two clear olefinic AX coupling systems, i.e. H-1 [$\delta_H$ 7.75, dd, $J_1$ = 10.6 Hz, $J_2$ = 0.7 Hz]/H-2 [$\delta_H$ 5.70, d, $J$ = 10.6 Hz] and H-5 [$\delta_H$ 7.11, dd, $J_1$ = 8.9 Hz, $J_2$ = 0.7 Hz]/H-6 [$\delta_H$ 8.22, d, $J$ = 8.9 Hz], together with an additional olefinic proton at $\delta_H$ 7.17 (1H, s, H-9). Closer inspection of the $^1$H NMR spectrum of compound **65** performed in benzene-$D_6$ (Figure 2.18b) revealed the presence of two OH groups at $\delta_H$ 12.01 (1H, broad s, COO<u>H</u>-8; 1H, broad s, OH-7). An absorption band at 3440 cm$^{-1}$, together with an other large and intense band between 3270 and 2517 cm$^{-1}$ (overlapping with the usual band of C-H) in the IR spectrum (Figure 2.19) are indicative of the presence of OH groups. This latter band between 3270 and 2517 cm$^{-1}$ is typical to the absorption of the OH groups of carboxylic acid dimers (121b). The small coupling constant ($J_2$ = 0.7 Hz) observed between H-1/H-5 is a characteristic of a "zigzag" configuration which results in a $^5J$ coupling (117,120). As a consequence of this coupling, the two doublets are flattened and their heights are near the half of their counterparts in the respective AX systems (Figure 2.18).

Apart from the protonated carbons assigned with the help of DEPT and HETCOR spectra, the $^{13}$C NMR spectrum (Figure 2.16) showed the presence of a carbonyl carbon $\delta_C$ 173.10 (<u>C</u>OOH-8), an oxygenated sp$^3$ carbon at $\delta_C$ 76.01 (C-3), together with seven additional sp$^2$ carbon quaternary carbons (Table 2.34). An absorption band at 1648 cm$^{-1}$ in the IR spectrum confirmed this C=O functional group, the absorption frequency of which is lessened by intramolecular hydrogen bond bridges (121b). The intense band at 259.1 nm observed in the UV spectrum can be attributed to the $\alpha,\beta$-unsaturated carbonyl of the carboxylic acid (141). The values of the $\delta_C$ 76.01 (C-3) is within the typical chemical shift range for the sp$^3$ $C_q$-O found in pyran rings. In addition, the "zigzag" coupling path observed between H-1 and H-5 indicated the fusion of this pyran ring (holding the AX system H-1/H-2) with the aromatic

one on which is located the other AX system (H-5/H-6), in a such a way that this type of coupling is allowed.

HMBC analysis (Figures 2.21-2.22, Table 2.34) allowed an unequivocal assignment for the quaternary carbons. One of the key quaternary carbons is C-10a ($\delta_C$ 127.66) whose correlations both with H-1, H-6 and H-9 located in the three different ring systems confirmed the arrangement proposed for the latter. Additional refinements came from the following observed correlations: H-2/C-10b, H-5/C-6a, H-6/C-4a, H-6/C-7, H-9/C-7 and H-9/COOH.

Finally, the skeletal arrangement proposed for compound **65** was confirmed by the comparison of its $^1$H spectral data, particularly concerning the two AX coupling systems, with those of teretifolione B **76** (120) isolated from the Australian shrub *Conospermum* sp. (Proteaceae). The two compounds showed an identical $^1$H spectrum pattern in the aromatic region (Figure 2.23a-b).

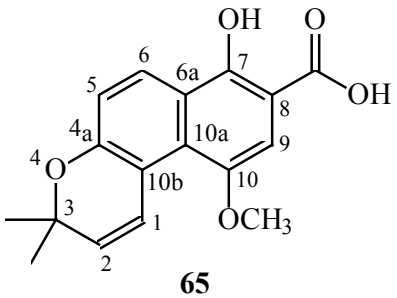



Teretifolione B **76**

Table 2.34. NMR Spectral data: $^{1}H$, $^{13}C$ NMR and observed HMBC (at 300 MHz for $^{1}H$ and 100 MHz for $^{13}C$, Acetone-$D_6$) of compound **65** (δ in ppm, *J* in Hz)

| Position | $\delta_H$*, multiplicity, (*J*) | $\delta_C$ | HMBC |
|---|---|---|---|
| 1 | 7.75 dd (10.6; 0.7) | 123.46 | |
| 2 | 5.70 d (10.6) | 128.66 | 2 x $CH_3$-3 |
| 3 | - | 76.01 | H-1, H-2, 2 x $CH_3$-3 |
| 4a | - | 155.90 | H-5, H-1, H-6 |
| 5 | 7.11 dd (8.9; 0.7) | 119.31 | |
| 6 | 8.22 d (8.9) | 126.04 | |
| 6a | - | 122.23 | H-5 |
| 7 | - | 156.87 | H-6, H-9 |
| 8 | - | 103.30 | H-9 |
| 9 | 7.17 s | 105.16 | |
| 10 | - | 150.06 | H-9, O<u>C</u>$H_3$-10 |
| 10a | - | 127.69 | H-9, H-1, H-6 |
| 10b | - | 115.76 | H-1, H-2, H-5 |
| 2 x $CH_3$-3 | 1.46 (each) | 27.60 (each) | H-2 |
| $OCH_3$-10 | 3.94 | 56.15 | |
| <u>C</u>OOH | - | 173.10 | H-9 |

*Signals of O<u>H</u>-7 and COO<u>H</u>-8 observed around $\delta_H$ 12.01(spectrum run in benzene-$D_6$)




**65**

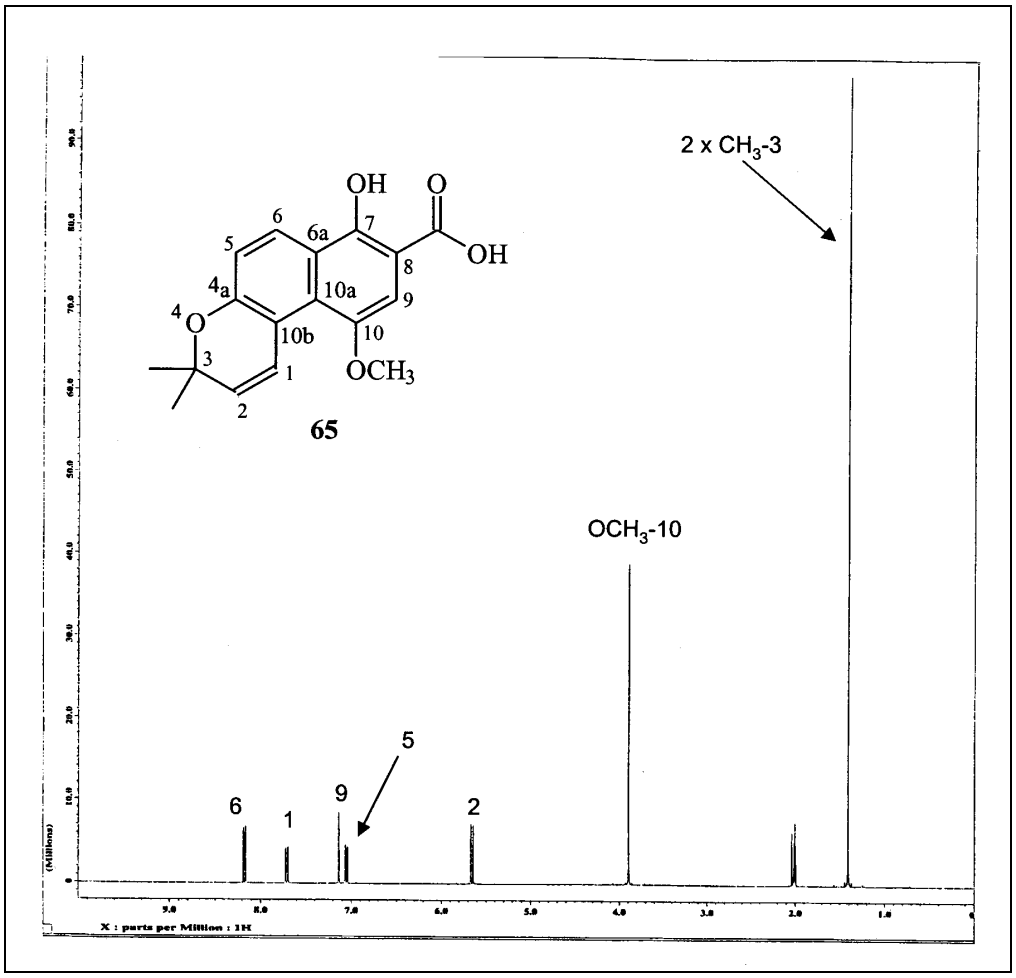



Figure 2.18a. $^{1}$H NMR Spectrum of Compound **65** (400 MHz, Acetone-$D_6$)

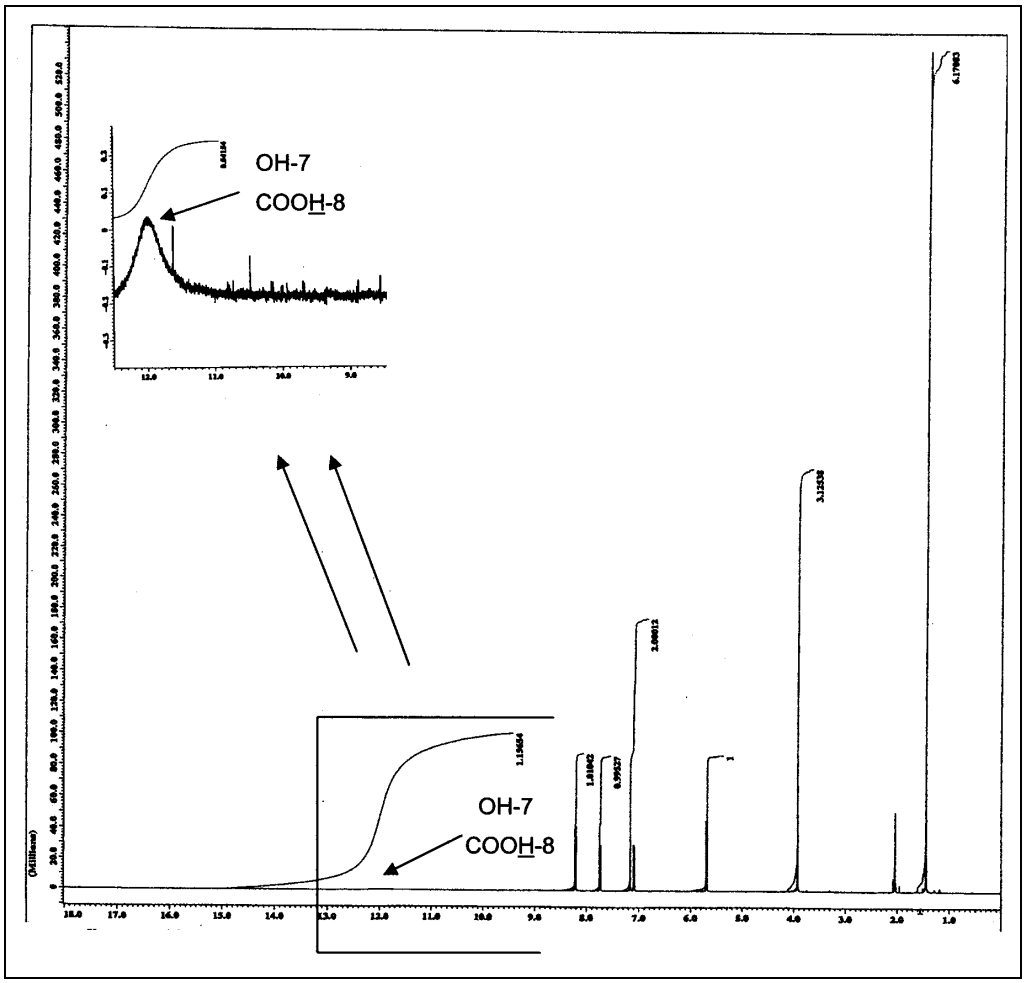
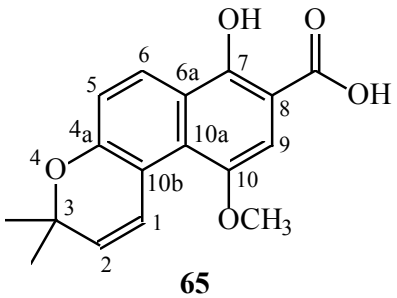



Figure 2.18b. $^{1}$H NMR Spectrum of Compound **65** (400 MHz, Benzene-$D_6$)




**65**

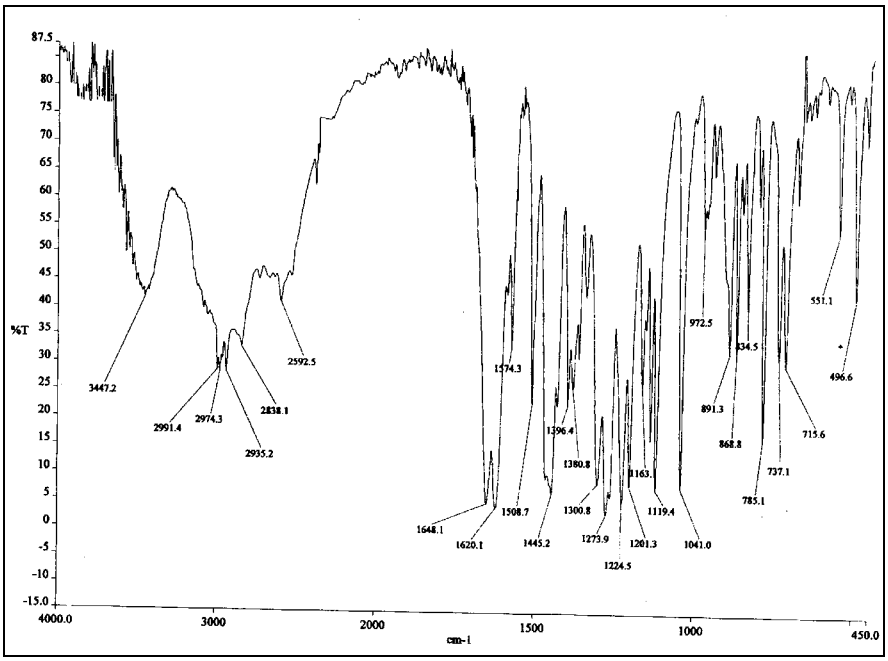



Figure 2.19. IR Spectrum (in KBr) of Compound **65**

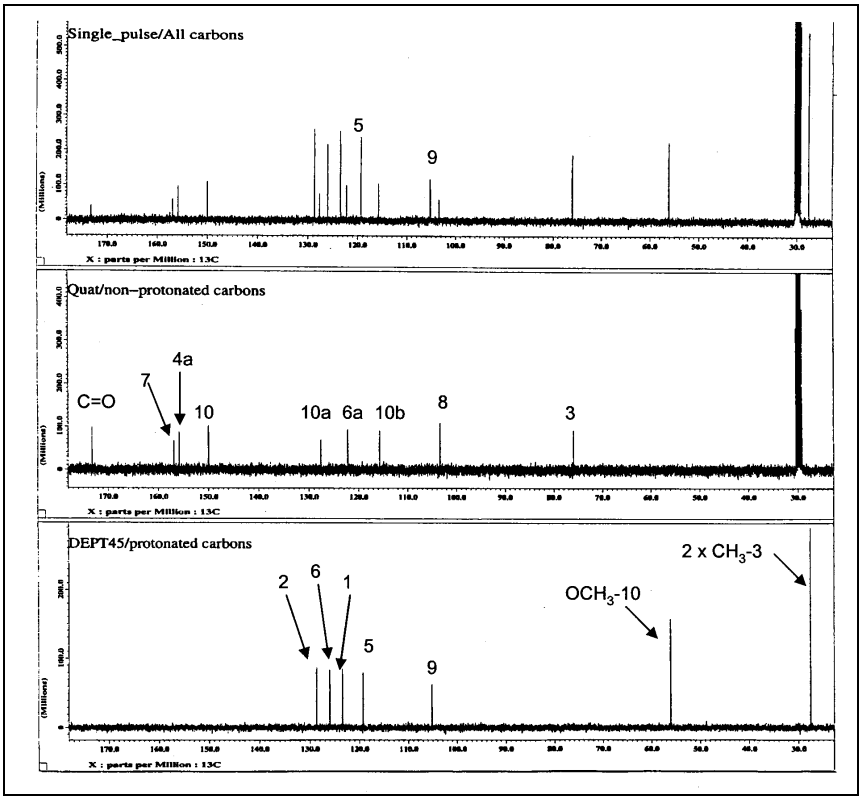



Figure 2.20. $^{13}C$ NMR Spectrum of Compound **65** (100 MHz, Acetone-$D_6$)
(Single Pulse Decoupling Experiments)

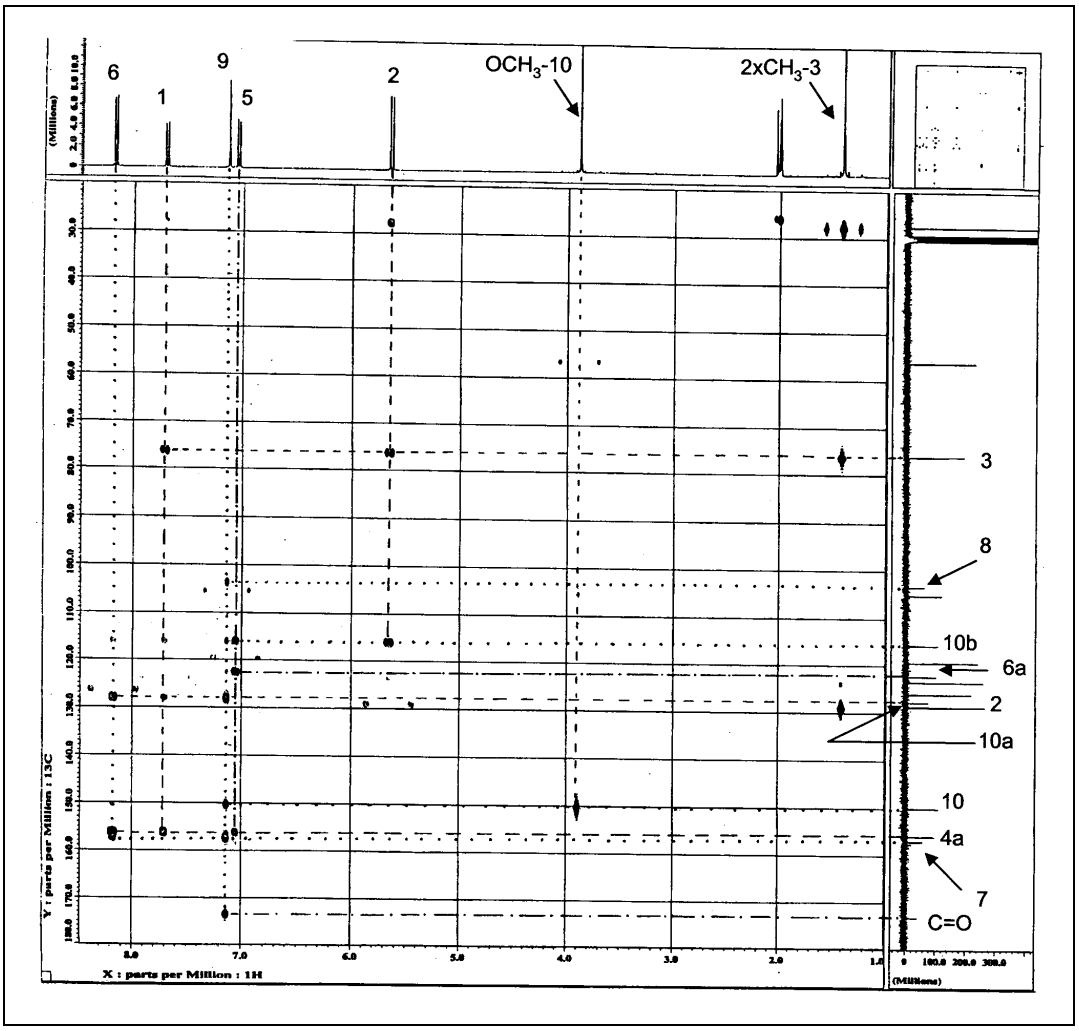



Figure 2.21. HMBC Spectrum of Compound **65** (400 MHz, Acetone-$D_6$)

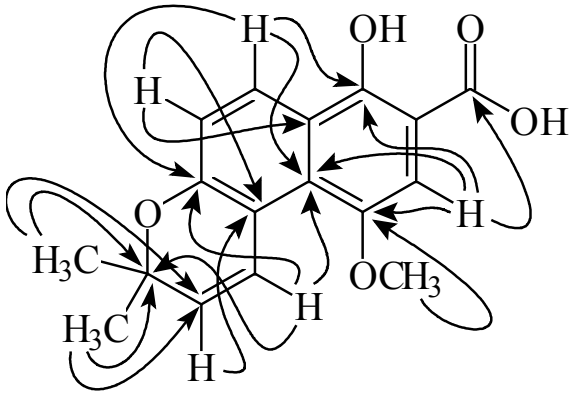



Figure 2.22. Key HMBC Correlations for Compound **65**

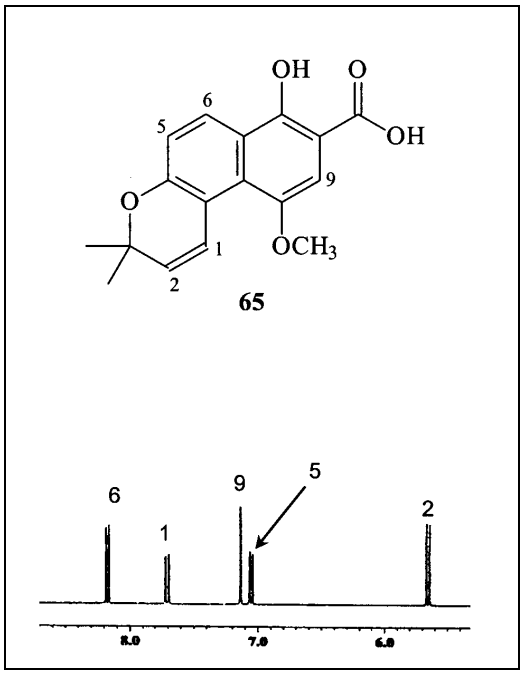



Figure 2.23a. AX Coupling Systems ($^1$H NMR spetra) for Compound **65** (at 400 MHz in Acetone-$D_6$)

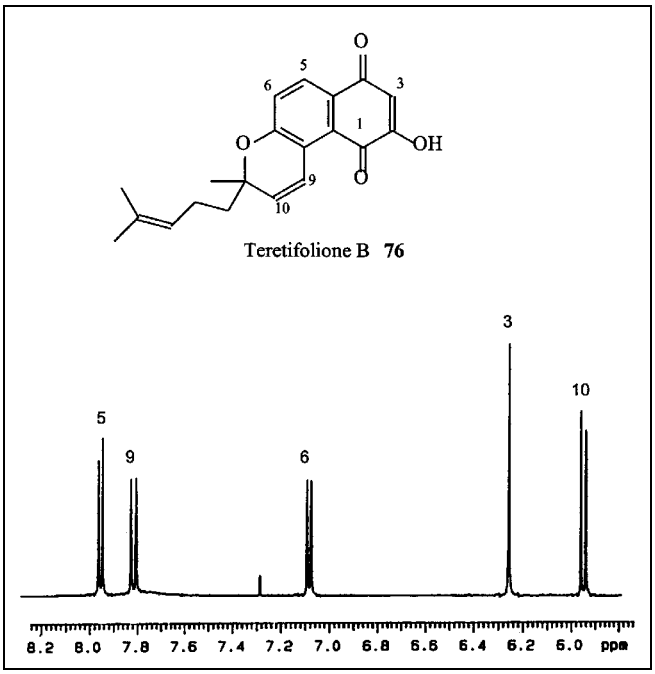
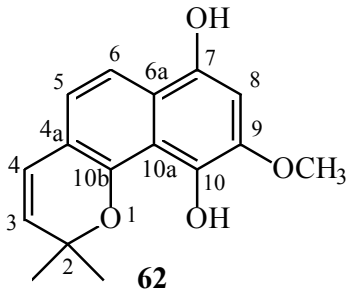



Figure 2.23b. AX Coupling Systems ($^1$H NMR spetra) for Teretifolione B **76** (at 500 MHz in $CDCl_3$, ref. 120).

II.3.1.1.4. 9-Methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol **62**

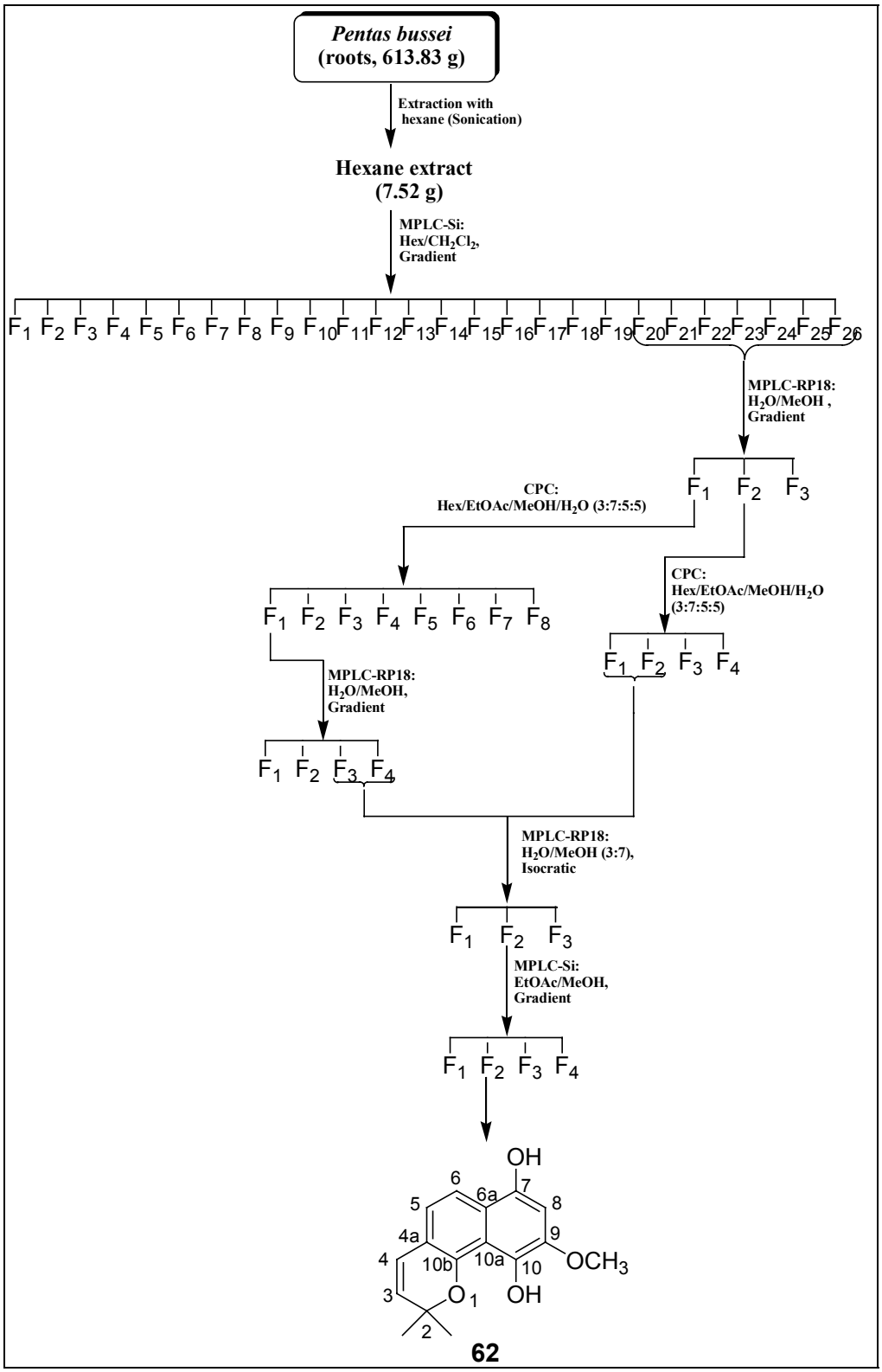
The fractionation of the mixture of the fractions 6:3, 6:4, 8:1 and 8:2 (Tables 2.10 and 2.13), both from the hexane extract of the roots of *P. bussei*, by MPLC on RP18 followed by further purification by MPLC on silica gel, gave rise to compound **62**. The complete separation of this new benzochromene is described in Scheme 2.7.

**9-Methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol 62**: White solid from hexane/acetone (1:1), mp 201.8-204.0 °C. A spectral pattern similar to that of compound **65** was observed in the UV spectrum of the benzochromene **62**, with maximum absorption bands at 255.0 nm (log $\varepsilon$ 4.12) and 360.0 nm (log $\varepsilon$ 3.63). These data are compatible with a naphthalene ring substituted with functional groups enhancing delocalisation of electrons (121).




**62**

The $^1$H NMR spectrum of compound **62** in $CD_3OD$ (Figure 2.24a, Table 2.35) showed the major features found in the benzochromene **65**, especially the spectral part concerning the aromatic area and the methoxy group. Two AX coupling systems, i.e. H-3 [$\delta_H$ 5.51, d, $J$ = 10.2 Hz]/ H-4 [$\delta_H$ 7.62, d, $J$ = 10.2 Hz] and H-5 [$\delta_H$ 8.07, d, $J$ = 8.9 Hz]/H-6 [$\delta_H$ 6.90, d, $J$ = 8.9 Hz] were observed also in compound **62**. However, the zigzag coupling pattern (with $^5J$ = 0.7 Hz) existing between the AX systems of the benzochromene **65** are not present in compound **62**. The methoxy ($OCH_3$-9) protons resonate at $\delta_H$ 3.79, while the two methyl groups gave a unique singlet at $\delta_H$ 1.39. In addition, a singlet at $\delta_H$ 7.36 was assigned to H-8.




**Scheme 2.7**: Isolation of 9-Methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol **62** from the Roots of *P. bussei*.

The location of H-8, in *ortho* position from the $OCH_3$ was confirmed by a DIFNOE experiment which showed effects of 6% on $OCH_3$ following the irradiation of H-8 and of 5% on H-8, following the irradiation of the $OCH_3$-9 protons (Figure 2.25). Although the $^1H$ NMR spectrum in $CD_3OD$ did not show OH signals, the $^1H$ spectrum run in acetone-$D_6$ (Figure 2.24b) displayed OH signals at $\delta_H$ 3.20-3.40 and 13.43, respectively. The very high value of $\delta_H$ 13.43 is consistent with an extended conjugation existing in the molecule. The presence of OH groups was additionally confirmed by the IR spectrum (Figure 2.26) which showed a particular large band at 3430 $cm^{-1}$, typical to intramolecular hydrogen bond bridges of phenolic hydroxy groups. Besides, another large band observed at 1605 $cm^{-1}$ (C=C) is compatible with an unsaturated, broadly conjugated system(121b).

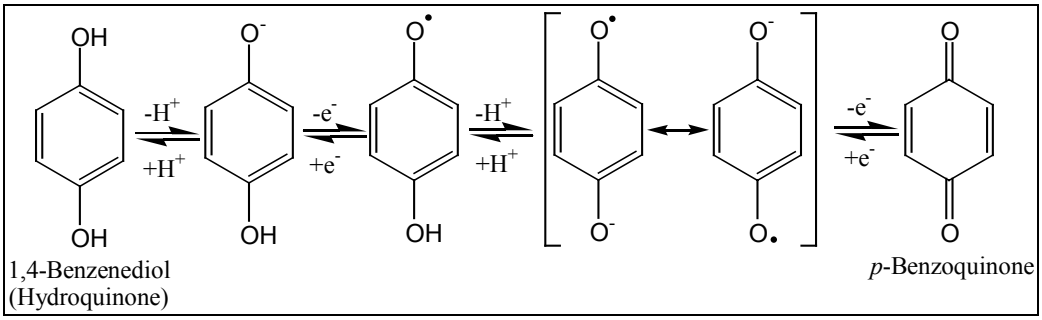
The $^{13}C$ NMR spectrum (Figure 2.27, Table 2.35) showed fourteen different signals. Combination of $^{13}C$ NMR, HETCOR and DEPT spectra allowed to unequivocally assign all protonated carbons. From all these spectra, it was clear that the two methyl groups are overlapping in the $^{13}C$ NMR spectrum ($\delta_C$ 28.89). The $OCH_3$-9 signal appears at $\delta_C$ 57.52, while the typical oxygenated quaternary carbon C-3 of the pyran ring resonates at $\delta_C$ 77.14. The high field position of C-8 ($\delta_C$ 109.56) is compatible with the double *ortho* substitution by the electron giving functional groups OH-7 and $OCH_3$-9. In addition to the rest of the protonated $sp^2$ carbons (see data in Table 2.35), in the aromatic region appeared signals of three non-oxygenated ($=C_q$) and three oxygenated ($=C_q$-O) quaternary carbons.

HMBC analysis (Figures 2.28-2.29) brought in some additional refinements for the structural elucidation of compound **62**, especially concerning the arrangement of the rings, together with the number of $=C_q$-O. Two observed correlations, i.e. H-3/C-4a and H-6/C-4a, were indicative of the fusion of the pyran and the benzene rings. Furthermore, the lack of the zigzag coupling relationship between the AX systems, as observed in compound **65** which is of the benzo[*f*]chromene type, helped to rule out this type of configuration, and therefore, led to the conclusion that the fusion of the pyran and benzene rings in compound **62** is of the benzo[*h*]chromene type. Concerning the number of $=C_q$-O, on the one hand, the correlation between $OCH_3$-9/C-9 allowed to unequivocally assign the $\delta_C$ 150.92 to C-9. On the other hand, all the four protons H-4, H-5, H-6 and H-8 displayed correlations with $=C_q$-O resonating in lower fields ($\delta_C$ 156.11 and 156.63) than C-9. Closer observation allowed to establish the correlation of H-6 with the most deshielded of the $=C_q$-O (dot located in lower

fields than the dots corresponding to H-4, H-5 and H-8) which is evidently C-7 consequently assigned with $\delta_C$ 156.63. As both the protons H-4, H-5 and H-8 are correlated to $=C_q$-O resonating at $\delta_C$ 156.11, it is unequivocally evident that this chemical shift may be attributed to C-10 and C-10b, the signals of which are therefore overlapping in the $^{13}C$ NMR spectrum.

The ESI mass spectrum of compound **62** displayed a pseudomolecular ion peak at *m*/*z* 295 ($[M + Na]^+$, 9%), consistent with the molecular composition of $C_{16}H_{16}O_4$ with an unsaturation index of 9.

Compound **62** structurally belongs to the class of 1,4-naphthohydroquinones. Its chemistry may therefore be similar to that of the elementary 1,4-benzenediol or hydroquinone. It is well known that because they are highly conjugated, quinones are rather closely balanced (Scheme 2.8), energetically, against the corresponding hydroquinones (131).




**Scheme 2.8**: Oxydation-Reduction Relationship Between Hydroquinone and *p*-Benzoquinone

The oxidation-reduction processes leading to the interconversion lie in a series of proton and electron transfers and have been largely documented (132). Many properties of quinones result from their tendency to form the aromatic hydroquinone system, and nature makes much use of this type of oxidation-reduction to transport a pair of electrons from one substance to another in enzyme-catalyzed reactions. The hydrogen transportation function may be the reason why *p*-benzoquinones have enzymatic activity on 5-lipoxygenase to reduce the biosynthesis of SRS-A (slow reacting substance of anaphylaxis) which will result in treatment of asthma (132c, 133) or against the porcine lens aldose reductase which would be effective in preventing cataract formation in diabetes (134).

Closer inspection of the $^{13}C$ NMR spectrum of the compound **62** confirmed the presence of the above mentioned tendency to balance between the hydroquinone and the quinone form. After repeated purification and spectral analysis, the spectrum of the compound always displayed two slight pseudo signals at $\delta_C$ 109-110 and 179-180, whereas a high variability of the shape (from sharp to flattened) was observed for the most deshielded $=C_q$-O ($\delta_C$ 156.63). On the one hand, the position of $\delta_C$ 109-110 corresponds to the chemical shift of CH-8, and on the other hand, $\delta_C$ 156.63 corresponds to C-7, whereas $\delta_C$ 179-180 is a suitable chemical shift for a conjugated ketone (such as in the case of the quinone form). Besides, when kept in solution, even light-protected, the compound slowly turned to a slight greenish colour. It is well-known that because of their high conjugation, quinones are coloured (131a). These findings constitute an additional argument supporting the structure proposed for compound **62**. It is the first time that a compound possessing this type of skeleton is isolated from a plant of the genus *Pentas*.

Table 2.35. NMR Spectral data: $^{1}H$ NMR (270 MHz, $CD_3OD$), $^{13}C$ NMR (67.5 MHz, $CD_3OD$) and observed HMBC (at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$) for compound **62** (δ in ppm, *J* in Hz).

| Position | $\delta_H$, multiplicity, (*J*) | $\delta_c$ | HMBC |
|---|---|---|---|
| 2 | - | 77.14 | H-3, H-4, 2 x C<u>H<sub>3</sub></u>-2 |
| 3 | 5.51 d (10.2) | 129.11 | 2 x C<u>H<sub>3</sub></u>-2 |
| 4 | 7.62 d (10.2) | 125.62 | H-5 |
| 4a | - | 116.96 | H-3, H-6 |
| 5 | 8.07 d (8.9) | 127.26 | |
| 6 | 6.90 d (8.9) | 119.65 | |
| 6a | - | 128.12 | H-5, H-8 |
| 7 | - | 156.63 | H-6 |
| 8 | 7.36 s | 109.56 | |
| 9 | - | 150.92 | OC<u>H<sub>3</sub></u>-9 |
| $OCH_3$-9 | 3.79 s | 57.52 | |
| 10 | - | 156.11 | H-8 |
| 10a | - | 124.33 | H-6 |
| 10b | - | 156.11 | H-4, H-5 |
| 2 x $CH_3$-2 | 1.39 s | 28.89 | |
| OH | 3.20-3.40 | - | - |
| OH | 13.43 | - | - |

Signal of OH protons observed at $\delta_H$ 3.20-3.40 and 13.43 ppm were displayed in the spectrum run in acetone-$D_6$

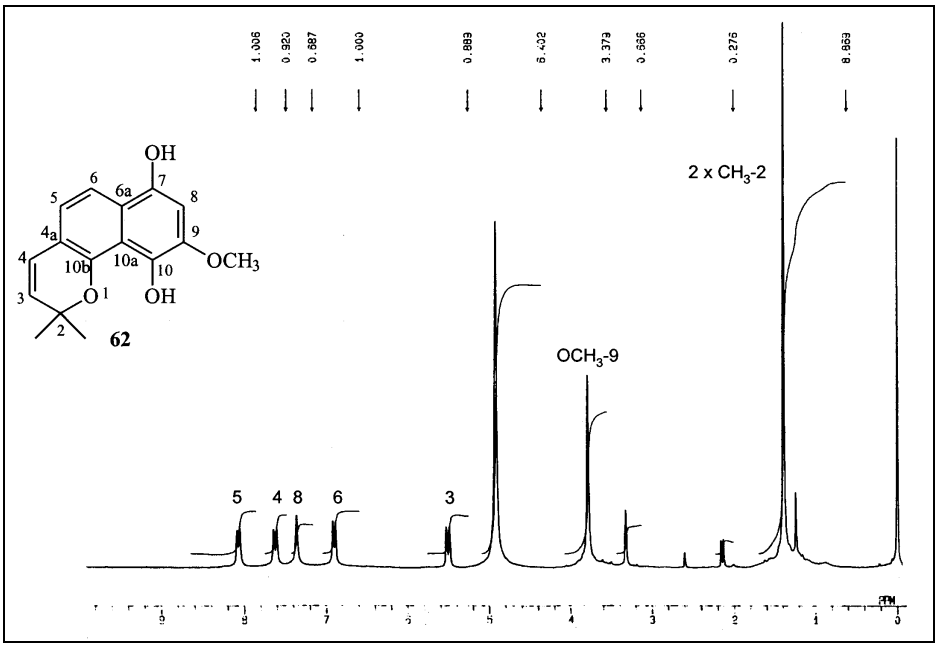



Figure 2.24a. $^{1}$H NMR Spectrum of Compound **62** (270 MHz, $CD_3OD$)

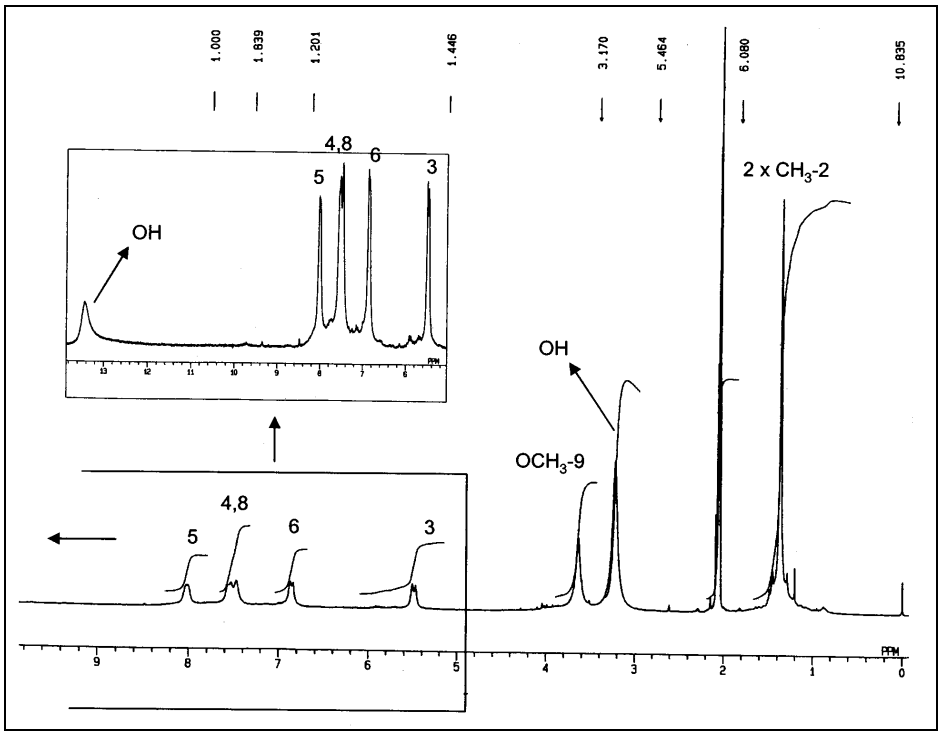



Figure 2.24b. $^{1}$H NMR Spectrum of Compound **62** (270 MHz, Acetone-$D_6$)

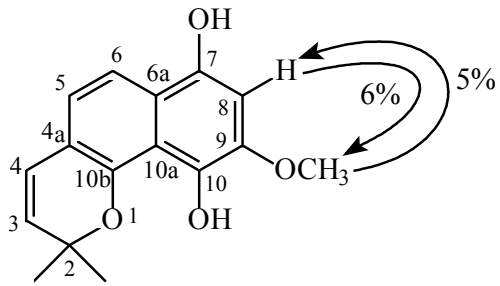



Figure 2.25. Pertinent DIFNOE Effects for Compound **62**

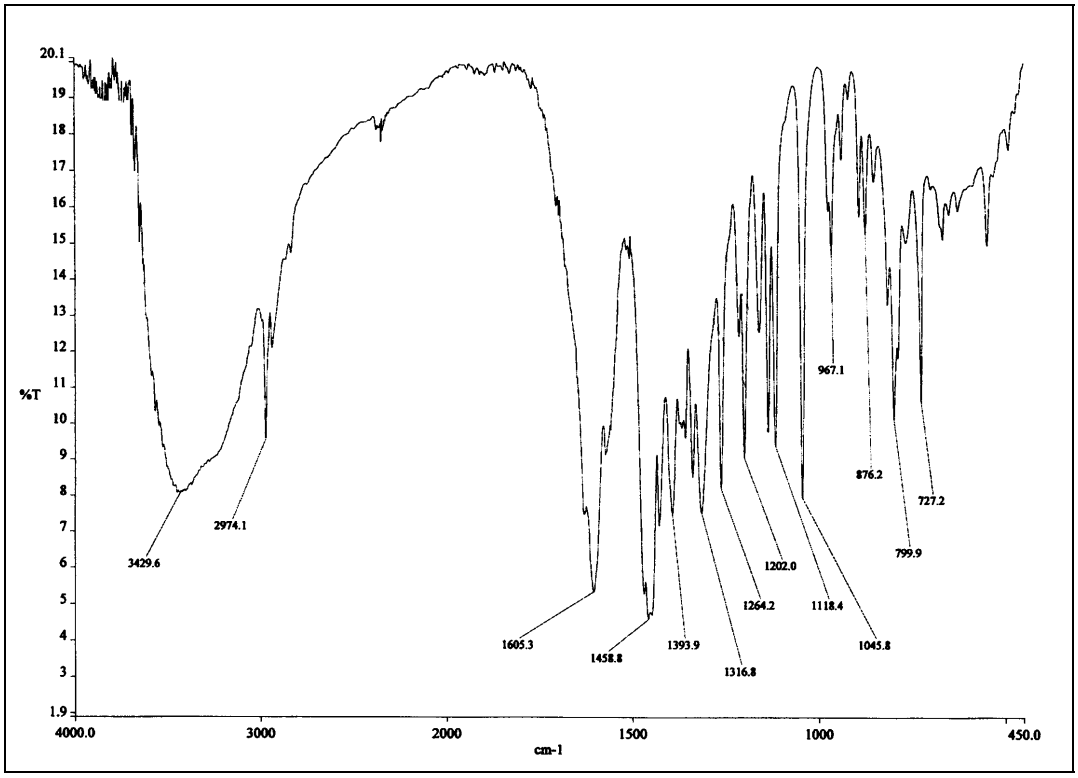



Figure 2.26. IR Spectrum of Compound **62** (in KBr)

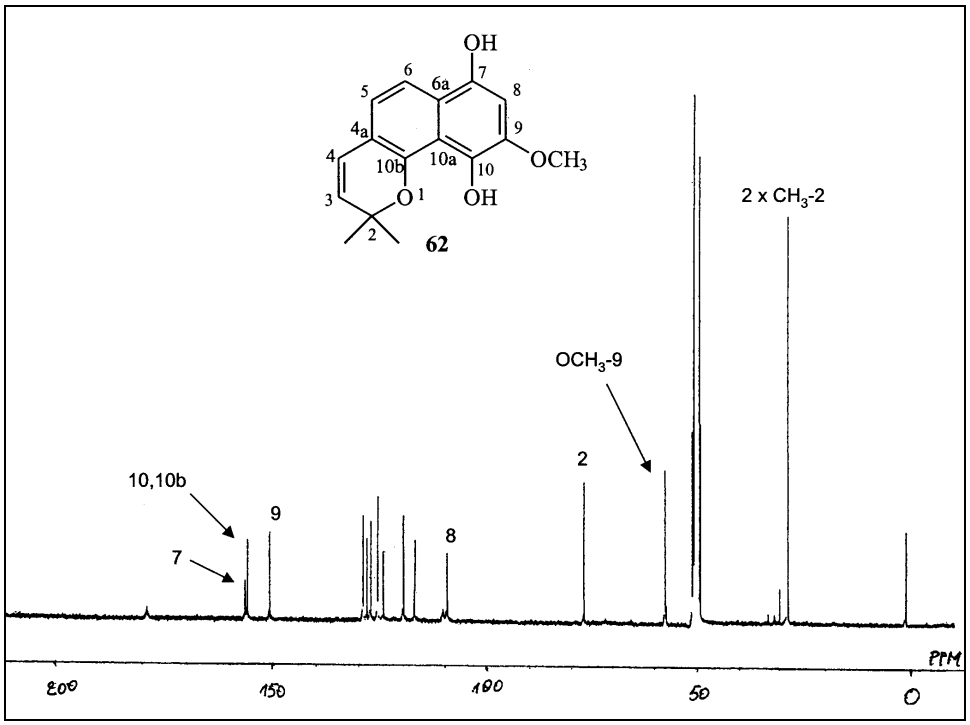



Figure 2.27. $^{13}C$ NMR Spectrum of Compound **62** (270 MHz, $CD_3OD$)

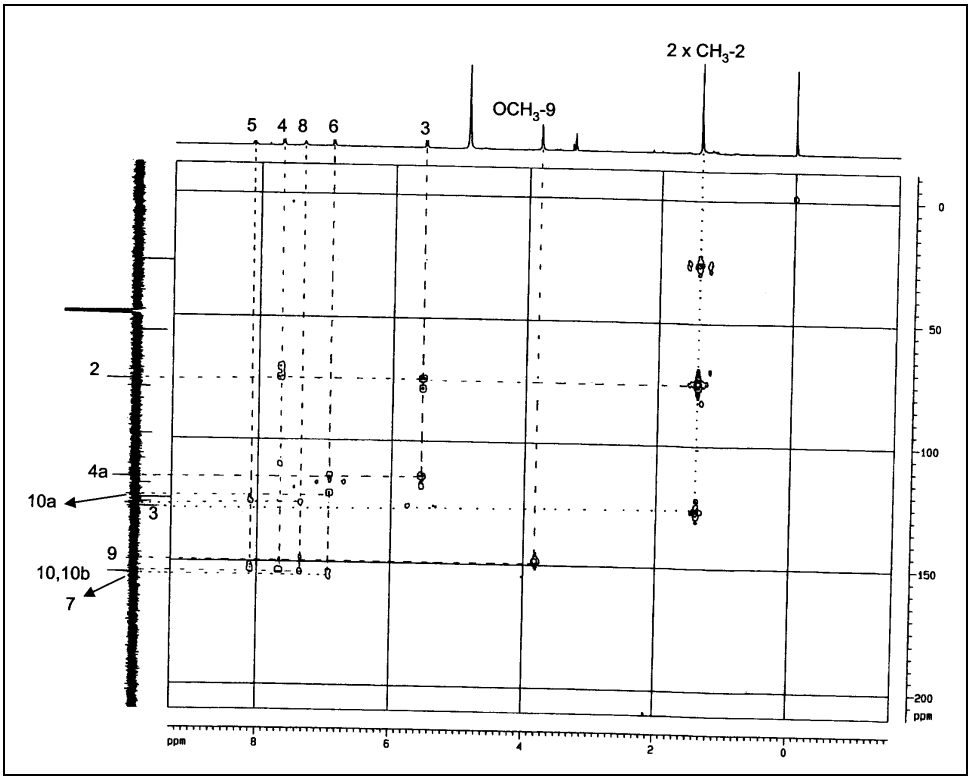



Figure 2.28. HMBC Spectrum of Compound **62** (400 MHz , CD3OD)

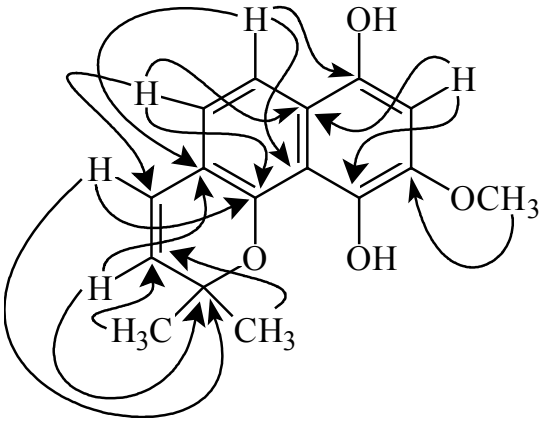
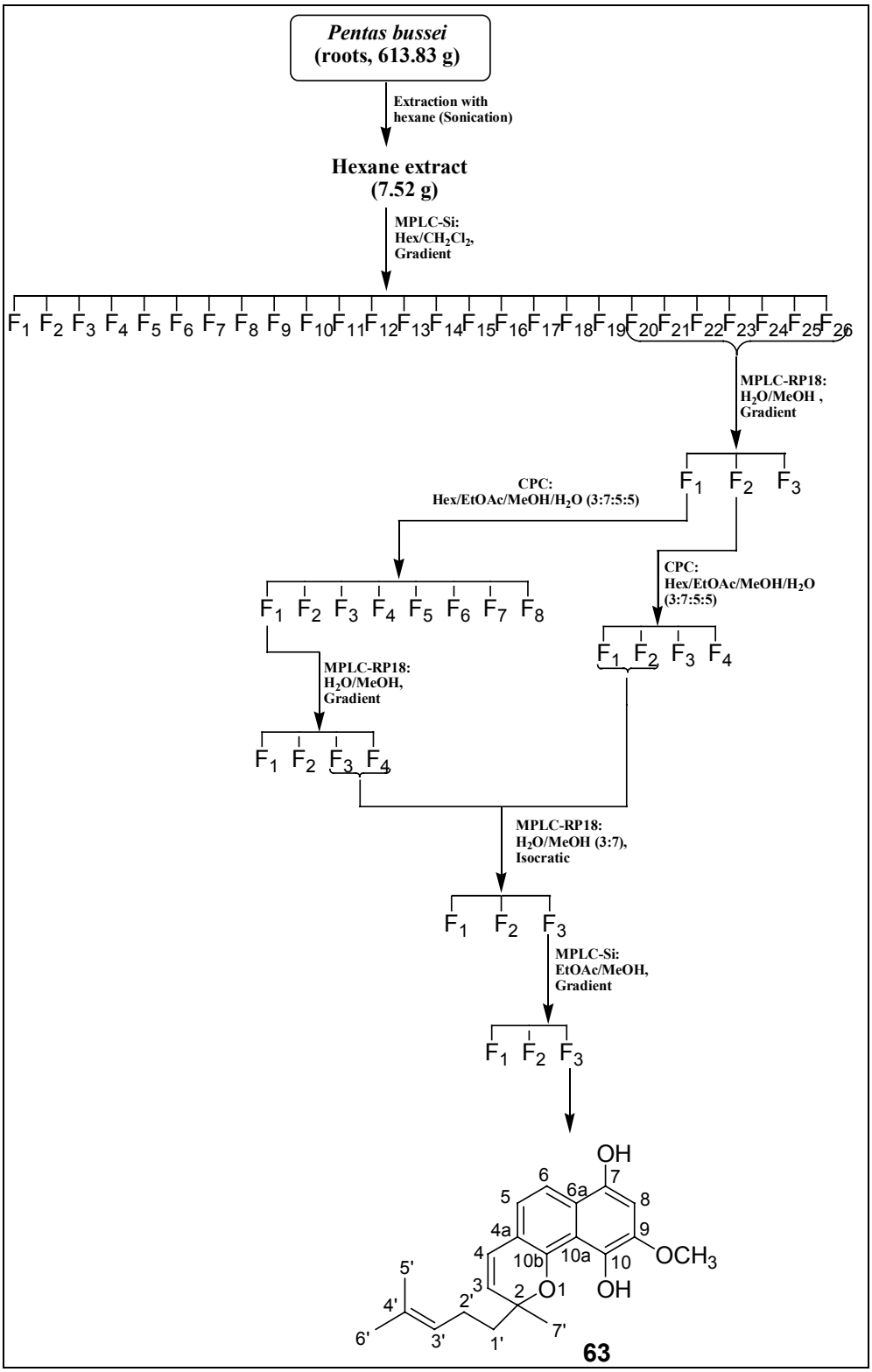



Figure 2.29. Key HMBC Correlations for Compound **62**

II.3.1.1.5. 9-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]chromene-7,10-diol **63**

The fractionation of the fraction 9:3 (Table 2.14) by MPLC on silica gel afforded compound **63** which is a new naphthohydroquinone of the benzochromene type. Scheme 2.9 describes the complete isolation of this new naphthohydroquinone of the benzochromene type from the roots of *P. bussei*.




**Scheme 2.9**: Isolation of 9-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]-chromene-7,10-diol **63** from the Roots of *P. bussei*.

**9-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]chromene-7,10-diol 63**, white solid from hexane/acetone (1:1), mp 174.5-176.4 °C. The UV spectrum of the compound **63** displayed exactly the same pattern as the one observed in the case of the benzochromene **62**, showing the maximum absorption bands at 254.1 nm (log $\varepsilon$ 4.25) and 369.0 nm (log $\varepsilon$ 3.70). This pattern is consistent with a conjugated naphthalene ring (121).

The IR spectrum of compound **63** (Figure 2.30) showed the typical OH absorption band already observed in the benzochromene **62** at 3423 $cm^{-1}$ (121b). Although the general pattern of the spectrum is similar to that of the latter compound, the C=C absorption band of compound **63** appeared rather complex with maxima at 1626, 1595 and 1560 $cm^{-1}$. This complexity is due to the presence of an additional olefinic double bond at C-3'=C-4'.

The $^1$H NMR spectrum in $CD_3OD$ (Figure 2.31a, Table 2.36) displayed three major features already observed in compound **62**, i.e. two AX systems, H-3 [$\delta_H$ 5.51, d, $J$ = 10.4 Hz]/H-4 [$\delta_H$ 7.72, d, $J$ = 10.4 Hz] and H-5 [$\delta_H$ 8.08, d, $J$ = 8.9 Hz]/H-6 [$\delta_H$ 6.92, d, 8.9 Hz], a singlet at $\delta_H$ 7.34 (H-8) and a singlet at $\delta_H$ 3.82 belonging to the $OCH_3$-9 protons. The differences with compound **62** originate from the observation of a total of three methyls at $\delta_H$ 1.37 ($H_3$-7'), 1.55 ($H_3$-6') and 1.62 ($H_3$-5'), three methylenes at $\delta_H$ 1.66-1.72 ($H_2$-1') and 2.11-2.17 ($H_2$-2'), together with a methine at $\delta_H$ 5.09 (H-3'). From these differences, it is evident that the structure of compound **63** contains a homoprenyl side chain. In addition, the $^1$H NMR spectrum in acetone-$D_6$ (Figure 2.31b) displayed two OH signals at $\delta_H$ 3.60-3.80 and 13.40, the latter high value being an indication of a broad conjugation in the molecule.

The $^{13}$C and DEPT NMR spectra (Figure 2.32, Table 2.36) confirmed the presence of the homoprenyl side chain, the data of which are $\delta_C$ 41.63 (C-1'), 23.84 (C-2'), 125.41 (C-3'), 132.36 (C-4'), 25.88 (C-5') and 17.70 (C-6'). The rest of the data match together in the two compounds **62** and **63**. Thus, the differences observed between the $^1$H and $^{13}$C NMR data of the two compound are due to the homoprenyl side chain.

The ESI mass spectrum of compound **63** displayed an ion peak at *m/z* 341 $[M + H]^+$ (5 %). The molecular formula was deduced as being $C_{21}H_{24}O_4$, with an unsaturation

index of 10.

In addition, the slight pseudo-signals observed in the $^{13}C$ NMR spectrum of the compound **62** and related to the hydroquinone/quinone balance, together with the tendency to turn to a green colour in solution, were also observed in the case of the compound **63**. All these findings lead to the establishment of the structure of the homoprenylated benzochromene **63** as proposed.

Table 2.36. $^{1}H$ NMR (270 MHz, $CD_3OD$) and $^{13}C$ NMR (67.5 MHz, $CD_3OD$) spectral data of compound **63** (δ in ppm, *J* in Hz).

| Position | $\delta_H$, multiplicity, (*J*) | $\delta_c$ |
|---|---|---|
| 2 | - | 78.27 |
| 3 | 5.51 d (10.2) | 127.06 |
| 4 | 7.62 d (10.2) | 124.78 |
| 4a | - | 115.56 |
| 5 | 8.07 d (8.9) | 126.05 |
| 6 | 6.90 d (8.9) | 118.36 |
| 6a | - | 126.88 |
| 7 | - | 155.54 |
| 8 | 7.36 s | 108.26 |
| 9 | - | 149.74 |
| $OCH_3$-9 | 3.79 s | 56.30 |
| 10 | - | 155.07 |
| 10a | - | 123.05 |
| 10b | - | 155.07 |
| 1' | 1.66-1.72 m | 41.63 |
| 2' | 2.11-2.17 m | 23.84 |
| 3' | 5.09 br t (6.9) | 125.41 |
| 4' | - | 132.36 |
| 5' | 1.62 s | 25.88 |
| 6' | 1.55 s | 17.70 |
| 7' | 1.37 s | 26.04 |
| OH | 3.60-3.80 | - |
| OH | 13.45 | - |

Signal of OH protons observed at $\delta_H$ 3.60-3.80 and 13.45 ppm were displayed in the spectrum run in acetone-$D_6$

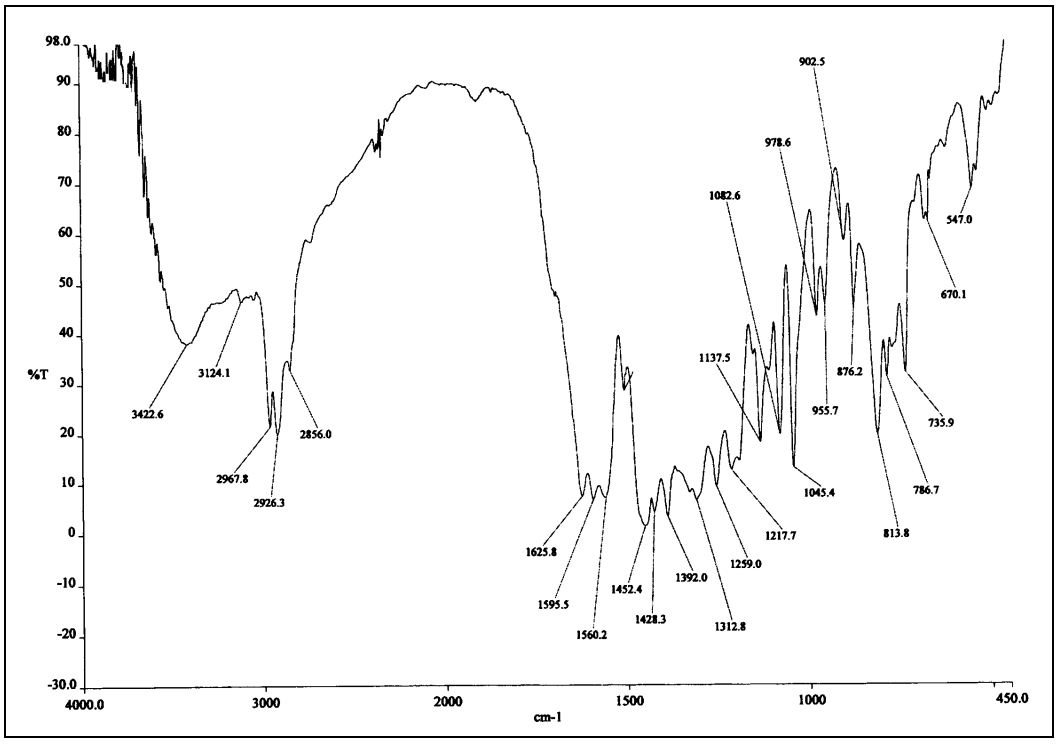



Figure 2.30. IR Spectrum of Compound **63** (in KBr)

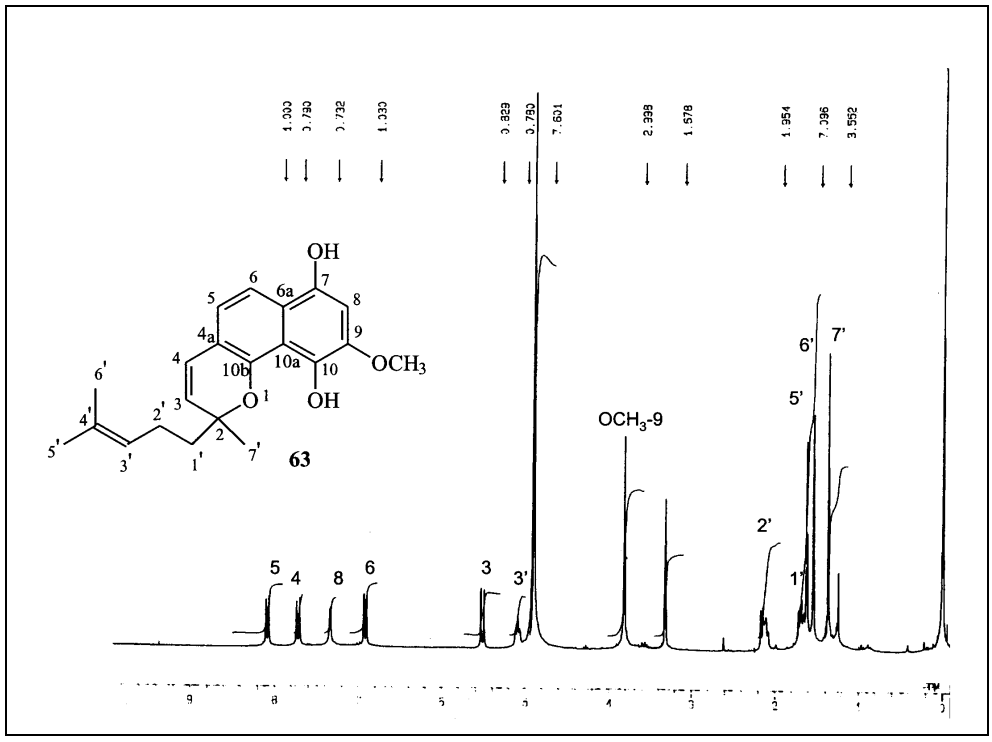



Figure 2.31a. $^{1}$H NMR Spectrum of Compound **63** (270 MHz, $CD_3OD$)

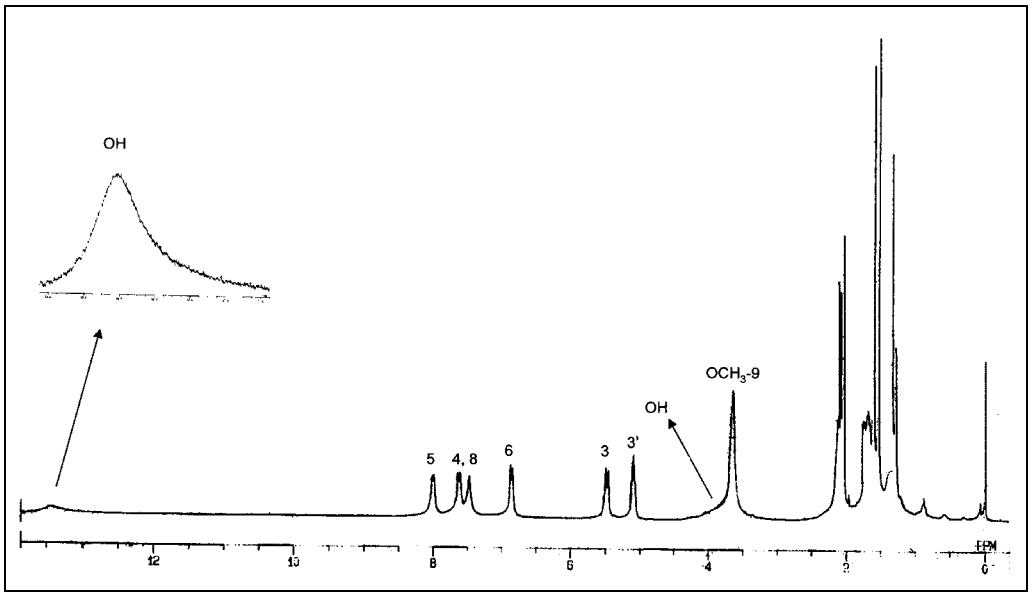



Figure 2.31b. $^{1}$H NMR Spectrum of Compound **63** (270 MHz, Acetone-$D_6$)

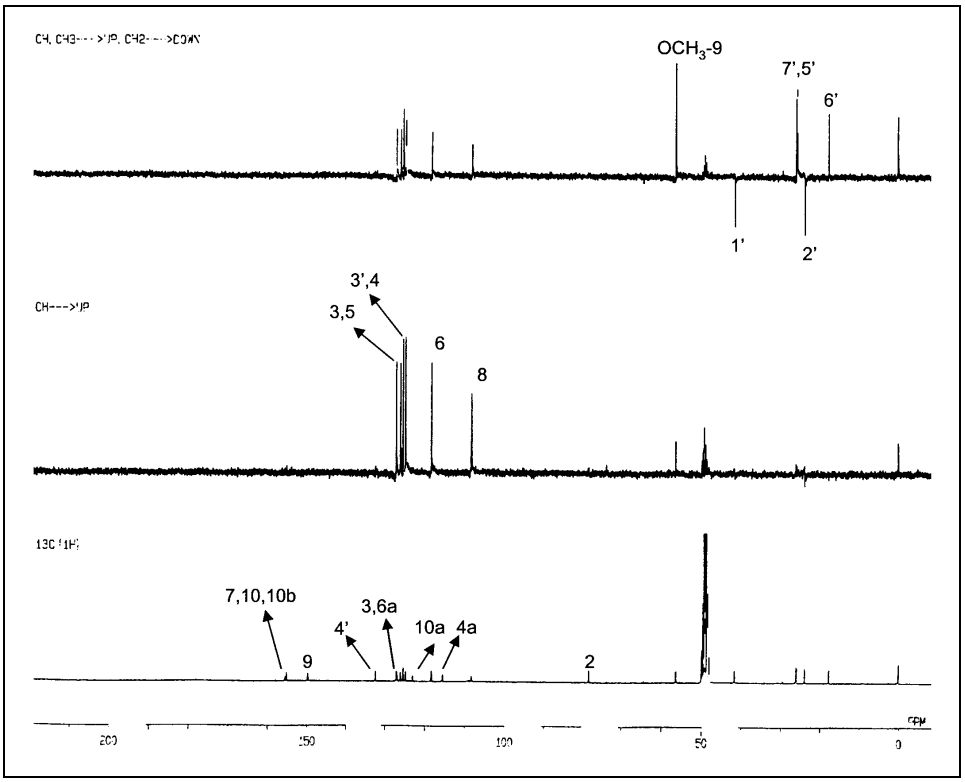
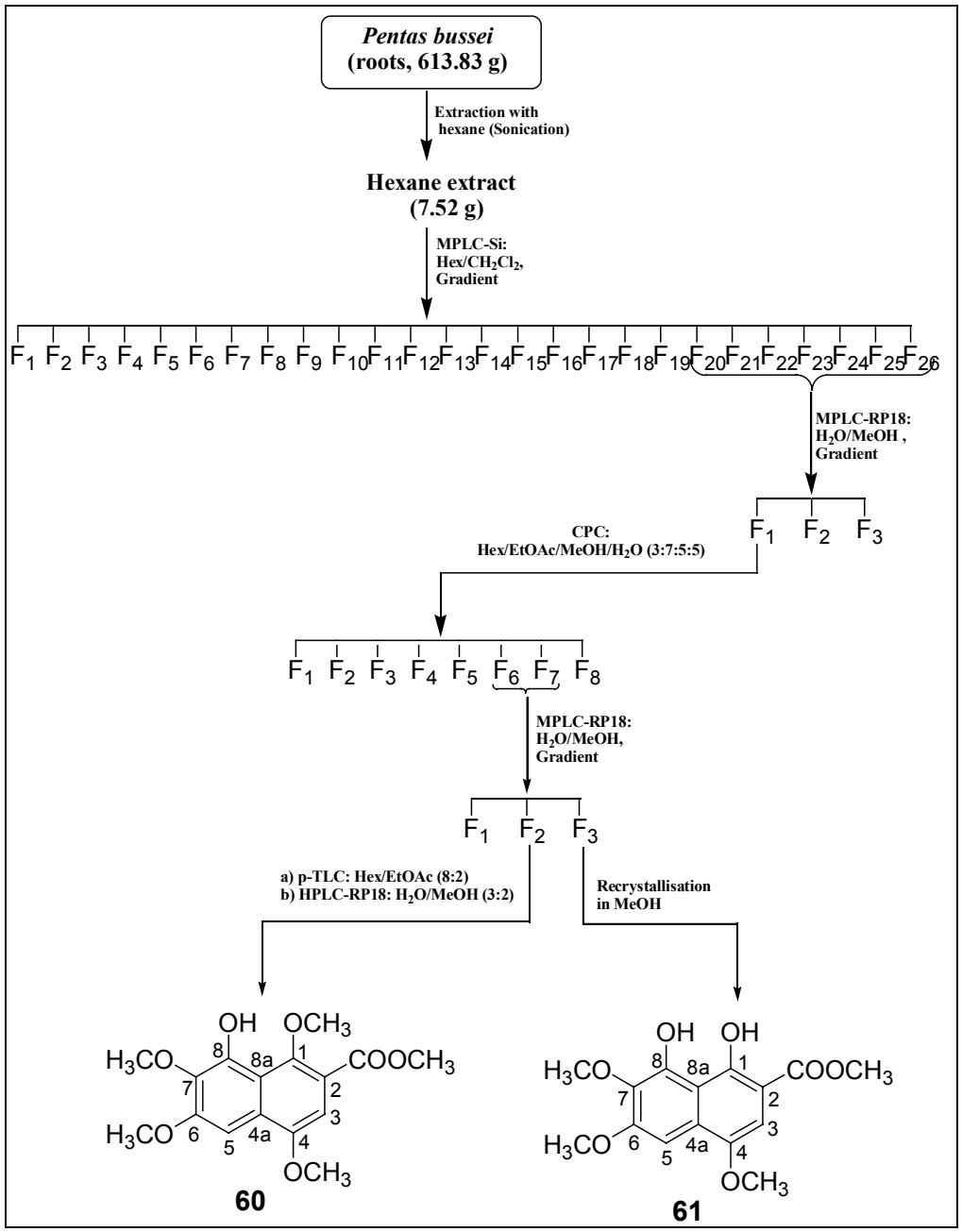



Figure 2.32. $^{13}$C and DEPT NMR Spectra of Compound **63** (67.5 MHz, $CD_3OD$)

Compounds **58**-**59**, **62**-**63** and **65** are novel natural products isolated for the first time from a plant species. Since the isolation of mollugin **38** and pentalongin **39** from the root bark of *P. longiflora* (92,93), these five compounds isolated from the roots of *P. bussei* form a new series of pyranonaphthoquinone derivativatives from the plant genus *Pentas*.

*II.3.1.2. Highly Oxygenated Naphthohydroquinones*

The fractions 5:6 and 5:7 (Table 2.8) were mixed and fractionated by MPLC, followed, on the one hand, by p-TLC and HPLC, and, on the other hand, by recrystallisation in MeOH, to afford the two compounds **60** and **61**. The complete separation of these two highly oxygenated naphthohydroquinones is described in Scheme 2.10.




**Scheme 2.10**: Isolation of Methyl 8-hydroxy-1,4,6,7-tetramethoxy-2-naphthoate **60** and Methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61** from the Roots of *P. bussei*.

II.3.1.2.1. Methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61**

Compound **61** crystallised as reddish needles from MeOH, mp 175.8-176.8 °C. The IR spectrum diplayed an OH absorption band at 3341 $cm^{-1}$. The band of the C=O appeared at the frequency of 1655 $cm^{-1}$, the value of which is lower than that usually observed for a normal ester (~ 1740 $cm^{-1}$). This low frequency is evidently due to hydrogen bond bridges between OH-1 and the C=O, but also to the conjugation of the latter with the ring system (121b).

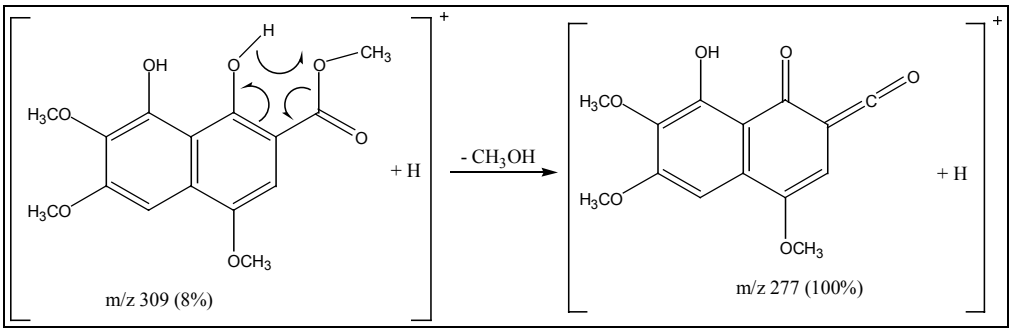
The $^{1}H$ NMR spectrum (Figure 2.33, Table 2.37) displayed a relatively simple pattern consisting of four singlets of the $OCH_3$ groups at $\delta_H$ 3.92, 3.95, 3.97 and 3.98 ppm, two singlets of aromatic protons at $\delta_H$ 6.92 and 7.06, and two OH protons signals at $\delta_H$ 9.67 and 12.95. The low field position of the latter OH is consistent with its *ortho* position next to the methoxycarbonyl group on the ring B of the α-naphthol moiety. The value of $\delta_H$ 12.95 was therefore assigned to OH-1, and this is compatible with hydrogen bond formation evoked in the IR analysis.

The $^{13}C$ NMR (Figure 2.34, Table 2.37) and DEPT spectra showed three non-oxygenated $sp^2$ hybridized carbons at $\delta_C$ 101.79, 110.7 and 127.81ppm, respectively, together with five oxygenated $sp^2$ hybridized carbons at $\delta_C$ 135.25, 147.13, 149.45, 155.79 and 156.04 ppm. In addition, the carbonyl carbon resonated at 171.71 ppm. Combination of $^{13}C$ NMR, DEPT and HETCOR spectra allowed to assign all protonated carbons. The four methoxy groups were assigned as $OCH_3$-4 [$\delta_H$ 3.92, s; $\delta_C$ 55.61], $OCH_3$-6 [$\delta_H$ 3.98, s; $\delta_C$ 55.92], $OCH_3$-7 [$\delta_H$ 3.95, s; $\delta_C$ 60.75] and COO$\underline{CH_3}$-2 [$\delta_H$ 3.97, s; $\delta_C$ 52.42], whereas, the two $sp^2$ methine groups were assigned as =CH-3 [$\delta_H$ 6.82, s; $\delta_C$ 99.67] and =CH-5 [$\delta_H$ 7.06, s; $\delta_C$ 93.74]. The high-field positions of these methine signals in the $^{13}C$ NMR spectrum is compatible with their *ortho* positions next to the methoxy groups (121b).

The HMBC NMR spectrum (Figures 2.35-2.37, Table 2.37) allowed to unequivocally attribute all $=C_q$-O bearing the hydroxy and the methoxy groups by displaying the following the six correlations OH-1/C-1, $OCH_3$-4/C-4, $OCH_3$-6/C-6, $OCH_3$-7/C-7, OH-8/C-8 and COO$\underline{CH_3}$-2/$\underline{C}$OO$CH_3$-2. Figure 2.34 shows particularly and distinctly all methoxy bearing $sp^2$ carbons. The chemical shifts $\delta_C$ 101.79 and 127.81 were attributed to C-2 and C-4a, following

the observation of the correlations OH-1/C-2 and H-3/C-4a, respectively. Besides, it was evident to attribute the $\delta_C$ 110.17 to C-8a due to the three observed correlation dots OH-1/C-8a, H-5/C-8a and OH-8/C-8a. DIFNOE experiments (Figure 2.38) confirmed the *ortho* positions of H-3 next to $OCH_3$-4 and H-5 next to $OCH_3$-6.

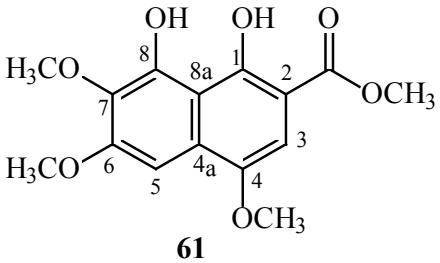
The ESI mass spectrum of compound **61** showed an ion peak at *m*/*z* 309 ($[M + H]^+$, 8%) which is compatible with a molecular formula of $C_{15}H_{16}O_7$. In addition, the base peak (100%) was displayed at *m*/*z* 277. The formation of this fragment can be explained by the expulsion of methanol from the ion $[M + H]^+$ by the "*ortho* effect" (121b), generating the ketene ion at *m*/*z* 277 (Scheme 2.11).




**Scheme 2.11**: Formation of the Mass Spectral Fragment at *m*/*z* 277 (100%) from Compound **61**

Table 2.37. $^{1}H$ NMR (270 MHz, $CDCl_3$) and $^{13}C$ NMR (67.5 MHz, $CDCl_3$) spectral data, with observed HMBC (at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$) for compound **61** ($\delta$ in ppm).

| Position | $\delta_H$, multiplicity | $\delta_c$ | HMBC |
|---|---|---|---|
| 1 | - | 155.79 | OH-1, H-3 |
| OH-1 | 12.95 s | - | |
| 2 | - | 101.79 | OH-1 |
| 3 | 6.82 s | 99.67 | |
| 4 | - | 147.13 | H-3, H-5, $OCH_3$-4 |
| 4a | - | 127.81 | H-3 |
| 5 | 7.06 s | 93.74 | |
| 6 | - | 156.04 | H-5, $OCH_3$-6 |
| 7 | - | 135.25 | H-5, OH-8, $OCH_3$-7 |
| 8 | - | 149.45 | OH-8 |
| OH-8 | 9.67 s | - | |
| 8a | - | 110.17 | OH-1, H-5, OH-8 |
| $COO\underline{C}H_3$-2 | 3.97 s | 52.42 | |
| $\underline{C}OOCH_3$-2 | - | 171.71 | H-3, $COO\underline{C}H_3$-2 |
| $OCH_3$-4 | 3.92 s | 55.61 | |
| $OCH_3$-6 | 3.98 s | 55.92 | |
| $OCH_3$-7 | 3.95 s | 60.75 | |




**61**

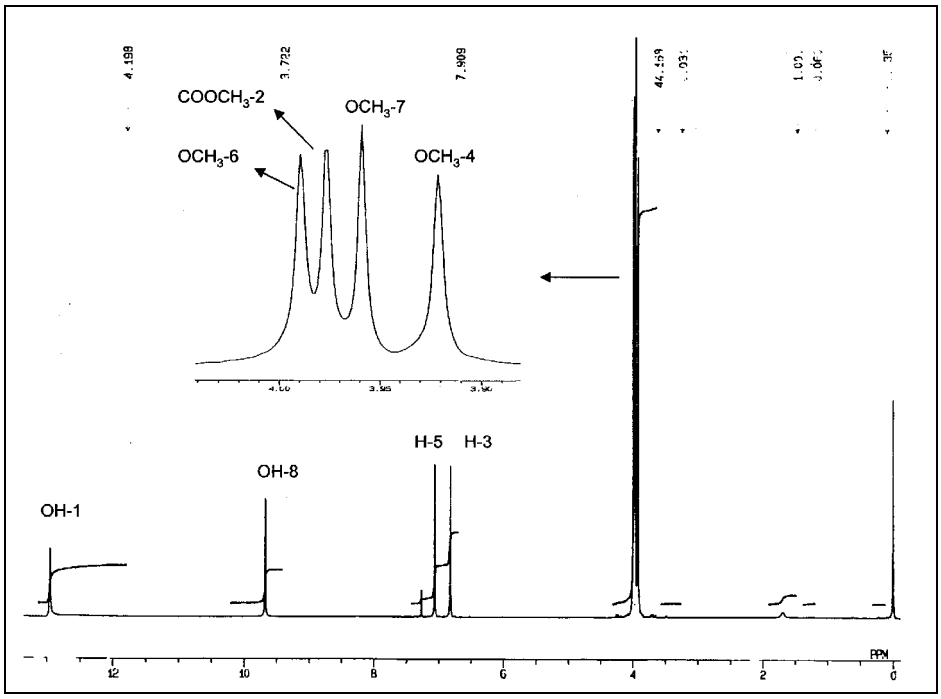



Figure 2.33. $^{1}$H NMR Spectrum of Compound **61** (270 MHz, $CDCl_3$)

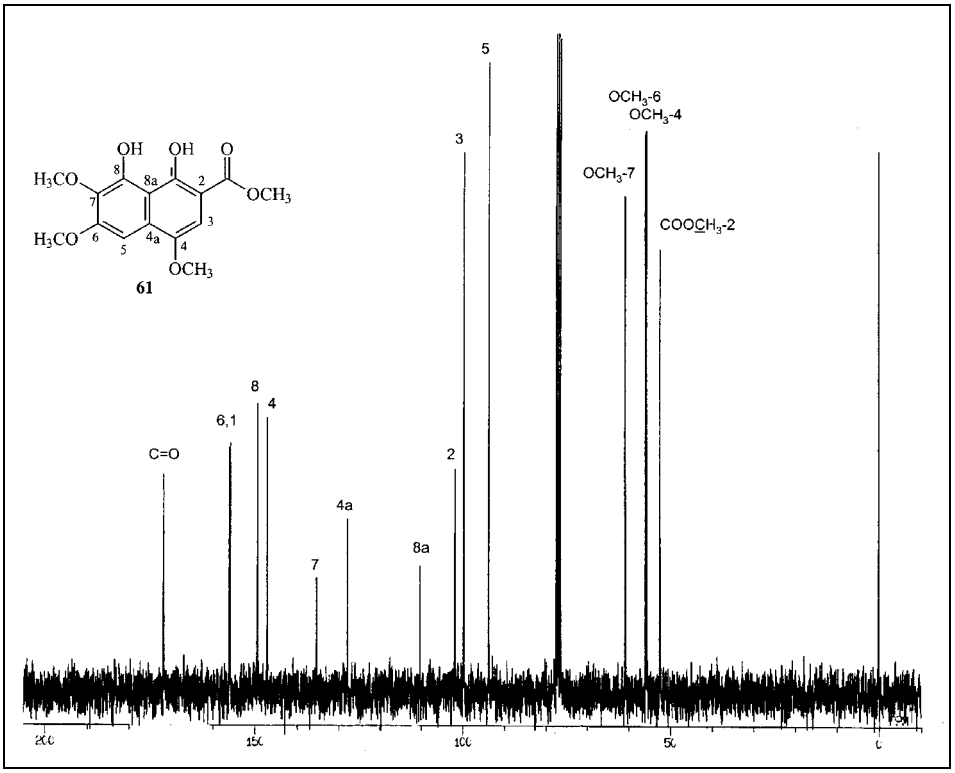



Figure 2.34. $^{13}$C NMR Spectrum of Compound **61** (67.5 MHz, $CDCl_3$)

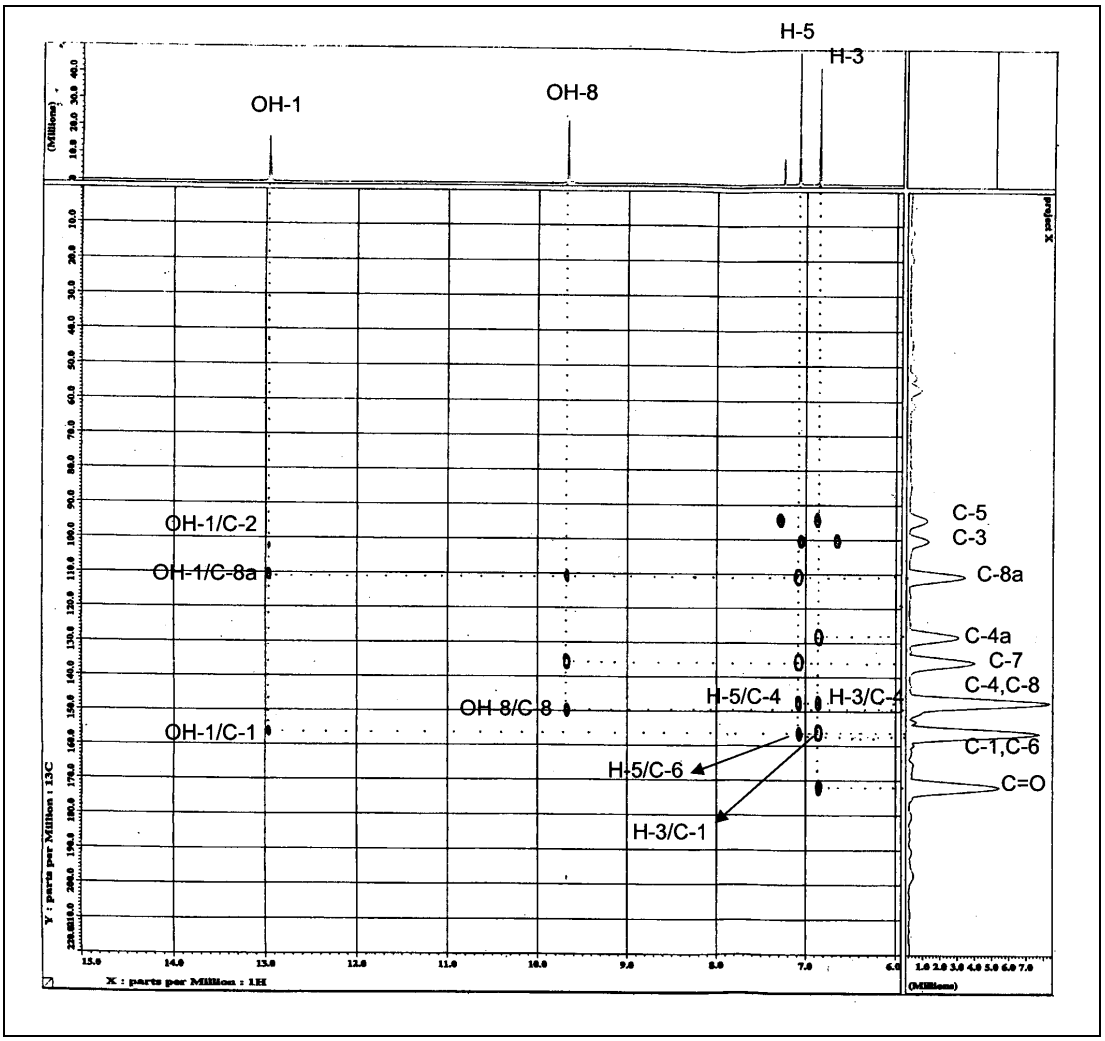
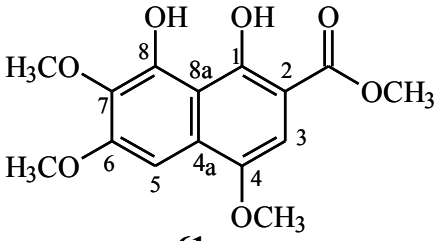



Figure 2.35. HMBC Spectrum of Compound **61** (400 MHz, $CDCl_3$): portion corresponding to the aromatic and low-field regions on the $^1H$ NMR spectrum




**61**

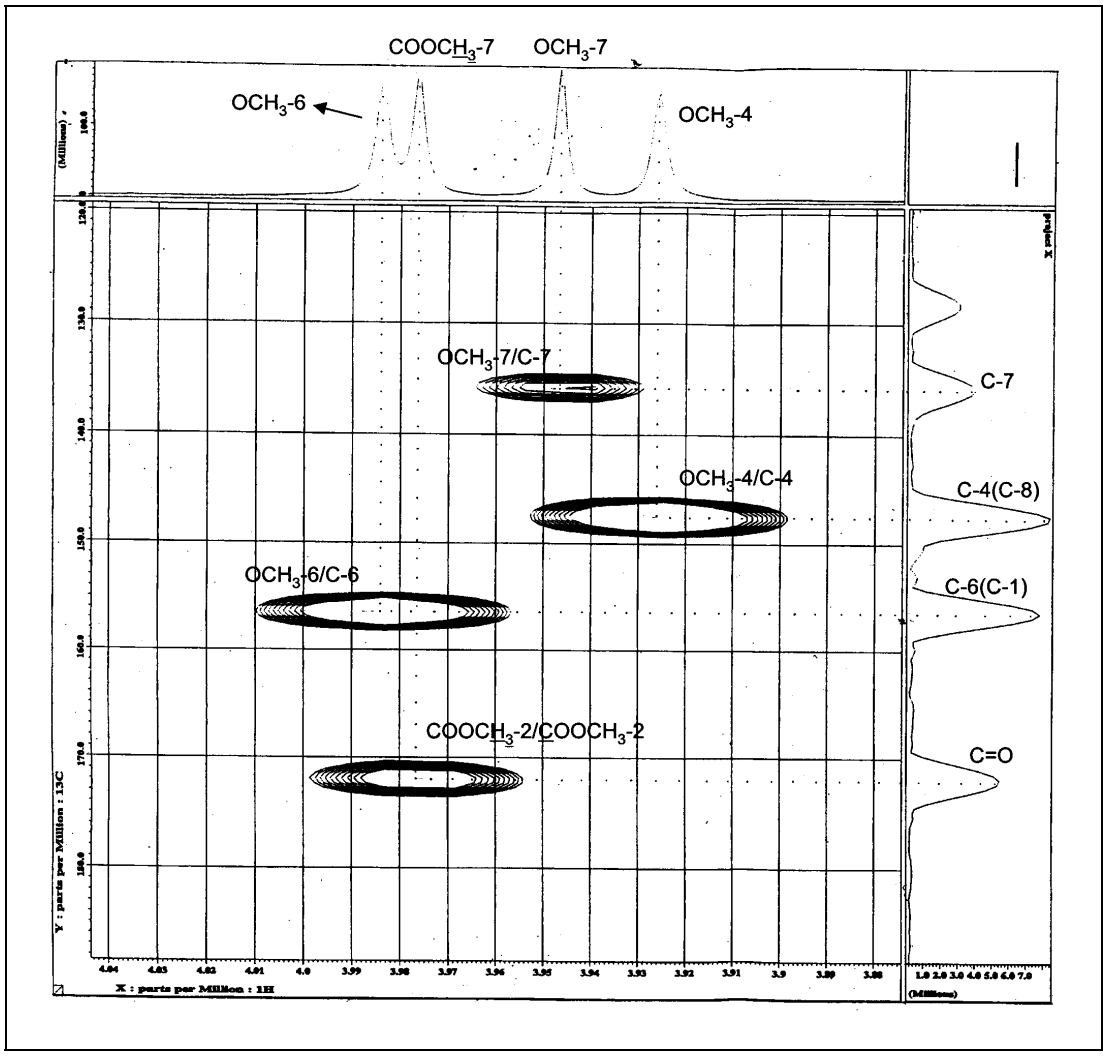



Figure 2.36. HMBC spectrum of compound **61** (400 MHz, $CDCl_3$): methoxy bearing $sp^2$ carbons

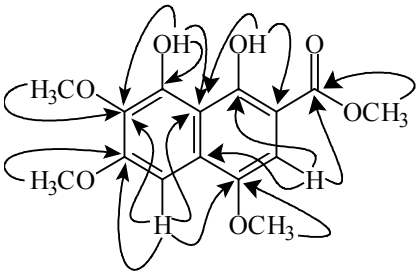



Figure 2.37. Main HMBC correlations of compound **61**

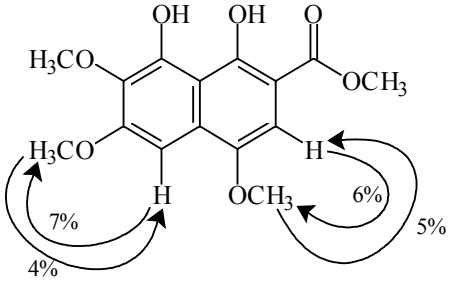



Figure 2.38. DIFNOE experiments of compound **61**

II.3.1.2.2. Methyl 8-hydroxy-1,4,6,7-tetramethoxy-2-naphthoate **60**

Compound **60** was isolated as an amorphous reddish solid, mp 167.9-169.4 °C. The spectral data of this compound were closely similar to those of compound **61**. The $^{1}H$ NMR spectrum did not show any highly deshielded OH proton, while displaying an additional $OCH_3$ group at $\delta_H$ 4.02, in comparison with compound **61**. The lack of this OH was confirmed by the IR spectrum which showed the C=O band at 1715 $cm^{-1}$, a frequency which is higher than that observed for compound **61** (1655 $cm^{-1}$) and perfectly compatible with the lack of OH group in *ortho* position. Such an OH may, indeed, form hydrogen bonds with the carbonyl oxygen, the final effect being the decreasing of the frequency of absorption of the carbonyl group (121b). Therefore, it is understandable that the unique difference between the two compounds lies in the substituent located at the 1-position and which is a methoxy group for compound **60** and a hydroxyl group in the case of compound **61**. However, the IR spectrum of compound **60** displayed a broad band at 3318 $cm^{-1}$, typical to phenolic hydroxy groups (121b).

The $^{13}C$ NMR spectrum, compared to that of compound **61**, confirmed the presence of an additional $OCH_3$ at $\delta_C$ 64.56. This very high chemical shift is completely located in the low-field part of the usual shift region (~ 51.3 – 67.1 ppm) for the methyl group in ethers (121b). Such a deshielding of this methoxy carbon can be understood as an effect due to the carbonyl group through resonance, and can be considered as an indirect confirmation of the location of the methoxy group in *ortho* position next to the methoxycarbonyl group.

In the same way, the high-field position observed for the carbonyl group ($\delta_C$ 165.99) can also be explained in terms of the shielding effect of the $OCH_3$-1 protons via hydrogen bond formation with the carbonyl oxygen. All $^{1}H$ and $^{13}C$ NMR data of the compound **60** are listed in Table 2.38.

From the ESI mass spectrum was deduced the molecular formula of $C_{16}H_{18}O_7$ following the observation of an ion peak at *m*/*z* 323 corresponding to $[M + H]^+$ (7%). The base peak (100%) observed at *m*/*z* 291 may derive from the ion $[M + H]^+$ by the loss of MeOH which is typical to *ortho*-substituted benzoates (121b). Compounds **60** and **61** are new natural products isolated for the first time from a plant source.

Table 2.38. $^{1}H$ NMR (270 MHz, $CDCl_3$) and $^{13}C$ NMR (67.5 MHz, $CDCl_3$)

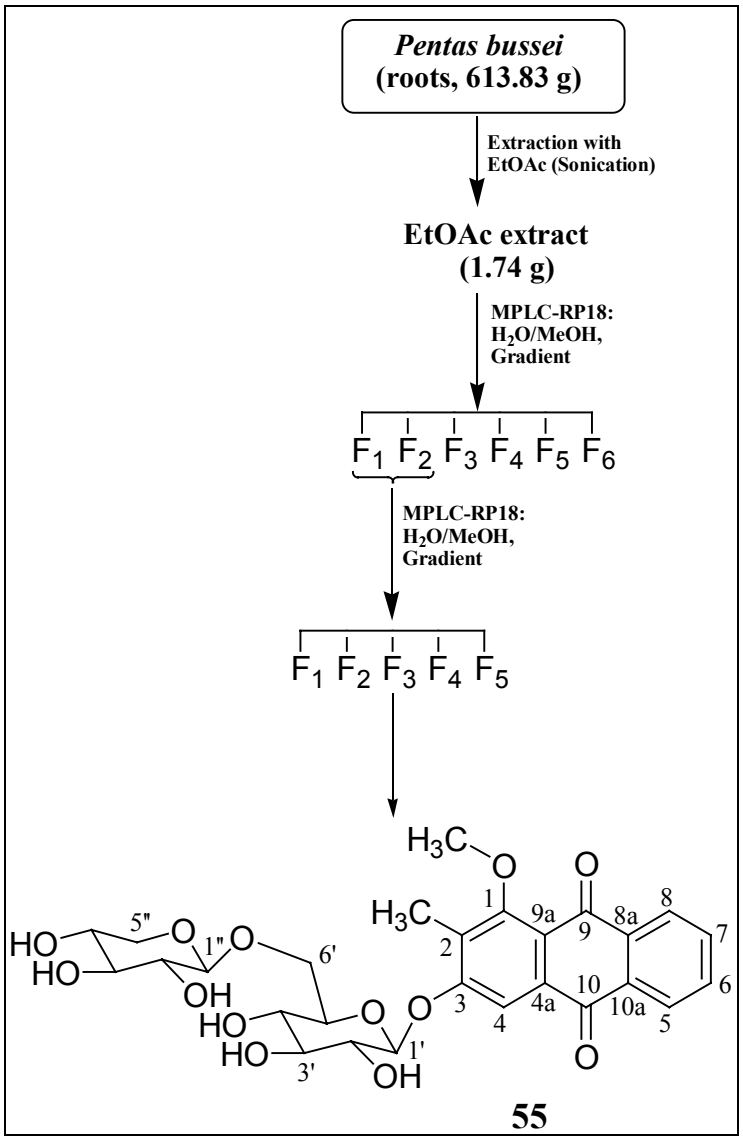
spectral data: for compound **60** (δ in ppm).

| Position | $\delta_H$, multiplicity | $\delta_c$ |
|---|---|---|
| 1 | - | 152.36 |
| 2 | - | 113.52 |
| 3 | 7.11 s | 103.59 |
| 4 | - | 147.99 |
| 4a | - | 126.56 |
| 5 | 7.14 s | 93.40 |
| 6 | - | 155.22 |
| 7 | - | 135.05 |
| 8 | - | 150.62 |
| OH-8 | 9.85 s | - |
| 8a | - | 115.11 |
| $OCH_3$-1 | 4.02 s | 64.56 |
| $COOCH_3$-2 | 3.98 s | 52.34 |
| $COOCH_3$-2 | - | 165.99 |
| $OCH_3$-4 | 3.96 s | 55.81 |
| $OCH_3$-6 | 4.00 s | 55.92 |
| $OCH_3$-7 | 3.97 s | 60.81 |

## II.3.2. Anthraquinone Glycosides

### *II.3.2.1. Rubiadin-1-methyl ether-3-O-β-primeveroside **55***

Fractionation of fractions 16:1 and 16:2 (Table 2.25) from the ethyl acetate extract of the roots of *P. bussei* by a couple of MPLC on RP18 afforded the anthraquinone glycoside rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55**. Scheme 2.12 describes the complete isolation of compound **55** from the plant.




**Scheme 2.12**: Isolation of Rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55** from the Roots of *P. bussei*.

**Rubiadin-1-methyl ether-3-*O*-*β*-primeveroside 55** was isolated as a yellowish solid, mp 161-163 °C, lit. mp. 158-160 °C (116). The IR spectrum of the compound **55** displayed two strong and broad bands at 3342 and 1079 $cm^{-1}$ indicating the presence of a carbohydrate moiety as already observed in various natural glycosides (116,135). In addition, an absorption band ascribed to an unchelated C=O was observed at 1672 $cm^{-1}$.

The $^{1}H$ NMR spectrum in $C_5D_5N$ (Figure 2.39) displayed signals for four aromatic signals in a symmetrical AA'BB' type pattern and one isolated aromatic proton at $\delta_H$ 7.56-7.70 (m), 8.30-8.32 (m) and 8.35 (s), respectively, together with one methoxy ($\delta_H$ 3.97, s) and one

methyl group ($\delta_H$ 2.36, s). Besides, the spectrum also disclosed the presence of the carbohydrate moiety by showing a typical complex pattern in between $\delta_H$ 3.60 – 6.00 ppm, together with two clearly separated anomeric protons at $\delta_H$ 4.93 (d, *J* = 6.3 Hz, H-1") and $\delta_H$ 5.84 (d, *J* = 6.3 Hz, H-1'). The $^{13}$C NMR (Figure 2.40) and HETCOR NMR spectra (in DMSO-$d_6$) showed a pair of two carbonyl signals at $\delta_C$ 180.52 and 182.19 which are typical of anthraquinones, two $sp^2$ $C_q$-O ($\delta_C$ 159.78 and 160.0), together with signals ascribed to the already observed (in the $^1$H NMR spectrum) $CH_3$ and $OCH_3$ groups at $\delta_C$ 9.22 and 60.77, respectively. Furthermore, the $^{13}$C NMR and DEPT NMR spectra confirmed the disaccharide nature of the sugar moiety which is composed of a hexose and a pentose by displaying eleven signals, nine of which range in between 65 – 77 ppm, whereas two methylenes ($\delta_C$ 65.52 and 67.92) and two anomeric carbons ($\delta_C$ 100.18 and 103.93) were also observed.

The $\delta_C$ 100.18 ppm ascribed to C-1' is typical of an anomeric carbon bonded to a *O*-aglycone in a phenolic glycoside (136). The $\delta_C$ 103.93 of C-1' is indicative of a primary alcoholic *β*-D-glucopyranoside (137) and is also in agreement with the observed $\delta_{H\text{-}1"}$ 4.93 ppm which strongly suggested that C-1" is linked to an alcoholic oxygen rather a phenolic one, i.e. $\delta_{H\text{-}1'}$ 5.84 ppm (116). Comparison of the sugar carbon resonances with published data revealed that the signal at $\delta_C$ 67.92 assigned to C-6' of glucose was downfield shifted by 7 ppm from that of methyl-*β*-D-glucose (138). Thus, the terminal xylosyl moiety should be attached to the glucosyl part at C-6' through (1→6)-β-linkage. Moreover, the diaxial coupling constant observed for the two anomeric protons H-1' and H-1", i.e. *J* = 6.3 Hz is consistent with a β-configuration of the anomeric linkages (135). From all the previous spectral observations and from the comparison of the observed data with those available in the literature (139), the sugar moiety was deduced as being the primeverosyl or xylopyranosyl-(1→6)-*O*-*β*-glucopyranosyl moiety.

Partial acid hydrolysis in an HCl atmosphere on TLC (140) of the anthraquinone glycoside (see Chapter I. Materials and Methods) readily liberates the xylose residue and glucose residue thereafter, therefore suggesting compound **55** to be an *O*-glycoside between the anthraquinone and the glucosyl moiety, and confirming the terminal position for xylose in the sugar moiety sequence. The presence of both glucose ($R_f$ 0.4) and xylose ($R_f$ 0.6) residues in the hydrolysate was monitored by comparison of their $R_f$ with reference samples.

The four aromatic protons in a symmetrical AA'BB' type pattern, the isolated aromatic proton, the $CH_3$, $OCH_3$ and primeverosyl substituents observed in the $^1H$ NMR spectrum, together with the presence of the two C=O and the two $=C_q$-O in the $^{13}C$ NMR spectrum, all these data were compatible with rubiadin-1-methyl ether-3-*O*-*β*-primeveroside as structure of compound **55**.

The aglycone rubiadin-1-methyl ether ($R_f$ 0.8) was also released in the acidic hydrolysate and confirmed by comparison of its $R_f$ with the one of a reference sample. The possible reversion of substituents at C-1 and C-3 was ruled out by the $^{13}C$ NMR chemical shift of the $OCH_3$ ($\delta_C$ 60.77 ppm, i.e. over 60 ppm) which is in agreement with an *ortho*,*ortho*-disubstituted arrangement (116), whereas the $CH_3$ was placed in position 2 on biogenetic grounds (27,71,141). The ESI mass spectrum of compound **55** displayed peaks at *m*/*z* 563 (18%) and 585 (57%) corresponding to $[M + H]^+$ and $[M + Na]^+$, respectively, and from which was deduced the molecular formula of $C_{27}H_{30}O_{13}$.

The spectral data of the isolated compound **55** are in full agreement with those available in the literature (112,116), confirming the structure proposed for the compound as mentioned above (Table 2.39). Rubiadin-1-methyl ether-3-*O*-*β*-primeveroside has previously been isolated from another *Pentas* species, i.e. *P. zanzibarica* (112).

Table 2.39. Comparison of NMR data of rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55** ($^1$H, 270 MHz, $C_5D_5N$; $^{13}$C, 67.5 MHz, DMSO-$d_6$; this work) with data from the literature ($^1$H/$^{13}$C, 400 MHz/100 MHz, DMSO-$d_6$; ref. 112): δ (ppm), *J* (Hz).

| Position | This work | | ref. 112 | |
|---|---|---|---|---|
| | $^1$H* | $^{13}$C | $^1$H* | $^{13}$C |
| 1 | - | 159.78[a] | - | 160.0[a] |
| 2 | - | 128.84 | - | 129.2 |
| 3 | - | 160.00[a] | - | 160.2[a] |
| 4 | 8.35 s | 108.10 | 7.69 s | 108.4 |
| 4a | - | 132.00[b] | - | 132.2[b] |
| 5 | 8.30-8.32 m | 126.16[c] | 8.15 dd (2,8) | 126.3[c] |
| 6 | 7.56-7.70 m | 133.55[d] | 7.89 dd (8, 8) | 133.7[d] |
| 7 | 7.56-7.70 m | 134.53[d] | 7.89 dd (8, 8) | 134.7[d] |
| 8 | 8.30-8.32 m | 126.59[c] | 8.15 dd (2, 8) | 126.8[c] |
| 8a | - | 133.80[b] | - | 134.0[b] |
| 9 | - | 180.52[e] | - | 180.2[e] |
| 9a | - | 120.05 | - | 120.3 |
| 10 | - | 182.19[e] | - | 182.4[e] |
| 10a | - | 134.19[b] | - | 134.4[b] |
| $CH_3$-2 | 2.36 s | 9.22 | 2.27 s | 9.4 |
| $OCH_3$-1 | 3.97 s | 60.77 | 3.83 s | 61.0 |
| 1' | 5.84 d (6.3) | 100.18 | 5.15 d (7.5) | 100.4 |
| 2' | | 73.06 | | 73.3 |
| 3' | | 76.01[f] | | 76.3[f] |
| 4' | | 69.04[g] | | 69.4[g] |
| 5' | | 75.56[f] | | 75.8[f] |
| 6' | | 67.92 | | 68.2 |
| 1" | 4.93 d (6.3) | 103.93 | 4.12 d (7.5) | 104.1 |
| 2" | | 73.21 | | 73.4 |
| 3" | | 76.30[f] | | 76.5[f] |
| 4" | | 69.38[g] | | 69.6[g] |
| 5" | | 65.52 | | 65.7 |

[a-g] Assignments may be reversed in the same column

* For the sugar moiety, only the anomeric $\delta_H$ values are shown

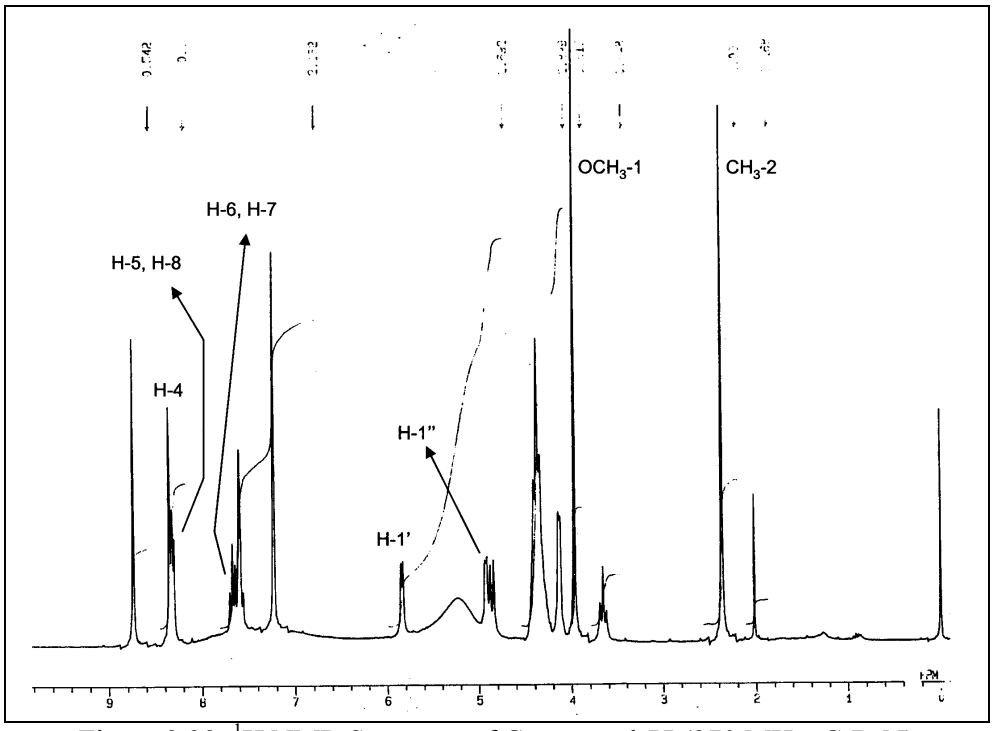



Figure 2.39. $^{1}H$ NMR Spectrum of Compound **55** (270 MHz, $C_5D_5N$)

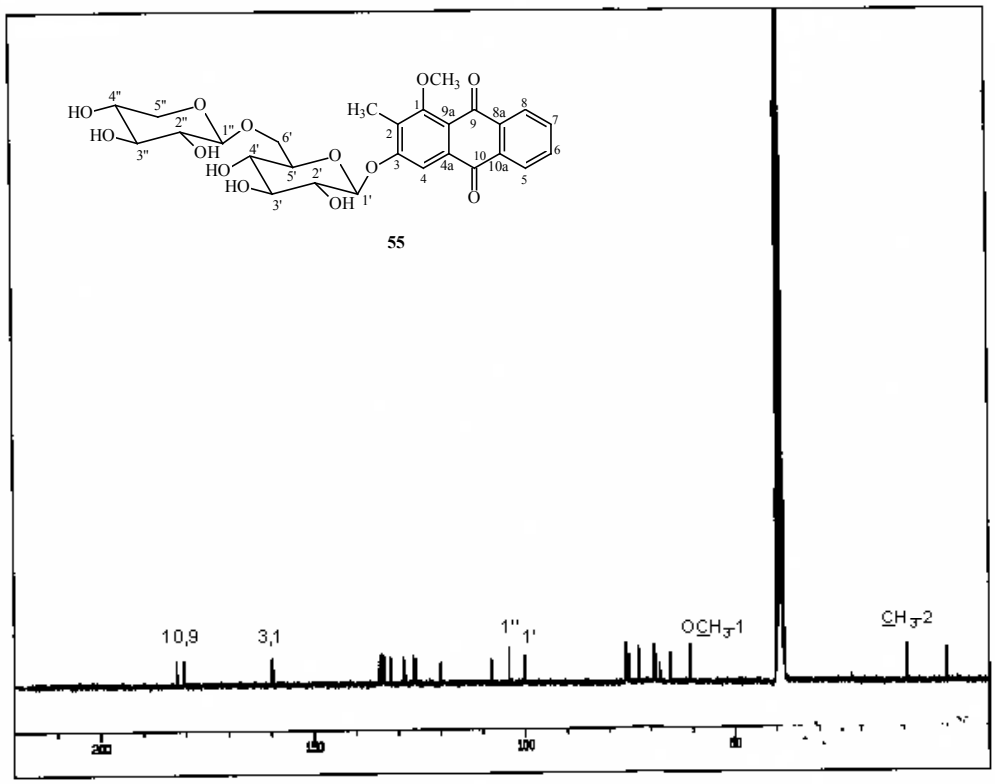
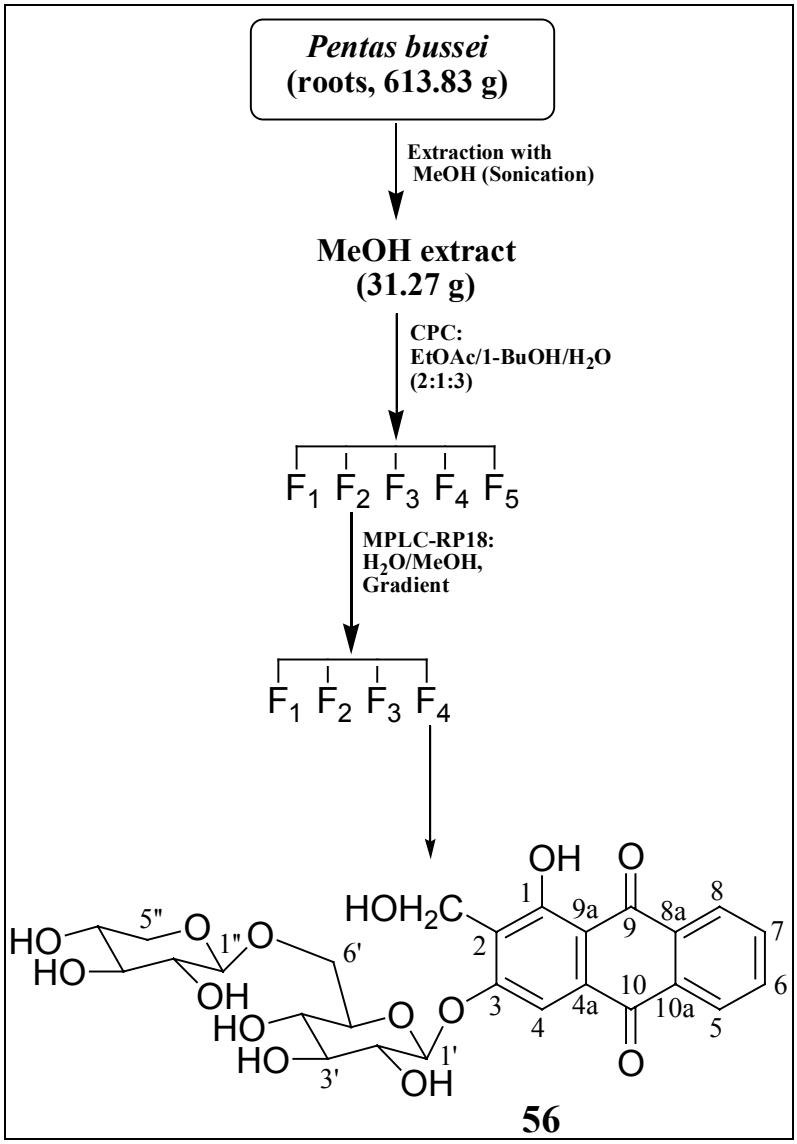



Figure 2.40. $^{13}C$ NMR Spectrum of Compound **55** (67.5 MHz, DMSO-$d_6$)

*II.3.2.2. Lucidin-3-O-β-primeveroside* ***56***

The CPC fractionation of the methanol extract of the roots of *P. bussei* followed by a MPLC fractionation of fraction 18:2 (Table 2.28) led to the isolation of lucidin-3-*O*-*β*-primeveroside **56** (fraction 19:4 of Table 2.30) as described on the Scheme 2.13.

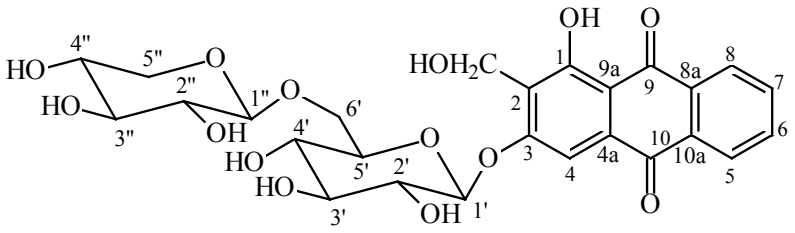



**Scheme 2.13**: Isolation of Lucidin-3-*O*-*β*-primeveroside **56** from the Roots of *P. bussei*.

**Lucidin-3-*O*-*β*-primeveroside 56** was isolated as an orange red solid, mp 206.5-208.3 °C, lit. mp 208-210 °C (116). The UV spectrum of this compound displayed an absorption band at

247.0 nm which is typical to anthraquinones (121a). The IR spectrum showed absorptions band of an unchelated C=O and a chelated one at 1677 and 1635 $cm^{-1}$, respectively. The presence of these bands is consistent with a quinone nature of the compound. Two broad bands ascribed to the sugar moiety at 3399 and 1079 $cm^{-1}$ were also observed.

The $^{1}H$, $^{13}C$ and 2D NMR data of compound **56** disclosed the presence of a primeverosyl moiety, the data of which were compared to and found matching with those observed in rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55**. The anomeric shifts were observed at $\delta_H$ 4.90-4.94 (1H, br d, $J$ = 6.3 Hz, H-1")/$\delta_C$ 103.97 (C-1") and $\delta_H$ 5.75 (1H, d, $J$ = 6.6 Hz, H-1')/ $\delta_C$ 100.66 (C-1'). Partial hydrolysis (see Chapter 1. Materials and Methods) in an HCl atmosphere on TLC (140) confirmed the release of xylose and glucose, the two monosaccharides constituting primeverose. The same pattern as in compound **55** was also observed for **56** in the $^{1}H$ NMR spectrum regarding the aromatic protons, i.e. four protons in a symmetrical AA'BB' type pattern and one isolated aromatic proton at $\delta_H$ 7.50-7.72 (m), 8.20-8.34 (m) and 8.16 (s), respectively.




**56**

However, compared to NMR spectral data of the anthraquinone glycoside **55**, compound **56** displayed two major distinct features. On one hand its $^{1}H$ NMR spectrum showed an AB coupling systems ascribed to the two protons of the hydroxymethyl group in 2-position at $\delta_H$ 5.17-5.25 and 5.34-5.41 (2H, d, $J$ = 11.5 Hz, each). The $\delta_H$ of the $\underline{C}H_2OH$-2 was observed at 51.36 ppm. On the other hand, the $^{13}C$ NMR spectrum displayed a large $\Delta\delta_C$ value of 5.41 ppm between the two carbonyl carbons ($\Delta\delta_C$ 1.67 for **55**), and this difference in chemical shift is consistent with hydrogen bonding (already observed in the IR) between the oxygen of one of the carbonyl (C-9) and a *peri*-hydroxyl proton. Consequently, the OH group must be located at the 1-position. No methoxy or methyl group was observed in the spectra of **56**.

The four aromatic protons in a symmetrical AA'BB' type pattern and the isolated one indicated that one aromatic ring in the anthraquinone was unsubstituted, whereas the other was trisubstituted. Therefore, this is compatible with the location of the OH, the hydroxymethyl and the *O*-primeverosyl substituents on the same aromatic ring with proton H-4 resonating at $\delta_H$ 8.16 (s)/$\delta_C$ 106.19.

The possibility of locating the primeverosyl moiety on the $CH_2OH$ was ruled out by two major reasons, namely 1) the $\underline{C}H_2O$ should have shifted (up to 60 ppm) to lower field, 2) the anomeric carbon C-1' ($\delta_C$ 100.66), thus linked to a primary alcohol should have shifted (i.e. to 104 ppm) to lower field (154), whereas the anomeric proton H-1' ($\delta_H$ 5.75) should have shifted to higher field (~ 4.70-4.90 ppm) (116).

The ESI mass spectrum of compound **56** displayed an ion peak at at *m*/*z* 547 (22 %) which may correspond to the fragment $[M + H - H_2O]^+$. The molecular formula was deduced as $C_{26}H_{28}O_{14}$.

Based on all the above spectral data, compound **56** was elucidated as being the known lucidin-3-*O*-*β*-primeveroside. The assigned structure was in full agreement (Table 2.40) with data found in the literature (112,116). The anthraquinone glycoside has also been isolated from *Pentas zanzibarica* (112) and from other Rubiaceae species (138a,142).

Table 2.40. Comparison of NMR data of lucidin-3-*O*-*β*-primeveroside **56** ($^1$H, 270 MHz, $C_5D_5N$; $^{13}$C, 67.5 MHz, DMSO-$d_6$; this work) with data from the literature ($^1$H/$^{13}$C, 400 MHz/100 MHz, DMSO-$d_6$; ref. 112): δ (ppm), *J* (Hz).

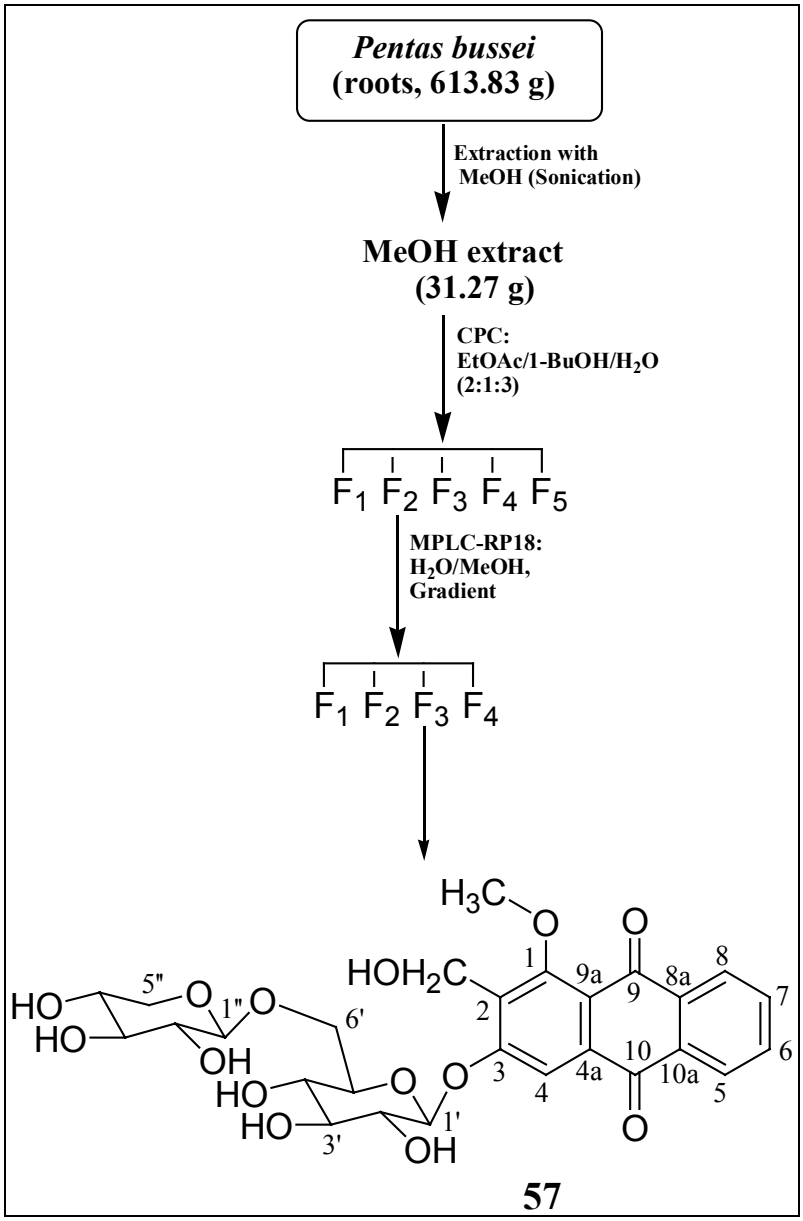
| Position | This work | | ref. 112 | |
|---|---|---|---|---|
| | $^1$H* | $^{13}$C | $^1$H* | $^{13}$C |
| 1 | - | 160.90[a] | - | 161.8[a] |
| 2 | - | 123.68 | - | 123.7 |
| 3 | - | 161.50[a] | - | 162.0[a] |
| 4 | 8.16 s | 106.19 | 7.40 s | 106.5 |
| 4a | - | 132.81[b] | - | 132.6[b] |
| 5 | 8.20-8.34 m | 126.45[c] | 8.12 dd (2,8) | 126.4[c] |
| 6 | 7.50-7.72 m | 134.52[d] | 7.88 dd (8, 8) | 134.6[d] |
| 7 | 7.50-7.72 m | 134.68[d] | 7.88 dd (8, 8) | 134.7[d] |
| 8 | 8.20-8.34 m | 126.81[c] | 8.12 dd (2,8) | 126.8[c] |
| 8a | - | 133.00[b] | - | 132.8[b] |
| 9 | - | 187.11 | - | 186.9 |
| 9a | - | 111.23 | - | 111.3 |
| 10 | - | 181.70 | - | 181.3 |
| 10a | - | 133.74[b] | - | 133.7[b] |
| $CH_2OH$-2 | 5.17-5.25 d (11.5) | 51.36 | 4.55 and 4.62 d (12) | 51.0 |
| | 5.34-5.41 d (11.5) | | | |
| 1' | 5.75 d (6.6) | 100.66 | 5.11 d (7.5) | 100.9 |
| 2' | | 73.19 | | 73.3 |
| 3' | | 75.81[e] | | 75.9[e] |
| 4' | | 69.40[f] | | 69.5[f] |
| 5' | | 76.30[e] | | 76.4[e] |
| 6' | | 67.92 | | 68.0 |
| 1" | 4.90-4.94 br d (6.3) | 103.97 | 4.14 d (7.5) | 104.0 |
| 2" | | 73.19 | | 73.3 |
| 3" | | 75.56[e] | | 75.8[e] |
| 4" | | 69.04[f] | | 69.3[f] |
| 5" | | 65.53 | | 65.6 |

[a-g] Assignments may be reversed in the same column

* For the sugar moiety, only the anomeric $\delta_H$ values are shown

*II.3.2.3. Damnacanthol-3-O-β-primeveroside* ***57***

The fractionation of fraction 18:2 (Table 2.28) by MPLC-RP18 additionally led to the isolation (Scheme 2.14) of the anthraquinone glycoside damnacanthol-3-*O*-*β*-primeveroside **57** (fraction 19:3 of Table 2.30).




**Scheme 2.14**: Isolation of Damnacanthol-3-*O*-*β*-primeveroside **57** from the Roots of *P. bussei*.

**Damnacanthol-3-*O*-$\beta$-primeveroside 57** was isolated as a yellow solid, mp 138.4-140.6 °C, lit. mp 140-142 °C (112). The UV spectrum of the compound displayed the typical absorption band of an anthraquinone at 246.0 nm (121a). In addition to the broad bands ascribed to the OH and the carbinyl carbon (C-O) groups of the sugar moiety at 3391 and 1069 $cm^{-1}$, respectively, the IR spectrum showed an unchelated C=O band at 1673 $cm^{-1}$. This lack of hydrogen bonding is consistent with the presence of a substituent other than a hydroxy group at 1-position.

As for the anthraquinone glycosides **55** and **56**, the $^{1}H$, $^{13}C$ and 2D NMR spectra disclosed the presence of the primeverosyl residue, the anomeric data of which were observed at $\delta_H$ 4.90-4.94 (1H, d, $J$ = 6.3 Hz, H-1")/$\delta_C$ 103.95 (C-1") and $\delta_H$ 5.77 (1H, d, $J$ = 6.6 Hz, H-1')/$\delta_C$ 100.77 (C-1'). Partial acid hydrolysis (see Chapter 1. Materials and Methods) in an HCl atmosphere on TLC (140) confirmed the presence of the two components of primeverose, i.e. xylose and glucose. The methoxy group was observed at $\delta_H$ 4.17 (3H, s, $OCH_3$-1)/$\delta_C$ 62.66. These low field shifts, both in $^{1}H$ and $^{13}C$ NMR, are compatible with the *peri* location of the $OCH_3$ from the oxygen of the carbonyl in 9 position. Moreover, the low $\Delta\delta_C$ between the two carbonyl carbons (1.74 ppm) is in agreement with this configuration. In addition, as the anthraquinone **56**, spectra of **57** displayed signals ascribed to the hydroxymethyl group with its typical AB coupling system at $\delta_H$ 5.15-5.19 and 5.22-5.28 (2H, d, $J$ = 11.2 Hz, each), whereas its $\delta_C$ was observed at 51.84 ppm. The latter low value indicated that the alcohol is not glycosylated (116). The aromatic protons displayed the pattern observed in the case of compounds **55** and **56**, i.e. four protons in a symmetrical AA'BB' type pattern and one isolated aromatic proton at $\delta_H$ 7.22-7.67 (m), 8.26-8.34 (m) and 8.42 (s), respectively, although differences in $\delta_H$ were observed, specially for H-4, due to the nature of the substituents.

The ESI mass spectrum of the compound displayed an ion peak at *m*/*z* 283 (7%) which may derive from the molecular ion (not seen) through the loss of the primeverosyl moiety. Therefore, the molecular formula $C_{27}H_{30}O_{14}$ was deduced for the compound.

All these spectral findings led to the elucidation of the compound as being damnacanthol-3-*O*-$\beta$-primeveroside **56**. The structure is in full agreement with data observed in the literature (Table 2.41) which show that the compound was isolated for the first time from *Pentas zanzibarica* (112).

Table 2.41. Comparison of NMR data of damnacanthol-3-*O*-*β*-primeveroside **57** ($^1$H, 270 MHz, $C_5D_5N$; $^{13}$C, 67.5 MHz, DMSO-$d_6$; this work) with data from the literature ($^1$H/$^{13}$C, 400 MHz/100 MHz, DMSO-$d_6$; ref. 112): δ (ppm), *J* (Hz).

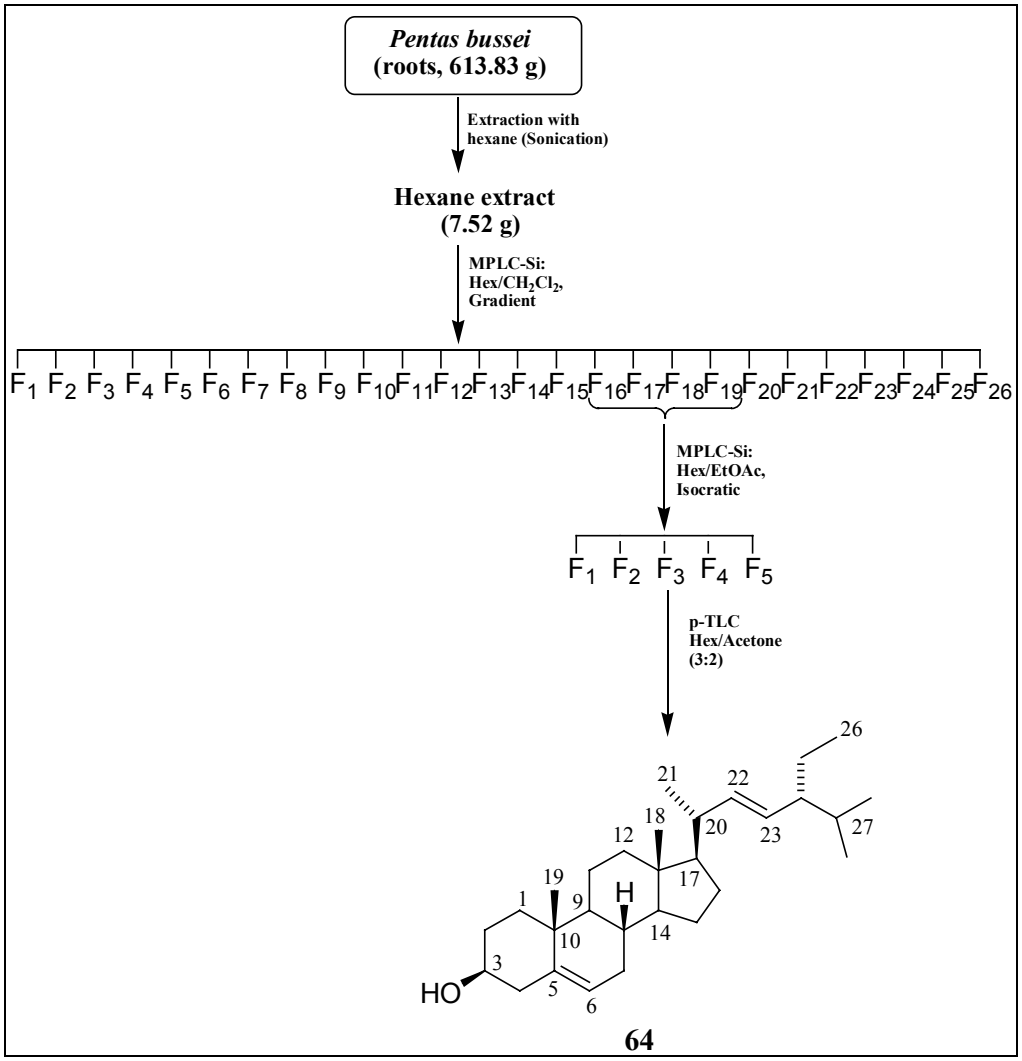
| Position | This work | | ref. 112 | |
|---|---|---|---|---|
| | $^1$H* | $^{13}$C | $^1$H* | $^{13}$C |
| 1 | - | 160.59 | - | 160.7[a] |
| 2 | - | 131.46 | - | 131.6 |
| 3 | - | 160.59 | - | 160.8[a] |
| 4 | 8.42 s | 108.98 | 7.71 s | 109.2 |
| 4a | - | 131.91[a] | - | 132.0[b] |
| 5 | 8.26-8.34 m | 126.22[b] | 8.14 dd (2,8) | 126.2[c] |
| 6 | 7.22-7.67 m | 133.58[c] | 7.88 dd (8, 8) | 133.6[d] |
| 7 | 7.22-7.67 m | 134.64[c] | 7.88 dd (8, 8) | 134.8[d] |
| 8 | 8.26-8.34 m | 126.63[b] | 8.14 dd (2,8) | 126.7[c] |
| 8a | - | 134.21[a] | - | 134.4[b] |
| 9 | - | 180.34[d] | - | 180.5[e] |
| 9a | - | 120.37 | - | 120.4 |
| 10 | - | 182.08[d] | - | 182.2[e] |
| 10a | - | 135.63[a] | - | 135.8[b] |
| $OCH_3$-1 | 4.17 s | 62.66 | 3.87 s | 62.8 |
| $CH_2OH$-2 | 5.12-5.17 d (11.2) | 51.84 | 4.60 d (12) | 52.0 |
| | 5.22-5.28 d (11.2) | | | |
| 1' | 5.77 d (6.6) | 100.77 | 5.10 d (7.5) | 101.0 |
| 2' | | 73.15 | | 73.4 |
| 3' | | 75.76[e] | | 75.8[f] |
| 4' | | 69.42[f] | | 69.6[g] |
| 5' | | 76.30[e] | | 76.4[f] |
| 6' | | 67.92 | | 68.0 |
| 1" | 4.90-4.92 br d (6.3) | 103.95 | 4.15 d (7.5) | 104.4 |
| 2" | | 73.22 | | 73.4 |
| 3" | | 75.60[e] | | 75.7[f] |
| 4" | | 69.07[f] | | 69.2[g] |
| 5" | | 65.53 | | 65.6 |

[a-g] Assignments may be reversed in the same column

* For the sugar moiety, only the anomeric $\delta_H$ values are shown

II.3.3. Steroidal Compound: *β*-Stigmasterol **64**

The application of MPLC-Si on the fractions 1:16-1:19 (Table 2.2) followed by preparative TLC gave rise to the steroid (24*S*)-24-ethylcholesta-5-(*E*)-22-dien-3*β*-ol (*β*-stigmasterol) **64**. Scheme 2.15 describes the isolation of compound **64** from the hexane extract of the roots of *P. bussei*.




**Scheme 2.15**: Isolation of (24*S*)-24-Ethylcholesta-5-(*E*)-22-dien-3*β*-ol (*β*-Stigmasterol) **64** from the Roots of *P. bussei*

**(24*S*)-24-Ethylcholesta-5-(*E*)-22-dien-3*β*-ol (*β*-stigmasterol) 64**: White solid (from hexane), mp 164.0-165.9 °C, lit. mp 164.2-166.0 °C (115). The IR spectrum of compound **64** showed the free OH group at 3406 $cm^{-1}$. The EI mass spectrum displayed a molecular ion peak $M^+$ at

*m*/*z* 412 (3%) which is compatible with the molecular composition of $C_{29}H_{48}O$ with an unsaturation index of 6.

The $^{1}H$ NMR spectrum disclosed two signals which are typical of steroidal compounds at $\delta_H$ 3.46-3.61 (1H, m, H-3) ascribed to the proton of the methine bearing the OH group, and at $\delta_H$ 5.35-5.38 (1H, m, H-6) attributed to the olefinic proton in the ring B. In addition, a particular AB coupling system with a Δν of 62.7 Hz was observed at 4.96-5.18 (2H, qd, $^{2}J$ = 15.2 Hz, $^{3}J$ = 8.7 Hz and 8.4 Hz, H-22 and H-23) and was consistent with the presence of an olefinic unit CH=CH in the side chain.

Combined analysis of the $^{1}H$, the DEPT and the HETCOR NMR spectra indicated the presence of three methyl groups having their protons resonating as doublet at $\delta_H$ 0.77 (3H, d, $J$ = 6.4 Hz, $CH_3$), 0.84 (3H, d, $J$ = 6.2 Hz, $CH_3$) and $\delta_H$ 1.04 (3H, d, $J$ = 6.8 Hz, $CH_3$), together with another one with protons resonating as triplet at $\delta_H$ 0.81 (3H, d, $J$ = 6.4 Hz, $CH_3$). Furthermore, the $^{13}C$ NMR and DEPT NMR spectra displayed signals for six methyl groups, nine $sp^3$ methylene groups, eight $sp^3$ methine groups, three $sp^2$ methine groups, two $sp^3$ quaternary carbons and one $sp^2$ quaternary carbon.

Comparison of all the above mentioned data with those of various steroidal compounds (143) led to the identification of the compound as (24*S*)-24-ethylcholesta-5-(*E*)-22-dien-3*β*-ol (*β*-stigmasterol) **64**. The spectral data of compound **64** are well matching (Tables 2.42 - 2.43) with those found in the literature (111,115).

Table 2.42. Comparison of characteristic $^1$H NMR data of $\beta$-stigmasterol **64** (270 MHz, $CDCl_3$; this work) with literature data (400 MHz, $CDCl_3$; ref. 111)

| | This work | δ (ppm) | ref. 111 |
|---|---|---|---|
| δ (ppm) | Proton, mult., *J* | δ (ppm) | Proton, mult., *J* |
| 0.68 | 3H, s, $CH_3$ | 0.69 | 3H, s, $CH_3$ |
| 0.77 | 3H, d, *J* = 6.4, $CH_3$ | 0.79 | 3H, d, *J* = 6.5, $CH_3$ |
| 0.81 | 3H, t, *J* = 7.0, $CH_3$ | 0.80 | 3H, t, *J* = 7.5, $CH_3$ |
| 0.84 | 3H, d, *J* = 6.2, $CH_3$ | 0.84 | 3H, d, *J* = 6.5, $CH_3$ |
| 1.01 | 3H, s, $CH_3$ | 1.01 | 3H, s, $CH_3$ |
| 1.04 | 3H, d, *J* = 6.8, $CH_3$ | 1.02 | 3H, d, *J* = 6.5, $CH_3$ |
| 3.46-3.61 | 1H, m, HO-C<u>H</u>-3 | 3.52 | 1H, m, HO-C<u>H</u>-3 |
| 4.96-5.18 | 2H, AB qd, | 5.01 and 5.15 | 2H, AB qd, |
| | Δν = 62.7 Hz | | Δν = 65 Hz |
| | $^2J$ = 15.2 Hz | | $^2J$ = 15 Hz |
| | $^3J$ = 8.7 Hz, 8.4 Hz, | | $^3J$ = 9 Hz, 9 Hz |
| | H-22 and H-23 | | H-22 and H-23 |
| 5.35-5.38 | 1H, m, H-6 | 5.35 | 1H, m, H-6 |

Table 2.43. Comparison of $^{13}C$ NMR data of *β*-stigmasterol **64** (67.5 MHz, $CDCl_3$; this work) with literature data (100 MHz, $CDCl_3$; ref. 111): δ (ppm)

| Carbon | This work | ref. 111 |
|---|---|---|
| $CH_3$-18 | 12.04 | 12.0 |
| $CH_3$-29 | 12.26 | 12.3 |
| CH-27 | 19.03 | 19.0 |
| $CH_3$-19 | 19.41 | 19.4 |
| $CH_2$-11 | 21.08 | 21.1 |
| $CH_3$-26 | 21.08 | 21.1 |
| $CH_2$-21 | 21.22 | 21.2 |
| $CH_2$-15 | 24.37 | 24.4 |
| $CH_3$-28 | 25.41 | 25.4 |
| $CH_2$-16 | 28.93 | 28.9 |
| $CH_2$-2 | 31.66 | 31.6 |
| $CH_2$-7 | 31.89 | 31.9 |
| CH-8 | 31.89 | 31.9 |
| $CH_2$-25 | 31.89 | 31.9 |
| $C_q$-10 | 36.51 | 36.5 |
| $CH_2$-1 | 37.25 | 37.2 |
| $CH_2$-12 | 39.68 | 39.7 |
| CH-20 | 40.50 | 40.5 |
| $C_q$-13 | 42.21 | 42.2 |
| $CH_2$-4 | 42.32 | 42.3 |
| CH-9 | 50.15 | 50.1 |
| CH-24 | 51.23 | 51.2 |
| CH-17 | 55.94 | 55.9 |
| CH-14 | 56.85 | 56.8 |
| HO-CH-3 | 71.82 | 71.8 |
| =CH-6 | 121.72 | 121.7 |
| =CH-23 | 129.27 | 129.2 |
| =CH-22 | 138.33 | 138.3 |
| =$C_q$-5 | 140.75 | 140.7 |

**Chapter III.**

# STUDY OF THE ROOTS OF *PENTAS LANCEOLATA* (Forsk.) Deflers (Rubiaceae)

# Chapter III. STUDY OF THE ROOTS OF *PENTAS LANCEOLATA*

## III.1. Introduction

*Pentas lanceolata* (Forsk.) Deflers is an erect branched shrub or woody herb of about 0.5-1.3 m high, with ovate-lanceolate leaves and mauve to white flowers in corymbs. Inflorescence with terminal and axillary components combined into a single cluster. Flowers often trimorphic, either with style exserted and anthers included, anthers exserted and style included or both. This very variable species is widespread in forest edges. There are two main population types, one that is dimorphic in its corolla tube length (as well as in the stamen and style length) and the other which is not. Occasionally these two may be found together. In Kenya where it was collected, the plant is mainly found in highlands (69,144). The plant also has an ornamental usage. No previous phytochemical study of these species is found in the literature. *P. suswaensis* Verdc. and *P. zanzibarica* (Klotzch) Vatke are two other species from the same genus and which are reported to be botanically similar to *P. lanceolata* (144).

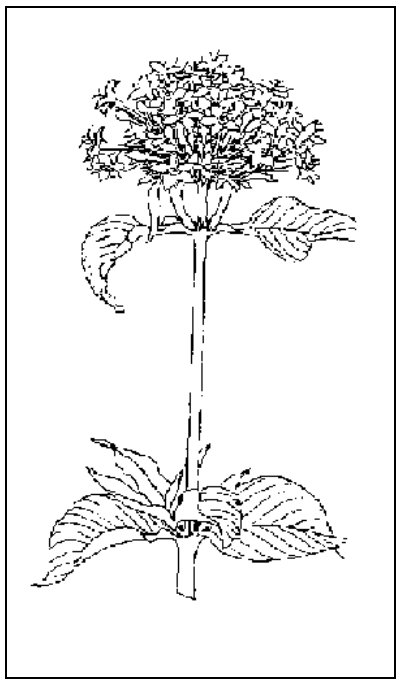


Figure 3.1. *Pentas lanceolata* (Forsk.) Deflers

## III.2. Experimental

### III.2.1. Extraction and Purification

#### *III.2.1.1. Extraction*

The ground dried roots of the plant (643.25 g) were extracted exhaustively (x 3) with *n*-hexane, dichloromethane ($CH_2Cl_2$), ethyl acetate (EtOAc) and methanol (MeOH), respectively. A volume of 0.75 litres of each solvent was used for each extraction. The solvent was removed and the sample dried before the extraction with a new solvent started. Extraction was executed in a sonication bath at room temperature. The filtration and the concentration of the extracts under reduced pressure with a rotavapor at 40 °C yielded a yellowish *n*-hexane residue (1.34 g, 0.21 % yield), a yellow $CH_2Cl_2$ residue (4.09 g, 0.64 % yield), a yellow EtOAc residue (6.87 g, 1.07 % yield), and a yellow red MeOH residue (76.46 g, 11.89 % yield).

#### *III.2.1.2. Study of the Hexane Extract of the Roots of Pentas lanceolata*

The *n*-hexane extract (1.34 g, 0.21 % yield) was submitted to MPLC on silica using an *n*-hexane/EtOAc/MeOH gradient (Table 3.1) to give 7 different fractions (Table 3.2) monitored by TLC. The MPLC conditions were: 2 coupled columns 460 x 49 mm i.d. (columns coupling aimed at the improvement of separation by increasing length); precolumn, 140 x 10 mm i.d.; sample adsorbed on 9.5 g of silica gel and packed in a 230 x 36 mm i.d. sample column; flow rate 40 ml/min at a back pressure < 40 bar; detection, LSD; collection 50 seconds per fraction.

Table 3.1. Programme for fractionation of the *n*-hexane extract of the roots of *P. lanceolata* on MPLC

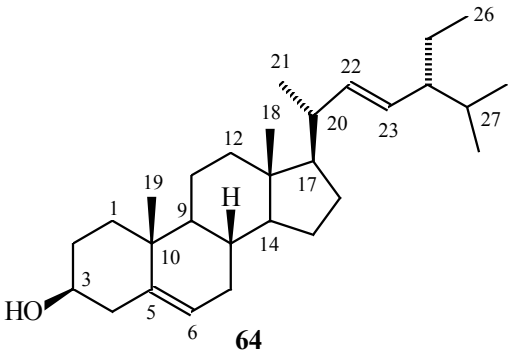
| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| *n*-Hexane-EtOAc | 100:0 | 100:0 | ---- | 5 |
| *n*-Hexane-EtOAc | 90:10 | 90:10 | ---- | 10 |
| *n*-Hexane-EtOAc | 80:20 | 0:100 | 10% | 180 |
| EtOAc- MeOH | 95:5 | 95:5 | ---- | 10 |

Table 3.2. MPLC fractionation of the *n*-hexane extract of the roots of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 1:1 | 1-19 | 299.5 |
| 1:2 | 22-30 | 220.9 |
| 1:3 | 31-39 | 80.6 |
| 1:4 | 40-61 | 281.2 |
| 1:5 | 62-75 | 30.0 |
| 1:6 | 76-162 | 246.3 |
| 1:7 | 163-246 | 157.6 |

III.2.1.2.1. Isolation of (24*S*)-24-Ethylcholesta-5-(*E*)-22-dien-3$\beta$-ol ($\beta$-Stigmasterol) **64**

Fraction 1:4 (281.2 mg, 0.044 % yield) was recrystallized in *n*-hexane (2% acetone) to yield a white solid. The compound was identified as of (24*S*)-24-ethylcholesta-5-(*E*)-22-dien-3$\beta$-ol ($\beta$-stigmasterol) according to the spectra and physical data analysis. The latter were totally identical to those obtained for the same compound isolated from *P. bussei*.




**64**

**(24*S*)-24-Ethylcholesta-5-(*E*)-22-dien-3$\beta$-ol ($\beta$-stigmasterol) 64**. White solid (from hexane); mp 164.1-166.0 °C, lit. mp 164.2-166.0 °C (115). The compound displayed spectral data (IR, $^{1}H$ and $^{13}C$, MS) similar to those observed in the case of the sample isolated from *P. bussei* (see Chapter II).

*III.2.1.3. Study of the Dichloromethane Extract of the Roots of Pentas lanceolata*

The dichloromethane extract (4.09 g, 0.64 % yield) was chromathographed on reversed phase MPLC under a gradient mode (Table 3.3). The eluates were combined together into 5 fractions (Table 3.4), the result of their monitoring by TLC with $H_2O$/MeOH (2:3). MPLC conditions were: column 460 x 70 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 15 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate, 30 ml/min at a back pressure < 40 bar; detection, LSD; collection, 80 seconds per fraction.

Table 3.3. Programme for fractionation of the dichloromethane extract of the roots of *Pentas lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 40:60 | 0:100 | 10% | 120 |

Table 3.4. MPLC fractionation of the dichloromethane extract of the roots of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 2:1 | 27-33 | 200.1 |
| 2:2 | 38-42 | 166.1 |
| 2:3 | 45-56 | 415.2 |
| 2:4 | 59-78 | 295.0 |
| 2:5 | 79-113 | 62.6 |

III.2.1.3.1. Isolation of Damnacanthol **77**

Fraction 2:2 (166.1 mg, 0.026 % yield) was submitted to reversed phase MPLC under a gradient mode (Table 3:5) to yield 3 different fractions (Table 3:6). Eluates were monitored by TLC on RP18 plates and eluted with $H_2O$/MeOH (5:5). MPLC conditions were: 2 coupled columns, 460 x 26 mm i.d. and 230 x 26 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 1 g of RP18 and packed in a 230 x 26 mm i.d. sample column; flow rate, 60 ml/min at a back pressure < 40 bar; detection, LSD; collection, 40 seconds per fraction.

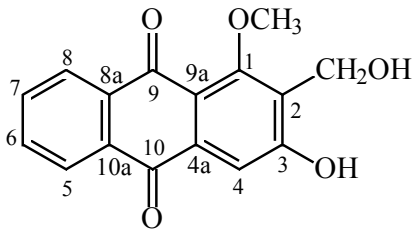
Table 3.5. Programme for fractionation of fraction 2:2 from the roots of *Pentas lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 40:60 | 0:100 | 10% | 60 |

Table 3.6. MPLC fractionation of fraction 2:2 from the roots of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 3:1 | 47-58 | 73.5 |
| 3:2 | 59-65 | 30.1 |
| 3:3 | 66-90 | 42.9 |

Fraction 3:1 was dried by solvent removal under reduced pressure to yield a yellow solid (73.5 mg, 0.011 % yield). Based on its spectral and physical data, the compound was identified as damnacanthol (3-hydroxy-2-hydroxymethyl-1-methoxy-9,10-anthraquinone) **77**.




**77**

**Damnacanthol (3-hydroxy-2-hydroxymethyl-1-methoxy-9,10-anthraquinone) 77**: Yellow solid ; mp 284.8-287.1 °C, lit. mp 286-288 °C (145); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 239.0 (3.39), 249.0 (3.30), 280.0 (3.42), 392.0 (2.70), 374.0 (2.68), 420.0 (2.45), 442.0 (2.40), 447.0 (2.39) nm; IR $\nu_{max}$ (KBr) 3308 (OH groups), 2939, 1673, 1651, 1567, 1425, 1339, 1282, 1119, 1069, 969, 715, 612 $cm^{-1}$; $^{1}H$ NMR (270 MHz, DMSO-$d_6$) δ (ppm) 8.09-8.17 (2H, m, H-5 and H-8), 7.84-7.90 (2H, m, H-6 and H-7), 7.49 (1H, s, H-4), 4.57 (2H, s, C$\underline{H_2}$OH-2), 3.86 (3H, s, OC$\underline{H_3}$-1) ; $^{13}C$ NMR (67.5 MHz, DMSO-$d_6$) δ (ppm) 182.58 (C-10), 179.82 (C-9), 162.75 (C-3), 161.54 (C-1), 135.27 (C-10a), 134.52 (C-7), 134.52 (C-8a), 133.26 (C-6), 131.95 (C-4a), 128.70 (C-2), 126.87 (C-8), 126.02 (C-5), 117.41 (C-9a), 109.93 (C-4), 62.26 (O$\underline{C}H_3$-1), 52.4 ($\underline{C}H_2$OH-2) ; ESIMS *m*/*z* (rel. int.) 307 $[M + Na]^+$ (80), 285 $[M + H]^+$ (45), 267 $[M-H_2O + H]^+$ (100).

III.2.1.3.2. Isolation of Rubiadin-1-methyl ether **36**

Fraction 2:3 (415.2 mg, 0.065 % yield) was rechromatographed by reversed phase MPLC under a gradient mode (Table 3.7). Eluates obtained were combined into 4 fractions (Table 3.8), based on their TLC monitoring on RP18 plates eluted with $H_2O$/MeOH (3:7). MPLC conditions were: 2 coupled columns: 460 x 26 mm i.d. and 230 x 26 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 3 g of RP18 and packed in a 230 x 26 mm i.d. sample column; flow rate, 45 ml/min at a back pressure < 40 bar; detection, LSD; collection, 15 seconds per fraction.

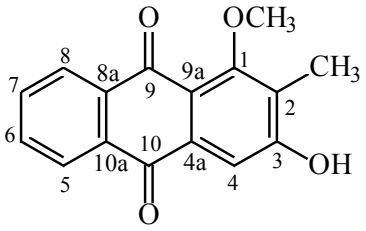
Table 3.7. Programme for fractionation of fraction 2:3 from the roots of *P. lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 30:70 | 0:100 | 10% | 40 |

Table 3.8. MPLC fractionation of fraction 2:3 from the roots of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 4:1 | 1-53 | 58.9 |
| 4:2 | 54-86 | 92.5 |
| 4:3 | 87-145 | 55.0 |
| 4:4 | 146-160 | 38.5 |

Evaporation of the solvent to dryness from fraction 4:2 afforded a yellow solid. The spectral data of the compound, together with its physical data, allowed to identify it as the known rubiadin-1-methyl ether (3-hydroxy-1-methoxy-2-methyl-9,10-anthraquinone) **36** (86-87,112).




**36**

**Rubiadin-1-methyl ether (3-hydroxy-1-methoxy-2-methyl-9,10-anthraquinone) 36**: Yellow crystals; mp 289.3-291.2 °C, lit. mp 292-294 °C (146a); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 239.9 (3.44), 250.0 (3.31), 280.0 (3.60), 313.9 (2.87), 317.1 (2.87), 350.0 (2.84), 353.0 (2.84), 355.0 (2.84), 360.0 (2.84), 374.0 (2.81), 413.0 (2.56) nm; IR $\nu_{max}$ (KBr) 3304 (OH), 2978, 1673 (C=O), 1651 (C=O), 1567, 1449, 1411, 1338, 1277, 1223, 1121, 1068, 981, 885, 757, 712 $cm^{-1}$; $^{1}H$ NMR (270 MHz, DMSO-$d_6$) $\delta$ (ppm) 8.08-8.16 (2H, m, H-5 and H-8), 7.83-7.92 (2H, m, H-6 and H-7), 7.50 (1H, s, H-4), 3.79 (3H, s, OC$\underline{H}_3$-1), 2.16 (3H, s, C$\underline{H}_3$-2); $^{13}C$ NMR (67.5 MHz, DMSO-$d_6$) $\delta$ (ppm) 182.51 (C-10), 180.10 (C-9), 161.60 (C-3), 160.50 (C-1), 134.43 (C-10a), 134.37 (C-7), 133.60 (C-8a), 133.26 (C-6), 131.93 (C-4a), 126.54 (C-8), 126.09 (C-2), 125.93 (C-5), 117.75 (C-9a), 108.91 (C-4), 60.48 (O$\underline{C}H_3$-1), 8.93 ($\underline{C}H_3$-2); EIMS *m/z* (rel. int.) 268 $[M]^+$ (6), 232 (3), 194 (72), 162 (85), 131 (100), 121 (37), 91 (49), 77 (10), 55 (8), 40 (9).

III.2.1.3.3. Isolation of Rubiadin **53**

Fraction 2:4 (295.0 mg, 0.046 % yield) was submitted to a normal phase MPLC separation under a gradient mode (Table 3.9) and the following MPLC conditions: 2 coupled columns 460 x 36 mm i.d. and 230 x 36 mm i.d.; precolumn 140 x 10 mm i.d.; sample adsorbed on 2 g of silica gel and packed in 230 x 26 mm i.d. sample column; flow rate, 35 ml/min at a back pressure < 40 bar; detection, LSD, collection, 30 seconds per fraction. The eluates were monitored by TLC on silica gel plates eluting with *n*-hexane/EtOAc (4:1) and combined into 2 fractions (Table 3.10).

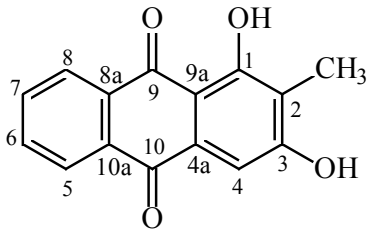
Table 3.9. Programme for fractionation of fraction 2:4 from the roots of *P. lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| *n*-Hexane/EtOAc | 80:20 | 50:50 | 10% | 90 |

Table 3.10. MPLC fractionation of fraction 2:4 from the roots of *P. lanceolata*.

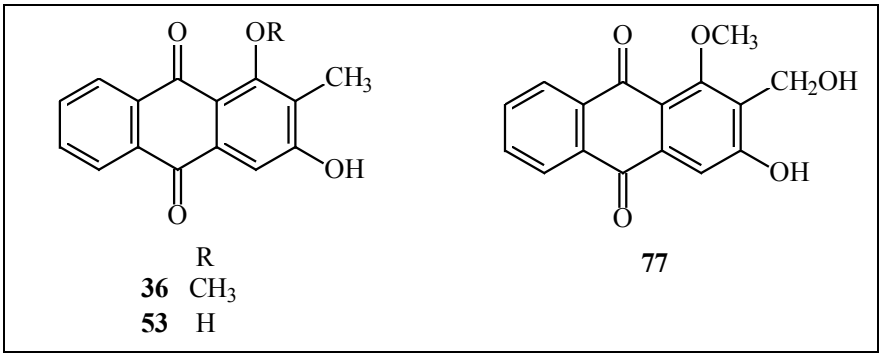
| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 5:1 | 1-88 | 60.7 |
| 5:2 | 89-184 | 113.4 |

After solvent evaporation to dryness, fraction 5:2 afforded a yellow solid (113.4 mg, 0.018 % yield) which was identified as the known rubiadin (1,3-dihydroxy-2-methyl-9,10-anthraquinone) **53** (112).




**53**

**Rubiadin (1,3-dihydroxy-2-methyl-9,10-anthraquinone) 53** : Yellow solid; mp 299.8-301.4 °C, lit. mp 300 °C (147); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 239.9 (3.37), 244.9 (3.36), 280.0 (3.37), 357.0 (2.83), 362.0 (2.83), 374.0 (2.86), 391.1 (2.87), 413.0 (2.89), 420.0 (2.89) nm; IR $\nu_{max}$ (KBr) 3392 (OH groups), 2915, 1661 (C=O, unchelated), 1624 (C=O, chelated), 1589, 1433, 1339, 1312, 1122, 866, 829, 806, 745, 711, 639, 595 $cm^{-1}$; $^1H$ NMR (270 MHz, DMSO-$d_6$) δ (ppm) 13.09 (1H, s, OH-1), 8.10-8.18 (2H, m, H-5 and H-8), 7.89 (2H, m, H-6 and H-7), 7.22 (1H, s, H-4), 2.05 (3H, s, C$\underline{H}_3$-2); $^{13}C$ NMR (67.5 MHz, DMSO-$d_6$) δ (ppm) 186.00 (C-9), 181.65 (C-10) ; 162.80 (C-3), 162.31 (C-1), 134.39 (C-7), 134.28 (C-6), 132.83 (C-8a), 132.70 (C-10a), 131.52 (C-4a), 126.54 (C-8), 126.20 (C-5), 117.21 (C-2), 108.73 (C-9a), 107.28 (C-4), 7.94 ($\underline{C}H_3$-2) ; ESIMS *m/z* (rel. Int.) 255 $[M + H]^+$ (42), 205 (27), 171 (40), 149 (100), 117 (40).




R
**36** $CH_3$
**53** H

**77**

Figure 3.2. Natural Products Isolated from the Dichloromethane Extract of the Roots of *P. lanceolata*

*III.2.1.4. Study of the Ethyl Acetate Extract of the Roots of Pentas lanceolata*

The ethyl acetate extract (6.87 g, 1.07 % yield) of the roots of *P. lanceolata* was subjected to a reversed phase MPLC separation under a gradient mode elution (Table 3.11) to afford four different fractions (Table 3.12) after combination of the eluates. The MPLC conditions were: Column, 460 x 36 mm i.d.; precolumn 140 x 10 mm i.d.; sample adsorbed on 30 g of RP18 (divided into 2 equal parts) and each part packed in 230 x 36 mm i.d. sample column; flow rate, 48 ml/min at a back pressure < 40 bar; detection, LSD, collection, 25 seconds per fraction. The combination of eluates was based on the TLC monitoring on RP18 plates using $H_2O$/MeOH (1:1) as eluent.

Table 3.11. Programme for fractionation of the ethyl acetate extract of the roots of *P.*

*lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 50:50 | 0:100 | 10% | 75 |

Table 3.12. MPLC fractionation of the ethyl acetate extract of the roots of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 6:1 | 1-6 | 42.9 |
| 6:2 | 9-20 | 498.6 |
| 6:3 | 22-62 | 2756.4 |
| 6:4 | 63-180 | 2903.2 |

Fraction 6:4 (2903.2 mg, 0.45 % yield) was rechromatographed by MPLC on reversed phase under a short gradient mode elution (Table 3.13) and the following conditions: 2 coupled columns 460 x 26 mm i.d.; sample adsorbed on 13 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate, 50 ml/min at a back pressure < 40 bar; detection, LSD, collection, 30 seconds per fraction. The eluates were monitored by TLC on RP18 plates utilizing $H_2O$/MeOH (2:3) as eluent and combined into 4 fractions (Table 3.14).

Table 3.13. Programme for fractionation of fraction 6:4 of *P. lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 40:60 | 20:80 | 10% | 105 |

Table 3.14. MPLC fractionation of fraction 6:4 of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 7:1 | 9-17 | 190.2 |
| 7:2 | 25-35 | 286.4 |
| 7:3 | 36-93 | 938.3 |
| 7:4 | 94-210 | 1475.9 |

Fractions 6:3 (Table 3.12) and 7:3 were mixed together (3694.7 mg, 0.57 % yield) and submitted to a MPLC separation on normal phase under a gradient mode elution (Table 3.15) and the following separation conditions: 2 coupled columns 460 x 49 mm i.d. and 230 x 49

mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 20 g of silica gel and packed in a 230 x 36 mm i.d. sample column; flow rate 45 ml/min at a back pressure < 40 bar; detection, LSD, collection, 50 seconds per fraction. After monitoring by TLC on silica gel plates utilizing *n*-hexane/EtOAc (1:4) as eluent the eluates were combined into 7 fractions (Table 3.16).

Table 3.15. Programme for fractionation of fraction 6:3 and 7:3 of *P. lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| *n*-Hexane/EtOAc | 80:20 | 40:60 | 10% | 150 |
| *n*-Hexane/EtOAc | 40:60 | 0:100 | 20% | 40 |
| EtOAc/MeOH | 100:0 | 90:10 | 5% | 30 |
| EtOAc/MeOH | 90:10 | 50:50 | 10% | 80 |

Table 3.16. MPLC fractionation of fraction 6:3 and 7:3 of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 8:1 | 79-116 | 56.2 |
| 8:2 | 117-136 | 84.5 |
| 8:3 | 137-197 | 434.1 |
| 8:4 | 198-252 | 375.6 |
| 8:5 | 253-280 | 114.0 |
| 8:6 | 281-306 | 350.5 |
| 8:7 | 307-364 | 745.1 |

III.2.1.4.1. Isolation of 1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone (Lucidin-ω-methyl ether) **78**

Fraction 8:7 (745.1 mg, 0.12 % yield) was submitted to a further MPLC separation on normal phase and under a gradient mode elution (Table 3.17). TLC monitoring on silica gel plates utilizing *n*-hexane/EtOAc (7:3) as eluent and combination of the eluates resulted in 9 different fractions listed in Table 3.18. The MPLC conditions were: 2 coupled columns 460 x 49 mm i.d. and 230 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 4 g of silica gel and packed in a 230 x 26 mm i.d. sample column; flow rate, 48 ml/min at a back pressure < 40 bar; detection, LSD, collection, 28 seconds per fraction.

Fraction 9:8 (Table 3.18) was further purified by preparative TLC (p-TLC) eluting with *n*-hexane/EtOAc (7:3). The sample was dissolved in 5 ml of EtOAc/EtOH (1:1) before its application to 3 p-TLC plates.

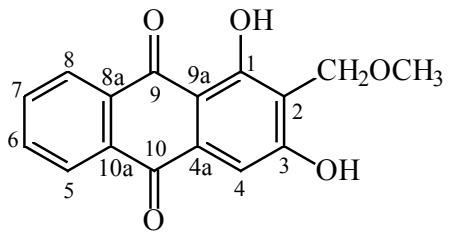
Table 3.17. Programme for fractionation of fraction 8:7 of *P. lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| *n*-Hexane/EtOAc | 100:0 | 50:50 | 10% | 120 |

Table 3.18. MPLC fractionation of fraction 8:7 of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 9:1 | 1-35 | 49.0 |
| 9:2 | 36-59 | 15.6 |
| 9:3 | 60-81 | 22.7 |
| 9:4 | 82-103 | 115.3 |
| 9:5 | 104-123 | 50.9 |
| 9:6 | 124-135 | 8.7 |
| 9:7 | 136-161 | 15.4 |
| 9:8 | 162-224 | 43.2 |
| 9:9 | 225-262 | 72.5 |

After the development of the plates, the spot of R*f* = 0.43 was scraped off and the compound extracted from silica gel with EtOAc. Solvent removal under reduced pressure yielded an amorphous yellow compound which was identified as 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (lucidin-ω-methyl ether) **78**, based on its spectral and physical data.




**78**

**1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone (lucidin-ω-methyl ether) 78**: Amorphous yellow powder; mp 169.8-172.4 °C, lit. mp 170 °C (148); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 242.0 (4.67), 246.0 (4.67), 280.0 (4.65), 420.0 (3.89); IR $\nu_{max}$ (KBr) 3393 (OH) 3196 (OH), 2925, 2851, 1673 (unchelated C=O), 1626 (chelated C=O), 1592, 1475, 1405, 1368, 1334, 1275 (C-O-C), 1159, 1130, 1099, 936, 874, 788, 746, 710, 620 $cm^{-1}$; $^{1}H$ NMR (270 MHz, $CDCl_3$) δ (ppm) 13.31 (1H, s, OH-1), 9.43 (1H, s, OH-3), 8.25-8.29 (2H, m, H-5 and H-8), 7.77-7.79 (2H, m, H-6 and H-7), 7.32 (1H, s, H-4), 4.94 (2H, s, $C\underline{H_2}OCH_3$-2), 3.58 (3H, s, $CH_2OC\underline{H_3}$-2); $^{13}C$ NMR (67.5 MHz, $CDCl_3$) δ (ppm) 186.88 (C-9), 182.23 (C-10), 164.08 (C-3), 161.88 (C-1), 134.12 (C-6), 134.12 (C-7), 134.12 (C-4a), 133.51 (C-10a), 132.43 (C-8a), 127.35 (C-8), 126.70 (C-5), 114.37 (C-2), 109.74 (C-4), 109.63 (C-9a), 68.89 ($\underline{C}H_2OCH_3$-2), 59.35 ($CH_2O\underline{C}H_3$-2); ESIMS *m*/*z* (rel. int.) 307 $[M + Na]^+$ (14), 275 (14), 253 (100).

III.2.1.4.2. Isolation of Damnacanthol-3-*O*-methyl ether **79**

Fraction 7:2 (286.4 mg, 0.044 % yield) (Table 3.14) was submitted to a MPLC separation on RP18 phase under a short gradient elution (Table 3.19). The MPLC conditions were: 2 coupled columns 460 x 26 mm i.d.; precolumn 140 x 10 mm i.d.; sample adsorbed on 2 g of RP18 and packed in a 230 x 26 mm i.d. sample column; flow rate, 40 ml/mn at a back pressure < 40 bar; detection, LSD; collection, 50 seconds per fraction. Based on the TLC monitoring on RP18 plates utilizing $H_2O$/MeOH (35:65), the eluates were combined into 3 different fractions (Table 3.20).

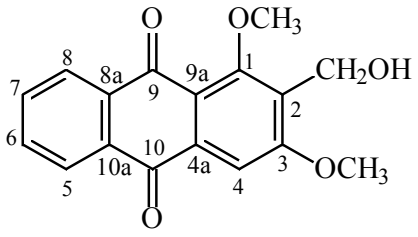
Table 3.19. Programme for fractionation of fraction 7:2 of *P. lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 40:60 | 30:70 | 5% | 45 |

Table 3.20. MPLC fractionation of fraction 7:2 of *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 10:1 | 1-12 | 47.1 |
| 10:2 | 13-21 | 97.2 |
| 10:3 | 27-55 | 89.8 |

Fraction 10:3 and 8:6 (Table 3.16) were combined (440.3 mg, 0.068 % yield) and repurified by an isocratic elution MPLC separation on reversed phase (RP18). Chromatographic conditions were: 2 coupled column 460 x 26 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 3 g of RP18 and packed in a 230 x 26 mm i.d. sample column; flow rate 30 ml/min at a back pressure < 40 bar; detection, LSD; collection, 80 seconds per fraction; eluting solvent system, $H_2O/CH_3CN$ (30:70); elution time, 240 min. Eluates 23-49 were put together. Solvent elimination by evaporation under reduced pressure gave rise to a yellow solid. The compound was identified as being the known anthraquinone 1,3-dimethoxy-2-hydroxymethyl-9,10-anthraquinone (damnacanthol-3-*O*-methyl ether) **79**.

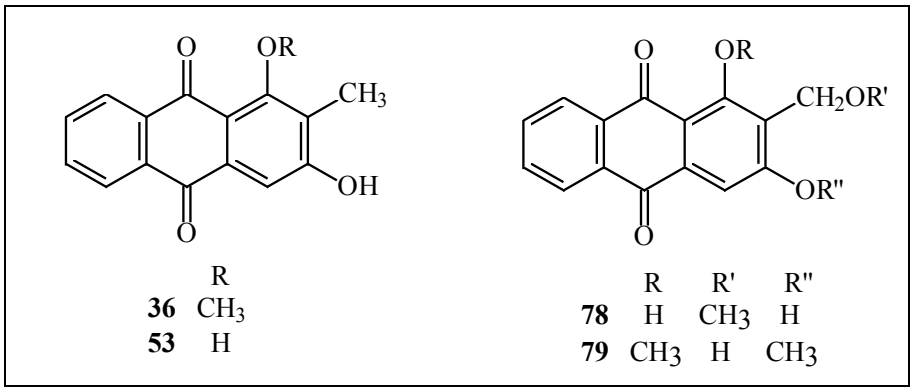



**79**

**1,3-Dimethoxy-2-hydroxymethyl-9,10-anthraquinone (damnacanthol-3-*O*-methyl ether) 79**: Amorphous yellow powder; mp 173.8-175.2 °C, lit. mp 173.5-174.0 °C (149); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 259.0 (4.09), 271.0 (4.12), 276.0 (4.13), 360.1 (3.77), 361.9 (3.77), 370.0 (3.76), 374.0 (3.75), 420.0 (3.62), 442.0 (3.60), 446.9 (3.59) nm; IR $\nu_{max}$ (KBr) 3445 (OH), 2927, 2855, 1673 (C=O), 1650 (C=O), 1568, 1282, 1100, 970, 804, 715, 577 $cm^{-1}$; $^1H$ NMR

(270 MHz, $C_5D_5N$) δ (ppm) 8.27-8.42 (2H, m, H-5 and H-8), 7.99 (1H, s, H-4), 7.63-7.71 (2H, m, H-6 and H-7), 5.37 (2H, s, C$\underline{H_2}$OH-2), 4.23 (3H, s, OC$\underline{H_3}$-1), 3.62 (3H, s, OC$\underline{H_3}$-3); $^{13}$C NMR (67.5 MHz, $C_5D_5N$) δ (ppm) 183.37 (C-9), 180.86 (C-10), 163.99 (C-3), 162.46 (C-1), 135.02 (C-10a), 134.86 (C-7), 134.46 (C-8a), 133.21 (C-6), 133.10 (C-4a), 129.97 (C-2), 127.29 (C-8), 126.59 (C-5), 118.96 (C-9a), 110.94 (C-4), 62.82 (O$\underline{C}$H$_3$-1), 54.73 ($\underline{C}$H$_2$OH-2), 49.68 (O$\underline{C}$H$_3$-3); ESIMS *m*/*z* (rel. int.) 285 (36), 267 (100).

In addition, further purification of fractions 7:4 (Table 3.14) and 8:3 (Table 3.16) gave rise to rubiadin-1-methyl ether **36** and rubiadin **53** already isolated from the dichloromethane extract of the roots of the plant (*vide supra*).




| | R |
|---|---|
| **36** | $CH_3$ |
| **53** | H |

| | R | R' | R" |
|---|---|---|---|
| **78** | H | $CH_3$ | H |
| **79** | $CH_3$ | H | $CH_3$ |

Figure 3.3. Natural Products Isolated from the Ethyl Acetate Extract of the Roots of *P. lanceolata*

*III.2.1.5. Study of the Methanol Extract of the Roots of Pentas lanceolata*

A sample amount of 3260.3 mg (4.26 % of the total extract) from the methanol extract of the roots of *P. lanceolata* (76.46 g, 11.89 % yield) was submitted to CPC using EtOAc/*n*-BuOH/$H_2O$ (2:1:3) as solvent system. The separation conditions were: column 320 ml x 3; elution mode, head to tail, the upper phase used as the stationary phase and the lower phase as mobile phase; flow rate 3 ml/min with a pressure ~ 70 psi; revolution speed, 1080 rpm; equilibrium volume 248 ml; sample loop 15 ml; detection, UV 254 nm and 280 nm; sample collection, 2 min per fraction; push out at 300 min with a flow rate of 7 ml/min dissolved into 42 ml of a mixture of the upper and the lower phase (1:1), divided into 3 equal parts partitioned separately. Based on the TLC monitoring on RP18 plates, developed with $H_2O$/MeOH (1:1), the eluates were combined into 6 fractions (Table 3.21).

Table 3.21. CPC fractionation of the Methanol extract of the roots of *P. lanceolata*

| Fraction | Eluate Number | Weight (mg) |
|---|---|---|
| 11:1 | 34-45 | 1921.1 |
| 11:2 | 46-53 | 77.2 |
| 11:3 | 54-72 | 110.6 |
| 11:4 | 74-95 | 174.4 |
| 11:5 | 110-134 | 84.0 |
| 11:6 | 135-156 | 325.1 |

III.2.1.5.1. Isolation of Rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55**

Fraction 11:4 (174.4 mg, 0.027 % yield) was submitted to a reversed phase MPLC separation under a gradient elution mode (Table 3.22) and under the following conditions: column, 460 x 26 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 2 g of RP18 and packed in a 230 x 26 mm i.d. sample column; flow rate 30 ml/min at a back pressure of < 40 bar; detection LSD; collection, 80 seconds per fraction. The eluates were monitored by TLC on RP18 plates developed in $H_2O$/MeOH (2:3) and combined into 4 different fractions (Table 3.23).

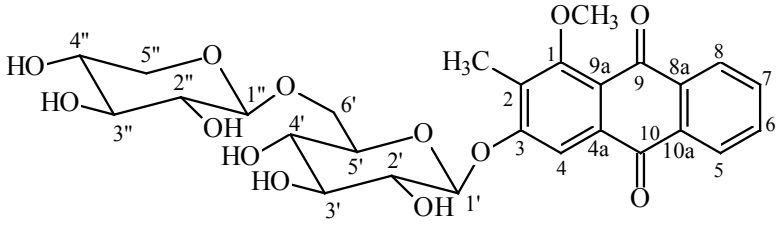
Table 3.22. Programme for fractionation of fraction 11:4 from *P. lanceolata* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 50:50 | 50:50 | --- | 20 |
| $H_2O$/MeOH | 40:60 | 0:100 | 20% | 30 |

Table 3.23. MPLC fractionation of fraction 11:4 from *P. lanceolata*.

| Fraction | Eluent number | Weight (mg) |
|---|---|---|
| 12:1 | 1-15 | 5.3 |
| 12:2 | 16-23 | 12.9 |
| 12:3 | 25-31 | 137.8 |
| 12:4 | 32-43 | 8.1 |

Solvent removal from fraction 12:3 under reduced pressure afforded a yellowish solid (137.8 mg, 0.021 % yield) which was identified as rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55**, according to its spectral and physical data. This compound has already been isolated from another species of the same plant genus, *P. zanzibarica* (112).




**55**

**Rubiadin-1-methyl ether 3-*O*-*ß*-primeveroside 55**: mp 160.7 – 162.5 °C, lit. mp 158-160 °C (116); $[\alpha]^{22}_{D}$ = -90 ° (*c* 0.1, $H_2O$). The anthraquinone glycoside displayed both the UV, the IR, the NMR ($^{1}H$, $^{13}C$, 2D) and the mass spectral data which were similar to those observed for the sample isolated from *P. bussei* (see Chapter II).

In addition, MPLC fractionation of fraction 11:6 (Table 3.21) gave rise to rubiadin-1-methyl ether **36** and rubiadin **53** already isolated from the dichloromethane and the EtOAc extracts. Also lucidin-ω-methyl ether **78**, already isolated from the EtOAc extract of the same plant, was isolated from *P. lanceolata*.

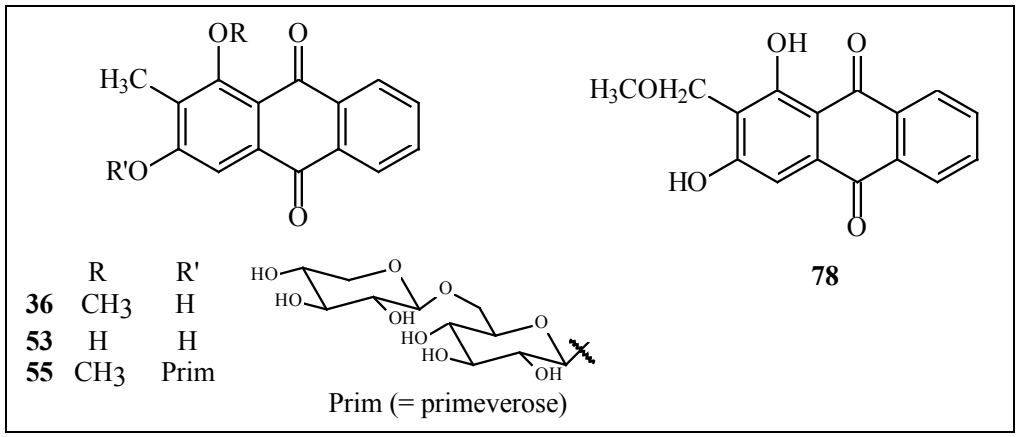



Figure 3.4. Natural Products Isolated from the Methanol Extract of the Roots of *P. lanceolata*

## III.3. Results and Discussion

The fractionation by various chromatographic methods of the dichloromethane, the ethyl acetate and the methanol extracts of the roots of *P. lanceolata* resulted in the isolation of the five anthraquinones rubiadin-1-methyl ether **36**, rubiadin **53**, damnacanthol **77**, lucidin-ω-methyl ether **78** and damnacanthol-3-methyl ether **79**, together with the anthraquinone glycoside rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55**.The hexane extract also afforded the steroid *β*-stigmasterol **64**.

### III.3.1. Anthraquinones

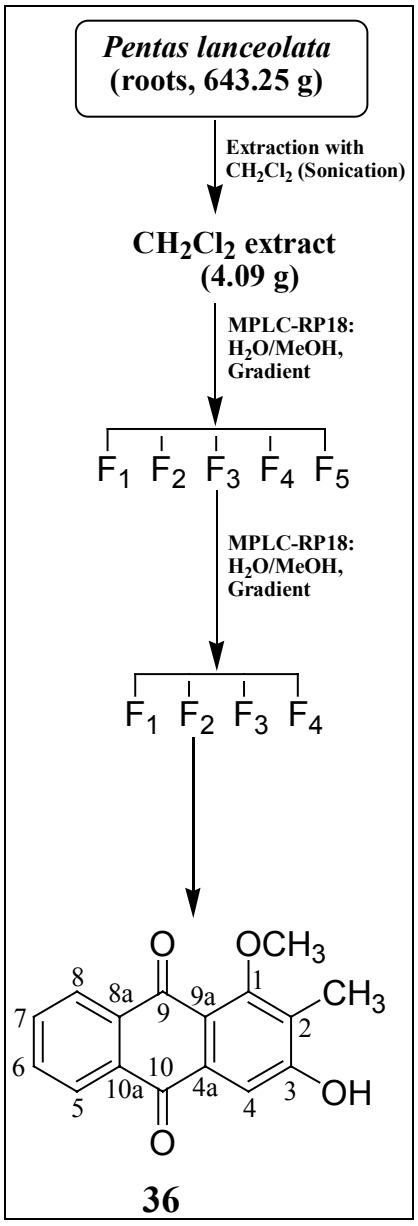
#### *III.3.1.1. Rubiadin-1-methyl ether **36***

The MPLC fractionation of the fraction 2:3 (Table 3.4) from the dichloromethane extract afforded the anthraquinone rubiadin-1-methyl ether **36**. Scheme 3.1 describes the isolation of the compound from the plant sample.

**Rubiadin-1-methyl ether (3-Hydroxy-1-methoxy-2-methyl-9,10-anthraquinone) 36**: Yellow crystals; mp 289.3-291.2 °C, lit. mp 292-294 °C (146a). The IR spectrum of compound **36** displayed an absorption band of a phenolic hydroxy group at 3304 $cm^{-1}$. The spectrum also showed bands of the carbonyl groups at 1673 and 1651 $cm^{-1}$, respectively.

The $^{1}H$ NMR spectrum of the compound in DMSO-$d_6$ displayed signals of a methyl group at $\delta_H$ 3.79 (3H, s, OC$\underline{H_3}$-1), whereas a singlet at $\delta_H$ 7.50 (1H, s, H-4) and an AA'BB' coupling system of four protons at $\delta_H$ 7.83-7.92 (2H, m, H-5 and H-8) were observed in the aromatic region. The latter system indicated that all the substituents were on one aryl ring. The $^{13}C$ NMR spectrum showed sixteen signals from which those of the two carbonyl carbons at $\delta_C$ 180.10 and 182.51 ppm. In addition, two =$C_q$-O signals were observed at $\delta_C$ 160.50 and 161.60 ppm, whereas the methoxy carbon resonated at $\delta_C$ 60.48 (OC$\underline{H_3}$-1). Such a high $\delta_C$ value for the methoxy carbon is consistent with its *peri* location (1-position) from the carbonyl in 9-position, as observed in several compounds with the same configuration (135). Based on biogenetic grounds, the methyl group may be located in the 2-position (27,71,141).

The EI mass spectrum of the compound displayed an ion peak at *m*/*z* 268 (6%) which is consistent with the molecular formula of $C_{16}H_{12}O_4$.




**Scheme 3.1**: Isolation of Rubiadin-1-methyl ether **36**
From the Roots of *P. lanceolata*

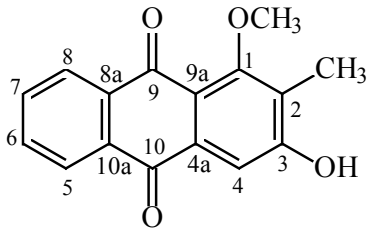
Based on all the above spectral data, the compound was identified as rubiadin-1-methyl ether (3-hydroxy-1-methoxy-2-methyl-9,10-anthraquinone) **36**. The assigned structure was confirmed by comparison (Table 3.24) of its spectral data with those observed in the literature (112). The compound has also been isolated from *Pentas zanzibarica* (112) and from various

other Rubiaceae plant species (146).

Table 3.24. Comparison of NMR data of rubiadin-1-methyl ether **36** ($^{1}$H, 270 MHz; $^{13}$C, 67.5 MHz; DMSO-$d_6$; this work) with data from the literature ($^{1}$H/$^{13}$C, 400 MHz/100 MHz, DMSO-$d_6$; ref. 112): δ (ppm).

| Position | This work | | ref. 112 | |
|---|---|---|---|---|
| | $^{1}$H | $^{13}$C | $^{1}$H | $^{13}$C |
| 1 | - | 160.50[a] | - | 160.4[a] |
| 2 | - | 126.09 | - | 126.0 |
| 3 | - | 161.60[a] | - | 161.5[a] |
| 4 | 7.50 s | 108.91 | 7.56 s | 109.1 |
| 4a | - | 131.93[b] | - | 131.9[b] |
| 5 | 8.08-8.16 m | 125.93[c] | 8.07 dd (2,8) | 125.6[c] |
| 6 | 7.83-7.92 m | 133.26[d] | 7.80 dd (8, 8) | 133.1[d] |
| 7 | 7.83-7.92 m | 134.37[d] | 7.80 dd (8, 8) | 134.3[d] |
| 8 | 8.08-8.16 m | 126.54[c] | 8.07 dd (2,8) | 126.3[c] |
| 8a | - | 133.60[b] | - | 133.6[b] |
| 9 | - | 180.10[e] | - | 180.1[e] |
| 9a | - | 117..75 | - | 117.7 |
| 10 | - | 182.51[e] | - | 182.4[e] |
| 10a | - | 134.43[b] | - | 134.4[b] |
| $OCH_3$-1 | 3.79 s | 60.48 | 3.86 s | 60.3 |
| $CH_3$-2 | 2.16 s | 8.93 | 2.20 s | 8.6 |

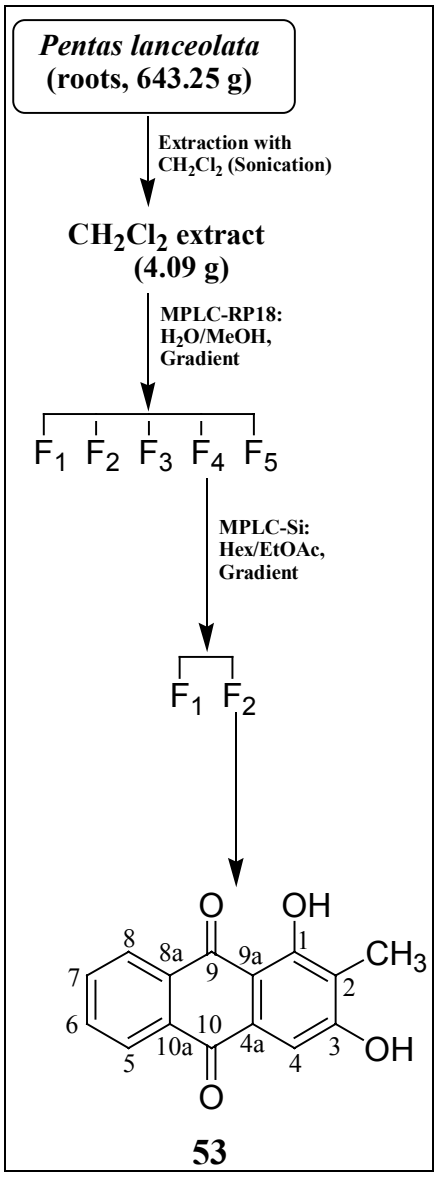
[a-e] Assignments may be reversed in the same column




**36**

*III.3.1.2. Rubiadin **53***

The application of a normal phase MPLC on the fraction 2:4 (Table 3.4) of the dichloromethane extract of the roots of *Pentas lanceolata* afforded the anthraquinone rubiadin (1,3-dihydroxy-2-methyl-9,10-anthraquinone) **53** as shown in Scheme 3.2.

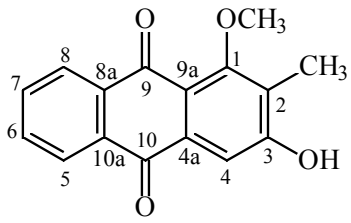



**Scheme 3.2**: Isolation of Rubiadin (1,3-Dihydroxy-2-methyl-9,10-anthraquinone) **53** from the Roots of *P. lanceolata*

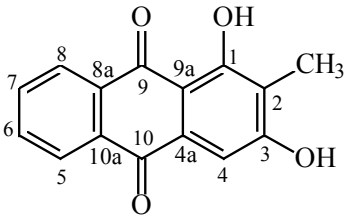
**Rubiadin (1,3-Dihydroxy-2-methyl-9,10-anthraquinone) 53**: Yellow solid; mp 299.8-301.4 °C, lit. mp 300 °C (147). The UV spectrum of the compound displayed a pattern

characterisitic of an anthraquinone with two absorption bands at 244.9, 280.0 and 413.0 nm (121a). The IR spectrum showed an absorption band of phenolic hydroxy groups at 3392 $cm^{-1}$, together with both non-hydrogen and hydrogen bonded carbonyl groups at 1661 and 1623 $cm^{-1}$, respectively.

The $^{1}H$ NMR spectrum of the compound displayed a low-field OH proton signal at $\delta_H$ 13.09 ppm, whereas the $^{13}C$ NMR spectrum showed that the chemical shifts of the two carbonyl carbons were largely separated at $\delta_C$ 181.65 (C-10) and $\delta_C$ 186.00 ppm (C-9) with a $\Delta\delta_C$ value of 4.35 ppm. Therefore, a hydroxy group is unequivocally located in the *peri* position from carbonyl in 9-position, and this is consistent with the chelation observed in the IR spectrum. In addition, the NMR spectra ($^{1}H$ NMR, $^{13}C$ NMR and HETCOR) showed one methyl at $\delta_H$ 2.05 (1H, s, $C\underline{H_3}$-2)/ $\delta_C$ 7.94 ($\underline{C}H_3$-2), a =CH at $\delta_H$ 7.2 (1H, s, H-4)/ $\delta_C$ 107.28 (C-4), together with two $=C_q$-O at $\delta_C$ 162.31 and 162.80 ppm. The $^{1}H$ NMR and COSY NMR spectra particularly disclosed a four protons AA'BB' coupling system at $\delta_H$ 7.89 (2H, m, H-6 and H-7) and 8.18 (2H, m, H-5 and H-8), which is consistent with the location of the aromatic proton H-4, the two hydroxy groups and the methyl group on one aryl ring. Comparison of compound **36** and compound **53** showed that, apart from the presence of the OH-1 in **53** ($OCH_3$-1 in **36**) which consequently brought in hydrogen bonding, the spectral data of the two compounds were closely related.




**36**




**53**

The ESI mass spectrum of compound **53** displayed a pseudomolecular ion peak at *m*/*z* 255 (42%) corresponding to the ion $[M + H]^+$ and from which was deduced the molecular formula $C_{15}H_{10}O_4$.

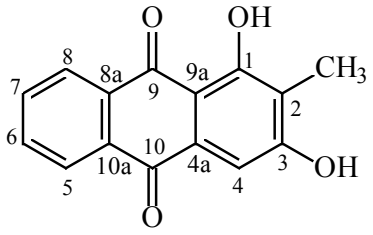
From all the above presented data, the structure of the compound was elucidated as being the known anthraquinone rubiadin (1,3-dihydroxy-2-methyl-9,10-anthraquinone) **53**. The spectral

data observed for the compound are in full agreement (Table 3.25) with those available in the literature (112,147). The compound has already been isolated from another *Pentas* species, i.e. *P. zanzibarica* (112) and from other plant species from the Rubiaceae (146a-b,147).

Table 3.25. Comparison of NMR data of rubiadin **53** ($^1$H, 270 MHz; $^{13}$C, 67.5 MHz; DMSO-$d_6$; this work) with data from the literature ($^1$H, 270 MHz, DMSO-$d_6$, ref. 147; $^{13}$C, 100 MHz, $CD_3OD$, ref. 112): δ (ppm).

| Position | This work | | Literature | |
|---|---|---|---|---|
| | $^1$H | $^{13}$C | $^1$H (ref. 147) | $^{13}$C (ref. 112) |
| 1 | - | 162.31[a] | - | 162.4[a] |
| 2 | - | 117.21 | - | 121.2 |
| 3 | - | 162.80[a] | - | 162.8[a] |
| 4 | 7.22 s | 107.28 | 7.20 s | 107.3 |
| 4a | - | 131.52[b] | - | 131.6[b] |
| 5 | 8.10-8.18 m | 126.20[c] | 8.10 m | 126.2[c] |
| 6 | 7.89 m | 134.28[d] | 7.82 m | 134.3[d] |
| 7 | 7.89 m | 134.39[d] | 7.82 m | 134.4[d] |
| 8 | 8.10-8.18 m | 126.54[c] | 8.10 m | 126.6[c] |
| 8a | - | 132.83[b] | - | 132.9[b] |
| 9 | - | 186.00 | - | 186.1 |
| 9a | - | 108.73 | - | 108.8 |
| 10 | - | 181.65 | - | 181.7 |
| 10a | - | 132.70[b] | - | 132.8[b] |
| OH-1 | 13.09 s | - | 12.90 s | - |
| $CH_3$-2 | 2.05 s | 7.94 | 2.05 s | 7.9 |
| OH-3 | | - | 10.02 s | - |

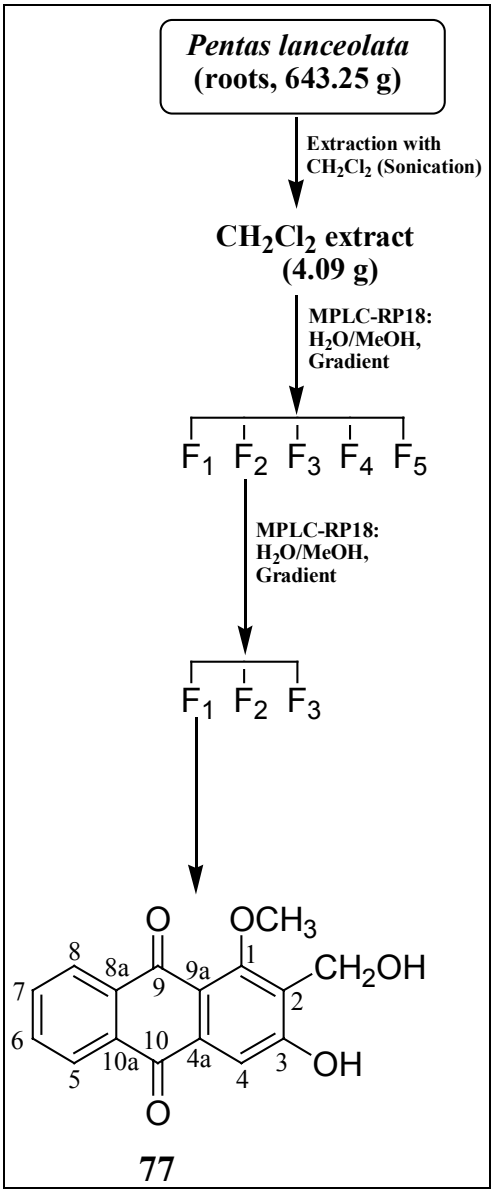
[a-d] Assignments may be reversed in the same column




**53**

*III.3.1.3. Damnacanthol 77*

Fraction 2:2 (Table 3.4) was subjected to a reversed phase MPLC to afford the anthraquinone damnacanthol (3-hydroxy-2-hydroxymethyl-1-methoxy-9,10-anthraquinone) **77**. Scheme 3.3 describes the complete isolation of the compound from the roots of *P. lanceolata*.




**Scheme 3.3**: Isolation of Damnacanthol (3-hydroxy-2-hydroxymethyl-1-methoxy-9,10-anthraquinone) **77** from the Roots of *P. lanceolata*

**Damnacanthol (3-hydroxy-2-hydroxymethyl-1-methoxy-9,10-anthraquinone) 77**: Yellow solid, mp 284.8-287.1 °C, lit. mp (synthetic compound) 286-288 °C (146). The UV spectrum

of the compound was compatible with an anthraquinone structure by showing absorption bands at 239.0, 249.0 and 280.0 nm (121a). The IR spectrum showed absorption bands of OH groups at 3308 $cm^{-1}$, together with bands ascribed to carbonyl groups at 1673 and 1651 $cm^{-1}$.

From combined analysis of the $^{1}H$, $^{13}C$ and the HETCOR NMR spectra were disclosed the methoxy group at $\delta_H$ 3.86 (3H, s, $OC\underline{H}_3$-1)/$\delta_C$ 62.26 ($O\underline{C}H_3$-1) and the hydroxymethylene group at $\delta_H$ 4.57 (2H, s, $C\underline{H}_2OH$-2)/$\delta_C$ 52.4 ($\underline{C}H_2OH$-2) and a $sp^2$ CH at $\delta_H$ 7.49 (1H, s, H-4)/$\delta_C$ 109.93 (C-4). The low-field $\delta_C$ value (62.26 ppm) of the $O\underline{C}H_3$-1 is compatible with its location at 1-position, a *peri* location from the carbonyl group in 9-position (135). Furthermore, no influence of the hydrogen bond was observed, neither in the IR spectrum, nor in the $^{13}C$ NMR $\delta_C$ values of the two carbonyl carbons ($\Delta\delta_C$ 2.76 ppm).

The $^{1}H$ and COSY NMR spectra showed a four proton AA'BB' coupling system corresponding to the four adjacent aryl protons. The $^{13}C$ NMR spectrum showed sixteen signals. Two carbonyl carbons were observed at $\delta_C$ 179.82 and 182.58 ppm, whereas two $=C_q$-O resonated at $\delta_C$ 161.54 and 162.75 ppm, respectively. It is obvious that the isolated aromatic proton is located on the trisubstituted ring. The ESI mass spectrum displayed ions at *m*/*z* 307 (80%), 285 (45%) and 267 (100%), corresponding to the fragments $[M + Na]^+$, $[M + H]^+$ and $[M - H_2O + H]^+$, respectively. From this pattern was deduced a molecular formula of $C_{16}H_{12}O_5$ for the compound.

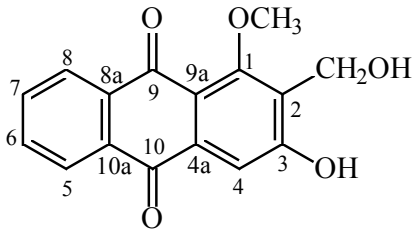
The above spectral data, together with literature search led to the identification of the compound as damnacanthol (3-hydroxy-2-hydroxymethyl-1-methoxy-9,10-anthraquinone) **77**. Although the compound has been isolated from various plants and particularly from several Rubiaceae species (146a,150), this is the first time it is isolated from a plant species of the *Pentas* genus. However, its 3-*O*-*β*-primeverosyl glycoside has been isolated from *P. zanzibarica* (112) and from *P. bussei* (see Chapter II of this work).

About its spectral characterization, only the $^{1}H$ NMR data (in acetone-$D_6$) of the synthesised product are reported in the literature (146). Here in this work, the $^{13}C$ NMR data of the natural compound **77** are reported for the first time. Table 3.26 provides a comparison of the $^{1}H$ NMR data of the isolated compound with those of the synthesised compound (145) (Table 3.26a), and of the $^{13}C$ NMR data of the isolated compound with those of the aglycone of the

corresponding damnacanthol-3-*O*-*β*-primeveroside **57** (112). The relatively large $\delta_H$ difference observed for the $OCH_3$-1 proton (Table 3.26a) may be due to a solvent effect. The upfield shift of C-2 and C-9a in damnacanthol **77** comparatively to damnacanthol-3-*O*-*β*-primeveroside **57** (Table 3.26b) is easily explained by the shielding effect of the free OH-3 on the *ortho* and *para* carbons, as already observed in such a case (116). Obviously, in the $^{13}C$ NMR spectrum, C-3 shifts to downfield in **77** relative to **57**. All the rest of the $^{13}C$ NMR data of damnacanthol **77** are compatible with those observed for the aglycone moiety of damnacanthol-3-*O*-*β*-primeveroside **57**.

Table 3.26a. Comparison of the $^1H$ NMR data of the isolated damnacanthol **77** (270 MHz, DMSO-$d_6$; this work) with those of the synthetised compound (100 MHz, DMSO-$d_6$; ref. 145)

| Proton | This work δ (ppm) | ref. 145 δ (ppm) |
|---|---|---|
| H-4 | 7.49 | 7.41 |
| H-5 | 8.09-8.17 | 8.26 |
| H-6 | 7.84-7.90 | 7.95 |
| H-7 | 7.84-7.90 | 7.95 |
| H-8 | 8.09-8.17 | 8.26 |
| $OC\underline{H}_3$-1 | 3.86 | 4.16 |
| $C\underline{H}_2OH$-2 | 4.57 | 4.67 |




**77**

Table 3.26b. Comparison of the $^{13}C$ NMR data of the isolated damnacanthol **77** (67.5 MHz, DMSO-$d_6$; this work) with data of the aglycone moiety of damnacanthol-3-*O*-*β*-primeveroside **57** (100 MHz, DMSO-$d_6$; ref. 112)

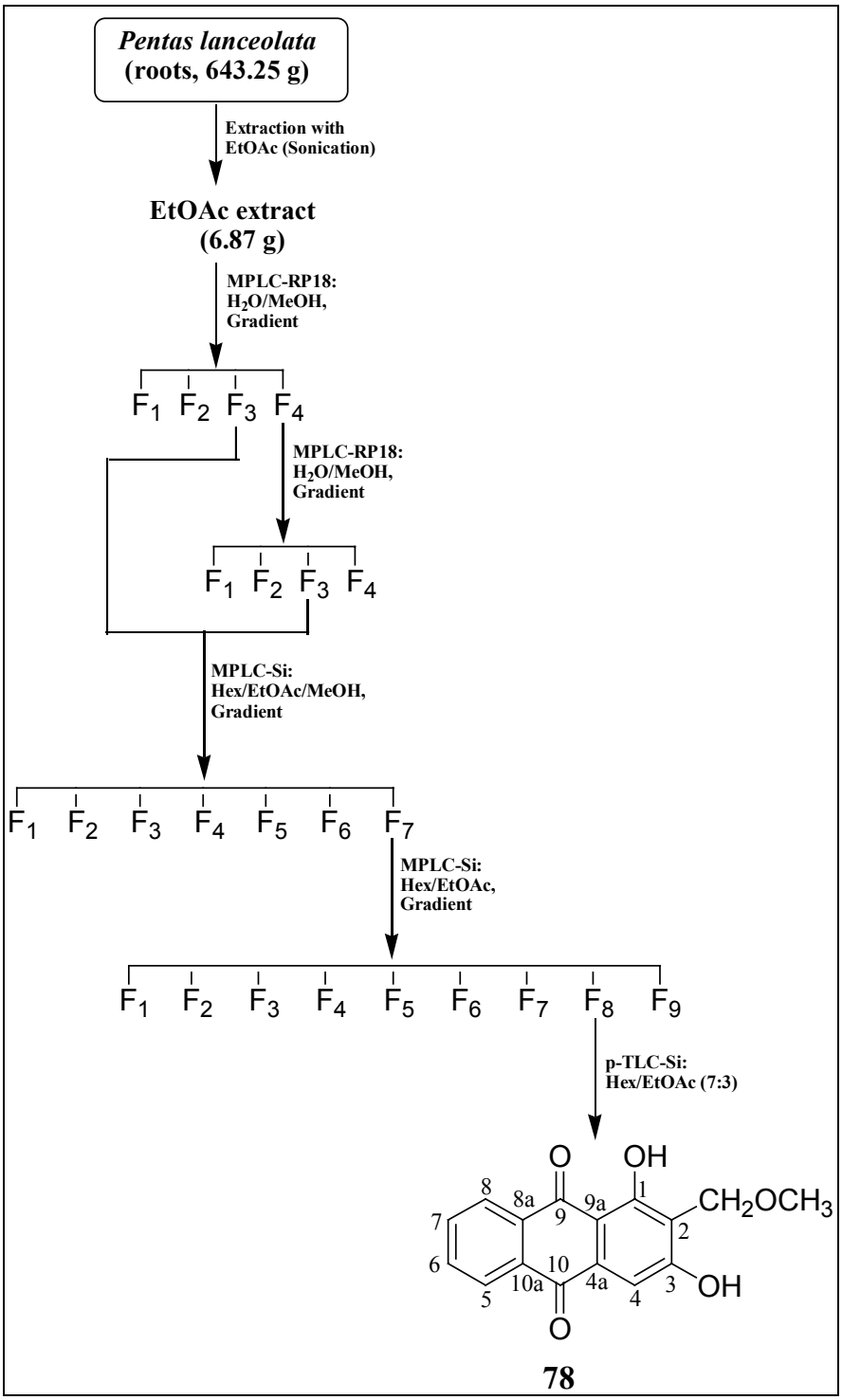
| This work | | ref. 112 (aglycone) | |
|---|---|---|---|
| Carbon | $\delta_C$ (ppm) | Carbon | $\delta_C$ (ppm) |
| 1 | 161.54[a] | 1 | 160.7[a] |
| 2 | **128.70** | 2 | **131.6** |
| 3 (=C-OH) | **162.75[a]** | 3 (=C-O-prim.)[*] | **160.8[a]** |
| 4 | 109.93 | 4 | 109.2 |
| 4a | 131.95[b] | 4a | 132.0[b] |
| 5 | 126.02[c] | 5 | 126.2[c] |
| 6 | 133.26[d] | 6 | 133.6[d] |
| 7 | 134.52[d] | 7 | 134.8[d] |
| 8 | 126.87[c] | 8 | 126.7[c] |
| 8a | 134.52[b] | 8a | 134.4[b] |
| 9 | 179.82[e] | 9 | 180.5[e] |
| 9a | **117.41** | 9a | **120.4** |
| 10 | 182.58[e] | 10 | 182.2[e] |
| 10a | 135.27[b] | 10a | 135.8[b] |
| $OCH_3$-1 | 62.26 | $OCH_3$-1 | 62.8 |
| $CH_2OH$-2 | 52.24 | $CH_2OH$-2 | 52.0 |

[a-e] Assignments may be reversed in the same column

[*] prim. = primeverose

*III.3.1.4. 1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone (Lucidin-ω-methyl ether)* ***78***

A series of chromatographic separations including MPLC-RP18, MPLC-Si and preparative TLC was applied on the fractions 6:3 and 6:4 (Table 3.12) from the ethyl acetate extract of the roots *P. lanceolata* (Scheme 3.4) to afford the anthraquinone 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (lucidin-ω-methyl ether) **78**.




**Scheme 3.4**: Isolation of 1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone (Lucidin-ω-methyl ether) **78** from the Roots of *P. lanceolata*

**1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone (lucidin-ω-methyl ether) 78**: Amorphous yellow powder; mp 169.8-172.4 °C, lit. mp 170 °C (148). The UV spectrum of

the compound showed absorption bands at 242.0, 246.0 and 280.0 nm which are consistent with an anthraquinone structure (121a). In the IR spectrum, the absorption bands of the OH groups were observed at 3393 and 3196 $cm^{-1}$. The absorption of carbonyl groups were observed at 1673 $cm^{-1}$ (unchelated C=O) and 1626 $cm^{-1}$ (chelated C=O), whereas a C-O-C group absorbed at 1275 $cm^{-1}$.

The $^{1}H$ NMR spectrum (in $CDCl_3$) displayed a hydroxy proton signal in low-field at $\delta_H$ 13.31 (1H, s, OH-1), obviously corresponding to the OH-1 in *peri* position from the carbonyl oxygen in 9-position. This OH-1 was associated with the hydrogen bonding observed in the IR spectrum. An additional hydroxy group was observed at $\delta_H$ 9.43 (1H, s, OH-3). Besides, the aromatic proton signals were typical of an anthraquinone having a non-substituted and a trisubstituted aryl ring by showing a symmetrical AA'BB' coupling system of four protons at $\delta_H$ 7.77-7.79 (2H, m, H-6 and H-8) and $\delta_H$ 8.25-8.29 (2H, m, H-5 and H-8), together with an aromatic proton singlet at $\delta_H$ 7.32 (1H, s, H-4). The methoxy and the oxymethylene protons were observed at 3.58 and 4.94 ppm respectively.

As expected from the conjugation and the hydrogen bonding effect of the OH-1, the $^{13}C$ NMR spectrum showed largely different chemical shifts for the two carbonyl carbons at $\delta_C$ 182.23 (C-10) and 186.88 (C-9). The two $=C_q$-O were observed at $\delta_C$ 161.88 and 164.08 ppm. The location of the methoxy group in 3-postion was ruled out by the presence of the OH-3 proton signal at $\delta_H$ 9.43 in the $^{1}H$ NMR spectrum. Moreover, the strongest evidence of the benzylalkyl ether nature (rather than an arylalkyl one) for compound **78** came from the $^{13}C$ NMR spectrum which showed the chemical shifts values $\delta_C$ 68.89 ($\underline{C}H_2OCH_3$-2) and $\delta_C$ 59.35 ($CH_2O\underline{C}H_3$-2). These values are typical of ethers for which the two alkoxy groups give rise to a reciprocal shift to down-field of more than 10 ppm in $^{13}C$ NMR, relatively to the corresponding alcohols. The two carbons exert to each other a reciprocal influence similar to that of a C-β in alkanes, the oxygen atom being considered as a C-α (121b). The fragment $[M + Na]^+$ was observed at *m*/*z* 307 (14%) in the ESI mass spectrum. Therefore, the molecular formula was deduced as being $C_{16}H_{12}O_5$.

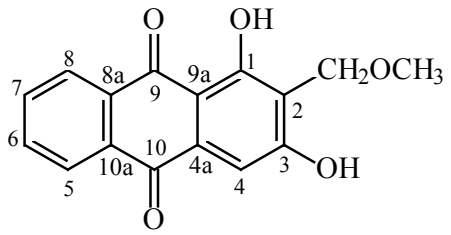
On the basis of all the above discussed spectroscopic data, the structure of the compound was established as lucidin-ω-methyl ether (1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone) **78**. The structure proposed was supported by the literature survey findings. The UV and IR

data were fully fitting with those already reported for the compound (150,151). The $^{1}H$ and $^{13}C$ NMR data were also in excellent agreement (Table 3.27a and 3.27b) with those already published (150,152).

Although the compound has been isolated from the roots, the rhizomes, the bark (or the whole plant) of many other Rubiaceae (153) and from the tissue and cell cultures of some rubiaceous plants (152,154), lucidin-ω-methyl ether **78** has been thought to be an artefact of the genuinely occurring lucidin as a result of the extraction and/or the isolation with methanol (71,155).

Table 3.27a. Comparison of the $^{1}H$ NMR data of the isolated lucidin-ω-methyl ether **78** (270 MHz, $CDCl_3$; this work) with data from the literature (200 MHz, $CDCl_3$, ref. 150)

| Proton | This work δ (ppm) | ref. 150 δ (ppm) |
|---|---|---|
| H-4 | 7.32 | 7.29 |
| H-5 | 8.25-8.29 | 8.24-8.32 |
| H-6 | 7.77-7.79 | 7.76-7.84 |
| H-7 | 7.77-7.79 | 7.76-7.84 |
| H-8 | 8.25-8.29 | 8.24-8.32 |
| OH-1 | 13.31 | 13.27 |
| $C\underline{H_2}OCH_3$-2 | 4.94 | 4.92 |
| $CH_2OC\underline{H_3}$-2 | 3.58 | 3.57 |
| OH-3 | 9.43 | 9.38 |




**78**

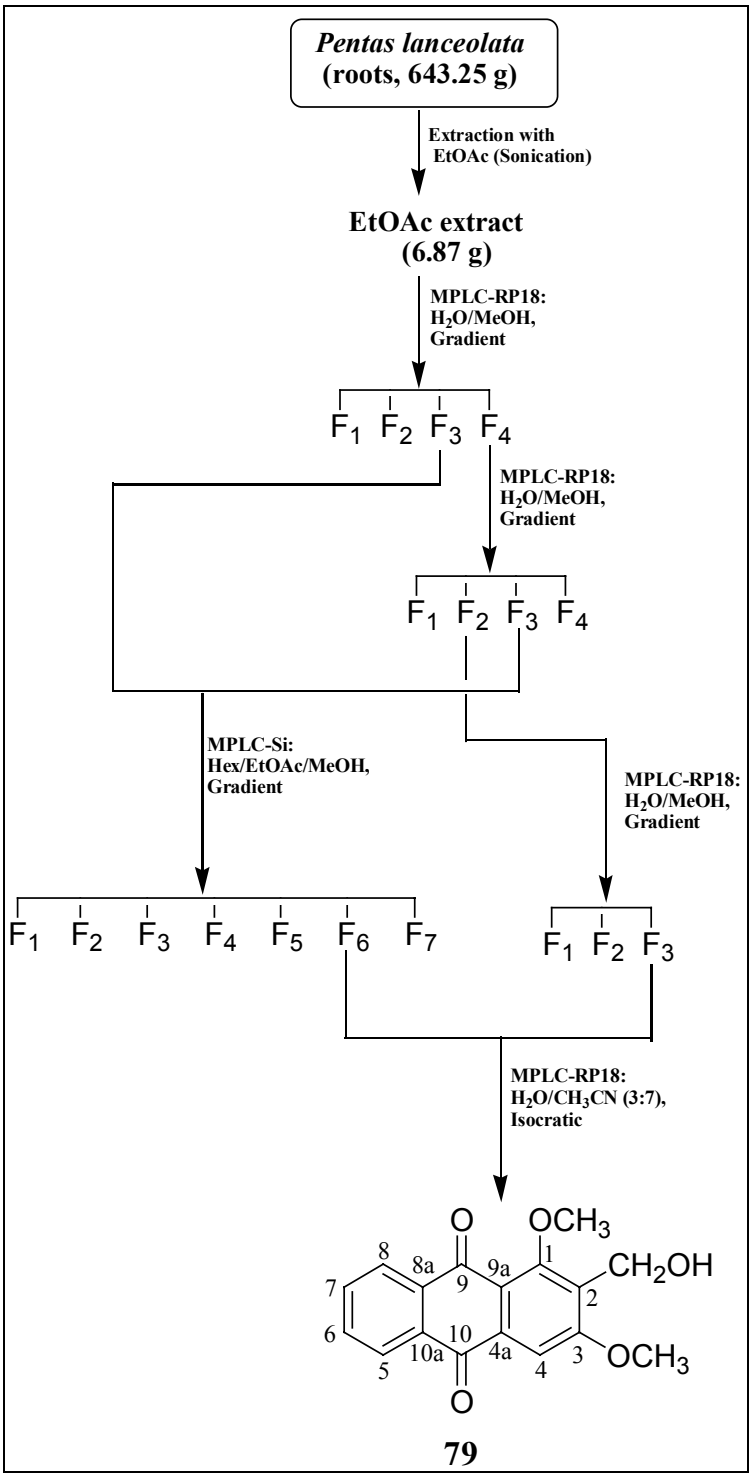
Table 3.27b. Comparison of the $^{13}C$ NMR data of the isolated lucidin-ω-methyl ether **78** (67.5 MHz, $CDCl_3$; this work) with data from the literature (100 MHz, $CDCl_3$, ref. 152)

| This work | | ref. 152 | |
|---|---|---|---|
| Carbon | $\delta_C$ (ppm) | Carbon | $\delta_C$ (ppm) |
| 1 | 161.88[a] | 1 | 161.8[a] |
| 2 | 114.37 | 2 | 114.3 |
| 3 | 164.08[a] | 3 | 164.0[a] |
| 4 | 109.74 | 4 | 109.8 |
| 4a | 134.12[b] | 4a | 134.1[b] |
| 5 | 126.70[c] | 5 | 126.7[c] |
| 6 | 134.12[d] | 6 | 134.1[d] |
| 7 | 134.12[d] | 7 | 134.0[d] |
| 8 | 127.35[c] | 8 | 127.3[c] |
| 8a | 132.43[b] | 8a | 133.5[b] |
| 9 | 186.88 | 9 | 186.9 |
| 9a | 109.63 | 9a | 109.6 |
| 10 | 182.23 | 10 | 182.2 |
| 10a | 133.51[b] | 10a | 133.5[b] |
| $\underline{C}H_2OCH_3$-2 | 68.89 | $\underline{C}H_2OCH_3$-2 | 68.9 |
| $CH_2O\underline{C}H_3$-2 | 59.35 | $CH_2O\underline{C}H_3$-2 | 59.4 |

[a-d] Assignments may be reversed in the same column

*III.3.1.5. Damnacanthol-3-O-methyl ether **79**.*

An isocratic elution using MPLC-RP18 with $H_2O$/ACN (3:7) as mobile phase was applied on the combined fraction 8:6 (Table 3.16) and 10:3 (Table 3.20) obtained from the EtOAc extract of the roots of *P. lanceolata*, as shown in Scheme 3.5. The separation gave rise to damnacanthol-3-*O*-methyl ether (1,3-dimethoxy-2-hydroxymethyl-9,10-anthraquinone) **79**.




**Scheme 3.5**: Isolation of Damnacanthol-3-*O*-methyl ether (1,3-dimethoxy-2-hydroxymethyl-9,10-anthraquinone) **79** from the Roots of *P. lanceolata*

**Damnacanthol-3-*O*-methyl ether (1,3-dimethoxy-2-hydroxymethyl-9,10-anthraquinone) 79**: Amorphous yellow powder; mp 173.8-175.2 °C, lit. mp 173.5-174.0 °C (149). The IR

spectrum of the compound displayed an OH absorption band at 3445 $cm^{-1}$. Two non-hydrogen bonded carbonyl absorption bands were observed at 1673 and 1650 $cm^{-1}$, respectively, therefore indicating that compound **79** was an anthraquinone without any OH group in the *peri* position.

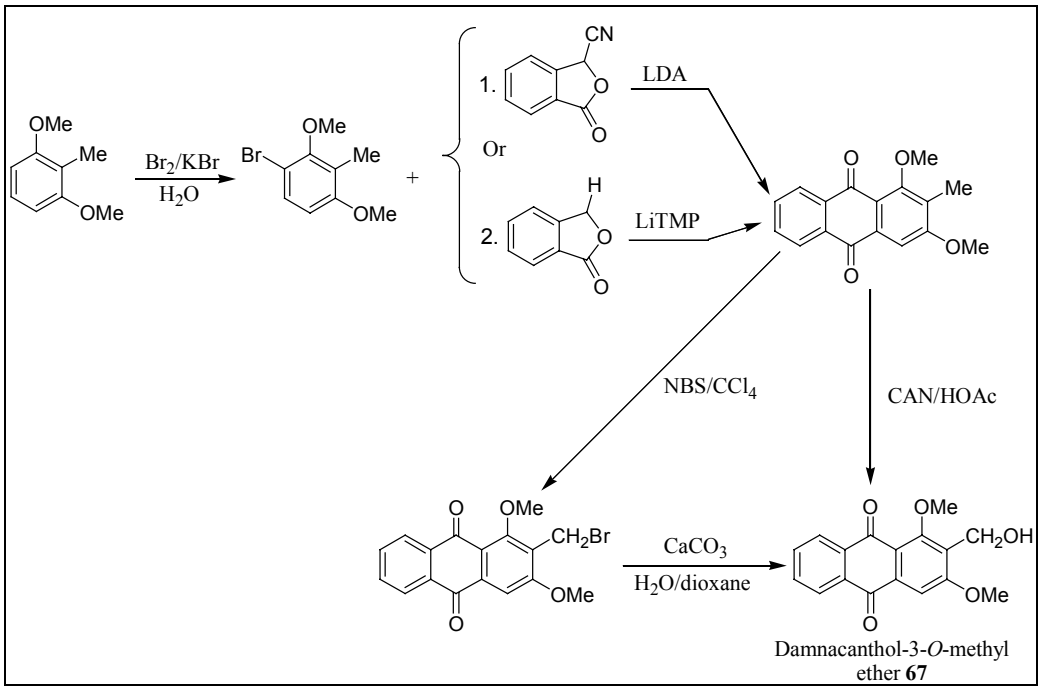
The $^{1}H$ NMR spectrum (in $C_5D_5N$) displayed the general pattern of a one-side-trisubstituted anthraquinone. Two multiplets (AA'BB' system) of the non-substituted aryl ring were observed at $\delta_H$ 7.63-7.71 (2H, m, H-6 and H-7) and $\delta_H$ 8.27-8.42 (2H, m, H-5 and H-8), whereas the isolated proton located at the trisubstituted ring resonated at $\delta_H$ 7.99 (1H, s, H-4).

The $^{13}C$ NMR spectrum showed the presence of non-chelated carbonyl groups which resonated at $\delta_C$ 183.37 and 180.86 ppm ($\Delta\delta_C$ 2.51 ppm). The two $=C_q$-O bearing the $OCH_3$ groups were observed at 163.99 and 162.46 ppm. The three substituents, two methoxy groups and a hydroxymethylene one, displayed the following NMR data: $\delta_H$ 3.62 (3H, s, OC<u>H</u>$_3$-3)/$\delta_C$ 49.68 (O<u>C</u>H$_3$-3), $\delta_H$ 4.23 (3H, s, OC<u>H</u>$_3$-1)/$\delta_C$ 62.82 (O<u>C</u>H$_3$-1) and $\delta_H$ 5.37 (2H, s, C<u>H</u>$_2$OH-2)/ $\delta_C$ 54.73 (<u>C</u>H$_2$OH-2). The low-field value of the O<u>C</u>H$_3$-1 (62.82 ppm) is consistent with its *peri* position from the carbonyl in C-9 as observed in similar compounds (135).

The possibility of having benzylalkyl ether (methylation of $CH_2OH$-2) was ruled out by the $^{13}C$ chemical shift of 54.73 ppm observed for the <u>C</u>H$_2$OH-2. The replacement of the OH by a $OCH_3$ group should have deshielded the methylene carbon about 10 ppm up to low field (121b).

The ESI mass spectrum showed a base peak (100%) at *m*/*z* 267. This fragment may derive from the ion $[M + H]^+$ *via* the loss of neutral MeOH. The molecular formula of $C_{17}H_{14}O_5$ was deduced for compound **79**.

From all these spectroscopic findings, the structure of the compound was established as damnacanthol-3-*O*-methyl ether (1,3-dimethoxy-2-hydroxymethyl-9,10-anthraquinone) **79**. To the best of our knowledge, it is the first time that this compound is isolated from a plant material. However, successful synthesis of the compound has been designed (145,149) as shown in Scheme 3.6.



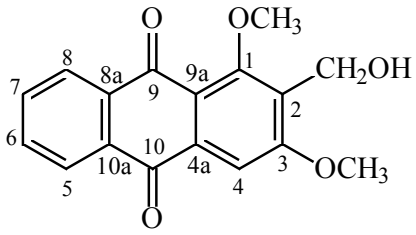

**Scheme 3.6**: Synthesis of Damnacanthol-3-*O*-methyl ether (1,3-dimethoxy-2-hydroxymethyl-9,10-anthraquinone) **79** (145)

The $^{1}$H NMR data of the isolated damnacanthol-3-*O*-methyl ether **79** were found consistent with those of the synthesised product (Table 3.28). The $^{13}$C NMR data are reported here for the first time.

Table 3.28. Damnacanthol-3-*O*-methyl ether **79**: Comparison of its $^{1}H$ NMR data (270 MHz, $C_5D_5N$; this work) with the $^{1}H$ NMR data from the literature (200 MHz, $CDCl_3$, ref. 145) and presentation of its $^{13}C$ NMR data (67.5 MHz, $C_5D_5N$, this work): δ (ppm)

| Position | $^{1}H$ | | $^{13}C$ |
|---|---|---|---|
| | This work (In $C_5D_5N$) | ref. 145 (In $CDCl_3$) | |
| 1 | - | - | 162.46[a] |
| 2 | - | - | 129.97 |
| 3 | - | - | 163.99[a] |
| 4 | 7.99 s | 7.67 | 110.94 |
| 4a | - | - | 133.10[b] |
| 5 | 8.27-8.42 m | 8.24 m | 126.59[c] |
| 6 | 7.63-7.71 m | 7.7 m | 133.21[d] |
| 7 | 7.63-7.71 m | 7.7 m | 134.86[d] |
| 8 | 8.27-8.42 m | 8.24 m | 127.29[c] |
| 8a | - | - | 134.46[b] |
| 9 | - | - | 183.37[e] |
| 9a | - | - | 118.96 |
| 10 | - | - | 180.86[e] |
| 10a | - | - | 135.02[b] |
| $OCH_3$-1 | 4.23 | 4.05 | 62.82 |
| $CH_2$OH-2 | 5.37 | 5.67 | 54.73 |
| $OCH_3$-3 | 3.62 | 3.98 | 49.68 |

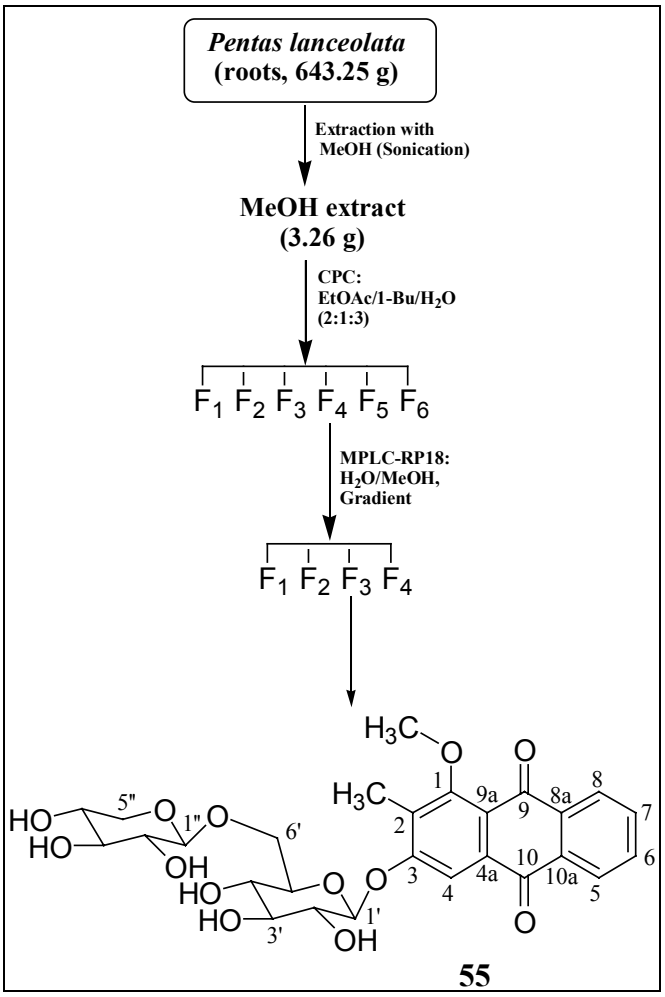
[a-e] Assignments may be reversed in the same column




**79**

III.3.2. Anthraquinone Glycoside: Rubiadin-1-methyl ether 3-*O*-*β*-primeveroside **55**

The partition by CPC followed by an MPLC fractionation of the MeOH extract of the roots of *P. lanceolata* as described in Scheme 3.7 afforded the anthraquinone glycoside rubiadin-1-methyl ether 3-*O*-*β*-primeveroside **55** (Fraction 12:3 of Table 3.23). Its physical and spectral data were compared to those observed for the sample isolated from *P. bussei*, and were found identical. The structure elucidation was also achieved on the basis of the comparison of these data with those found in the literature (112,121b). The structural discussion on the compound is provided in Chapter II, Section II.3.1.3.

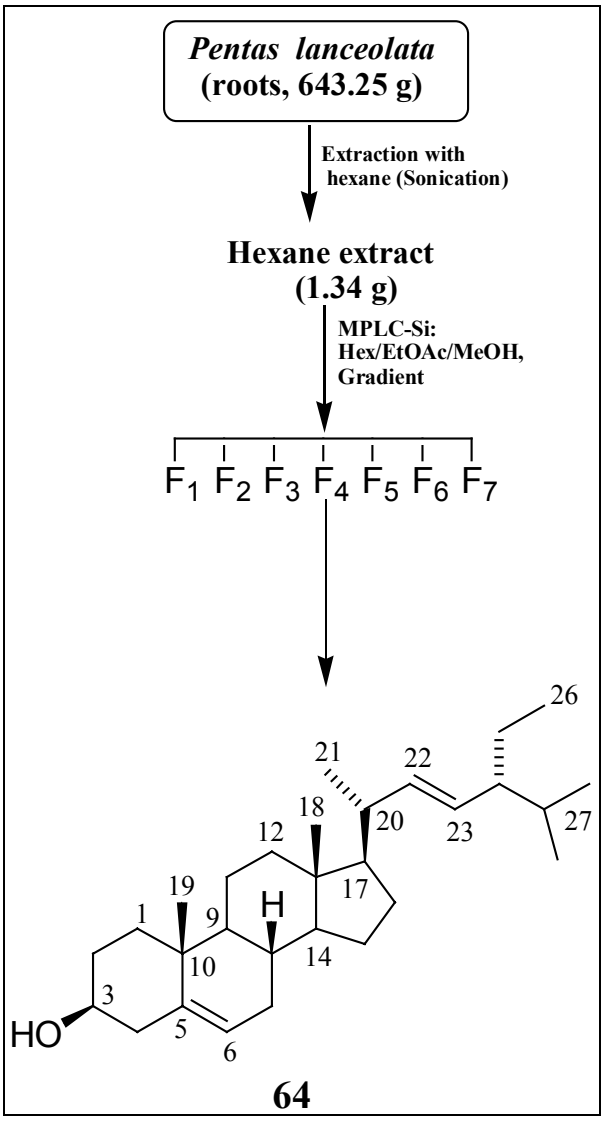



**Scheme 3.7**: Isolation of Rubiadin-1-methyl ether 3-*O*-*β*-primeveroside **55** from the Roots of *P. lanceolata*

*P. suswaensis* Verdc. and *P. zanzibarica* (Klotzch) Vatke are two other species from the same genus and which are reported to be botanically similar to *P. lanceolata* (144). The anthraquinones (**35**, **36**, **53**) and anthraquinone glycosides (**54-57**) have been isolated from *P. zanzibarica* (112). The several anthraquinones (**36**, **53**, **77-79**) and the anthraquinone glycoside (**55**) isolated from *P. lanceolata* through the present investigation may be regarded as a chemotaxonomical evidence for the resemblance (144) observed between the two *Pentas* species, *P. zanzibarica* and *P. lanceolata*.

III.3.3. Steroidal Compound: *β*-Stigmasterol **64**

The ubiquitous steroid (24*S*)-24-ethylcholesta-5-(*E*)-22-dien-3*β*-ol (*β*-stigmasterol) **64** was isolated from the hexane extract of the roots of *P. lanceolata* trough a one-step fractionation by MPLC on silica gel (Scheme 3.8). The physical and spectroscopical data of the compound were found fitting with those of the sample isolated from the roots of *P. bussei* and with the data from the literature (111,115). The structural discussion is given in Chapter II, Section II.3.3.




**Scheme 3.8**: Isolation of (24*S*)-24-Ethylcholesta-5-(*E*)-22-dien-3*β*-ol (*β*-stigmasterol) **64** from the Roots of *P. lanceolata*

**Chapter IV.**

# STUDY OF
# THE ROOTS OF
# *PENTAS PARVIFOLIA* Hiern
# (Rubiaceae)

# Chapter IV. STUDY OF THE ROOTS OF *PENTAS PARVIFOLIA*

## IV.1. Introduction

*Pentas parvifolia* Hiern is an erect pubescent shrub up to 0.5-2.5 m high, sometimes scrambling to 3.5 m, often having irregularly branched stems but not a true Liane. Stems are pale or purplish brown, woody, pubescent with white or ferruginous hairs above, glabrous below and the epidermis often peeling. Leaves are ovate or elliptic, base cuneate, apex acute, 1-10.5 by 0.3-2 cm, sparsely pubescent above, fine-pubescent beneath. Dense or lax corymbose heads of bright red flowers, in (sub)terminal few-to many-flowered cymes; flowers scarlet, rarely pink and white, mostly dimorphic, but sometimes particularly trimorphic corolla tube 7-18, lobes 2.5-10 mm long. Fruit oblong, 3-6 mm long (113).

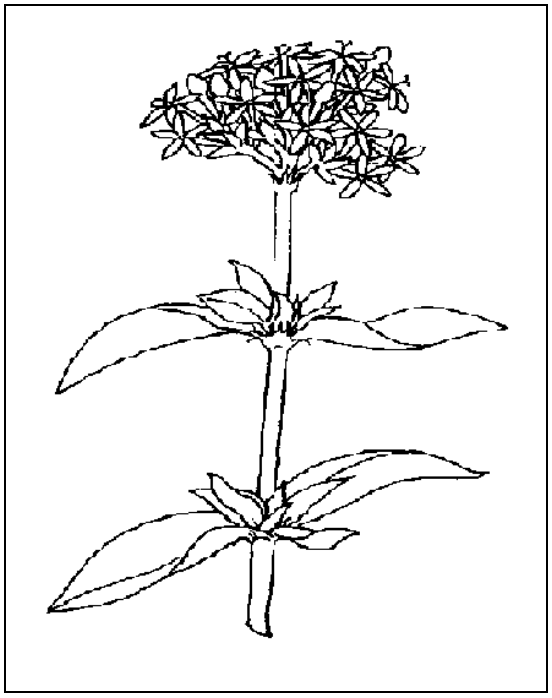


Figure 4.1. *Pentas parvifolia* Hiern

The plant is common in rocky sites in dry bushland and wooded grassland, on the coast usually in dry forest, in between 1-2400 m. Different names are given to the plant in Kenya by local tribes, such as "Kakalla", "Kirere", "Gora" or "Kinyeri" (113). No phytochemical investigation of this species is reported in the literature.

## IV.2. Experimental

### IV.2.1. Extraction and Purification

#### *IV.2.1.1. Extraction*

The ground dried roots of the plant (451.49 g) were extracted exhaustively (x 3) with *n*-hexane, dichloromethane ($CH_2Cl_2$), ethyl acetate (EtOAc) and methanol (MeOH), respectively. A volume of 0.50 litres of each solvent was used for each extraction. The solvent was removed and the sample dried before the extraction with a new solvent started. Extraction was executed under sonication at room temperature. The extracts were filtered and concentrated under reduced pressure on a rotavapor at 40 °C to afford a dark green *n*-hexane residue (4.24 g, 0.94 % yield), a dark $CH_2Cl_2$ residue (8.33 g, 1.84 % yield), a dark EtOAc residue (1.59 g, 0.35 % yield), and a dark brown MeOH residue (14.05 g, 3.11 % yield).

#### *IV.2.1.2. Study of the Hexane Extract of the Roots of Pentas parvifolia*

The *n*-hexane extract (4.24 g, 0.94 % yield) was partitioned between the biphasic solvent system *n*-hexane/EtOAc/MeOH/$H_2O$ (9:1:7:1). After decantation and removal of the solvent, 2 residues were obtained from the light and heavy phases, respectively, namely "hexane-1" (1.44g, 0.32 % yield) and "hexane-2" (2.76 g, 0.61 % yield).

##### IV.2.1.2.1. Isolation of (24 *S*)-24-Ethylcholesta-5-(*E*)-22-dien-3-*β*-ol (Stigmasterol) **64**

The "hexane-1" fraction residue (1.44 g) was subjected to MPLC over a normal phase elution using a *n*-hexane/acetone/MeOH gradient (Table 4.1) (column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 10.2 g of silica gel and packed in a 230 x 36 mm i.d. sample column; flow rate 50 ml/min at a back pressure < 40 bar; detection, LSD; collection 70 seconds per fraction) to afford 7 fractions monitored by TLC (Table 4.2).

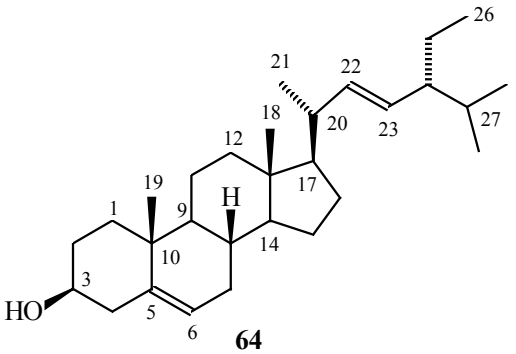
Table 4.1. Programme for fractionation of the "hexane-1" fraction of the roots of *P. parvifolia* on MPLC

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| *n*-Hexane/Acetone/MeOH | 100:0:0 | 95:5:0 | 5% | 55 |
| *n*-Hexane/Acetone/MeOH | 90:10:0 | 70:30:0 | 10% | 60 |
| *n*-Hexane/Acetone/MeOH | 50:50:0 | 50:50:0 | ---- | 40 |
| *n*-Hexane/Acetone/MeOH | 50:45:5 | 50:45:5 | ---- | 40 |

Table 4.2. MPLC fractionation of the "hexane-1" fraction of the roots of *P. parvifolia*.

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 1:1 | 1-44 | 706.5 |
| 1:2 | 45-85 | 52.4 |
| 1:3 | 86-102 | 44.3 |
| 1:4 | 103-115 | 117.3 |
| 1:5 | 116-126 | 94.0 |
| 1:6 | 127-155 | 86.4 |
| 1:7 | 156-168 | 144.4 |

Fraction 1:4 (117.3 mg, 0.026 % yield) was submitted to p-TLC developed with *n*-hexane/ acetone (3:2). The sample was dissolved in 5 ml of a mixture of *n*-hexane and acetone (1:1) and spotted on the plate, in a line 3 mm wide and 19 cm length at 1.5 cm from the bottom edge of the plate. The compound which, after development showed a $R_f$ 0.63 was scraped off and extracted from silica gel with acetone, followed by filtration. According to the spectral data, it was identified as *β*-stigmasterol **64** (47.6 mg, 0.01 % yield). Spectral data were completely identical to those obtained for the same compound isolated from *P. bussei*, and *P. lanceolata* (Chapters II and III).




**64**

**(24*S*)-24-Ethylcholesta-5-(*E*)-22-dien-3$\beta$-ol ($\beta$-stigmasterol) 64**. White solid (from hexane); mp 164.4-166.1 °C, lit. mp 164.2-166.0 °C (115). The compound displayed spectral data (IR, $^{1}$H and $^{13}$C, MS) similar to those observed in the case of the sample isolated from *P. bussei* (see Chapter II).

IV.2.1.2.2. Isolation of Methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate **58**

The "hexane-2" fraction residue was submitted to a further fractionation by CPC (3 x run, 0.92 g sample weight each time) with *n*-hexane/EtOAc/MeOH/$H_2O$ (7:7:8:2) to give 10 fractions (Table 4.3). The CPC conditions were: column 120 ml x 3; elution mode, head to tail, the upper phase as the stationary phase and the lower phase as the mobile phase; flow rate 1 ml/min with a pressure ≈ 80 psi; revolution speed, 1069 rpm; equilibrium volume, 80 ml; sample loop, 14 ml; detection, UV 254 nm and 280 nm; sample collection, 5 min per fraction; push out at 330 min with a flow rate of 8ml/min collected at 16 ml per tube.

Fractions 2:1 to 2:3 were mixed together (320.5 mg, 0.07 % yield) and the total sample submitted to reversed phase MPLC under a gradient mode (Table 4.4) to yield 5 fractions (Table 4.5) monitored by TLC (RP-18 plates, $H_2O$/MeOH (2:3) as eluting solvent system). MPLC conditions were: column, 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 2.27 g of RP-18 and packed in a 230 x 36 mm i.d. sample column; flow 35 ml/min at a back pressure < 40 bar; detection, LSD; collection 36 seconds per fraction.

Table 4.3. CPC fractionation of the "hexane-2" fraction of the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 2:1 | 1-18 | 133.7 |
| 2:2 | 19-26 | 77.5 |
| 2:3 | 27-32 | 109.3 |
| 2:4 | 33-41 | 318.4 |
| 2:5 | 42-47 | 565.3 |
| 2:6 | 48-53 | 49.9 |
| 2:7 | 54-61 | 43.9 |

| | | |
|---|---|---|
| 2:8 | 62-83 | 1132.2 |
| 2:9 | 84-89 | 286..2 |
| 2:10 | 90-97 | 124.7 |

Table 4.4. Programme for separation of fraction 1 :1-1 :3 of *P. parvifolia*

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 100:0 | 90 :10 | 5% | 15 |
| $H_2O$/MeOH | 90:10 | 0 :100 | 10% | 153 |

Table 4.5. MPLC fractionation of fractions 1 :1-1 :3 of the roots of *P. parvifolia*.

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 3:1 | 72-165 | 46.5 |
| 3:2 | 167-187 | 18.5 |
| 3:3 | 189-214 | 30.8 |
| 3:4 | 215-255 | 58.7 |
| 3:5 | 267-280 | 143.1 |

Fraction 3:5 (143.1 mg, 0.032 % yield) was submitted to a normal phase MPLC separation under a gradient mode (Table 4.6) to afford 5 fractions (Table 4.7) monitored by TLC on silica gel. MPLC conditions were: column 460 x 26 mm i.d.; precolumn, 140 x 10 mm i.d., sample adsorbed on 1.0 g of silica and packed in a 230 x 26 mm i.d. sample column; flow, 50 ml/min at a back pressure < 40 bar; detection, LSD; collection 30 seconds per fraction.

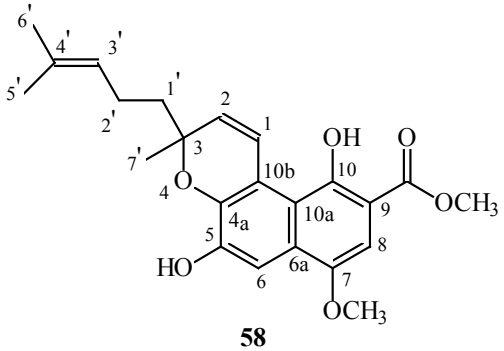
Table 4.6. Programme for separation of fraction 3:5 from the roots of *P. parvifolia*

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | stepwise | Start |
| *n*-Hexane/EtOAc | 100:0 | 60:40 | 20% | 20 |
| *n*-Hexane/EtOAc | 50:50 | 50:50 | ---- | 10 |
| *n*-Hexane/EtOAc | 40:60 | 40:60 | ---- | 10 |
| *n*-Hexane/EtOAc | 20:80 | 0:100 | 10% | 20 |
| *n*-Hexane/EtOAc | 95:5 | 80:20 | 5% | 65 |

Table 4.7. MPLC fractionation of fraction 3:5 from the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 4:1 | 12-25 | 11.6 |
| 4:2 | 37-57 | 24.3 |
| 4:3 | 152-177 | 15.0 |
| 4:4 | 178-198 | 26.9 |
| 4:5 | 199-250 | 40.7 |

Fraction 4:2 (24.3 mg, 0.005 %) was subjected to p-TLC eluted with *n*-hexane/EtOAc (3:2). The spot at $R_f$ = 0.65 (yellow and blue band under UV light at 254 and 365 nm, respectively) was scraped off and the compound removed from the adsorbent by extraction with acetone. The solvent was evaporated to dryness to yield 11.4 mg (0.0025 % yield) of a yellow solid. Based on its spectral and physical data, the compound was identified as methyl-5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate **58**, already isolated from the hexane extracts of the roots of *P. bussei* (see Chapter II).

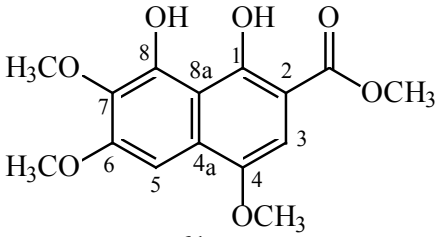



**58**

**Methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate 58**: Yellow solid (from hexane); mp 89.9-92.1 °C; $[\alpha]^{21}_D$ +34.8° (*c* 0.05, $CHCl_3$). Both the UV, the IR, the NMR ($^1H$, $^{13}C$, 2D) and the MS spectral data observed for the compound **58** were similar to those observed in the case of the sample isolated from *P. bussei* (see Chapter II).

IV.2.1.2.3. Isolation of Methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61**

After removal of the solvent to dryness under reduced pressure, fraction 2:4, yielded an amorphous dark white solid, which was recrystallized in MeOH to afford brown fine needles. The compound was identified as methyl-1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61**, based on its physical and spectral data, and was already isolated from the roots of *P. bussei* as described in Chapter II.




**61**

**Methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate 61**: Brown fine needles (from MeOH); mp 176.2-177.8 °C. The spectral data observed for the compound **61**, i.e. the UV, the IR, the NMR ($^{1}H$, $^{13}C$, 2D) and the MS, were similar to those observed for the same compound isolated from *P. bussei* (see Chapter II).

IV.2.1.2.4. Isolation of 9-Methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol **62**

Fraction 2:8 (1132.2 mg, 0.25 % yield) of Table 4.3 was submitted to an isocratic MPLC separation on reversed phase eluting with MeOH/$H_2O$ (9:1) for 90 min under the following conditions: column 460 x 70 mm i.d.; precolumn, 140 x 10 mm, sample adsorbed on 12 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate, 50 ml/min at a back pressure < 40 bar; detection, LSD; collection 36 seconds per fraction. The separation afforded 5 fractions, after TLC monitoring on RP18 plates and combination of the eluates (Table 4.8).

Table 4.8. MPLC fractionation of fraction 2:8 from the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 5:1 | 15-20 | 167.6 |
| 5:2 | 21-27 | 130.8 |
| 5:3 | 28-48 | 253.6 |
| 5:4 | 49-69 | 223.4 |

| | | |
|---|---|---|
| 5:5 | 70-115 | 266.6 |

On the one hand, fractions 5:2 and 5:3 were mixed together (384.4 mg, 0.085 % yield) and rechromatographed on reversed phase MPLC under an isocratic elution with MeOH/$H_2O$ (85:15) to afford 5 differents fractions (Table 4.9), while, on the other hand, fractions 5:4 and 5:5 were also mixed together (490 mg, 0.11 % yield) and submitted to a MPLC under the same separation conditions to give rise to the 5 fractions listed in Table 4.10. MPLC conditions were: column, 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 2.7 g and 3.5 g of RP18, respectively, and packed in a 230 x 26 mm i.d. sample column in each case; flow rate, 60 ml/min at a back pressure < 40 bar; detection, LSD; collection 10 seconds per fraction. Eluates were monitored by TLC on RP18 plates eluting with MeOH/$H_2O$ (7:3) before combination.

Table 4.9. MPLC fractionation of fractions 5:2 and 5:3 from the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 6:1 | 11-17 | 49.7 |
| 6:2 | 18-27 | 88.2 |
| 6:3 | 28-38 | 147.1 |
| 6:4 | 39-62 | 14.9 |
| 6:5 | 63-97 | 58.1 |

Table 4.10. MPLC fractionation of fractions 5:4 and 5:5 from the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 7:1 | 17-23 | 74.7 |
| 7:2 | 24-32 | 35.8 |
| 7:3 | 33-46 | 183.9 |
| 7:4 | 47-95 | 69.4 |
| 7:5 | 96-156 | 104.3 |

Fraction 6:1 and 7:1 were mixed together (124.4 mg, 0.027 % yield) and submitted to a reversed phase MPLC under gradient elution mode (Table 4.11). The eluates were monitored by TLC on RP18 plates eluted with MeOH/$H_2O$ (7:3) and combined into 3 fractions (Table 4.12). MPLC conditions were: short column, 230 x 36 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 0.9 g of RP18 and packed in a 230 x 26 mm i.d. sample column; flow rate, 45 ml/min at a back pressure < 40 bar; detection LSD; collection 20 seconds per fraction.

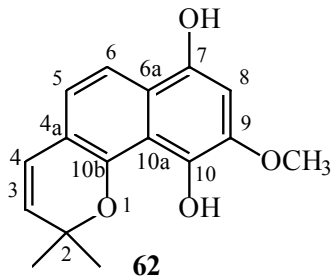
Table 4.11. Programme for separation of fraction 6:1 and 7:1 from the roots of *P. parvifolia*

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | stepwise | |
| $H_2O$/MeOH | 50:50 | 30:70 | 10% | 30 |

Table 4.12. MPLC fractionation of fractions 6:1 and 7:1 from the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 8:1 | 8-16 | 6.2 |
| 8:2 | 18-27 | 45.6 |
| 8:3 | 28-47 | 14.4 |

Evaporation of the solvent to dryness under reduced pressure from fraction 8:3 afforded an amorphous solid (14.4 mg, 0.0031 % yield). Based on its spectral data and physical properties, the compound was identified as 9-methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol **62**, which was already isolated from the roots of the species *P. bussei* and described in Chapter II.




**62**

**9-Methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol 62**: White solid (from hexane/ acetone (1:1)); mp 201.8-204.0 °C. Both the UV, the IR, the NMR ($^{1}$H, $^{13}$C, 2D) and the MS spectral data observed for the compound **62** were similar to those observed in the case of the sample isolated from *P. bussei* (see Chapter II).

IV.2.1.2.5. Isolation of 9-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]-chromene-7,10-diol **63**

Fractions 6:2 to 6:4 (Table 4.9) and 7:2 (Table 4.10) were mixed together (286.0 mg, 0.06 % yield) and rechromatographed on normal phase MPLC under a gradient mode elution (Table 4.13) and with the following conditions: column, 460 x 10 mm i.d. sample column; flow rate, 48 ml/min at a back pressure < 30 bar; detection LSD; collection, 15 seconds per fraction. The eluates were monitored by TLC on silica gel plates eluting with EtOAc/MeOH (4:1) and combined into 4 fractions (Table 4.14).

Table 4.13. Programme for separation of fraction 6:2 to 6:4 and 7:2 from the roots of *P. parvifolia*

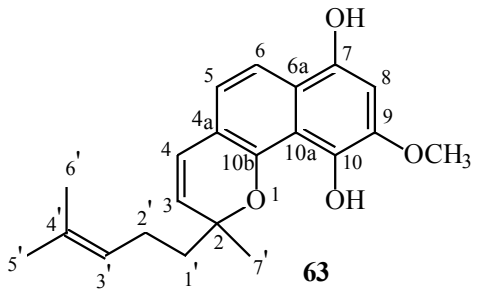
| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | stepwise | |
| EtOAc/MeOH | 100:0 | 70:30 | 10% | 75 |

Table 4.14. MPLC fractionation of fractions 6:2 to 6:4 and 7:2 from the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 9:1 | 1-104 | 27.1 |
| 9:2 | 105-135 | 37.9 |
| 9:3 | 136-210 | 108.3 |
| 9:4 | 211-232 | 12.2 |

Fraction 9:3 (108.3 mg, 0.024 % yield) was additionally purified by p-TLC eluting with EtOAc/MeOH (4:1). A solution of the sample in EtOAc/MeOH (9:1) was prepared and spotted on 3 preparative TLC plates (silica gel). After development, the band with R*f* 0.35 was scraped off and the compound extracted from the adsorbent by extraction (3 x) with EtOAc to afford an amorphous white solid (44.4 mg, 0.01 % yield). Further purification of the compound by HPLC was conducted on a RP18 column.

The analytical HPLC conditions were: column, a Kromasil C18, 5µm, 10 x 0.4 cm i.d., Teknokroma (Barcelona); elution, gradient mode with $H_2O$/MeOH (50:50 to 0:100); flow rate, 1 ml/min; detection, UV 254 nm. The preparative HPLC conditions were: column, a Kromasil C18, 5 µm, 25 x 2 cm i.d., Teknokroma (Barcelona); elution, isocratic mode with $H_2O$/MeOH (20:80); flow rate, 9 ml/min corresponding to a back pressure ≈ 179 bar; detection, UV 254 nm; collection 90 seconds per fraction; running time, 45 min. The sample was dissolved in 3 ml $H_2O$/MeOH (1:99) and filtered through a 0.45 µm filter (Acrodisc 13 CR PTFE, 13 mm, Gelman) before injection. The eluates in between 6.8-9.2 min (retention time) were mixed and dried under vacuum to give rise to a final pure compound (19.3 mg, 0.0043 % yield). Based on its physical and spectral data, the compound was identified as 9-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*f*]chromene-7,10-diol **63**, which was already isolated from the roots of the species *P. bussei* and described in Chapter II.




**63**

**9-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]chromene-7,10-diol 63**: White solid (from hexane/$(CH_3)_2CO$ (1:1)); mp 174.5-176.4 °C. The UV, IR, NMR ($^1H$, $^{13}C$, 2D) and MS spectral data observed for the compound **63** were similar to those observed in the case of the sample isolated from *P. bussei* (see Chapter II).

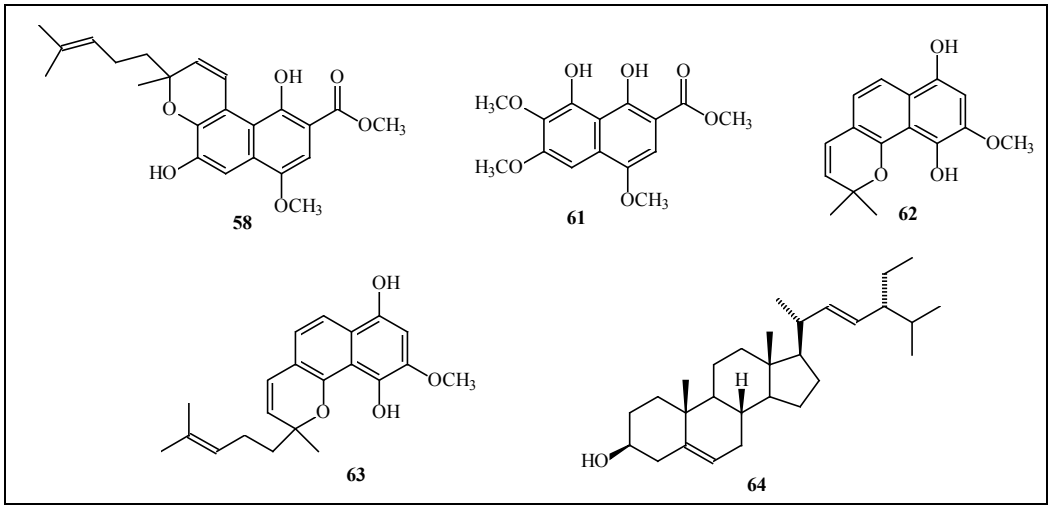



Figure 4.2. Natural Products Isolated from the Hexane Extract of the Roots of *P. parvifolia*

*IV.2.1.3. Study of the Dichloromethane Extract of the Roots of Pentas parvifolia*

A sample weight of 5964.3 mg from the dichloromethane extract of the roots of *P. parvifolia* was submitted to a reversed phase MPLC separation under a gradient mode elution (Table 4.15). The MPLC conditions were: column, 460 x 70 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 42 g of RP18, divided into 3 parts chromatographed separately and each of which was packed in a 230 x 36 mm i.d. sample column; flow rate, 30 ml/min at a back pressure < 40 bar; detection, LSD; collection 80 seconds per fraction. The eluates were monitored by TLC on RP18 plates eluting with MeOH/$H_2O$ (4:1) and combined into 8 fractions listed in Table 4.16.

Table 4.15. Programme for fractionation of the dichloromethane extract of the roots of *P. parvifolia* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| $H_2O$/MeOH | 40:60 | 0:100 | 10% | 175 |

Table 4.16. MPLC fractionation of the dichloromethane extract of the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 10:1 | 1-31 | 233.4 |
| 10:2 | 32-54 | 509.6 |
| 10:3 | 55-59 | 391.6 |
| 10:4 | 70-75 | 1199.8 |
| 10:5 | 76-95 | 147.8 |
| 10:6 | 96-104 | 1175.1 |
| 10:7 | 105-111 | 266.2 |
| 10:8 | 112-130 | 790.7 |

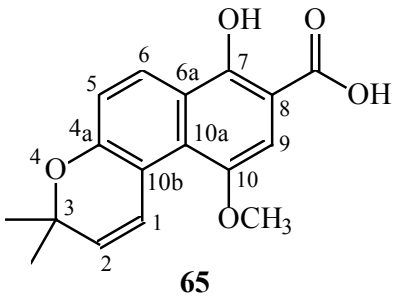
IV.2.1.3.1. Isolation of 7-Hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromene-8-carboxylic acid **65**

Fraction 10:5 (147.8 mg, 0.033 % yield) was fractionated by CPC with the sytem *n*-hexane/ EtOAc/MeOH/$H_2O$ (3:3:2:2) to afford 5 fractions (Table 4.17). The CPC conditions were: column 120 ml x 3; elution mode, head to tail, the upper phase as the stationary phase and the lower phase as the mobile phase; flow rate 1 ml/min with a pressure ~ 82 psi; revolution speed, 1067 rpm; equilibrium volume, 68 ml; sample loop, 14 ml; detection, UV 254 nm and 280 nm; sample collection, 5 min per fraction; push out at 315 min with a flow rate of 6 ml/min collected at 18 ml per tube.

Table 4.17. CPC fractionation of fraction 10:5 from the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 11:1 | 1-18 | 16.1 |
| 11:2 | 19-30 | 9.6 |
| 11:3 | 31-46 | 20.7 |
| 11:4 | 47-66 | 6.3 |
| 11:5 | 67-86 | 86.9 |

Fraction 11:5 was dried through a removal of the solvent under reduced pressure to afford a yellow solid (86.9 mg, 0.019 % yield). Based on its spectral and physical data, the compound was identified as 7-hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromene-8-carboxylic acid **65**, a new natural product already isolated from *Pentas bussei*, studied in the present research work, as described in Chapter II.




**65**

**7-Hydroxy-3,3-dimethyl-10-methoxy-*3H*-benzo[*f*]chromene-8-carboxylic acid 65**: Yellow fine crystals (from $CHCl_3$); mp 204.4-205.4 °C. Both the UV, the IR, the NMR ($^1H$, $^{13}C$, 2D) and the MS spectral data observed for the compound **65** were similar to those observed in the case of the sample isolated from *P. bussei* (see Chapter II).

In addition, from a CPC fractionation of fraction 10:3 (391.6 mg, 0.087 % yield) was isolated compound **61** which was already isolated from the *n*-hexane extracts of the roots of both *P. parvifolia* (this Chapter) and *P. bussei* (Chapter II).

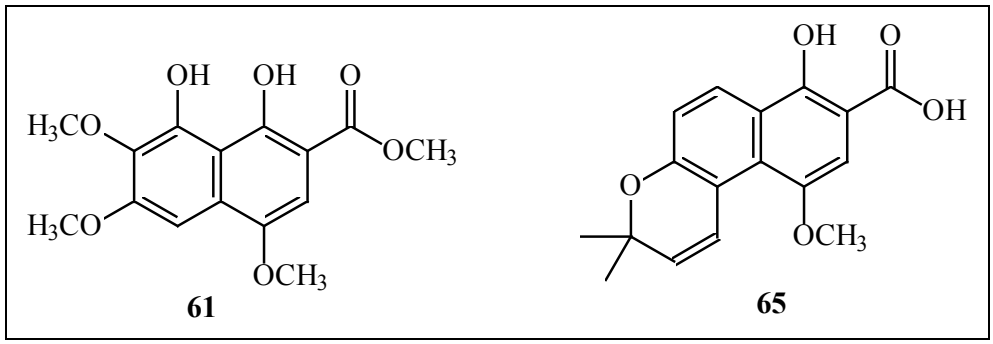



Figure 4.3. Natural Products Isolated from the Dichloromethane Extract of the Roots of *P. parvifolia*

*IV.2.1.4. Study of the Ethyl Acetate Extract of the Roots of Pentas parvifolia*

The ethyl acetate extract of the roots of *P. parvifolia* (1.59 g, 0.35 % yield), was subjected to a reversed phase MPLC under a gradient elution mode (Table 4.18) and the following separation conditions: column, 460 x 49 mm i.d.; sample adsorbed on 11 g of RP18 and packed in a 230 x 36 mm i.d. sample column; flow rate 60 ml/min at a back pressure < 40 bar; detection, LSD; collection 30 seconds per fraction. All the eluates were monitored by TLC on RP18 plates eluting with $H_2O/CH_3CN$ (3:2) and $H_2O/CH_3CN$ (3:7) for more polar and less polar fractions, respectively. The separation gave rise to 8 different fractions (Table 4.19).

Table 4.18. Programme for fractionation of the ethyl acetate extract of the roots of *P. parvifolia* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | stepwise | |
| $H_2O/CH_3CN$ | 100:0 | 0:100 | 10% | 200 |

Table 4.19. MPLC fractionation of the ethyl acetate extract of the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 12:1 | 38-68 | 164.0 |
| 12:2 | 69-86 | 57.6 |
| 12:3 | 87-161 | 207.1 |
| 12:4 | 163-203 | 213.9 |
| 12:5 | 205-267 | 236.7 |
| 12:6 | 269-293 | 54.2 |
| 12:7 | 295-325 | 48.3 |
| 12:8 | 357-381 | 509.5 |

IV.2.1.4.1. Isolation of 1,4,5-Trihydroxy-3-methoxy-6-(3,7,11,15,19-pentamethyleicosa-2,6,10,14,18-pentaenyl)naphthalene **80**

Fraction 12:8 (509.2 mg, 0.11 % yield) was submitted to a normal phase MPLC separation under a gradient mode elution (Table 4.20) and the following separation conditions: 2 coupled columns, 460 x 49 mm i.d. and 230 x 36 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 3.6 g of silica gel and packed in a 230 x 26 mm i.d. sample column; flow rate 48 ml/min at a back pressure < 40 bar; detection LSD; collection 50 seconds per fraction. The eluates were monitored by TLC on silica gel plates, developed with EtOAc/MeOH (99:1) and combined into 7 fractions (Table 4.21).

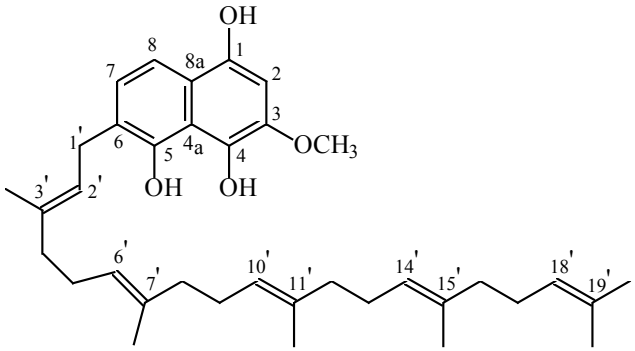
Table 4.20. Programme for fractionation of fraction 12:8 from the roots of *P. parvifolia* on MPLC.

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | |
| n-Hexane/EtOAc/MeOH | 20:80:0 | 0:100:0 | 10% | 45 |
| n-Hexane/EtOAc/MeOH | 0:90:10 | 0:80:20 | ---- | 30 |

Table 4.21. MPLC fractionation of fraction 12:8 from the roots of *P. parvifolia*

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 13:1 | 1-15 | 25.2 |
| 13:2 | 16-27 | 33.7 |
| 13:3 | 28-39 | 56.4 |
| 13:4 | 40-53 | 15.9 |
| 13:5 | 54-62 | 60.5 |
| 13:6 | 63-71 | 47.3 |
| 13:7 | 72-112 | 143.8 |

Fraction 13:7 (143.8 mg, 0.032 % yield) was subjected to a further purification on preparative TLC. The sample was dissolved in 6 ml of acetone/MeOH (7:3) and spotted onto 4 preparative silica gel plates. The upper phase of the solvent mixture *n*-hexane/ EtOAc/MeOH/$H_2O$ (1:1:1:1) was used to irrigate and develop the plates. The compound appearing (UV, 254 nm) at R*f* 0.22 was scraped off and separated from silica gel by extraction with acetone/MeOH (9:1). Evaporation of the solvent under reduced pressure afforded an amorphous compound (14.5 mg, 0.0032 % yield) which was identified as 1,4,5-trihydroxy-3-methoxy-6-(3,7,11,15,19-pentamethyleicosa-2,6,10,14,18-pentaenyl)naphthalene **80**, based on its spectral and physical data. The compound is a new natural product isolated for the first time from a plant species.




**80**

**1,4,5-Trihydroxy-3-methoxy-6-(3,7,11,15,19-pentamethyleicosa-2,6,10,14,18-pentaenyl)-naphthalene 80**: Amorphous solid; mp 185.3-186.7 °C; UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 264.9 (4.27), 270.0 (4.26), 360.0 (3.76), 502.0 (2.42), 516.1 (2.33) nm; IR $\nu_{max}$ (KBr) 3403 (OH), 2922, 1623 (C=C), 1511, 1453, 1384, 1268, 1223, 1158, 1126, 1026, 894, 796 $cm^{-1}$; $^1H$ NMR (270

MHz, $CDCl_3$) δ (ppm) 8.24 (1H, d, *J* = 8.7 Hz, H-7), 7.11 (1H, d, *J* = 8.7 Hz, H-8), 7.05 (1H, s, H-2), 5.32 (1H, dd, $J_1$ = 5.4 Hz, $J_2$ = 4.9 Hz, H-2'), 5.08 (4H, br t, *J* = 6.1 Hz, H-6', H-10', H-14' and H-18'), 4.09 (2H, d, *J* = 4.9 Hz, $H_2$-1'), 3.87 (3H, s, $OCH_3$-3), 1.90-2.15 (16H, m, $H_2$-4', $H_2$-5', $H_2$-8', $H_2$-9', $H_2$-12', $H_2$-13', $H_2$-16'and $H_2$-17'), 1.87 (3H, s, $CH_3$), 1.67 (3H, s, $CH_3$), 1.58 (9H, s, 3 x $CH_3$), 1.57 (3H, s, $CH_3$); $^{13}$C NMR (67.5 MHz, $CDCl_3$) δ (ppm) 156.73 (3 x $=C_q$-O), 149.39 ($=C_q$-O), 136.60 ($=C_q$), 135.43 ($=C_q$), 134.91 ($=C_q$), 131.26 ($=C_q$), 130.78 ($=C_q$), 128.82 ($=C_q$), 124.38 (=CH-7), 124.31 (=CH), 124.20 (2 x =CH), 123.75 (=CH), 123.38 (=CH-7), 121.74 ($=C_q$), 120.43 ($=C_q$), 117.64 (=CH-8), 103.11 (=CH-2), 55.72 ($OCH_3$), 39.71 (2 x $CH_2$), 39.66 (2 x $CH_2$), 26.76 (2 x $CH_2$), 26.58 (2 x $CH_2$), 26.50 ($CH_2$), 25.70 ($CH_3$), 17.68 ($CH_3$), 16.32 (2 x $CH_3$), 16.06 ($CH_3$), 15.99 ($CH_3$); ESIMS *m*/*z* (rel. int.) 547 $[M + H]^+$ (3), 507 (100), 247 (92), 229 (30).

In addition, chromatography (MPLC) of fractions 12:3 and 12:4, together with the CPC of fraction 12:5 (Table 4.19) gave rise to the two benzochromenes **63** and **65**, already isolated from, the *n*-hexane and the dichloromethane extracts, respectively, of the roots of *P. parvifolia*.

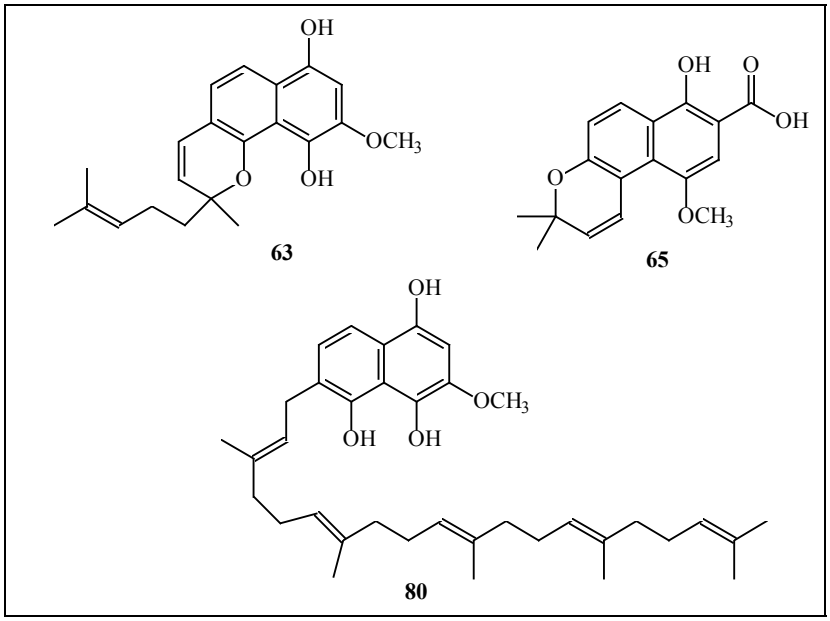



Figure 4.4. Natural Products Isolated from the Ethyl Acetate Extract of the Roots of *P. parvifolia*

*IV.2.1.5. Study of the Methanol Extract of the Roots of Pentas parvifolia*

An amount of 7020.9 mg weight from the methanol extract of *P. parvifolia* (14.05 g, 3.11 % yield) was subjected to CPC using EtOAc/*n*-BuOH/$H_2O$ (2:1:3) as solvent system and under the following conditions: column 320 ml x 3; elution mode, head to tail, the upper phase as the stationary phase and the lower phase as mobile phase; flow rate 3 ml/min, with a pressure ~ 70 psi; revolution speed 1064 rpm; equilibrium volume 250 ml, sample loop, 14 ml; detection, UV 254 nm and 280 nm; sample collection, 4 min per fraction; push out at 360 min with a flow rate of 8 ml/min collected at 16 ml per tube; the sample was divided into 2 parts partitioned separately. The eluates were monitored by TLC (on RP18 plates) and combined into 5 fractions (Table 4.22).

Table 4.22. CPC fractionation of the methanol extract of the roots of *P. parvifolia*.

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 14:1 | 44-50 | 4898.8 |
| 14:2 | 51-61 | 446.9 |
| 14:3 | 62-81 | 229.6 |
| 14:4 | 111-131 | 100.2 |
| 14:5 | 151-170 | 1135.5 |

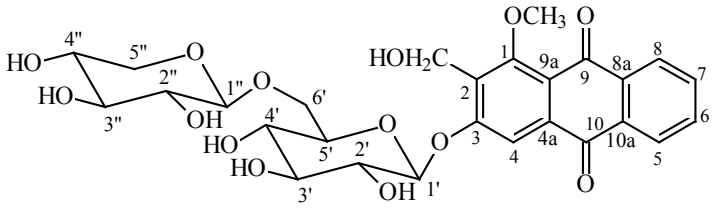
IV.2.1.5.1. Isolation of Damnacanthol-3-*O*-*β*-primeveroside **57**

Fractions 14:2 and 14:3 were combined together (676.5 mg, 0.15 % yield) and chromatographed isocratically on reversed phase MPLC eluting with $H_2O$/MeOH (7:3) to give rise to 3 different fractions (Table 4.23). MPLC conditions were: column 460 x 36 mm i.d.; sample dissolved in 12 ml of acetonitrile/water (1:9), filtered through a 0.45 µm filter (25 mm syringe filter, PVDF, Alltech Associates, Inc.) and introduced into a 15 ml sample loop; flow rate 45 ml/min at a back pressure < 40 bar; detection LSD; collection 50 seconds per fraction.

Table 4.23. MPLC fractionation of the fraction 14:2-14:3 from the roots of *P. parvifolia*.

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 15:1 | 10-14 | 26.5 |
| 15:2 | 79-107 | 136.8 |
| 15:3 | 112-176 | 183.3 |

The solvent was removed from fraction 15:2 (136.8 mg, 0.030 % yield) by evaporation under reduced pressure to yield a yellow solid, identified as damnacanthol-3-*O*-*β*-primeveroside **57**, according to its spectral and physical data, together with comparison of the latter with data provided in the literature (112). The compound was already isolated from the MeOH extract of the roots *P. bussei* (Chapter II).

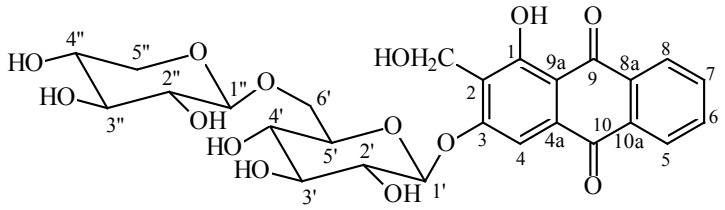



**57**

**Damnacanthol-3-*O*-*β*-primeveroside 57**: mp 138.4-140.6 °C, lit. mp 140-142 °C (120); $[\alpha]^{16}_{D}$ = -130 ° (*c* 0.1, $H_2O$), lit. $[\alpha]^{20}_{D}$ = -94.8 ° (*c* 0.18, MeOH) (112). Both the UV, the IR, the NMR ($^{1}H$, $^{13}C$, 2D) and the MS spectral data observed for the compound **57** were similar to those observed in the case of the sample isolated from *P. bussei* (see Chapter II).

IV.2.1.5.2. Isolation of Lucidin-3-*O*-*β*-primeveroside **56**

Removal of solvent from fraction 15:3 (183.3 mg, 0.041 % yield) under reduced pressure yielded an orange red solid which was identified as lucidin-3-*O*-*β*-primeveroside **56**, based on the spectral and physical data of the compound, together with comparison with data of the literature (112). The anthraquinone glycoside was already isolated from the MeOH extract of the roots of *P. bussei* (Chapter II).



**56**

**Lucidin-3-*O*-*ß*-primeveroside 56**: mp 206.5-208.3 °C, lit. mp 208-210 °C (116); $[\alpha]^{17}_{D}$ = -140 ° (*c* 0.05, $H_2O$). The UV, the IR, the NMR ($^1H$, $^{13}C$, 2D) and the MS spectral data observed for the compound **56** were similar to those observed in the case of the compound isolated from *P. bussei* (see Chapter II).

IV.2.1.5.3. Isolation of Rubiadin-3-*O*-*ß*-primeveroside **54**

Fractions 14:4 and 14:5 (Table 4.22) were mixed together (1235.7 mg, 0.27 % yield) and submitted to a reversed phase MPLC separation under a gradient mode (Table 4.24) to give rise to 3 different fractions (Table 4.25) after TLC (RP18) monitoring of all the eluates obtained. MPLC conditions were: column 460 x 49 mm i.d.; precolumn, 140 x 10 mm i.d.; sample adsorbed on 8 g of RP18 and packed in a 230 x 26 mm i.d. sample column; flow rate 30 ml/min at a back pressure < 40 bar; detection LSD; collection, 90 seconds per fraction.

Table 4:24. Programme for separation of fractions 14:4-14:5 from the roots of *P. parvifolia*

| Mobile phase | Gradient elution | | | Time (min) |
|---|---|---|---|---|
| | Start | End | Stepwise | Start |
| $H_2O/CH_3CN$ | 100:0 | 0:100 | 10% | 180 |

Table 4.25. MPLC fractionation of the fraction 14:4-14:5 from the roots of *P. parvifolia*.

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 16:1 | 1-24 | 244.4 |
| 16:2 | 27-96 | 499.3 |
| 16:3 | 97-170 | 175.0 |

Fractions 16:1 and 16:2 (743.7 mg, 0.16 % yield) was rechromatographed using the previous conditions (Table 4.24) slightly modified (coupled columns; gradient elution running time,

140 min). The global MPLC conditions were: 2 coupled columns, 460 x 49 mm i.d. and 230 x 36 mm i.d.; precolumn 140 x 10 mm i.d.; sample adsorbed on 5 g of RP-18 and packed in a 230 x 26 mm i.d. sample column; flow rate, 48 ml/min at a back pressure < 40 bar; detection, LSD; collection, 40 seconds per fraction. The eluates were monitored by TLC (on RP18 plates) and combined into 4 different fractions (Table 4.26).

Table 4.26. MPLC fractionation of fractions 16:1-16:2 from the roots of *P. parvifolia*

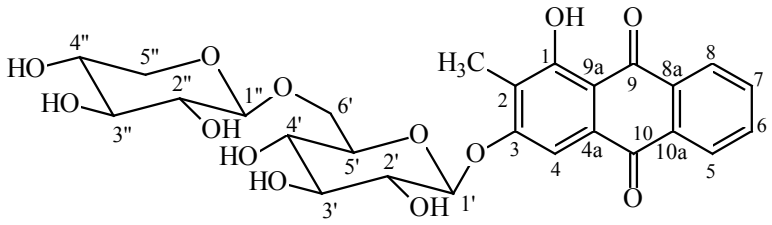
| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 17:1 | 14-18 | 73.1 |
| 17:2 | 31-50 | 410.0 |
| 17:3 | 51-97 | 101.4 |
| 17:4 | 98-211 | 123.7 |

Fraction 17:2 (410.0 mg, 0.091 % yield) was submitted to a CPC with EtOAc/1-BuOH/$H_2O$ (2:1:3) as solvent system under the following conditions: column, 320 x 3; elution mode, head to tail, the upper phase as the stationary phase and the lower phase as mobile phase; flow rate 1 ml/min with a pressure ~ 70 psi; revolution speed 1070 rpm; equilibrium volume, 188 ml; sample loop, 14 ml; detection, UV 254 nm and 280 nm; sample collection, 5 min per fraction; push out at 300 min with a flow rate of 2 ml/min; collection, 4 ml per tube. The eluates were monitored by TLC on RP18 plates and combined into 5 differents fractions (Table 4.27).

Table 4.27. CPC fractionation of the fraction 17:2 from the roots of *P. parvifolia*.

| Fraction | Eluate number | Weight (mg) |
|---|---|---|
| 18:1 | 1-43 | 61.3 |
| 18:2 | 44-78 | 32.1 |
| 18:3 | 79-101 | 24.5 |
| 18:4 | 102-116 | 41.7 |
| 18:5 | 117-128 | 101.1 |

Solvent evaporation to dryness from fraction 18:4 (41.7 mg, 0.0092 % yield) afforded a pale yellow solid. The compound was identified as rubiadin-3-*O*-*β*-primeveroside **54**, based on the analysis of its spectral and physical data, together with their comparison with literature data (112).



**54**

**Rubiadin-3-*O*-*ß*-primeveroside 54**: Yellow solid; mp 251.2-253.0 °C, lit. mp 250-252 °C (116); $[\alpha]^{22}_{D}$ = -80 ° (*c* 0.1, $H_2O$); UV ($H_2O$) $\lambda_{max}$ (log$\varepsilon$) 246.0 (4.03), 271.1 (4.14), 273.0 (4.14), 327.9 (4.09), 364.0 (4.10), 412.0 (3.92), 416.9 (3.92), 456.0 (3.89) nm; IR (KBr) $\nu_{max}$ 3367 (broad, OH), 2934, 1673 (unchelated C=O), 1582 (chelated C=O), 1343, 1289, 1050 (broad), 895, 775, 756, 715, 603 $cm^{-1}$. $^1H$ NMR (270 MHz, $C_5D_5N$) δ (ppm) 8.30 (2H, m, H-5 and H-8), 8.09 (1H, s, H-4), 7.63 (2H, m, H-6 and H-7), 5.80 (1H, d, *J* = 6.6 Hz), H-1'), 5.10-5.36 (OH protons), 4.91 (1H, broad d, *J* = 6.6 Hz, H-1"), 3.62-4.84 (the rest of sugar protons), 2.40 (3H, s, C<u>H</u>$_3$-2); $^{13}C$ NMR (67.5 MHz, DMSO-$d_6$) δ (ppm) 187.12 (C-9), 182.07 (C-10), 161.50 (C-3), 160.90 (C-1), 134.63 (C-7), 134.49 (C-6), 133.20 (C-10a), 132.94 (C-8a), 132.03 (C-4a), 127.10 (C-5), 126.94 (C-8), 120.56 (C-2), 111.41 (C-9a), 105.57 (C-4), 103.98 (C-1"), 100.07 (C-1'), 76.42 (C-3"), 76.07 (C-3'), 75.66 (C-5'), 73.19 (C-2"), 73.07 (C-2'), 69.45 (C-4"), 69.11 (C-4'), 68.02 (C-6'), 65.52 (C-5"), 8.46 ($CH_3$-2); ESIMS *m*/*z* (rel. int.) 553 (5), 505 (15), 390 (6), 295 (8), 253 (100).

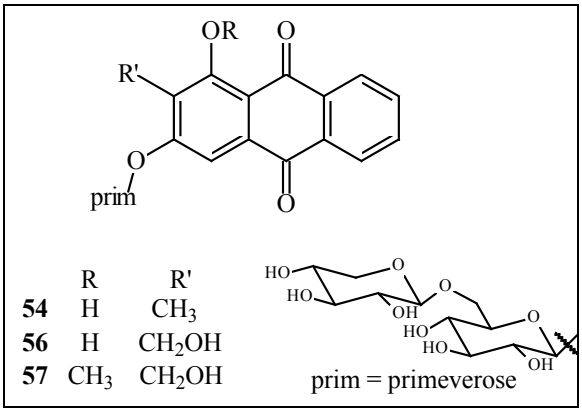
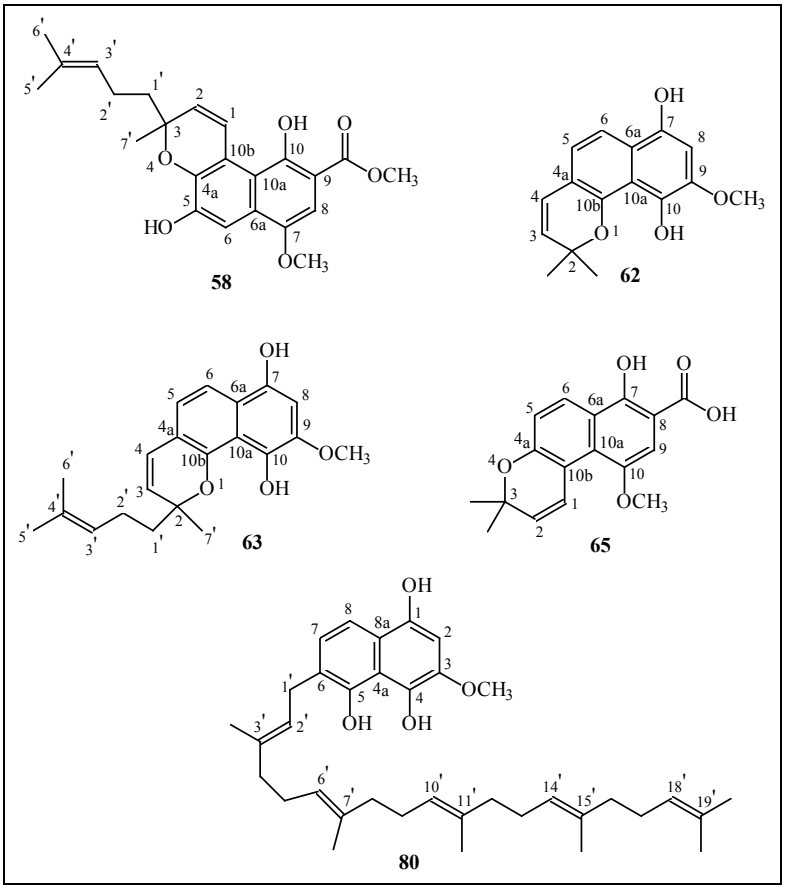



Figure 4.5. Natural Products Isolated from the Methanol Extract of the Roots of *P. parvifolia*

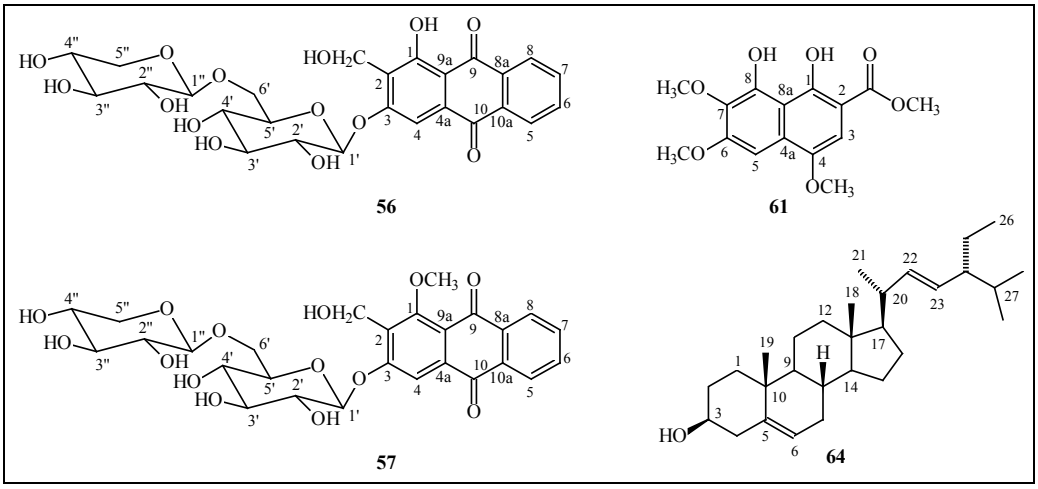
## IV.3. Results and Discussion

No phytochemical investigation of *P. parvifolia* was done before. The chemistry of the plant was found similar to that of *P. bussei* (Chapter II). Four novel naphthohydroquinones of the benzochromene type (**58**, **62**, **63** and **65**) already isolated from *P. bussei*, were also reisolated from *P. parvifolia*, and are, therefore, common to the two plant species. The long-chain-branched naphthohydroquinone **80** can be considered as a metabolic benzochromene ring precursor through the cyclisation of the side chain which may result in the formation of the pyran ring. Compound **80** was consequently attached to the class of the naphthohydroquinone of the benzochromene type in the following structural discussion.




Three additional compounds, common to the two species, are the highly oxygenated naphthohydroquinone **61** and the two anthraquinone glycosides lucidin-3-*O*-*β*-primeveroside **56** and damnacanthol-3-*O*-*β*-primeveroside **57** which were also isolated from the roots of *P. bussei*. However, *P. parvifolia* afforded, in addition, the anthraquinone glycoside rubiadin-3-

*O*-*β*-primeveroside **54**. The ubiquitous steroid *β*-stigmasterol **64** was also isolated from *P. parvifolia*.



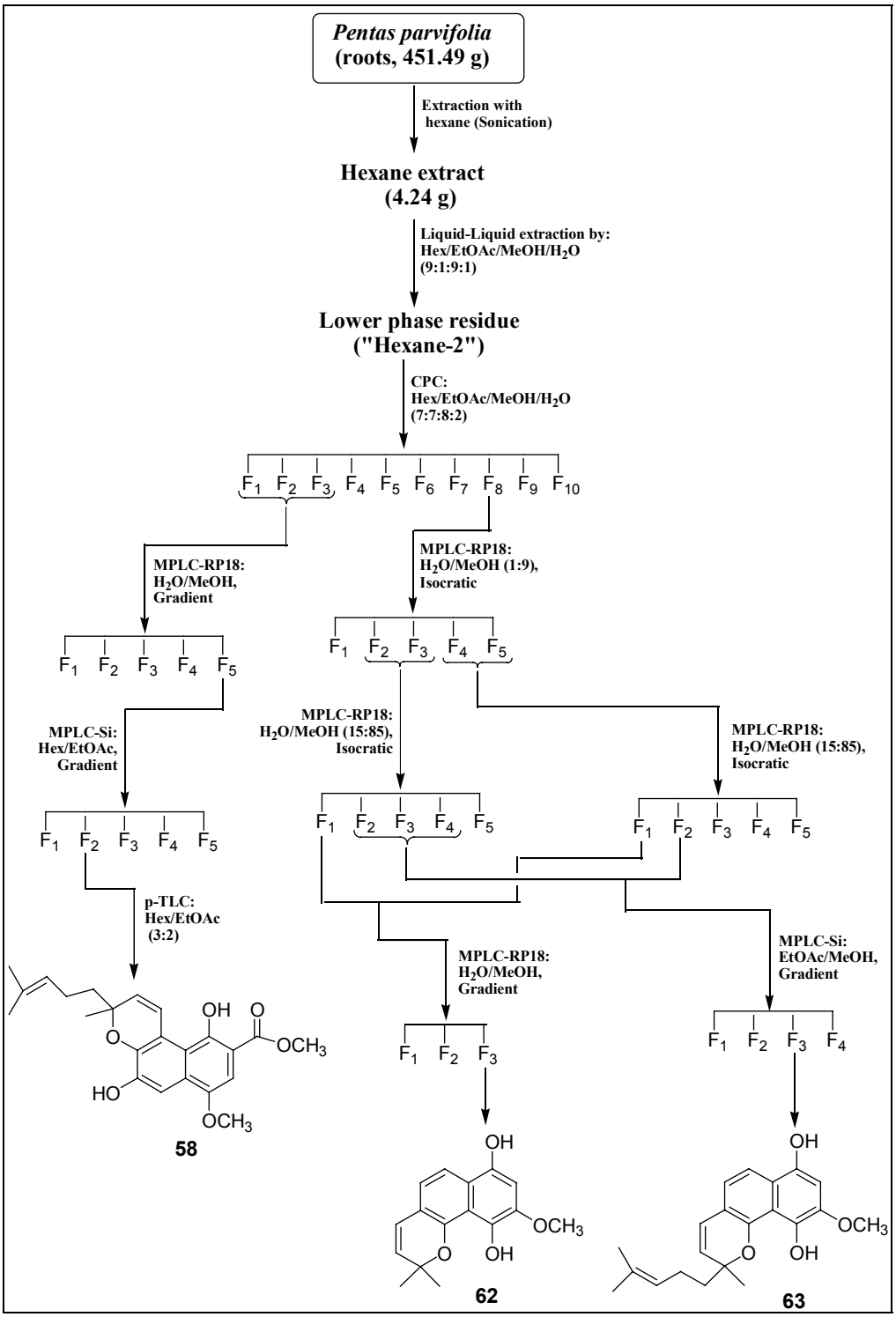

IV.3.1. Novel Natural Products from the Roots of *P. parvifolia*

*IV.3.1.1. Naphthohydroquinones of the Benzochromene Type*

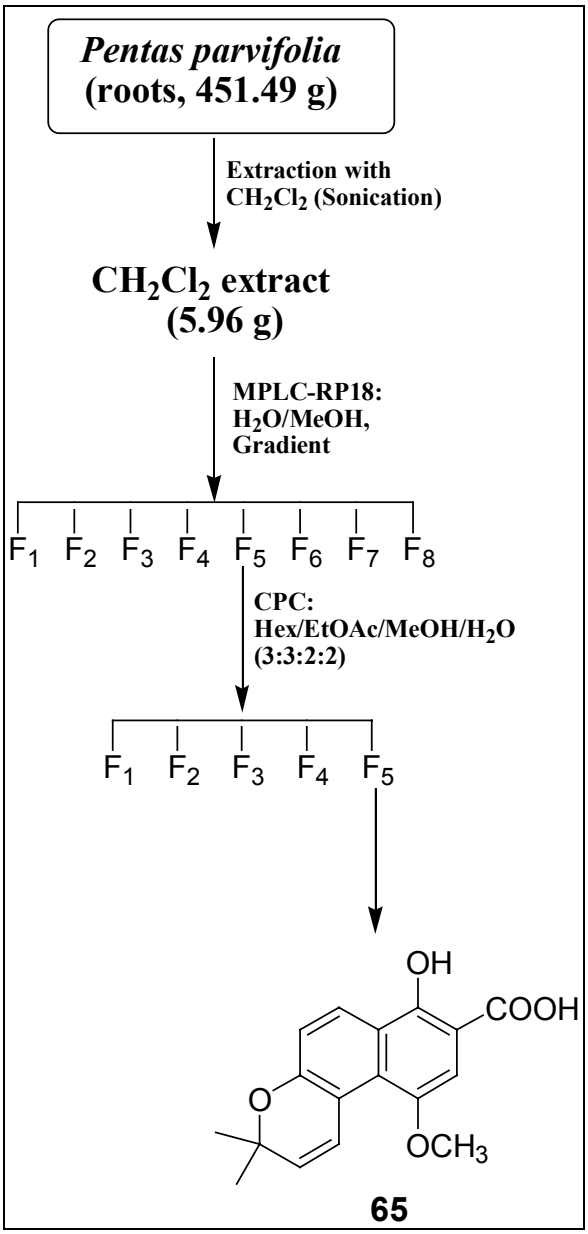
IV.3.1.1.1. Common Compounds of *P. bussei* and *P. parvifolia*

On the one hand, the hexane extract of the roots of *P. parvifolia* was partitioned between the lower and the upper phases of the system Hex/EtOAc/MeOH/$H_2O$ (9:1:7:1). Compounds **58**, **62** and **63** (fractions 4:2 of Table 4.7, 8:3 of Table 4.12 and 9:3 of Table 4.14, respectively) were isolated from the lower phase residue by the use of CPC, MPLC-RP18, MPLC-Si and p-TLC techniques, as described on the Scheme 4.1. On the other hand, the benzochromene carboxylic acid **65** was isolated from the $CH_2Cl_2$ extract by the application of MPLC-RP18 and CPC separations (Scheme 4.2). This compound came out of the fraction 11:5 (Table 4.17) as a yellow solid upon evaporation of the solvent.

The physical and spectroscopic data of these four compounds were found identical to those of the respective samples isolated from *P. bussei*. Their full structural elucidation is discussed in Chapter II, Paragraph II.3.



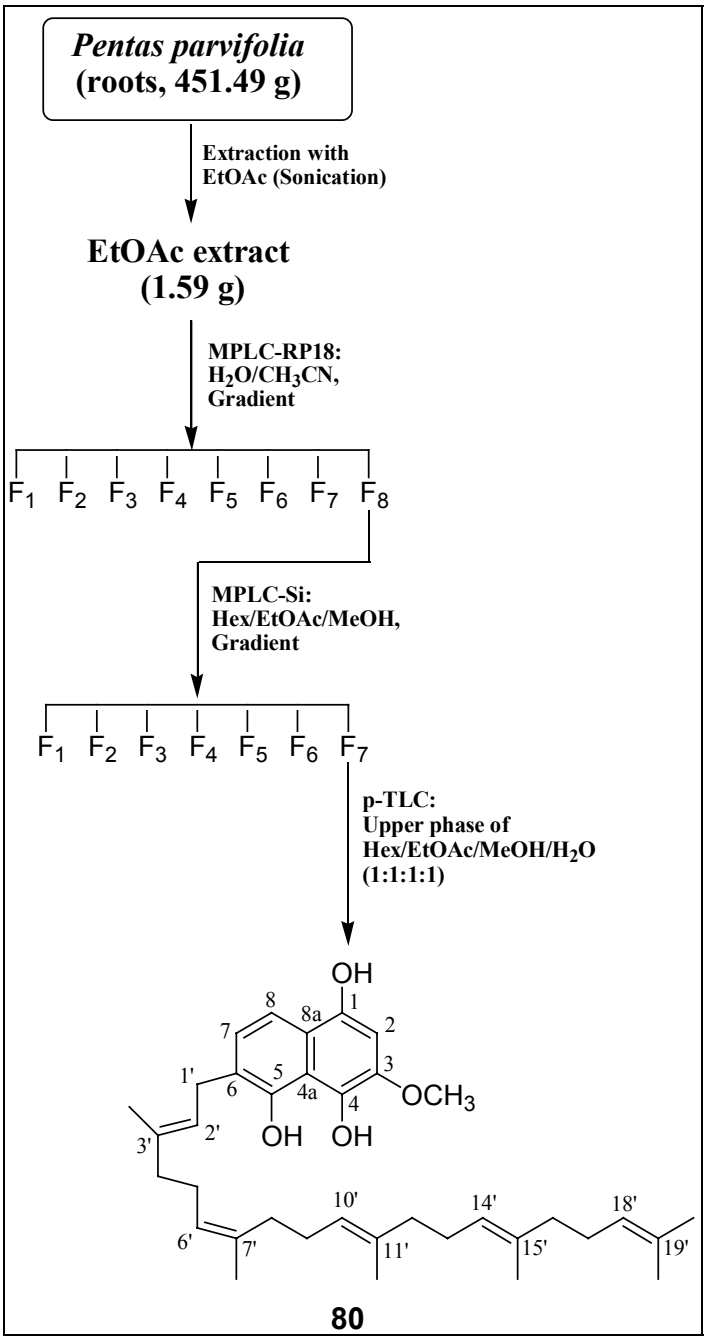

**Scheme 4.1**: Isolation of Compounds **58**, **62** and **63** from the Roots of *P. parvifolia*




**Scheme 4.2**: Isolation of Compound **65** from the Roots of *P. parvifolia*

IV.3.1.1.2. 1,4,5-Trihydroxy-3-methoxy-6-(3,7,11,15,19-pentamethyleicosa-2,6,10, 14, 18-pentaenyl)naphthalene **80**

The fractionation of the EtOAc extract of the roots of *P. parvifolia* by MPLC-RP18, followed by the application of MPLC-Si on the fraction 12:8 (Table 4.19) and the p-TLC of the fraction 13:7 (Table 4:21) resulted in the separation of compound **80** (Scheme 4.3).




**Scheme 4.3**: Isolation of the compound **80** from the Roots of *P. parvifolia*

**1,4,5-Trihydroxy-3-methoxy-6-(3,7,11,15,19-pentamethyleicosa-2,6,10,14,18-pentaenyl)naphthalene 80**: Amorphous solid; mp 185.3-186.7 °C. The UV spectrum of this compound showed intense absorption bands at 264.9 nm (log $\varepsilon$ 4.27), 270.0 nm (log $\varepsilon$ 4.26) and 360.0 nm (log $\varepsilon$ 3.76). The pattern observed is typical to naphthalene compounds, substituted with functional groups enhancing the delocalisation of electrons (121). The

spectrum (Figure 4.6) displayed a large absorption band at 3403 $cm^{-1}$, typical to phenolic hydroxy groups. The absorption band at 1623 $cm^{-1}$ (C=C) was unusually broadened as a result of the contributions of the double bonds of the side chain.

The $^1H$ NMR spectrum (in $CDCl_3$) of the compound (Figure 4.7) showed an AB system in the aromatic region at $\delta_H$ 7.11 (1H, d, $J$ = 8.7 Hz, H-8) and $\delta_H$ 8.24 (1H, d, $J$ = 8.7 Hz, H-7). Another aromatic proton resonated at $\delta_H$ 7.05 (1H, s, H-2) as a singlet, and this is in agreement with its location in a trisubstituted aromatic ring. The $OCH_3$ was observed at $\delta_H$ 3.87 (3H, s, $OCH_3$-3). The $^1H$ NMR spectrum also showed signals ascribed to the terpenic side chain moiety. Two types of olefinic protons were observed in this side chain at $\delta_H$ 5.32 (1H, dd, $J_1$ = 5.4 Hz, $J_2$ = 4.9 Hz H-2') and $\delta_H$ 5.08 (1H, br t, $J$ = 6.1 Hz, H-6', H-10', H-14' and H-18'). A particular methylene was observed at $\delta_H$ 4.09 (2H, d, $J$ = 4.9 Hz, $H_2$-1') which is obviously the $CH_2$-1' under the influence of the aromatic ring. The rest of the signals observed in the $^1H$ NMR spectrum belong to methylene groups overlapping at $\delta_H$ 1.90-2.15 ppm and to methyl groups.

The $^1H$-$^1H$ COSY spectrum (Figure 4.8) showed that the sp methine groups of the side chain are involved in two types of coupling systems. The methylene protons $H_2$-1' were coupled to the olefinic proton H-2', whereas the other overlapping olefinic protons are coupled to corresponding vicinal $CH_2$ protons. The arrangement of the side chain obviously shows that the double bond C2'=C3' corresponds to the prenylic double bonds often involved in the pyran ring formation.

Apart from the signals which can be attributed to the side chain, the $^{13}C$ NMR spectrum (Figure 4.9) displayed the pattern already observed for the benzochromenes **62** and **63**, specially concerning the =CH-2 [$\delta_H$ 7.05 (s)/$\delta_C$ 103.11], the =$C_q$-O region and the $OCH_3$-3 [$\delta_H$ 3.87 (s)/$\delta_C$ 55.72] units.

The presence of the AB system in the aromatic ring, the $OCH_3$-3 group, the evident trisubstituted ring and the side chain containing a prenylic double bond which can be involved in the pyran ring formation, all these findings indicated that compound **80** has the same ring substitution pattern as that of compounds **62** and **63**.

The ESI mass spectrum of the compound displayed a pseudomolecular ion peak at at *m*/*z* 547

$[M + H]^+$ (3) and which was fitting with the molecular formula $C_{36}H_{50}O_4$, corresponding to an unsaturation index of 12.

From all the above discussed spectral data, the structure of the compound was elucidated as 1,4,5-trihydroxy-3-methoxy-6-(3,7,11,15,19-pentamethyl-eicosa-2,6,10,14,18-pentaenyl)-naphthalene **80**. This compound is a novel natural product extracted for the first time from a plant source.

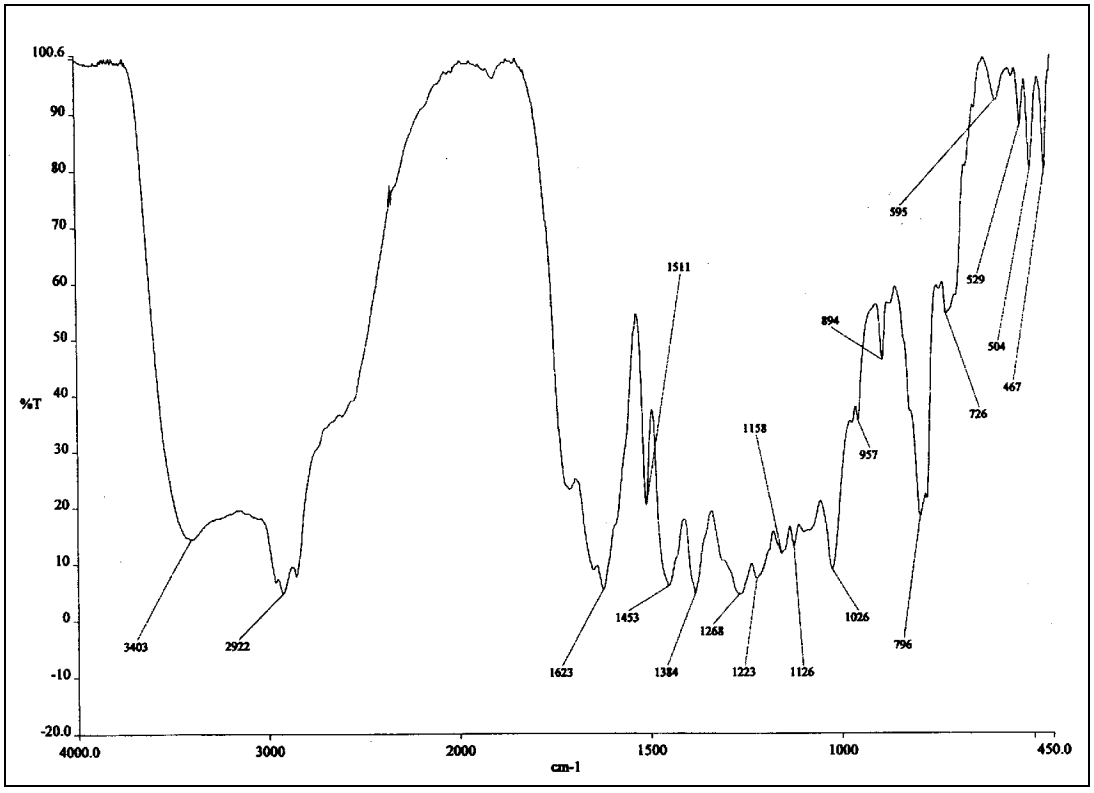



Figure 4.6. IR Spectrum of Compound **80**

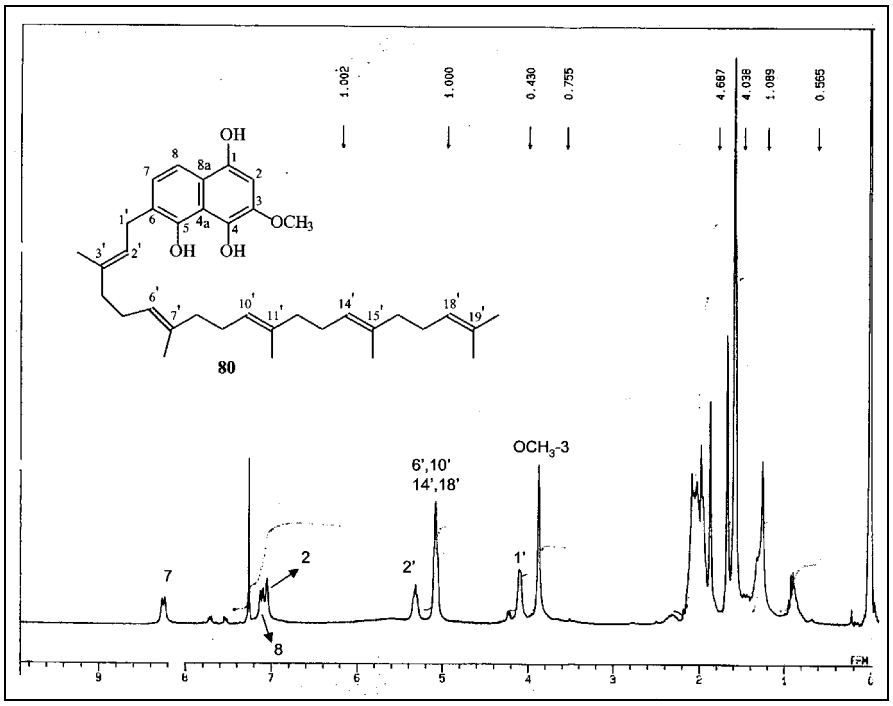



Figure 4.7. $^{1}H$ NMR Spectrum of Compound **80** (270 MHz, in $CDCl_3$)

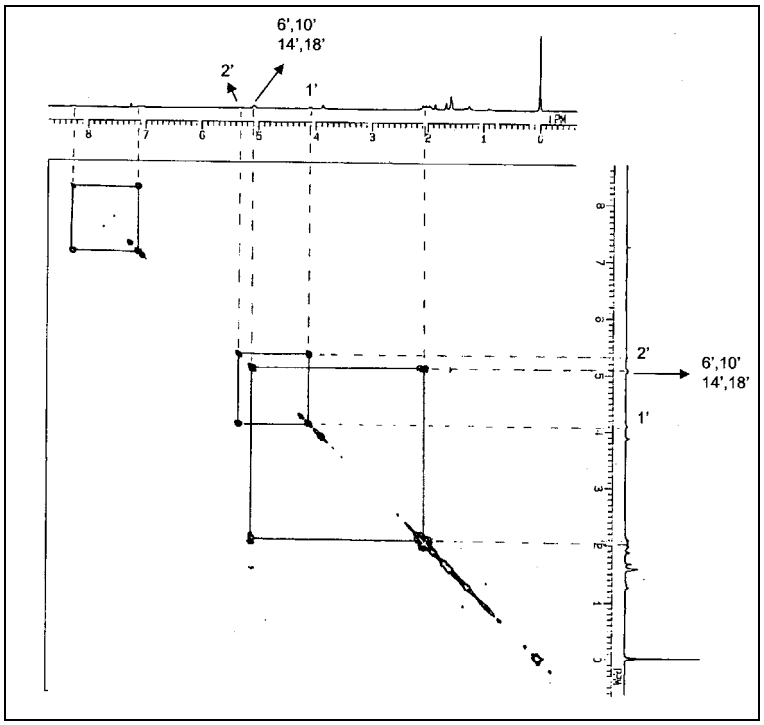



Figure 4.8. $^{1}H$-$^{1}H$ COSY Spectrum of Compound **80** (270 MHz, in $CDCl_3$)

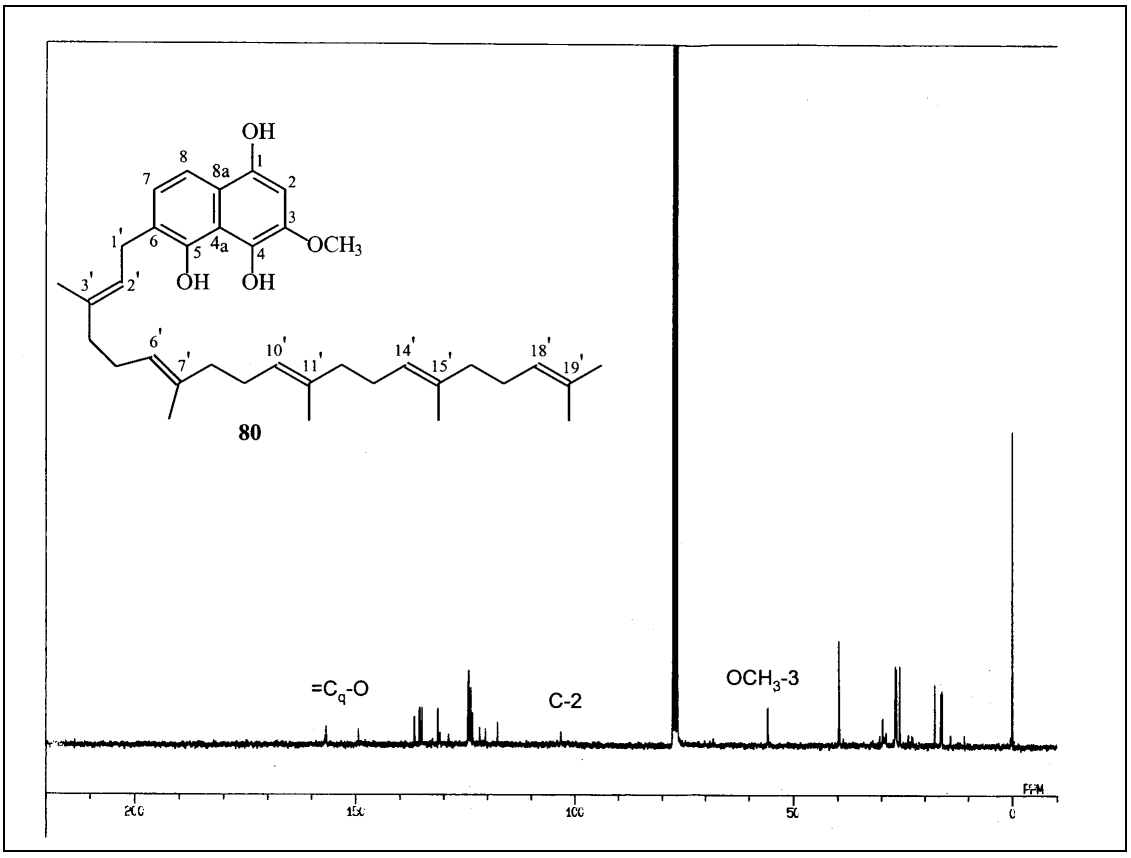


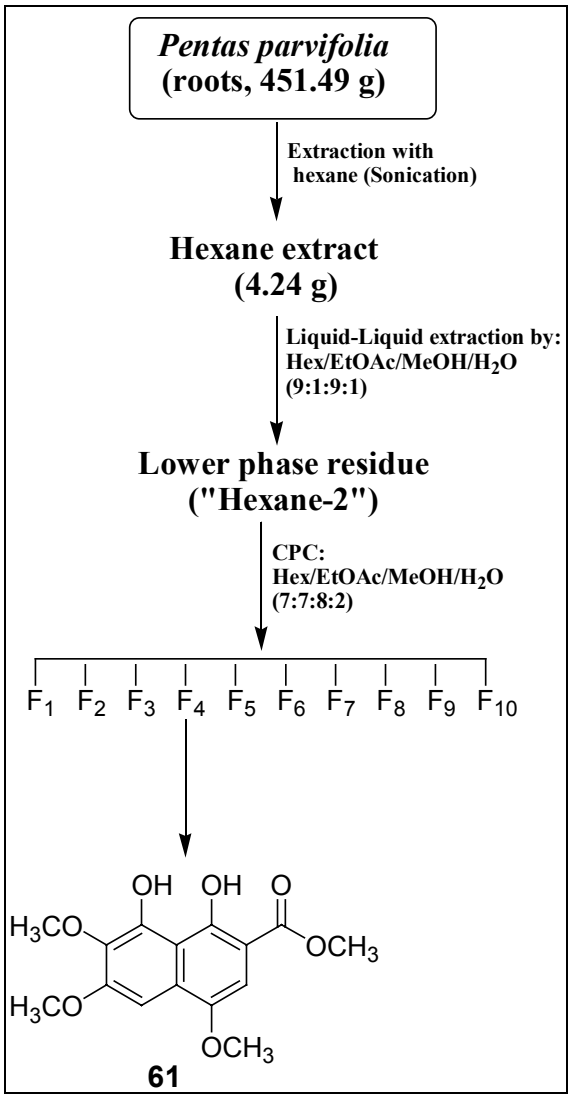

Figure 4.9. $^{13}C$ Spectrum of Compound **80** (67.5 MHz, in $CDCl_3$)

*IV.3.1.2. Highly Oxygenated Naphthohydroquinone*

IV.3.1.2.1. Methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61**

The lower phase residue of the hexane extract from the roots of *P. parvifolia* was submitted to a CPC separation (Scheme 4.4) to readily afford compound **61** (fraction 2:4 of Table 4.3) which was already isolated from the roots of *P. bussei*.

The compound displayed both physical and spectroscopic data identical to those obtained for the sample from the roots of *P. bussei*. The discussion about the structural elucidation of the compound is provided in Chapter II, Section II.3.



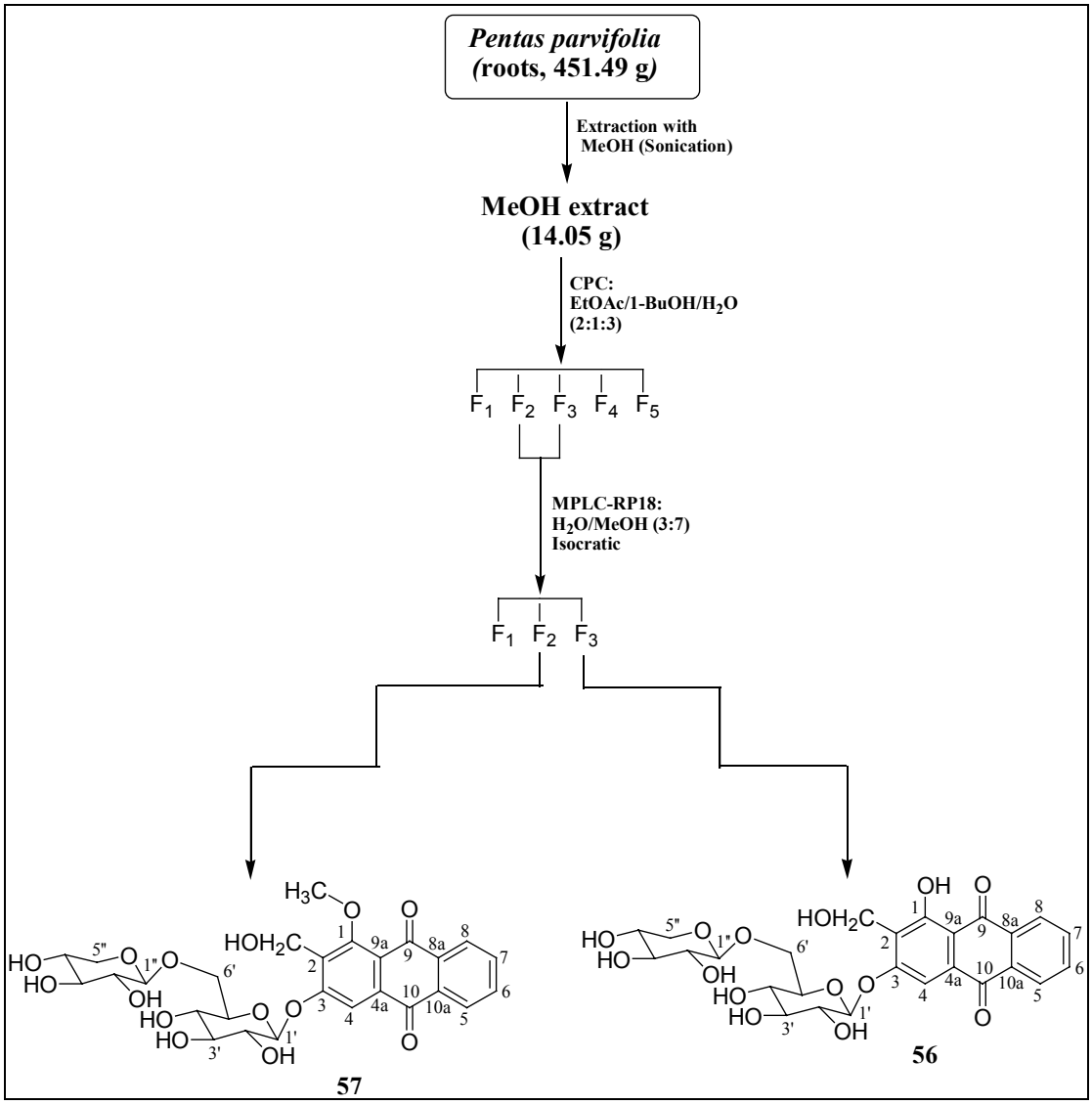

**Scheme 4.4**: Isolation of Methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61** from the Roots of *P. parvifolia*

IV.3.2. Anthraquinone Glycosides

*IV.3.2.1. Common Anthraquinone Glycosides of P. bussei and P. parvifolia*

The CPC fractionation of the MeOH extract of the roots of *P. parvifolia*, followed by the application of the MPLC-RP18 on the fractions 14:2 and 14:3 (Table 4.22) gave rise to the anthraquinone glycosides lucidin-3-*O*-*β*-primeveroside **56** and damnacanthol-3-*O*-*β*-primeveroside **57** (Scheme 4.5), already isolated from the roots of *P. bussei*. The characterisation of their sugar moiety was done by acid hydrolysis in an HCl atmosphere on

TLC (140) as described in "Materials and Methods". The two anthraquinone glycosides displayed physical and spectroscopic data identical to the data observed for the sample isolated from the roots of *P. bussei*. Their structural elucidation is discussed in Chapter II, Section II.3.

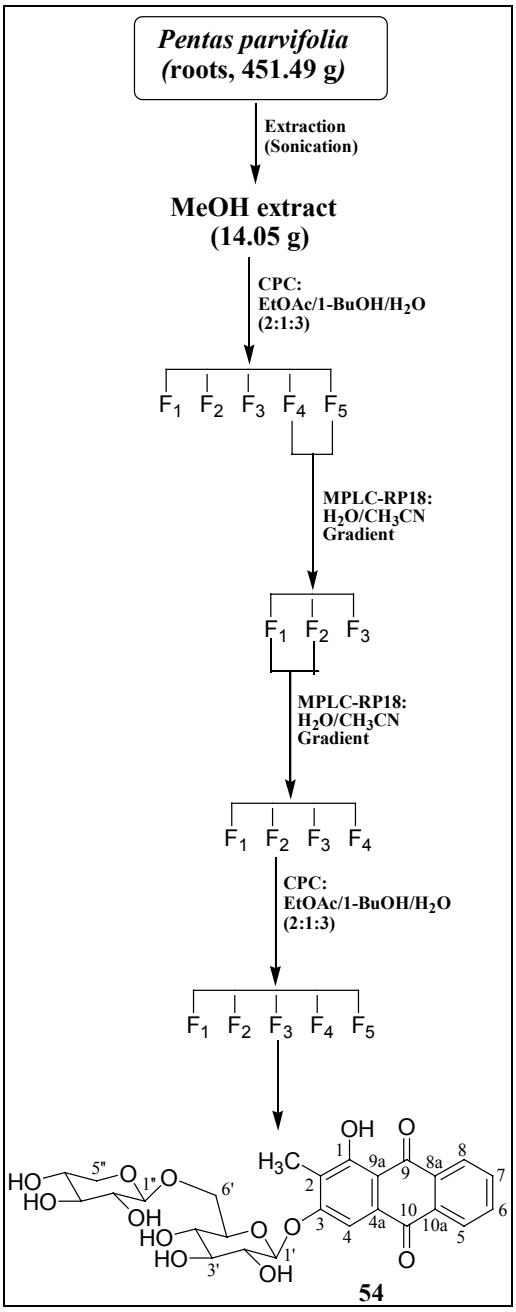



**Scheme 4.5**: Isolation of Lucidin-3-*O*-*β*-primeveroside **56** and Damnacanthol-3-*O*-*β*-primeveroside **57** from the Roots of *P. parvifolia*

*IV.3.2.2. Rubiadin-3-O-β-primeveroside **54***

The MPLC-RP18 application on the fractions 14:4 - 14:5 (Table 4.22) from the MeOH extract of the roots of *P. parvifolia*, followed by the MPLC-RP18 and CPC separations applied on the

deriving fractions (Scheme 4.6) led to the isolation of the anthraquinone glycoside **54**.




**Scheme 4.6**: Isolation of Rubiadin-3-*O*-*β*-primeveroside **54** from the Roots of *P. parvifolia*

**Rubiadin-3-*O*-*β*-primeveroside 54**: Yellow solid; mp 251.2-253.0 °C, lit. mp 250-252 °C (116). The UV spectrum of the compound displayed to pattern typical to anthraquinones with the absorption bands observed at 246.0 nm (log $\varepsilon$ 4.03), 271.1 nm (log $\varepsilon$ 4.14) and 273.0 nm

(log $\varepsilon$ 4.14). The IR spectrum showed a broad band at 3367 $cm^{-1}$, which was ascribed to an OH group, and two carbonyl bands at 1673 and 1621 $cm^{-1}$, corresponding to an unchelated and chelated C=O, respectively. The latter hydrogen bonding was indicative of the presence of an OH group in the *peri* position.

The $^{1}H$ and $^{13}C$ NMR spectra of compound **54** were different to those of the anthraquinone glycoside rubiadin-1-methyl ether-3-*O*-$\beta$-primeveroside **55** (isolated from *P. bussei* and *P. lanceolata*, this work) only by the presence of OH-1 in **54** instead of $OCH_3$ in **55** (Chapter II, Section II.3). The $^{13}C$ NMR spectrum of compound **54** showed the two carbonyl carbons at $\delta_C$ 187.12 (C-9) and $\delta_C$ 182.07 (C-10) ppm, with a large $\Delta\delta_C$ value of 5.05 ppm which is consistent with the presence of an OH group in 1-position. In addition, no $OCH_3$ was observed in the $^{1}H$ NMR spectrum. The biose nature of the sugar moiety was determined by partial hydrolysis in an HCl atmosphere on TLC (140) of the anthraquinone glycoside (see "Materials and Methods") which readily released xylose and glucose.

The ESI mass spectrum of this compound displayed the base peak at *m*/*z* 553 (4 %) which may correspond to the ion $[M + Na - H_2O]^+$. The molecular formula of the compound was deduced as being $C_{26}H_{28}O_{13}$.

On the basis of the spectroscopic data observed and their comparison (Table 4.28) with data available in the literature (112,116), the compound was elucidated as being the known anthraquinone glycoside rubiadin-3-*O*-$\beta$-primeveroside **54**. The compound has already been isolated from another *P.* species, i. e. *P. zanzibarica* (112).

Table 4.28. Comparison of NMR data of rubiadin-3-*O*-*β*-primeveroside **54** ($^1$H, 270 MHz, $C_5D_5N$; $^{13}$C, 67.5 MHz, DMSO-$d_6$; this work) with data from the literature ($^1$H/$^{13}$C, 400 MHz/100 MHz, DMSO-$d_6$; ref. 112): δ (ppm), *J* (Hz).

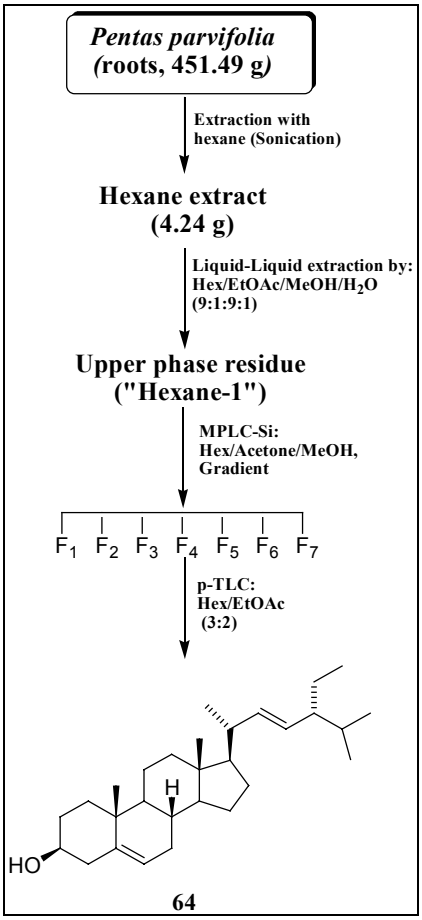
| Position | This work | | Ref. 112 | |
|---|---|---|---|---|
| | $^1$H* | $^{13}$C | $^1$H* | $^{13}$C |
| 1 | - | 160.90[a] | - | 161.2[a] |
| 2 | - | 120.56 | - | 120.8 |
| 3 | - | 161.50[a] | - | 161.4[a] |
| 4 | 8.09 s | 105.57 | 7.44 s | 106.0 |
| 4a | - | 132.03[b] | - | 131.5[b] |
| 5 | 8.30 m | 127.10[c] | 8.17 dd (2,8) | 126.5[c] |
| 6 | 7.63 m | 134.49[d] | 7.88 dd (8, 8) | 134.6[d] |
| 7 | 7.63 m | 134.63[d] | 7.88 dd (8, 8) | 134.8[d] |
| 8 | 8.30 m | 126.94[c] | 8.17 dd (2, 8) | 126.9[c] |
| 8a | - | 132.94[b] | - | 131.9[b] |
| 9 | - | 187.12 | - | 187.1 |
| 9a | - | 111.41 | - | 110.9 |
| 10 | - | 182.07 | - | 181.5 |
| 10a | - | 133.20[b] | - | 132.9[b] |
| $CH_3$-2 | 2.40 s | 8.46 | 2.18 s | 8.4 |
| OH-1 | - | - | 13.00 | - |
| 1' | 5.80 d (6.6) | 100.07 | 5.15 d (7.5) | 100.3 |
| 2' | | 73.07 | | 73.2 |
| 3' | | 76.07[e] | | 76.1[e] |
| 4' | | 69.11[f] | | 69.2[f] |
| 5' | | 75.66[e] | | 75.7[e] |
| 6' | | 68.02 | | 68.0 |
| 1" | 4.91 d (6.6) | 103.98 | 4.14 d (7.5) | 104.0 |
| 2" | | 73.19 | | 73.2 |
| 3" | | 76.42[e] | | 76.4[e] |
| 4" | | 69.45[f] | | 69.5[f] |
| 5" | | 65.52 | | 65.6 |

[a-f] Assignments may be reversed in the same column

* For the sugar moiety, only anomeric $\delta_H$ are shown

IV.3.3. Steroidal Compound: *β*-Stigmasterol **64**

The partition of the hexane extract of the roots of *P. parvifolia* between the two phases of the solvent system Hex/EtOAc/MeOH/$H_2O$ (9:1:7:1) resulted in the two residues "hexane-1" and "hexane-2" from the upper and lower phases, respectively. The steroid *β*-stigmasterol **64** was isolated from the "hexane-1" residue which was subjected to MPLC-Si followed by p-TLC of fraction 1:4 (Table 4.2) as shown on the Scheme 4.7.




**Scheme 4.7**: Isolation of *β*-Stigmasterol **64**
from the Roots of *P. parvifolia*

*β*-Stigmasterol **64** is an ubiquitous steroid present in several plant species. The physical and spectroscopic data of the isolated compound were identical to those already obtained for the samples extracted from *P. bussei* and *P. lanceolata* (for the structural elucidation, see Chapter II, Section II.3).

**Chapter V.**

# SYNTHESIS OF TWO NATURAL PRODUCTS ISOLATED FROM *PENTAS LONGIFLORA* Oliver (Rubiaceae)

# Chapter V. SYNTHESIS OF TWO NATURAL PRODUCTS ISOLATED FROM *PENTAS LONGIFLORA*

## V.1. Introduction

*Pentas longiflora* Oliver is an erect stemmed woody herb up to 3 m high. Stipules are divided into thread-like segments. Corolla lobs 4. Leaves are ovate, with base cuneate 2 – 3, and with corolla tube of 2 – 4.5 cm long and 0.5 – 1.5 mm wide. Flowers are white and located in terminal. The fruit is a capsule. *P. longiflora* originates from Oriental, Intertropical Africa (90a), and is reputed to possess several medicinal properties.

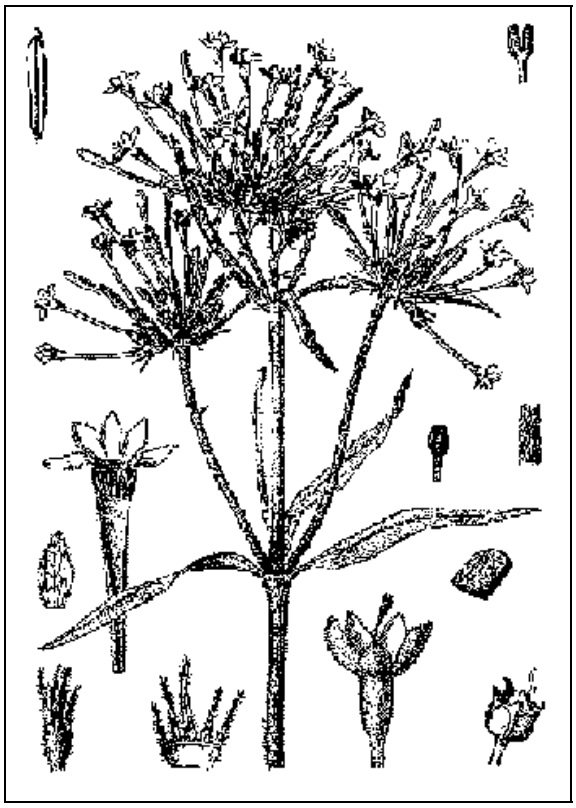
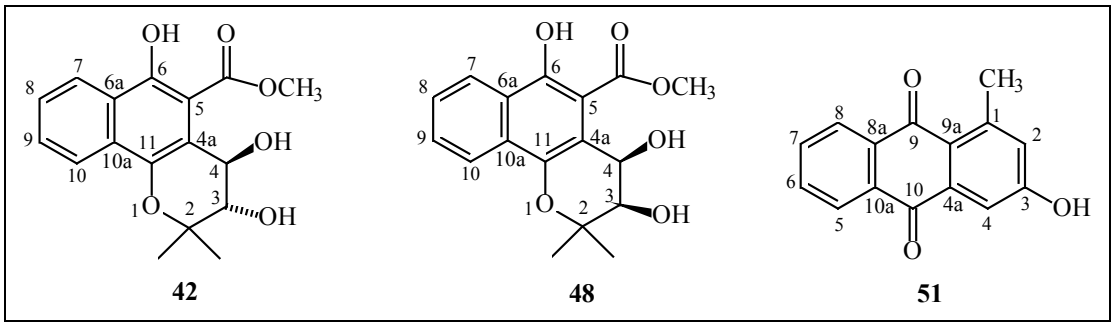


Figure 5.1. *Pentas longiflora* Oliver

In Kenya, where it is known as "Nekilango" or "Segimbe", the roots are used as a cure for tapeworm, for itchy rashes and for pimples. A decoction of the roots is mixed with milk and taken as a cure for malaria, but causes acute diarrhoea, and thus acts as a purgative (91).

In Rwanda, where the plant is called "Isagara", the traditional healer uses the powder of the roots, mixed with butter, as an ointment to treat scabies and the skin disease pityriasis versicolor (91,92).

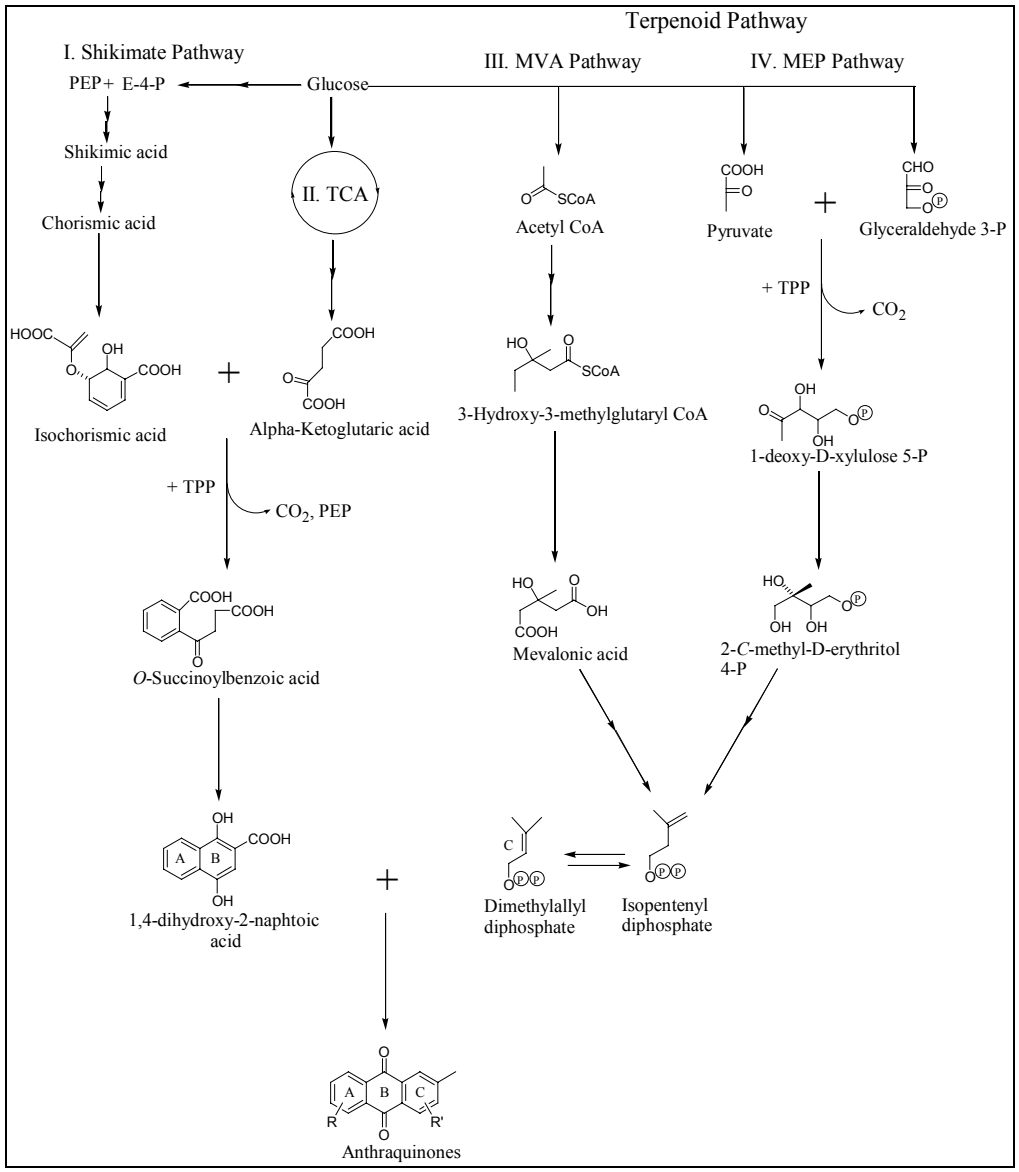
During a detailed phytochemical investigation of the plant (96), which was aiming at the identification of minor constituents and new physiologically active compounds, the two isomeric pyranonaphthohydroquinones, *trans*-3,4-dihydroxy-3,4-dihydromollugin **42** and *cis*-3,4-dihydroxy-3,4-dihydromollugin **48**, together with the anthraquinone 3-hydroxy-1-methyl-9,10-anthraquinone **51** were isolated and identified.




*Trans*-3,4-dihydroxy-3,4-dihydromollugin **42** was previously isolated from another rubiaceous plant, *Rubia cordifolia* (76a), although its stereochemistry was not established. C*is*-3,4-dihydroxy-3,4-dihydromollugin **48** was a novel natural product (104). The discrimination of the two stereoisomers was based on the comparison of their physical and spectral data. Particularly, the important observed difference between the two $^1$H NMR spectra of the two compounds was the coupling constant (*J*) of the two protons of the O-CH-CH-O unit at $\delta_H$ 3.79 (1H, d, *J* = 6.4 Hz, CH-3) and 5.06 (1H, d, *J* = 6.4 Hz, CH-4) for the *trans*-compound (**42**), and at $\delta_H$ 3.85 (1H, d, *J* = 4.9 Hz, CH-3) and 5.22 (1H, d, *J* = 4.9 Hz, CH-4) for the *cis*-compound (**48**).

The anthraquinone 3-hydroxy-1-methyl-9,10-anthraquinone **51** was also found as a novel natural product. A strong argument which could be argued against the structure assigned to this anthraquinone was the location of the methyl group at the 1-position which is contradictory to the biogenesis of anthraquinones in Rubiaceae (Scheme 5.1). From this biosynthetic route, it is obvious that a methyl group or an oxidised methyl group is always found at the 2- or 6-position which are equivalent from a biogenetic point of view (71). As

shown in Scheme 5.1., rings A and B of anthraquinones are derived from chorismic or isochorismic acid and α-ketoglutarate *via* *O*-succinylbenzoic acid through a so called "shikimate pathway (I and II)" (156). The origin of ring C is not yet known, either *via* the MVA or acetate/mevalonic acid pathway (III) or the MEP or 2-*C*-methyl-D-erythritol 4-phosphate pathway (IV).




**Scheme 5.1**: Biosynthetic pathway leading to anthraquinones in Rubiaceae (141): E-4-P = erythrose 4-phosphate, P = phosphate residue, PEP = phosphoenolpyruvate, "TCA = tricarboxylic acid", TPP = thiamine diphosphate

In order to confirm the structural assignment proposed for *cis*-3,4-dihydroxy-3,4-dihydromollugin **48** and for 3-hydroxy-1-methyl-9,10-anthraquinone **51**, the synthesis of the two compounds was undertaken.

## V.2. Experimental

Melting points measurements were carried out on a Büchi melting point apparatus. Optical rotations were obtained on an AA-10 automatic polarimeter. The IR spectra were recorded on a Perkin-Elmer 1310 infrared spectrometer. The $^{1}H$ and $^{13}C$ NMR spectra were recorded on a JEOL-JNM-Ex 270 MHz FT NMR spectrometer. The mass spectra were measured at 70 eV, with a Varian MAT 112 mass spectrometer, using a direct inlet system.

### V.2.1. Synthesis of *Cis*-3,4-dihydroxy-3,4-dihydromollugin **48**

Mollugin **38** (100 mg, 0.0003 mol) was dissolved in 50 ml of dry THF and N-methylmorpholine N-oxide (NMO) (50 mg, 0.0004 mol) was added (solution I). A solution of $OsO_4$ (0.3 mg, $10^{-6}$ mol) in 2 ml of water was prepared (solution II). Solutions I and II were mixed at 0 °C and kept stirring at 0 °C for 10 min. The reaction mixture was brought to room temperature and stirred for 24 h. Workup involved the reduction of excess of $OsO_4$ by saturated $NaHSO_3$ (the reaction mixture was poured into the $NaHSO_3$ solution and stirred for 30 min), and the extraction with EtOAc (157). The EtOAc solution was dried ($MgSO_4$), and evaporated in vacuo to afford 67.5 mg of a crude residue which was subjected to flash chromatography (silica, eluent: hexane/EtOAc, 6:4) to give rise to *cis*-3,4-dihydroxy-3,4-dihydromollugin **48** (50.4 mg, 50% yield).

*Cis*-3,4-dihydroxy-3,4-dihydromollugin **48**: Colourless amorphous powder; mp 106.3 – 107.7 °C; lit. mp 107.6 – 108.0 °C (96); $[\alpha]^{21}_{D}$ –13.7 ° (*c* 0.42, $CHCl_3$); IR (KBr) $\nu_{max}$ 3565-3190 (OH), 1665 (C=O), 1590 (C=C), 1455, 1237, 1170, 1100 $cm^{-1}$; $^{1}H$ NMR (270 MHz, $CDCl_3$) δ (ppm) 11.15 (1H, s, OH-6), 8.11-8.31 (2H, m, H-7 and H-10), 7.46-7.60 (2H, m, H-8 and H-10), 5.15 (1H, d, *J* = 4.9 Hz, H-4), 3.99 (3H, s, $COOC\underline{H}_3$), 3.78 (1H, d, *J* = 4.9 Hz, H-3), 1.44 (3H, s, $CH_3$-2), 1.43 (3H, s, $CH_3$-2); $^{13}C$ NMR (67.5 MHz, $CDCl_3$) δ (ppm) 171.21 ($\underline{C}OOCH_3$-5), 155.63 (C-6), 140.72 (C-11), 129.43 (C-9), 128.75 (C-10a), 126.95 (C-8), 125.53 (C-6a), 123.81 (C-10), 122.46 (C-7), 112.76 (C-4a), 104.56 (C-5), 77.70 (C-2, overlapping $CDCl_3$), 72.09 (C-3), 64.31 (C-4), 52.80 ($COO\underline{C}H_3$), 24.60 ($CH_3$-2), 22.05 ($CH_3$-2); EIMS *m*/*z* (rel. int.) 318 $[M]^+$ (48), 301 (56), 288 (69), 270 (69), 216 (100), 199 (20), 187 (20), 171 (11), 159 (40), 87 (12).

V.2.2. Synthesis of 3-Hydroxy-1-methyl-9,10-anthraquinone **51**

*V.2.2.1. Synthesis of (E)-4-Methoxy-3-penten-2-one **81***

A dry acetone solution (350 ml) containing equimolar amounts of acetylacetone **82** and dimethyl sulfate (0.55 mol of each) was heated under reflux for 24 h in the presence of anhydrous potassium carbonate. Inorganic solids were filtered off and the filtrate and acetone washings were concentrated in vacuo. The crude oily mixture was distilled under reduced pressure (26 mm Hg). Two fractions were collected at 82-88 °C and 95-98 °C. To the fraction collected at 82-88 °C (12.62 g) were added 20 ml of dry ether, and 20 ml of 10 N NaOH. The mixture was stirred at room temperature for 1 h, and the ethereal phase was separated. The aqueous phase was washed three times with dry ether. These washings were then added to the initial ethereal phase. Evaporation of the combined ether phases gave 14.13 g (23% yield) of (*E*)-4-methoxy-3-penten-2-one **81** as a yellow oil (158,159).

*V.2.2.2. Synthesis of (E and Z)-4-Methoxy-2-trimethylsilyloxy-1,3-pentadiene **83***

To a suspension of anhydrous zinc chloride (77.5 mg) in triethylamine (4.37g, 0.042 mol) was added 1.2 equivalents of (*E*)-4-methoxy-3-penten-2-one **81** in 5.7 ml of benzene, followed by the addition of $(CH_3)_3SiCl$ (4.19 g, 0.038 mole). An exothermic reaction was noted. After 30 min, the temperature was raised to 40° C and the reaction mixture was stirred overnight. After cooling, it was added to 40 ml of dry ether and filtered. The filtrate and the combined ether washings were concentrated in vacuo to give a brown oil. Distillation through a Vigreux column gave 2.2 g (39 % yield) of a mixture of the dienes (*E* and *Z*)-4-methoxy-2-trimethylsilyloxy-1,3-pentadiene **83** (bp 63-70 °C/3 mm Hg) (158,160).

*V.2.2.3. Synthesis of 3-Hydroxy-1-methyl-9,10-anthraquinone **51** and Its Methyl ether **84***

To a solution of 2-bromo-1,4-naphthoquinone **85** (1252 mg, 0.005 mol) in 10 ml of toluene was added a solution of the mixture of (*E* and *Z*)-4-methoxy-2-trimethylsilyloxy-1,3-pentadiene **83** (655 mg, 5 ml) in 10 ml of toluene. The mixture was stirred for 30 min at 0 °C. It was then brought to room temperature and continuously stirred overnight. $SiO_2$ (700 mg) was added and the mixture was stirred for 4 h (160). The filtered and concentrated material

(1680 mg) was chromatographed by MPLC on silica gel. The chromatography conditions were: column, 460 x 49 mm i.d. ; precolumn, 140 x 10 mm i.d.; sample adsorbed on 9 g of silica gel and packed in a 230 x 36 mm i.d. sample column; flow 42 ml/min at a back pressure < 40 bars; detection, LSD ; collection 50 seconds per fraction; elution: gradient with hexane/EtOAc 100:0 to 0:100 (10 %, stepwise) for 135 min and with wash out by EtOAc/MeOH 100:0 to 95:5 for 20 min. The eluates 76 to 95 were combined together and the solution was dried in vacuo to afford 3-methoxy-1-methyl-9,10-anthraquinone **84** (341.2 mg, 52 %), whereas the combination of the eluates 149 to 171 gave rise to 3-hydroxy-1-methyl-9,10-anthraquinone **51** (313.8 mg, 45 % yield) after evaporation of the solvent under reduced pressure.

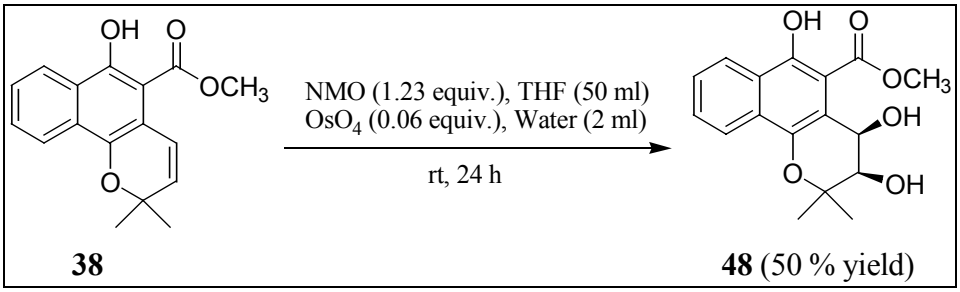
**3-Hydroxy-1-methyl-9,10-anthraquinone 51**: Yellow powder; mp 291.1-293.2 °C; mp reported for the "isolated natural anthraquinone" (96): 270.5 – 271.8 °C; IR (KBr) $\nu_{max}$ 3347 (OH), 1673 (C=O), 1649 (C=O), 1610, 1592, 1560, 1463, 1368, 1338, 1296, 1276, 1141, 993 $cm^{-1}$; $^{1}H$ NMR (270 MHz, $C_5D_5N$) δ (ppm) 8.37-8.40 (1H, broad d, *J* = 7.0 Hz, H-8), 8.30-8.33 (1H, broad d, *J* = 7.0 Hz, H-5), 8.08 (1H, d, *J* = 2.1 Hz, H-4), 7.61-7.71 (2H, m, H-6 and H-7), 7.30 (1H, d, *J* = 2.1 Hz, H-2), 5.47 (1H, br, OH-3), 2.89 (3H, s, $CH_3$-1); $^{13}C$ NMR (67.5 MHz, $C_5D_5N$) δ (ppm) 182.95 (C=O), 182.46 (C=O), 162.79 (C-3), 144.60 (C-1), 136.89 (=$C_q$), 133.39 (C-6), 132.31 (=$C_q$), 132.26 (C-7), 130.53 (=$C_q$), 128.36 (=$C_q$), 126.34 (C-8), 125.62 (C-5), 124.46 (C-2), 111.81 (C-4), 22.91 ($\underline{C}H_3$-1); EIMS *m/z* (rel. int.) 238 $[M]^+$ (100%), 210 (17), 181 (18), 149 (26), 97 (11), 84 (59), 56 (33).

**3-Methoxy-1-methyl-9,10-anthraquinone 83**: Pale white powder; mp 138.4-139.4 °C, IR (KBr) $\nu_{max}$: 1671 (C=O), 1592, 1472, 1436, 1413, 1346, 1283, 1208, 1138, 1018, 993 $cm^{-1}$; $^{1}H$ NMR (270 MHz, $CDCl_3$) δ (ppm) 8.14-8.19 (2H, m, H-5 and H-8), 7.65-7.75 (2H, m, H-6 and H-7), 7.57 (1H, d, *J* = 3.0 Hz, H-4), 6.92 (1H, d, *J* = 3.0 Hz, H-2), 3.91 (3H, s, O$\underline{C}H_3$-3), 2.74 (3H, s, $\underline{C}H_3$-1); $^{13}C$ NMR (67.5 MHz, $CDCl_3$) δ (ppm) 182.48 (C=O), 182.41 (C=O), 162.79 (C-3), 144.87 (C-1), 137.02 (=$C_q$), 134.71 (=$C_q$), 134.05 (C-6), 132.97 (C-7), 132.59 (=$C_q$), 127.41 (C-8), 126.47 (C-5), 124.89 (=$C_q$), 124.09 (C-2), 109.18 (C-4), 55.65 (O$\underline{C}H_3$-3), 23.68 ($\underline{C}H_3$-1); EIMS *m/z* (rel. int.): 252 $[M]^+$ (10), 166 (5), 150 (3), 118 (5), 83 (100), 47 (17).

## V.3. Results and Discussion

### V.3.1. *Cis*-3,4-dihydroxy-3,4-dihydromollugin **48**

The *cis*-dihydroxylation of mollugin **38** with osmium tetroxide and the use of N-methyl morpholine N-oxide (NMO) as cooxidant (Scheme 5.2) in THF in the presence of water at room temperature was exploited for the synthesis of *cis*-3,4-dihydroxy-3,4-dihydromollugin **48**. The coupling of the two oxidants has proved its effectiveness for the hydroxylation of less hindered olefins (161). The *cis*-dihydroxylation of mollugin **38** gave rise to *cis*-3,4-dihydroxy-3,4-dihydromollugin **48** in 50 % yield.




**Scheme 5.2**: Synthesis of *Cis*-3,4-dihydroxy-3,4-dihydromollugin **48**

The IR spectrum of the synthetic diol **48** was identical to the one observed for the naturally occurring diol (96). The mp observed for the synthetic compound (106.3-107.7 °C) was quite identical to the mp of the plant-derived diol (107.6-108.3 °C). Sofar the *cis*-dihydroxylation has not been performed enantioselectively. Also at present, the absolute stereochemistry of the *cis*-3,4-dihydroxy-3,4-dihydromollugin from the plant source (*P. longiflora*) is not known.

The comparison of the $^{1}H$ and $^{13}C$ NMR of the synthetic versus the natural compound (Tables 5.1 and 5.2) showed that the two derivatives displayed the same spectroscopic data for the O-CH-CH-O unit in 3- and 4-position. The chemical shifts observed were $\delta_H$ 5.15 (1H, d, $J$ = 4.9 Hz, H-4)[synthetic]/$\delta_H$ 5.22 (1H, d, $J$ = 4.9 Hz, H-4) [natural] and $\delta_H$ 3.78 (1H, d, $J$ = 4.9 Hz, H-3) [synthetic]/$\delta_H$ 3.85 (1H, d, $J$ = 4.9 Hz, H-3) [natural]. The $J_{H\text{-}3/H\text{-}4}$ = 4.9 Hz and $\Delta\delta_H = \delta_{H\text{-}4} - \delta_{H\text{-}3}$ = 1.37 ppm are, respectively, the same in both the two compounds. The $^{13}C$ NMR spectra displayed also the data for the O-CH-CH-O unit at $\delta_C$ 72.09 (C-3) [synthetic]/$\delta_C$ 72.21 (C-3) [natural] and $\delta_C$ 64.31 (C-4) [synthetic]/$\delta_C$ 64.42 (C-4) [natural].

The data observed confirmed that the diol **48** isolated from the roots of *Pentas longiflora* (96) was *cis*-3,4-dihydroxy-3,4-dihydromollugin which is quite different from the *trans*-form **42** (76a,96). The differences from the *trans*-form are particularly observed in the $\delta_C$ of the unit O-CH-CH-O and of the two methyl groups at the 2-position (Table 5.2).

Table 5.1. Comparison of $^1$H NMR data of the synthetic (this work) and the naturally occurring (ref. 96) c*is*-3,4-dihydroxy-3,4-dihydromollugin **48** (270 MHz, $CDCl_3$) with data of the *trans*-3,4-dihydroxy-3,4-dihydromollugin **42** (270 MHz, $CDCl_3$, ref. 96; 400 MHz, $CDCl_3$, ref. 76a)

| | δ (ppm) multiplicity (*J* in Hz) | | | |
|---|---|---|---|---|
| Proton | **48** Synthetic *cis*-form (this work) | **48** Naturally ocurring *cis*-form (ref. 96) | **42** *trans*-form (ref. 96) | **42** *trans*-form (ref. 76a) |
| $CH_3$-2 | 1.43 s | 1.40 s | 1.40 s | 1.40 s |
| $CH_3$-2 | 1.44 s | 1.51 s | 1.58 s | 1.58 s |
| **3** | **3.78 d (4.9)** | **3.85 d (4.9)** | **3.79 d (6.4)** | **3.80 d (6.4)** |
| **4** | **5.15 d (4.9)** | **5.22 d (4.9)** | **5.06 d (6.4)** | **5.06 d (6.4)** |
| COOC$\underline{H_3}$-5 | 3.99 s | 4.06 s | 4.05 s | 4.06 s |
| OH-6 | 11.15 s | 11.20 s | 11.46 s | 11.44 s |
| 7 | 8.11-8.31 m | 8.20-8.37 m | 8.19-8.36 m | 8.37 d (8.0) |
| 8 | 7.46-7.60 m | 7.58-7.62 m | 7.57-7.62 m | 7.56 t (8.0) |
| 9 | 7.46-7.60 m | 7.58-7.62 m | 7.57-7.62 m | 7.64 t (8.0) |
| 10 | 8.11-8.31 m | 8.20-8.37 m | 8.19-8.36 m | 8.19 d (8.0) |

Table 5.2. Comparison of $^{13}C$ NMR data of the synthetic (this work) and the naturally occurring (ref. 96) c*is*-3,4-dihydroxy-3,4-dihydromollugin **48** (67.5 MHz, $CDCl_3$) with data of the *trans*-3,4-dihydroxy-3,4-dihydromollugin **42** (67.5 MHz, $CDCl_3$, ref. 96; 100 MHz, $CDCl_3$, 76a)

| | δ (ppm) | | | |
|---|---|---|---|---|
| Carbon | **48** Synthetic *cis*-form (this work) | **48** Naturally ocurring *cis*-form (ref. 96) | **42** *trans*-form (ref. 96) | **42** *trans*-form (ref. 76a) |
| 2 | 77.70 (overlap with $CDCl_3$) | Overlap with $CDCl_3$ | overlap with $CDCl_3$ | Overlap with $CDCl_3$ |
| **$CH_3$-2** | **22.05** | **21.97** | **19.62** | **19.77** |
| **$CH_3$-2** | **24.60** | **24.81** | **25.49** | **25.57** |
| **3** | **72.09** | **72.27** | **69.77** | **69.87** |
| **4** | **64.31** | **64.42** | **76.06** | **76.18** |
| 4a | 112.76 | 112.90 | 112.84 | 112.96 |
| 5 | 104.56 | 104.63 | 103.84 | 103.96 |
| $\underline{C}OOCH_3$-5 | 171.21 | 171.21 | 171.30 | 171.35 |
| $COO\underline{C}H_3$-5 | 52.80 | 52.81 | 52.81 | 52.86 |
| 6 | 155.63 | 155.79 | 156.43 | 156.52 |
| 6a | 125.53 | 125.67 | 125.60 | 125.27 |
| 7 | 122.46 | 122.56 | 122.49 | 122.58 |
| 8 | 126.95 | 127.04 | 126.95 | 127.05 |
| 9 | 129.43 | 129.50 | 129.59 | 129.66 |
| 10 | 123.81 | 123.91 | 123.95 | 124.04 |
| 10a | 128.75 | 128.92 | 128.96 | 129.09 |
| 11 | 140.72 | 140.84 | 140.96 | 141.07 |

V.3.2. 3-Hydroxy-1-methyl-9,10-anthraquinone **51**

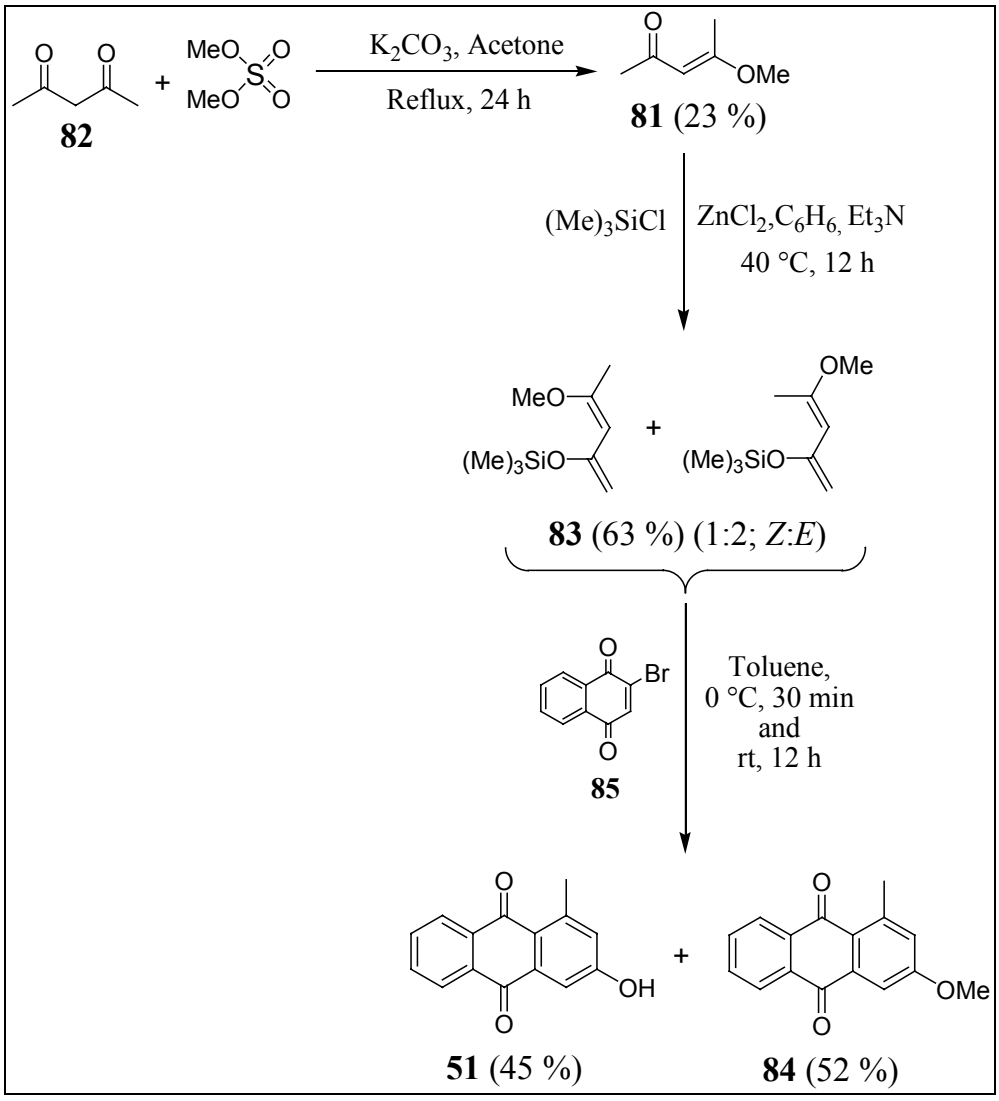
The anthraquinone **51** was isolated as a yellow powder (mp 270.5-271.8 °C) from the dichloromethane extract of the roots of *P. longiflora* (96). To its structural assignment as a 1,3-disubstitued anthraquinone, with the particular location of the methyl group at the 1-position, could be opposed the fact that the methyl group should be located at the 2-position according to what is widely observed in the biosynthesis of anthraquinones in rubiaceous plants (71,141). Therefore, the regioselective synthesis of the anthraquinone **51** was performed in order to confirm the structural assignment of the naturally occurring

anthraquinone (96) through comparison of its spectral and physical data with those of the synthetic one.

The synthesis of 3-hydroxy-1-methyl-9,10-anthraquinone **51** was performed *via* the successful use of conjugated ketene acetals in cycloaddition and through a three-step process (Scheme 5.3). The use of trimethylsilyloxydienes was chosen as the most promising approach (158). The trimethylsilyloxydiene **83** was derived from the methyl enol ether (**81**) of pentane-2,4-dione **82** (159). 3-Hydroxy-1-methyl-9,10-anthraquinone **51** (45 % yield) was obtained by condensation (Diels-Alder reaction) of the latter diene with 2-bromo-1,4-naphthoquinone **85** (160). 3-Methoxy-1-methyl-9,10-anthraquinone **84** (3-*O*-methyl ether of **51**; 52 % yield) was also obtained as a final product of the Diels-Alder condensation. Compound **84** is a novel synthetic anthraquinone.

**3-Hydroxy-1-methyl-9,10-anthraquinone 51** was obtained as a yellow powder. The mp of the compound, 291.1-293.2 °C, was largely different from that (270.5-271.8 °C) of the anthraquinone isolated from the roots of *P. longiflora* (96). However, the IR spectrum of the synthetic anthraquinone displayed the same pattern of peaks as the naturally occurring one. The absorption band of the hydroxy group was observed at 3347 $cm^{-1}$, whereas the carbonyl groups absorbed at 1673 and 1649 $cm^{-1}$. The EI mass spectrum displayed a molecular ion peak at *m*/*z* 238 (100%) corresponding to the molecular formula of $C_{15}H_{10}O_3$.

The $^1H$ NMR spectrum (in $C_5H_5N$) of the synthetic anthraquinone **51** showed an unsubstituted aromatic ring by displaying an AA'BB' system of four adjacent protons at $\delta_H$ 8.37-8.40 and 8.30-8.33 (1H each, br d, $J$ = 7.3 Hz, H-5 and H-8) and at $\delta_H$ 7.61-7.71 (2H, m, H-6 and H-7). A clear *meta* coupling system was observed in the disubstituted ring at $\delta_H$ 8.08 (1H, d, $J$ = 2.3 Hz, H-4) and 7.30 (1H, d, $J$ = 2.3 Hz, H-2). The methyl protons resonated at $\delta_H$ 2.29 (3H, s, $CH_3$-1), whereas the hydroxy proton resonated at $\delta_H$ 5.47 (1H, br s, OH-3).




**Scheme 5.3**: Synthesis of 3-Hydroxy-1-methyl-9,10-anthraquinone **51**
(and its 3-*O*-methyl ether **84**)

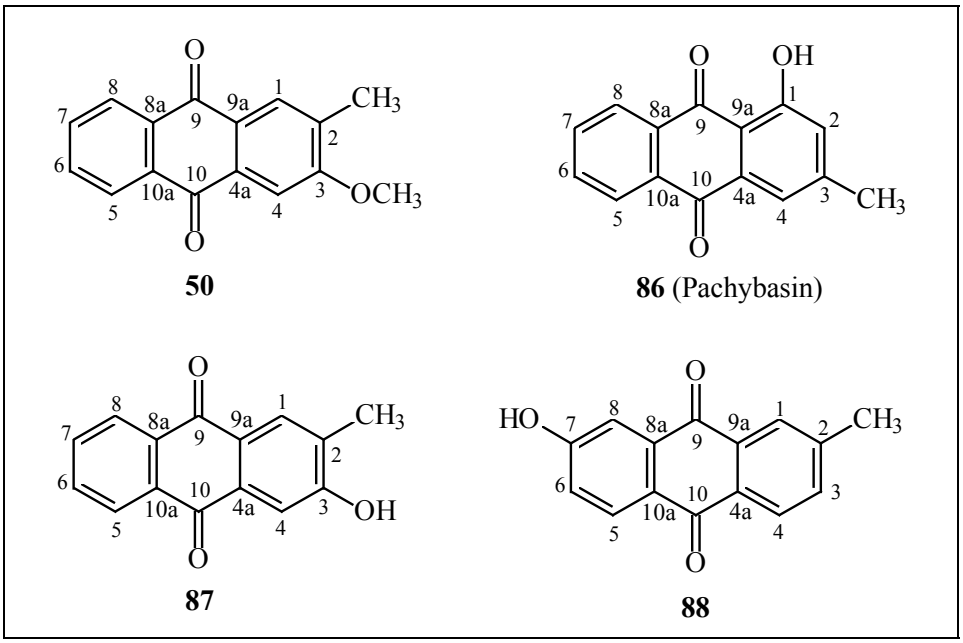
The $^{13}C$ NMR spectrum showed key signals at $\delta_C$ 182.95 and 182.46 (C-9 and C-10), $\delta_C$ 162.79 (C-3), $\delta_C$ 144.60 (C-1) and $\delta_C$ 22.91 ($\underline{C}H_3$-1). Comparison of the $^1H$ and $^{13}C$ NMR data of the synthetic and the naturally occurring anthraquinone (Table 5.3) showed very important differences. The protons and the carbon of the methyl group were more deshielded (in the $^1H$ and $^{13}C$ NMR spectra, respectively) in the synthetic anthraquinone than in the natural one, and this was in agreement with the *peri* position of the $CH_3$-1 in the case of the synthetic anthraquinone. Evidently, the $CH_3$ of the anthraquinone isolated from the roots of *Pentas longiflora* (96), was not located at 1-position. The structure of this naturally occurring anthraquinone could also not correspond to 1-hydroxy-3-methyl-9,10-anthraquinone or

pachybasin **86** (108b) for two major reasons which are the lack of hydrogen bonding effect (chelation) in the IR spectrum of the compound, together with the lack of a deshielding effect of a *peri* hydroxy group (an OH at 1-position) on one of the carbonyl carbons in the $^{13}C$ NMR. Besides, the melting point of the latter compound (pachybasin **86**) is 179-180 °C and this value is very different from that of the melting point (270.5-271.8 °C) measured for the anthraquinone isolated from *P. longiflora* (96). The possibility of locating the two substituents, i.e. the $CH_3$ and the OH groups, in different rings for the naturally occurring anthraquinone was ruled out by the presence of an AA'BB' coupling system of four aromatic protons (96). Finally, closer inspection of the $^{1}H$ NMR spectrum of this naturally occurring anthraquinone did not show the clear *meta* coupling system for the disubstituted ring observed for the synthetic anthraquinone **51**, but instead two sharp singlets.

According to all the pertinent features discussed above, the right structure which fits with the data observed for the naturally occurring anthraquinone isolated from the dichloromethane extract of the roots of *Pentas longiflora* (96), and previously elucidated as 3-hydroxy-1-methyl-9,10-anthraquinone **51** (96), is 3-hydroxy-2-methyl-9,10-anthraquinone **87**.

The $^{1}H$ and $^{13}C$ NMR data of the newly assigned anthraquinone **87** were compared (Tables 5.4 and 5.5, respectively) to its 3-*O*-methyl ether (3-methoxy-2-methyl-9,10-anthraquinone) **50** which was isolated from the EtOAc extract of the roots of *P. longiflora* during the same investigation (96) and to its isomer 7-hydroxy-2-methyl-9,10-anthraquinone **88** (109).

On the one hand, Table 5.4 showed that the proton chemical shifts of the anthraquinone **87** could be related to its 3-*O*-methyl ether **50**. The differences observed in the disubstituted ring may be attributed to the substitution of the OH group in the anthraquinone **87** by a $OCH_3$ in the anthraquinone **50** (96). On the other hand, both the chemical shifts and the coupling patterns observed in the anthraquinone **87** were different from those observed in the anthraquinone **88** (109). Particularly, the lack of the *meta* coupling systems in the anthraquinone **87** (whereas they were observed in **88**) ruled out the possibility of locating the two substituents (OH and $CH_3$) in two different rings for the anthraquinone **87**.




In addition, the $^{13}C$ NMR data of the anthraquinone **87** were found consistent (Table 5.5) with those of its 3-*O*-methyl ether (3-methoxy-2-methyl-9,10-anthraquinone) **50**. The remarkable $\delta_C$ difference observed for C-4 in the two anthraquinones, 111.79 ppm for **87** and 106.84 ppm for **50**, could be attributed to the difference of shielding effect of the OH and the $OCH_3$ groups in **87** and **50**, respectively. The Table 5.5 also compares the $^{13}C$ NMR data of the newly assigned anthraquinone **87** with those of its isomer 7-hydroxy-2-methyl-9,10-anthraquinone **88** (109). In the anthraquinone **87**, C-1 and C-4a were more deshielded by the OH-3, $\delta_C$ 130.75 ppm (C-1) and 135.78 ppm (C-4a), respectively, than in the anthraquinone **88** for which the signals of C-1 and C-4a were displayed at $\delta_C$ 127.1 ppm and 131.3 ppm, respectively. Besides, the *ortho* position of the OH from the $CH_3$ was also confirmed by the higher field position of the C-2 ($\delta_C$ 125.99) for the anthraquinone **87** than for the anthraquinone **88** for which the C-2 resonated at $\delta_C$ 145.0 ppm. All these findings reinforce the structural arrangement proposed for the anthraquinone **87**.

The data observed for the $CH_3$-2 group of the newly assigned anthraquinone **87** were, somehow, comparable to those found for other 2-methyl substituted anthraquinones, such as the anthraquinones **36**, **52** and **54** (see Chapter III), although in the latter compounds, an additional alkoxy substitution in *ortho* position to the $CH_3$-2 exerts a significant influence in the chemical shifts.

The anthraquinone **87** was reported to occur in varous rubiaceous plants. It has been isolated from the root bark of *Coprosma* species (*C. ternuicaulis* and *C. linariifolia*) (146a), from the whole plant MeOH extract of *Hedyotis diffusa* (162), and from the benzene extract of the heartwood of *Faramea cyanea* (153c). The compound was also isolated from *Ophiorrhiza pumila* tissue and cell cultures (154).

3-Hydroxy-2-methyl-9,10-anthraquinone **87** has also been successfully synthesised, both regiospecifically through the use of trimethylsilyloxydienes cycloaddition (Diels-Alder reaction) to halonaphthoquinones (163), and non-regiospecifically by condensing benzoic acid with 3-methoxy-4-methylbenzoic acid in hot concentrated sulfuric acid (164).

Although the anthraquinone **87** is reported in the literature as mentioned above, this is, however, the first time that its $^{1}H$ and $^{13}C$ NMR data are reported (Tables 5.4 and 5.5).

Table 5.3. Comparison of NMR data of the synthetic anthraquinone **51** (this work) and the naturally occurring anthraquinone (ref. 96): $^{1}H$ and $^{13}C$, 270 MHz/67.5 MHz, $C_5D_5N$

| | δ (ppm) | | | | |
|---|---|---|---|---|---|
| | Synthetic Anthraquinone **51** (this work) | | | Naturally occurring anthraquinone (ref. 96) | |
| Proton | $^{1}H$ multiplicity (*J* in Hz) | $^{13}C$ | Position | $^{1}H$ multiplicity (*J* in Hz) | $^{13}C$ |
| **1** | **-** | **144.60** | 1 | 8.29 s | 130.75 |
| **$CH_3$-1** | **2.89 s** | **22.91** | **2** | **-** | **125.99** |
| **2** | **7.30 d (2.3)** | **124.46** | **$CH_3$-2** | **2.44 s** | **16.72** |
| 3 | - | 162.79 | 3 | - | 162.79 |
| OH-3 | 5.47 s | - | OH-3 | 5.11 s | - |
| **4** | **8.08 d (2.3)** | **111.81** | 4 | 7.97 s | 111.79 |
| 4a | - | 136.89 | 4a | - | 135.78[a] |
| 5 | 8.30-8.33 br d (7.0) | 125.62[a] | 5 | 8.28-8.31 m | 126.91[b] |
| 6 | 7.61-7.71 m | 133.31[b] | 6 | 7.61-7.67 m | 134.08[c] |
| 7 | 7.61-7.71 m | 132.26[b] | 7 | 7.61-7.67 m | 133.57[c] |
| 8 | 8.37-8.40 br d (7.0) | 126.34[a] | 8 | 8.28-8.31 m | 127.01[b] |
| 8a | - | 130.53[c] | 8a | - | 132.93[a] |
| 9 | - | 182.95[d] | 9 | - | 182.13[d] |
| 9a | - | 128.36[c] | 9a | - | 134.24[a] |
| 10 | - | 182.46[d] | 10 | - | 183.35[d] |
| 10a | - | 132.31[c] | 10a | - | 134.27[a] |

[a-d] Assignments may be reversed in the same column

Table 5.4. Comparison of the $^{1}$H NMR data of the naturally occurring anthraquinone **87** (270 MHz, $C_5D_5N$; structural revision of anthraquinone **51** and novel data assignment from ref. 96) with those of its methyl ether **50** (270 MHz, $CDCl_3$, ref. 96) and its isomer **88** (300 MHz, DMSO-$d_6$, ref. 109): $\delta_H$ in ppm, multiplicity and (*J* in Hz).

| | Anthraquinone | | |
|---|---|---|---|
| Proton | **87** (data of **51** according to ref. 96) | **50** (ref. 96) | **88** ( ref.109)* |
| **1** | **8.29 s** | **8.06 s** | **7.47 d (2.4)**[a] |
| 2 | - | - | - |
| **$CH_3$-2** | **2.44 s** | **2.35 s** | **2.50 s** |
| **3** | **-** | **-** | **7.23 dd (8.5, 2.4)**[b] |
| OH-3 | 5.11 s | - | - |
| $OCH_3$-3 | - | 4.02 s | - |
| **4** | **7.97 s** | **7.64 s** | **8.06 d (8.5)**[c] |
| 4a | - | - | - |
| 5 | 8.28-8.31 m | 8.25-8.29 m | 8.03 d (7.8)[c] |
| 6 | 7.61-7.67 m | 7.74-7.75 m | 7.68 br d (7.8)[b] |
| 7 | 7.61-7.67 m | 7.74-7.75 m | - |
| OH-7 | - | - | 11.0 s |
| 8 | 8.28-8.31 m | 8.25-8.29 m | 7.92 br s[a] |
| 8a | - | - | - |
| 9 | - | - | - |
| 9a | - | - | - |
| 10 | - | - | - |
| 10a | - | - | - |

* Protons assignment is done by us according to coupling systems and coupling constants given in ref. 109;

[a-c] Assignments may be reversed in the same column

Table 5.5. Comparison of the $^{13}C$ NMR data of the naturally occurring anthraquinone **87** (67.5 MHz, $C_5D_5N$; structure revision of anthraquinone **51** and novel data assignment from ref. 96) with those of its methyl ether **50** (67.5 MHz, $CDCl_3$, ref. 96) and its isomer **88** (75 MHz, DMSO-$d_6$, ref. 109): $\delta_C$ in ppm.

| | Anthraquinone | | |
|---|---|---|---|
| Carbon | **87** (data of **51** according to ref. 96) | **50** (ref. 96) | **88** ( ref.109) |
| **1** | **130.75** | **129.77** | **127.1** |
| **2** | **125.99** | **126.79** | **145.0** |
| **$CH_3$-2** | **16.72** | **16.71** | **21.3** |
| 3 | 162.79 | 162.62 | 135.5 |
| $OCH_3$-3 | - | 56.06 | - |
| **4** | **111.79** | **106.84** | 127.1 |
| **4a** | **135.78**[a] | **134.19**[a] | **131.3**[a] |
| 5 | 126.91[b] | 127.06[b] | 130.1 |
| 6 | 134.08[c] | 133.97[c] | 121.8 |
| 7 | 133.57[c] | 133.56[c] | 163.5 |
| 8 | 127.01[b] | 127.02[b] | 112.5 |
| 8a | 132.93[a] | 134.19[a] | 135.6[a] |
| 9 | 182.13[d] | 182.53[d] | 183.3[b] |
| 9a | 134.24[a] | 133.67[a] | 133.3[a] |
| 10 | 183.35[d] | 183.37[d] | 181.5[b] |
| 10a | 134.27[a] | 133.76[a] | 125.5 |

[a-d] Assignments may be reversed in the same column

**Chapter VI.**

# SCREENING OF THE BIOLOGICAL ACTIVITY OF THE NATURAL PRODUCTS ISOLATED FROM *PENTAS BUSSEI* K. Krause, *PENTAS LANCEOLATA* (Forsk.) Deflers AND *PENTAS PARVIFOLIA* Hiern

# Chapter VI. SCREENING OF THE BIOLOGICAL ACTIVITY OF THE NATURAL PRODUCTS ISOLATED FROM *PENTAS BUSSEI*, *PENTAS LANCEOLATA* AND *PENTAS PARVIFOLIA*

## VI.1. Introduction

All the natural products isolated from *Pentas bussei*, *Pentas lanceolata* and *Pentas parvifolia* were evaluated externally for antiviral and for their potential anticancer activity through their interaction with DNA. Antiviral activity was tested against two viruses: HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C virus).

HIV is the RNA-virus that causes AIDS, Acquired Immuno Deficiency Syndrome, a disease that was first described in the early 80's. Since then AIDS has spread all over the world and more than 40 million people are infected with HIV.

HCV is a flavivirus which causes a chronic infection of the liver. About 200 million patients worldwide are infected with the virus. Over the years, more than 20 % of the patients develop cirrhosis and about 10 % develop liver cancer.

## VI.2. Antiviral Screening

### VI.2.1. HIV Antiviral and Toxicity Assay

The HIV antiviral activity has been determined with a cell-based replication assay. The antiviral assay directly measures the ongoing replication of the virus in MT4 (gene "membrane type-4") cells *via* the specific interaction of HIV-tat with LTR (Long Terminal Repeat) coupled to GFP (Green Fluorescent Protein). In the toxicity assay, a reduced expression of the GFP reporter protein serves as a marker for cellular toxicity of a compound.

Briefly, various concentrations of the test compounds are brought in a 384-well-microtiter plate. Subsequently, HIV (wild type) and MT4 cells are added to the plate. In order to determine the toxicity of the test compound, mock-infected cell cultures containing an

identical compound concentration range, are incubated for 3 days (37 °C, 5 % $CO_2$) in parallel with the HIV-infected cell cultures. Based on the calculated percent inhibitions, the dose response curves are plotted. The $EC_{50}$ (Effective Concentration) and the $CC_{50}$ (Cytotoxic Concentration) values are calculated.

VI.2.2. HCV Cell Culture Assay

The HCV cell culture system is based on the autonomous replication of subgenomic selectable HCV-RNAs, called replicons. In brief, the structural genes of the HCV genome were replaced by heterologues sequences, namely the gene encoding the neomycin phosphotransferase (NPT), the internal ribosome entry site (IRES) of the encephalomyocarditis virus and the luciferase reporter gene. The synthetic RNAs were introduced into the human hepatoma cell line HuH-7 (human hepatocellular carcinoma cell line) and after treatment with G418 (antibiotic), only cells supporting RNA replication high enough to confer resistance could survive and were selected.

By this method, the luciferase reporter gene was correlated with the replicon status. Huh-7 cells are exposed to various concentrations of the test compounds in 384-well plates. After 3 days, substrate is added to the plates and the replicon status is measured by luminescence. Based on the calculated percent inhibitons, the dose response curves are plotted and $EC_{50}$ and $CC_{50}$ values are calculated. The compounds are tested in 384 well plates at the following concentrations: 32 μM - 8 μM - 2 μM - 0.5 μM - 0.125 μM - 0.031 μM - 0.0078 μM - 0.00050 μM (4 replicates each).

## VI.3. Screening of the Natural Products for Potential Anticancer Activity Through Interaction with DNA

Genetic toxicology studies play a central role in the development and marketing of new chemicals for pharmaceutical, agricultural, industrial, and consumer use. During the discovery phase of product development, rapid screening tests that require minimal amounts of test materials are used to assist in the design and prioritization of new compounds (165).

What kind of test(s) should be performed is greatly dependent on the destination of the

compound or the risk related to human exposure but as a general rule, a battery of tests is required in order to allow detection of different genotoxic events (gene, chromosome, genome mutations).

The following tests were conducted to evaluate the genotoxic properties of the compounds isolated from *P. bussei*, *P. lanceolata* and *P. parvifolia*: the bacterial Ames and VITOTOX® tests, together with the micronucleus test on human white blood cells.

VI.3.1. The Bacterial Ames Test

The Ames *Salmonella*/microsome mutagenicity assay (*Salmonella* Test; Ames test) is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations. The test employs several histidine dependent *Salmonella* strains each carrying different mutations in various genes in the histidine operon. These mutations act as hot spots for mutagens that cause DNA damage *via* different mechanisms. The Ames test is the most widely and validated genotoxicity test.

When the *Salmonella* tester strains are grown on a minimal media agar plate containing a trace of histidine, only those bacteria that revert to histidine independence ($his^+$) are able to form colonies. The number of spontaneously induced revertant colonies per plate is relatively constant. However, when a mutagen (the compound) is added to the plate, the number of revertant colonies per plate is increased, usually in a dose-related manner (166).

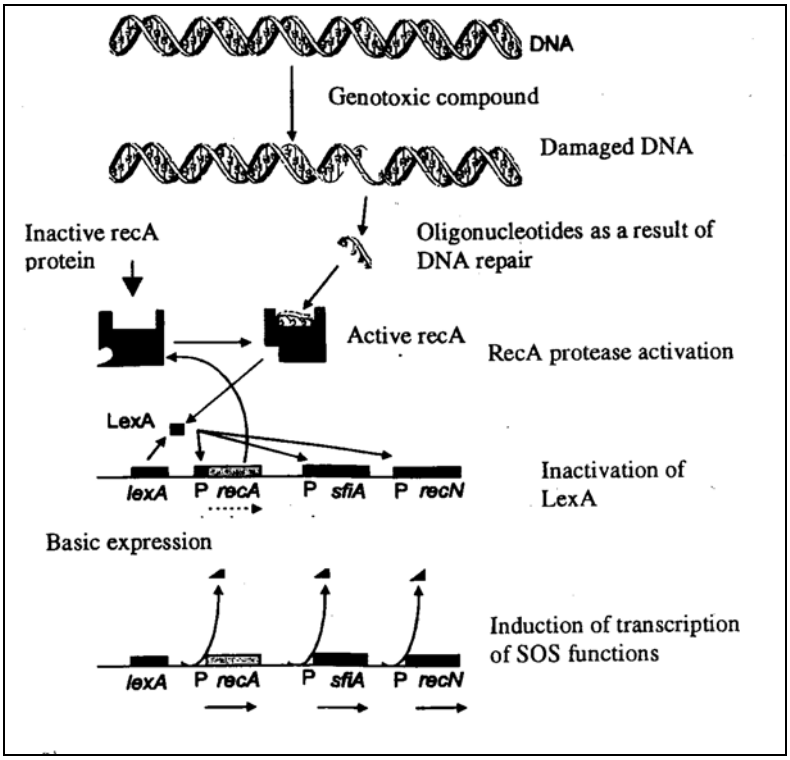
The sensitivity of the test is enhanced by the use of particular mutant strains preventing, e.g., adequate DNA repair or increasing resistance to toxic compounds. Bacteria are grown on a selective medium in the presence of the tested compound. After 48 h of incubation at 37 °C, mutant colonies are counted and compared to the number of colonies formed in unexposed cultures (spontaneous back mutatons).

A compound is considered genotoxic when the mean number of revertants is the double of that found in the solvent control culture (+ dose effect relationship). Negative controls are normally between approximatively 5-50 revertants for TA98 and 100-150 for TA100.

Positive controls must show a clear increase over the solvent controls.

VI.3.2. The VITOTOX® Test

The VITOTOX® (167) test is a test which is more rapid and less expensive than the "classical" Ames test. The test is also at least as sensitive as the Ames test. It is based on bacteria that contain the *lux* operon of *Vibrio fischeri* under transcriptional control of the *rec*N gene, that is part of the SOS-system (Scheme 6.1). This gene is normally not transcribed (no light production) but it will be "switched on" when the bacteria are exposed to a genotoxic compound (mutagen or "SOS-inducing" substance). Genotoxicity is thus expressed as light production.




**Scheme 6.1**: Schematic representation of the principle of SOS-induction on which the VITOTOX® test is based (insertion of an operon-less "*lux*" gene next to *rec*N results in light production when the bacterial DNA is damaged) (167).

Although any bacterial strain can be used, *Salmonella typhimurium* strains were chosen as these are well known for mutagenicity testing and because the same bacteria can also be used

for a classical Ames test. However, as all *Salmonella* constructs gave very comparable results, only the TA104 construct [called T104 (*rec*N2-4)] is used as it was shown to be sometimes a little bit more sensitive than the other hybrid strains.

As it was realised that some compounds act directly on the light production (e.g., aldehydes) or enhance the metabolism of the bacteria creating false-positive results, a constitutive light producing strain with a *lux* operon under control of the strong promoter *pr1* was also incorporated. This is used as an internal control system, which also gives important information on the toxicity of the test compound.

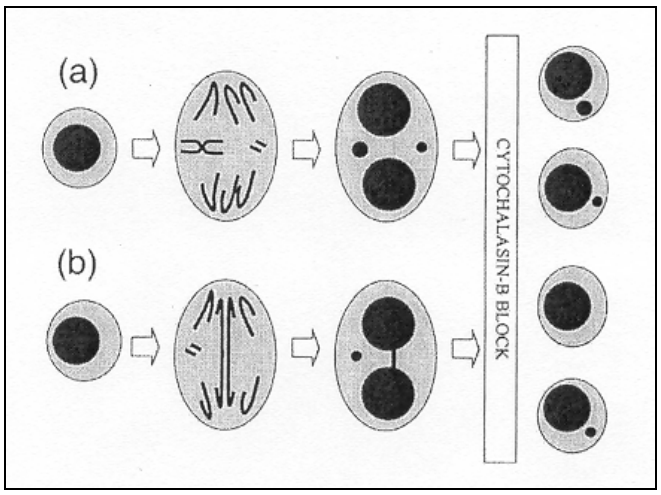
### VI.3.3. The *in vitro* Micronucleus Test

The study of DNA damage at the chromosome level is an essential part of the genetic toxicology because chromosomal mutation is an important event in carcinogenesis. The micronucleus (168) assays have emerged as one of the preferred methods for assessing chromosome damage because they enable both chromosome loss and chromosome breakage to be measured reliably.

A micronucleus is formed when during cell division a chromosome or a chromosome fragment becomes separated from the spindle, and therefore is not incorporated into one of the daughter nuclei (Scheme 6.2). The test can thus detect both clastogenic (chromosome breaking) and aneugenic (e.g., spindle disturbances, genome mutations) events, which can be distinguished using either anti-kinetochore antibodies (CREST-staining), centromere banding (C-banding) or fluorescent *in situ* hybridisation (FISH).

In the *in vitro* micronucleus test, usually human peripheral blood lymphocytes are used. To distinguish cells that divided just once in culture, the cultures are treated with cytochalasin-B, a chemical that blocks actin polymerisation and as such also cytokinesis.

After one cell cycle, binucleated cells, eventually with one or more micronuclei are obtained. It is necessary to distinguish the cells that divided in culture, as a cell division is required before formation of a micronucleus and as a very small fraction of lymphocytes has micronuclei acquired *in vivo*.




**Scheme 6.2**: (a) The origin of micronuclei from lagging whole chromosomes and acentric chromosome fragments at anaphase. (b) Formation of a nucleoplasmic bridge from a dicentric chromosome, formation of a micronucleus from the accompanying acentric chromosome fragment, the critical role of cytochalasin-B in blocking dividing cells at the binucleate stage (168a).

## VI.4. Results and Discussion

At the moment of the finalisation of this research work on natural products, the biological screening of all the natural compounds isolated from the three *Pentas* plant species is still going on. Therefore, the results of the biological activity tests are not reported here.

# VII.

# SUMMARY

## VII. 1. SUMMARY

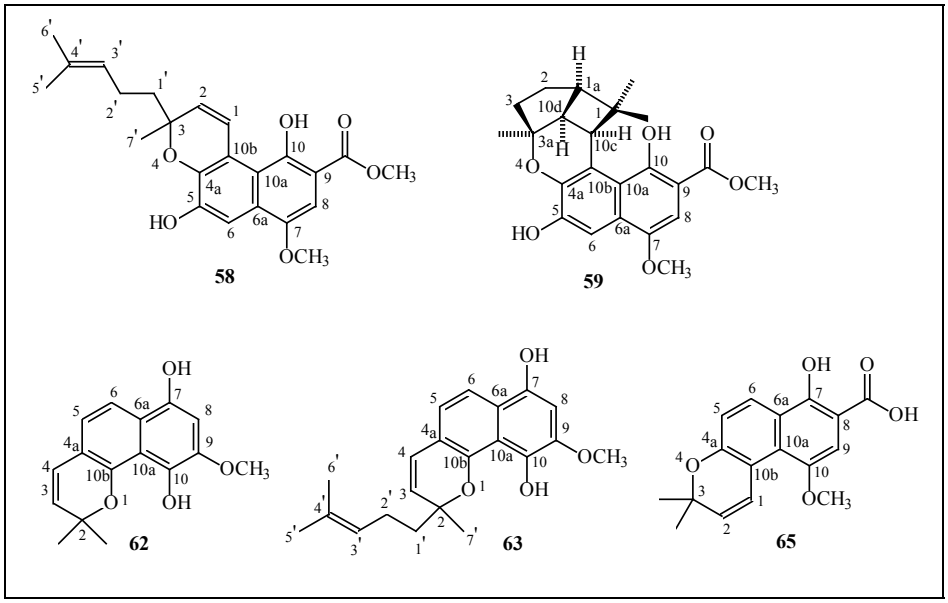
Since decades, plant-based drugs have played an essential role in the primary health care of a large part of the world's population. Several chemotherapeutic agents have been discovered as a result of chemical studies directed towards the isolation of the active substances from plants used in traditional medicine.

Several plant species from the family Rubiaceae are widely used in African Folk medicines. Among them, *Pentas* species display a broad ranges of uses. However, the phytochemistry of these species is only scarcely investigated. From the about 40 known species, only *Pentas longiflora* Oliver and *Pentas zanzibarica* (Klotsch) Vatke have been studied. Aiming at the search for novel naturally occurring compounds which may be used as "lead" for the synthesis of potentially active compounds, this work describes the isolation and structural elucidation of natural products from *Pentas bussei* K. Krause, *Pentas lanceolata* (Forsk.) Deflers and *Pentas parvifolia* Hiern (Chapters II, III, IV). In addition, all the isolated natural compounds are being subjected to a bioactivity screening for antiviral (HIV and HCV) and anticancer activity (Chapter VI). Chapter V has been devoted to the synthesis and structural assignment confirmation of two natural products isolated from *P. longiflora* a species already investigated.

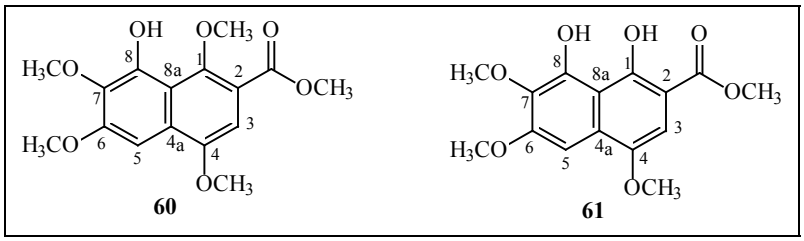
The fractionation, the isolation and the purification of the natural products were achieved by means of medium pressure liquid chromatography (MPLC), centrifugal counter current chromatography (CPC), high pressure liquid chromatography (HPLC), liquid-liquid extraction (partition) and preparative thin layer chromatography (p-TLC). The structural elucidation of the natural products was performed by $^{1}H$ NMR and $^{13}C$ NMR spectrometry (including $^{1}H$-$^{1}H$ COSY, $^{1}H$-$^{13}C$ COSY, DEPT, HMBC and DIFNOE techniques), mass spectrometry (MS), infrared (IR) and ultra violet (UV) spectrometry.

Chapter II describes the phytochemical investigation of *Pentas bussei*. The plant is a woody herb or shrub, of about 0.5-4 m high, and sometimes scrambling. The plant was collected in Kenya where a decoction of the roots is taken as a remedy against gonorrhoea, syphilis and dysentery. The investigation of the hexane, the $CH_2Cl_2$, the EtOAc and the MeOH extracts of the roots of the plant resulted in the isolation of five naphthohydroquinones of the

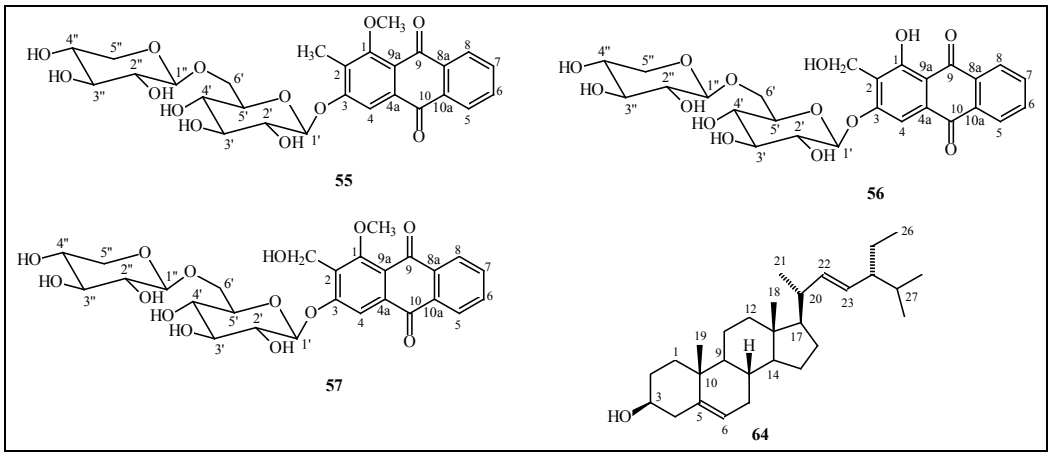
benzochromene type, e. g. methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate **58**, methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a,10c,10d-hexahydro-1*H*-4-oxacyclobuta[3,4]indeno[5,6-*a*]naphthalene-9-carboxylate **59**, 9-methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-7,10-diol **62**, 9-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]chromene-7,10-diol **63** and 7-hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromene-8-carboxylic acid **65**. All these compounds were new natural products.



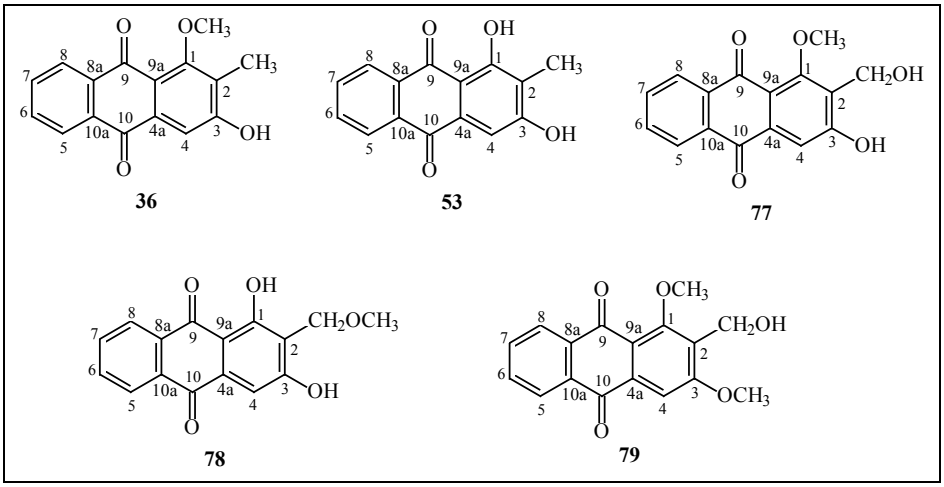

In addition, two highly oxygenated naphthohydroquinones, e. g. methyl 8-hydroxy-1,4,6,7-tetramethoxy-2-naphthoate **60** and methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61**. These two compounds were also novel natural products.




Besides, four known compounds were also isolated, e. g. the anthraquinone glycosides rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55**, lucidin-3-*O*-*β*-primeveroside **56** and damnacanthol-3-*O*-*β*-primeveroside **57**, together with the ubiquitous *β*-stigmasterol **64**.



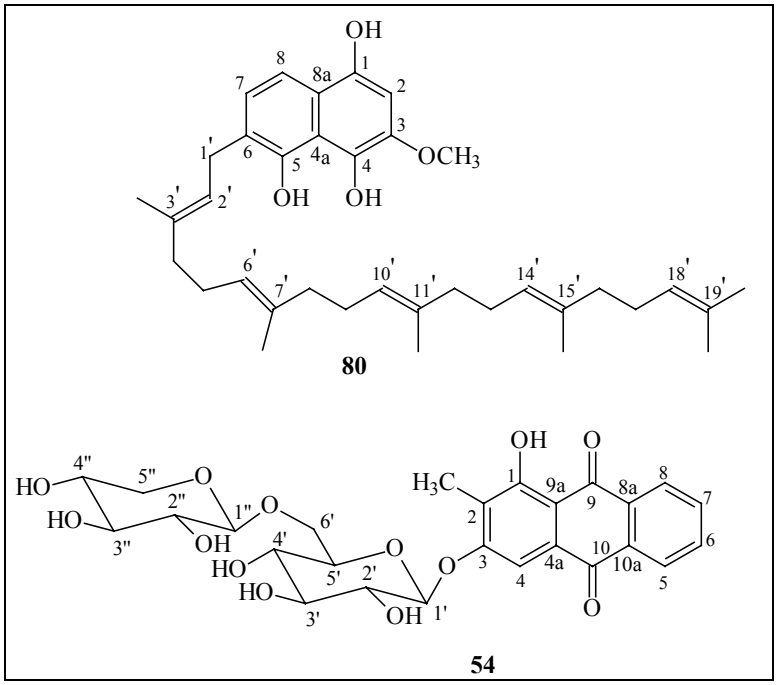

The phytochemical investigation of natural products of *Pentas lanceolata* (Chapter III) mainly led to the isolation of five known anthraquinones, e. g. rubiadin-1-methyl ether **36**, rubiadin **53**, damnacanthol **77**, 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (lucidin-*ω*-methyl ether) **78**, damnacanthol-3-*O*-methyl ether **79**, together with the anthraquinone glycoside rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55** (which was already isolated from *P. bussei*). *β*-Stigmasterol **64** was also isolated from *Pentas lanceolata*.



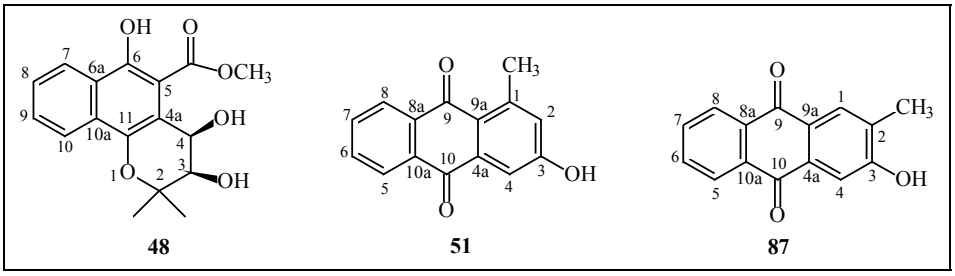

Chapter IV describes the investigation of constituents of *Pentas parvifolia*. The chemistry of the plant was mainly found similar to that of *P. bussei*. The following four novel naphthohydroquinones of the benzochromene type, which were already isolated from *P. bussei*, were isolated from *P. parvifolia*: methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromene-9-carboxylate **58**, 9-methoxy-2,2-dimethyl-2*H*-

benzo[*h*]chro-mene-7,10-diol **62**, 9-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo[*h*]chromene-7,10-diol **63** and 7-hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromene-8-carboxylic acid **65**. The highly oxygenated naphthohydroquinone methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naphthoate **61**, the two anthraquinone glycosides lucidin-3-*O*-*β*-primeveroside **56** and damnacanthol-3-*O*-*β*-primeveroside **57**, together with the ubiquitous *β*-stigmasterol **64** were also isolated from *Pentas parvifolia*.

In addition, the long-chain-branched naphthohydroquinone 1,4,5-trihydroxy-3-methoxy-6-(3,7,11,15,19-pentamethyleicosa-2,6,10,14,18-pentaenyl)naphthalene **80**, together with an additional anthraquinone glycoside, i. e. rubiadin-3-*O*-*β*-primeveroside **54**, were isolated from *Pentas parvifolia*.




The synthesis of two natural products isolated from *Pentas longiflora*, i. e. *cis*-3,4-dihydroxy-3,4-dihydromollugin **48** and the anthraquinone **51** allowed, on the one hand, to confirm the structure already assigned to the diol **48**, and, on the other hand, to revise the structure erroneously assigned to the anthraquinone **51** from 3-hydroxy-1-methyl-9,10-anthraquinone (1-methyl substituted) **51** to 3-hydroxy-2-methyl-9,10-anthraquinone (2-methyl substituted) **87**. The latter structural arrangement is in agreement with the biosynthesis of anthraquinones in the Rubiaceae.




**48** **51** **87**

Plants continue to be the most important source of potentially useful chemical agents which can either be used directly or serve as "lead" compound for the synthesis of more potent derivatives.

## VII. 2. SAMENVATTING

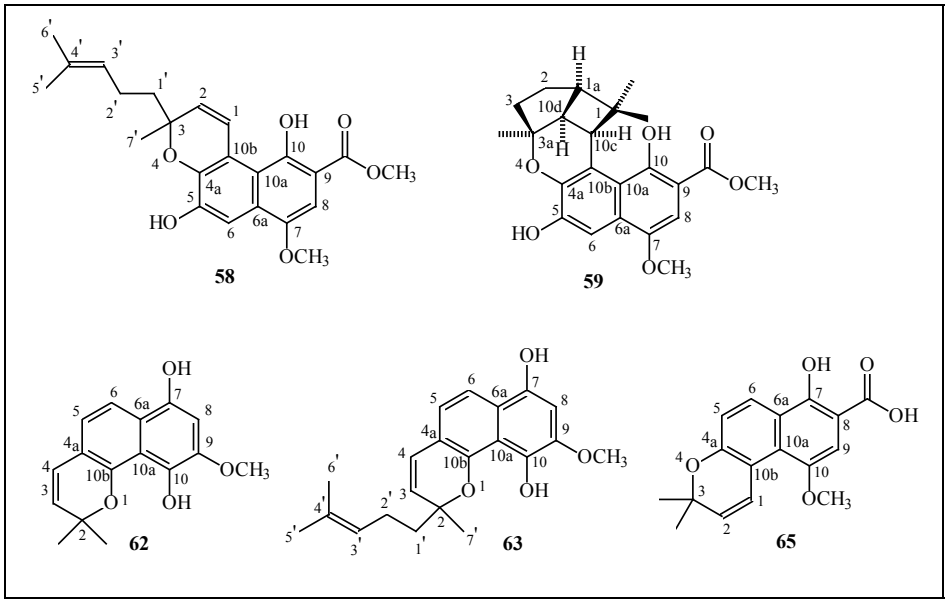
Gedurende vele tientallen jaren spelen plantgebaseerde systemen een stijgende essentiële rol in de eerste hulpverlening bij een groot deel van de wereldbevolking. Verschillende chemotherapeutische stoffen werden ontdekt dankzij chemische studies over de isolatie van actieve bestanddelen afkomstig van traditionele medicinale planten.

Verschillende plantensoorten van de familie Rubiaceae worden wijdverspreid gebruikt in de Afrikaanse traditionele geneeskunde. Onder deze familie ressorteren de *Pentas* soorten, welke voor verschillende doeleinden worden gebruikt. Ondanks dit gegeven is de fytochemie van deze soorten amper onderzocht. Van de ongeveer 40 gekende soorten werden enkel *Pentas longiflora* and *Pentas zanzibarica* bestudeerd. Met het doel nieuwe natuurproducten te vinden die kunnen gebruikt worden als leidraad voor de synthese van potentieel actieve stoffen, wordt in dit werk de isolatie en de structuuropheldering van natuurproducten afkomstig van *Pentas bussei* K. Krause, *Pentas lanceolata* (Forsk.) Deflers en *Pentas parvifolia* beschreven (hoofdstukken II, III, IV). Alle geïsoleerde natuurproducten werden onderworpen aan een (lopende) bioactieve screening voor antivirale (HIV and HCV) en antikanker activiteit (hoofdstuk VI). Hoofdstuk V is gewijd aan de synthese en bevestiging van de structuur van twee natuurproducten geïsoleerd uit *Pentas longiflora* Oliver, een reeds onderzochte plantensoort.

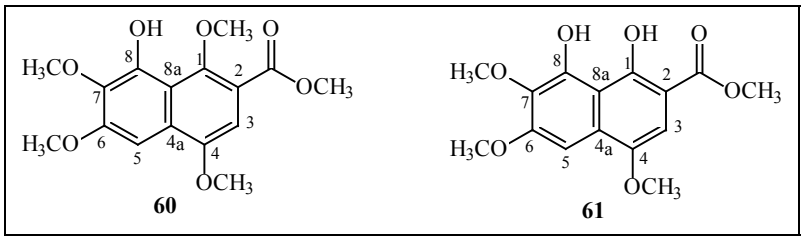
De fractionering, de isolatie en de opzuivering van de natuurproducten werden uitgevoerd door middel van 'medium pressure' vloeistofchromatografie (MPLC), centrifugale tegenstroom chromatografie (CPC), hoge druk vloeistofchromatografie (HPLC), vloeistof-vloeistof extractie (partitie) en preparatieve dunne laag chromatografie (p-TLC). De structuuropheldering van de natuurproducten werd gerealiseerd door $^{1}$H-NMR en $^{13}$C-NMR spectrometrie (inclusief $^{1}$H-$^{1}$H COSY, $^{1}$H-$^{13}$C COSY, DEPT, HMBC en DIFNOE technieken), massaspectrometrie (MS), infrarood (IR) en ultraviolet (UV) spectrometrie.

Hoofdstuk II beschrijft het fytochemisch onderzoek van *Pentas bussei*. Deze plant is een houtachtig kruid of struik, ongeveer 0,5 tot 4 meter hoog, en soms klimmend. De planten werden verzameld in Kenia waar een afkooksel van de wortels gebruikt wordt als een remedie tegen gonorroe, syfilis en dysenterie. Onderzoek van de hexaan-, dichloormethaan-,

ethylacetaat- en methanolextracten van de wortels van deze plant resulteerde in de isolatie van vijf naftohydrochinonen van het benzochromeen-type, nl. methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromeen-9-carboxylaat **58**, methyl 5,10-dihydroxy-7-methoxy-1,1,3a-trimethyl-1a,2,3,3a,10c,10d-hexahydro-1*H*-4-oxacyclobuta[3, 4]indeno[5,6-*a*]naftaleen-9-carboxylaat **59**, 9-methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromeen-7,10-diol **62**, 9-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-benzo-[*h*]chromeen-7,10-diol **63** en 7-hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo[*f*]chromeen-8-carbonzuur **65**. Deze verbindingen zijn allemaal nieuwe natuurproducten.

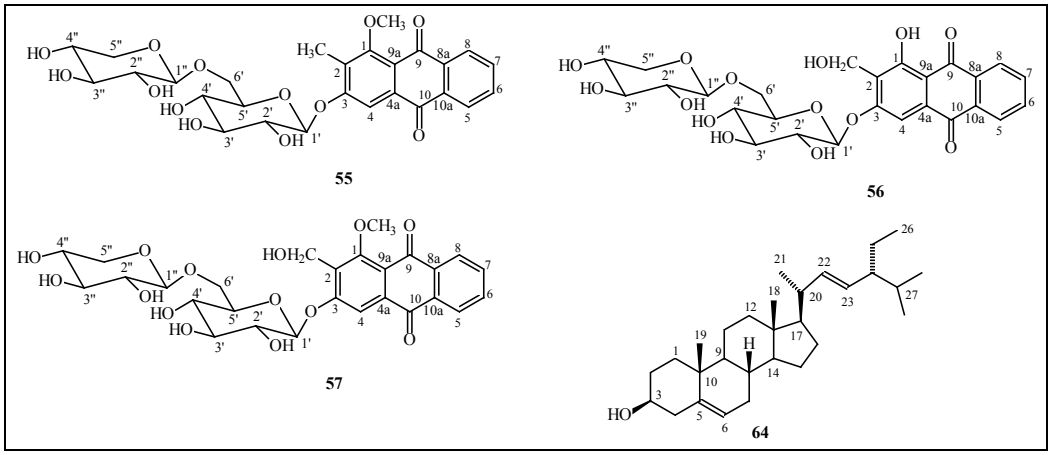



Bovendien werden twee sterk geoxygeneerde naftohydrochinonen geïsoleerd, namelijk methyl 8-hydroxy-1,4,6,7-tetramethoxy-2-naftoaat **60** en methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naftoaat **61**. Deze twee verbindingen zijn eveneens nieuw ontdekte natuurstoffen.

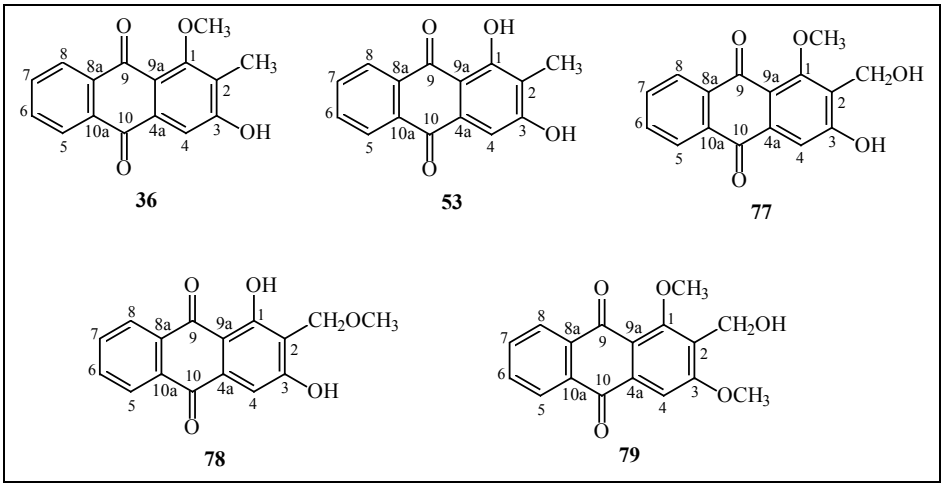



Bovendien werden vier gekende verbindingen geïsoleerd, namelijk de antrachinon glycosiden

rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55**, lucidin-3-*O*-*β*-primeveroside **56** en damnacanthol-3-*O*-*β*-primeveroside **57**, samen met het veel voorkomende *β*-stigmasterol **64**.



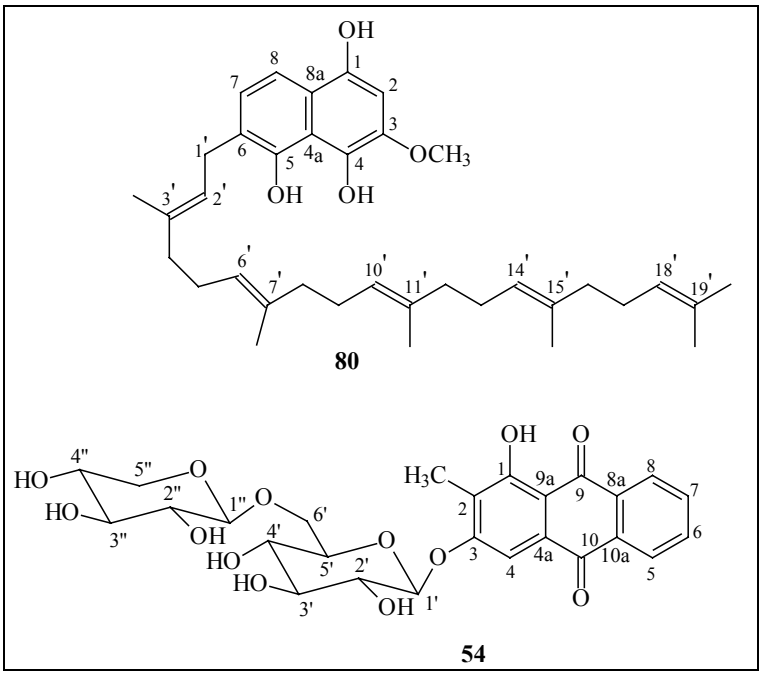

Het fytochemisch onderzoek van natuurproducten van *Pentas lanceolata* (Hoofdstuk III) leidde hoofdzakelijk tot de isolatie van vijf gekende antrachinonen, namelijk rubiadin-1-methyl ether **36**, rubiadin **53**, damnacanthol **77**, 1,3-dihydroxy-2-methoxymethyl-9,10-antrachinon (lucidin-ω-methyl ether) **78**, damnacanthol-3-*O*-methyl ether **79**, samen met het antrachinon glycoside rubiadin-1-methyl ether-3-*O*-*β*-primeveroside **55** (dat reeds geïsoleerd was uit *P. bussei*). *β*-Stigmasterol **64** werd eveneens geïsoleerd uit *Pentas lanceolata*.




Hoodstuk IV beschrijft het onderzoek naar verschillende bestanddelen van *Pentas parvifolia*. De chemische opbouw van de plant was analoog met deze van *P. bussei*. De volgende vier nieuwe naftohydrochinonen van het benzochromeen type, die vroeger reeds geïsoleerd

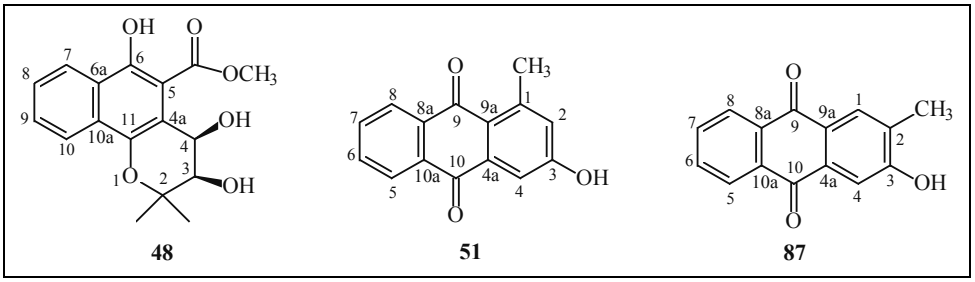
werden uit *P. bussei*, werden afgezonderd uit *P. parvifolia*: methyl 5,10-dihydroxy-7-methoxy-3-methyl-3-(4-methyl-3-pentenyl)-3*H*-benzo[*f*]chromeen-9-carboxylaat **58**, 9-methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromeen-7,10-diol **62**, 9-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-ben-zo[*h*]chromeen-7,10-diol **63** en 7-hydroxy-3,3-dimethyl-10-methoxy-3*H*-benzo-[*f*]chromeen-8-carbonzuur **65**. Het meervoudig geoxygeneerde naftochinon methyl 1,8-dihydroxy-4,6,7-trimethoxy-2-naftoaat **61**, de twee antrachinon glycosiden, lucidin-3-*O*-*β*-primeveroside **56** en damnacanthol-3-*O*-*β*-primeveroside **57**, werden samen met het alomtegenwoordige *β*-stigmasterol **64** geïsoleerd uit *Pentas parvifolia*.

Naast bovenvermelde verbindingen werd eveneens het naftochinon 1,4,5-trihydroxy-3-methoxy-6-(3,7,11,15,19-pentamethyleicosa-2,6,10,14,18-pentaenyl)naftaleen **80**, samen met een antrachinon glycoside, i.e. rubiadin-3-*O*-*β*-primeveroside **54**, geïsoleerd uit *Pentas parvifolia*.




De synthese van twee natuurproducten geïsoleerd uit *Pentas longiflora*, meer bepaald *cis*-3,4-dihydroxy-3,4-dihydromollugin **48** en het antrachinon **51** geven aan de ene hand de bevestiging van de structuur reeds toegewezen aan het diol **48** en aan de andere hand de herziening van de verkeerd toegewezen structuur aan het antrachinon **51** van 3-hydroxy-1-methyl-9,10-antrachinon (1-methyl gesubstitueerd) **51** naar 3-hydroxy-2-methyl-9,10-

antrachinon (2-methyl gesubstitueerd) **87**. De laatste structuurbepaling is in overeenkomst met de biosynthese van antrachinonen in de Rubiaceae.




**48** **51** **87**

Planten blijven de belangrijkste bron van potentieel bruikbare chemische stoffen welke rechtstreeks of als leidraad voor de synthese van meer actieve derivaten kunnen gebruikt worden.

**VIII.**

# BIBLIOGRAPHY

## VIII. BIBLIOGRAPHY


1. (a) Cragg, G. M.; Newman, D. J. and Weiss, R. B. (1997) Coral Reefs, Forests, and Thermals Vents: The Worldwide Exploration of Nature for Novel Antitumor Agents. Seminars in Oncology 24 (2): 156-163. (b) Carlson, T. J.; Cooper, R.; King S. R. and Rozhon, E. J. (1997) Modern Science and Traditional Healing. In: Wrigley, S.; Hayes, M.; Thomas, R. and Chrystal, E. (eds.) *Phytochemical Diversity: A Source of New Industrial Products*. The Royal Society of Chemistry, Cambridge, United Kingdom, p. 84.
2. McConnell, O.; Longley, R. E. and Koehn, F. E. (1994) The Discovery of Marine Natural Products with Therapeutic Potential. In: Gullo, V. P. (ed.) *The Discovery of Natural Products with Therapeutic Potential*. Boston, MA, Butterworth-Heinemann, pp. 109-174.
3. Cragg, G. M.; Newman, D. J. and Snader, K. M. (1997) Natural Products in Drug Discovery and Development. J. Nat. Prod. 60 (1): 52-60.
4. Farnsworth, N. R.; Akerele, O.; Bingel, A. S.; Soejarto, D. D. and Guo, Z. (1985) Medicinal Plants in Therapy. Bull WHO 63: 965-981.
5. Kinghorn, A. D. (1994) The Discovery of Drugs from Higher Plants. In: Gullo, V. P. (ed.) *The Discovery of Natural Products with Therapeutic Potential*. Boston, MA, Butterworth-Heinemann, pp. 81-108.
6. a) Neuss, N.; Gorman, M.; Svoboda, G. H.; Maciak, G. and Beer, C. T. (1959) *Vinca* alkaloids. III. Characterization of Leurosine and Vincaleukoblastine, New Alkaloids from *Vinca rosea*. J. Am. Chem. Soc. (1959), 81: 4754-4755. b) Johnson, I. S.; Wright, H. F.; Svoboda, G. H. and Vlantis, J. (1960) Antitumor Principles Derived from *Vinca rosea* Linn. I. Vincaleukoblastine and Leurosine. Cancer Res. 20: 1016-1022. c) Neuss, N.; Gorman, M. and Cone, N. J. (1964) The Structures of the Oncolytic Alkaloids Vinblastine (VLB) and Vincristine (VCR) from *Vinca rosea* (*Catharanthus roseus*). Lloydia 27(4): 389-392. d) Neuss, N.; Gorman, M.; Hargrove, W. Cone, N. J.; Biemann, K.; Buechi, G. and Manning, R. E. (1964) *Vinca* Alkaloids XXI. Structures of the Oncolytic Alkaloids Vinblastine and Vincristine. J. Am. Chem. Soc. 86(7): 1440-1442.
7. Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellina, II, J. H.; McMahon, J. B.; Currens, M. J., Buckheit, R. W.; Hughes, S. H.; Cragg, G. M. and Boyd, M. R. (1992)

The Calanolides, a Novel HIV-Inhibitory Class of Coumarin Derivatives from the Tropical Rainforest Tree, *Calophyllum lanigerum*. J. Med. Chem. 35(15): 2735-2743.

8. Shu, Y.-Z. (1998) Recent Natural Products Based Dug Development: A Pharmaceutical Industry Perspective. J. Nat. Prod. 61 (8): 1053-1071.
9. Currens, M. J.; Gulakowski, R. J.; Mariner, J. M.; Moran, R. A.; Buckheit Jr., R. W.; Gustafson, K. R.; McMahon, J. B. and Boyd, M. R. (1996) Antiviral Activity and Mechanism of Action of Calanolide A Against the Human Immunodeficiency Virus Type-1. J. Pharmacol. Exp. Ther. 279(2): 645-651.
10. Hizi, A.; Tal, R.; Shaharabany, M.; Currens, M. J.; Boyd, M. R.; Hughes, S. H. and McMahon, J. B. (1993) Specific Inhibition of the Reverse Transcriptase of Human Immunodeficiency Virus Type 1 and the Chimeric Enzymes of Human Immunodeficiency Virus Type1 and Type 2 by Nonnucleoside Inhibitors. Antimicrobial Agents and Chemotherapy 37 (5): 1037-1042.
11. Buckheit, Jr., R. W.; White, E. L.; Fliakas-Boltz, V.; Russel, J.; Stup, T. L.; Kinjerski, T. L.; Osterling, M. C.; Weigand, A. and Bader, J. P. (1999) Unique Anti-Human Immunodeficiency Virus Activities of the Nonnucleoside Reverse Transcriptase Inhibitors Calonolide A, Costatolide, and Dihydrocostatolide. Antimicrobial Agents and Chemotherapy 43 (8): 1827-1834.
12. Buckheit, R. W.; Fliakas-Boltz, V.; Decker, W. D.; Roberson, J. L.; Stup, T. L.; Pyle, C. A.; White, E. L.; McMahon, J. B.; Currens, M. J.; Boyd, M. R. and Bader, J. P. (1995) Comparative Anti-HIV Evaluation of Diverse HIV-1-specific Reverse Transcriptase Inhibitor-resistant Virus Isolates Demonstrates the Existence of Distinct Phenotypic Subgroups. Antiviral Res. 26: 117-132.
13. McKee, T. C.; Covington, C. D.; Fuller, R. W.; Bokesch, H. R.; Young, S.; Cardellina II, J. H.; Kadushin, M. R.; Soejarto, D. D.; Stevens, P. F.; Cragg, G. M. and Boyd, M. R. (1998) Pyranocoumarins from Tropical Species of the Genus *Calophyllum*: A Chemotaxonomic Study of Extracts in the National Cancer Institute Collection. J. Nat. Prod. 61 (10): 1252-1256.
14. Pengsuparp, T. (1996) Specific Inhibition of Human Immunodeficiency Virus Type 1 Reverse Transcriptase Mediated by Soulattrolide, a Coumarin Isolated from the Latex of *Calophyllum teysmannii*. J. Nat. Prod. 59 (9): 839-842.
15. Patil, A. D.; Freyer A. J.; Eggleston, D. S. Haltiwanger R. C.; Bean, M. F.; Taylor, P. B.; Caranfa, M. J.; Breen, A. L.; Bartus, H. R.; Johnson, R. K.; Hertzberg, R. P. and

Westley, J. W. (1993) The Inophyllums, Novel Inhibitors of HIV-1 Reverse Transcriptase Isolated from Malaysian Tree, *Calophyllum inophyllum* Linn. J. Med. Chem. 36 (26): 4131-4138.

16. Boyd, M. R.; Hallock, Y. F.; Cardellina II, J. H.; Manfredi, K. P., Blunt, J. W.; MacMahon, J. B.; Buckheit, Jr., R. W.; Bringmann, G.; Schäffer, M.; Cragg, G. M.; Thomas, D. W. and Jato, J. G. (1994) Anti-HIV Michellamines from *Ancistrocladus korupensis*. J. Med. Chem. 37 (12): 1740-1745.
17. McMahon, J. B.; Currens, M. J.; Gulakowski, R. J.; Buckheit, Jr., R. W.; Lackman-Smith, C.; Hallock, Y. F. and Boyd, M. R. (1995) Michellamine B, a Novel Plant Alkaloid, Inhibits Human Immunodeficiency Virus-Induced Cell Killing by at Least Two Distinct Mechanisms. Antimicrobial Agents and Chemotherapy 39 (2): 484-488.
18. Vlietinck, A. J.; De Bruyne, T.; Apers, S. and Pieters, L. A. (1998) Plant-Derived Leading Compounds for Chemotherapy of Human Immunodeficiency Virus (HIV) Infection. Planta Med. 64: 97-109.
19. Houghton, P. J.; Woldemarian, T. Z.; Khan, A. I.; Burke, A. and Mahmood, N. (1994) Antiviral Activity of Natural and Semi-synthetic Chromone Alkaloids. Antiviral Res. 25 (3-4): 235-244.
20. Pettit, G. R.; Gaddamidi, V.; Herald, D. L.; Singh, S. B.; Cragg, G. M.and Schmidt, J. M. (1986) Antineoplastic Agents, 120. *Pancratium littorale*. J. Nat. Prod. 49 (6): 995-1002.
21. Gabrelsen, B.; Monath, T. P.; Huggins, J. W. and Kefauver, D. F. (1992). Antiviral (RNA) Activity of Selected Amaryllidaceae Isoquinoline Constituents and Synthesis of Related Substances. J. Nat. Prod. 55 (11): 1569-1581.
22. Klayman, D. L. (1985). Qinghaosu (artemisinin): An Antiviral Drug from China. Science 228: 1049-1055.
23. Luo, X.-D., Shen, C.-C. (1987). The Chemistry, Pharmacology, and Clinical Applications of Qinghaosu (Artemisinin) and Its Derivatives. Med. Res. Rev. 7: 29-52.
24. De Vries, P. J. and Dien, T. K. (1996). Clinical Pharmacology and Therapeutic Potential of Artemisinin and Its Derivatives in the Treatment of Malaria. Drugs 52 (6): 818-836.
25. Allen, J. R. F. and Holmstedt, B. R. (1980). The Simple ß-Carboline Alkaloids. Phytochemistry 19: 1573-1582.
26. Lutomski, V. J.; Malek, B. and Raybacka, L. (1975) Pharmacochemical Investigation of the Raw Materials from *Passiflora* Genus. Planta Med. 27: 112-121.

27. Bruneton, J. (1999) *Pharmacognosie: Phytochimie, Plantes Médicinales*. 3è Edition, Editions TEC & DOC, Paris Cedex 08, pp. 409-973.
28. Cavin, J. C.; Krasmer, S. M. and Rodriguez E. (1987). Plant-Derived Alkaloids Active Against *Trypanosoma cruzi*. J. Ethnopharmacol. 19: 89-94.
29. (a) Proskurnina, N F. and Yakovleva, A. P. (1952) Alkaloids of *Galanthus woronowi*. II. Isolation of a New Alkaloid. Zh. Obshch. Khim. (J. Gen. Chem.) 22, 1899-1902. (Chem. Abstr. 47, 6959, 1953). (b) Boit, H.-G. (1954) Uber die Alkaloide der Zwiebeln von *Galanthus nivalis* (III. Mitteil. Uber Amaryllidaceen-Alkaloide). Chem. Ber. 87: 724-725. (c) Tanahshi, T.; Poulev, A. and Zenk, M. H. (1990) Radioimmunoassay for the Quantitative Determination of Galanthamine. Planta Med. 56: 77-81.
30. Paskov, D. S. (1986) Galanthamine. In: Kharkevich, D. A. (ed.) *New Neuromuscular Blocking Agents*, Vol. 79, Handbook of Experimental Pharmacology, Springer-Verlag, Berlin, pp. 653-672.
31. (a) Dal-Bianco, P.; Maly, J.; Wober, C.; Lind, C.; Koch, G.; Hufgard, J.; Marshall, I.; Mraz, M. and Deecke, L. (1991). Galanthamine Treatment in Alzheimer's Disease. J. Neural Transm. 33 (suppl.): 59-63. (b) Thomsen, T., Bickel, U., Fisher, J. P. and Kewitz, H. (1990a) Galanthamine Hydrobromide in a Long-term Treatment of Alzheimer's Disease. Dementia 1: 46-51. (c) Harvey, A. L. (1995) The Pharmacology of Galanthamine and Its Analogues. Pharmac. Ther. 68(1): 113-128.
32. Rainer, M. and Mucke, H. A. M. (1998) Long-term Cognitive Benefit from Galanthamine in Alzheimer's Disease. International Journal of Geriatric Psychopharmacology 1(4): 197-201.
33. (a) Liu, J. S.; Zhu, Y. L.; Yu, C. M.; Zhou, Y. Z.; Han, Y. Y.; Wu, F. W. and Qi, B. F. (1986) The Structures of Huperzine A and B, Two New Alkaloids Exhibiting Marked Anticholinesterase Activity. Can. J. Chem. 64(4): 837-839. (b) Liu, J. S.; Yu, C.; Zhou, Y.; Han, Y.; Wu, F.; Qi, B. and Zhu, Y. (1986) Chemistry of Huperzine A and B. Huaxue Xuebao 44(10): 1035-1040. (c) Zhou, B.-N.; Zhu, D.-Y.; Huang, M.-F.; Lin, L.-J.; Lin, L.-Z.; Yue-Yi-Han and Cordell, G. A. (1993) NMR Assignments of Huperzine A, Serratinine and Lucidioline. Phytochemistry 34(5): 1425-1428.
34. Wang, Y.; Yue, D. and Tang, X. (1986) Anticholinesterase Activity of Huperzine A. Zhongguo Yaoli Xuebao 7(2): 110-113.
35. Prous, J. R. (1996) Drugs Future 21: 651-652.

36. (a) Qian, L. and Ji, R. (1989) A Total Synthesis of (+/-) Huperzine A. Tetrahedron Lett. 30(16): 2089-2090. (b) Chen, W. and Yang, F. (1995) Asymmetric Total Synthesis of Optically Active Huperzine A. Zhongguo Yaowu Huaxue Zazhi 5(1): 10-17.
37. Luo, J. (1998) In: *Natural Products II, New Technology to Increase Efficiency and Speed*; Sapienza, D. M. (ed.), IBC Library Series N° 982, International Business Communications, Southborough, MA, pp. 119-145.
38. (a) Bhat, S. V.; Bajwa, B. S.; Dornauer, H. and deSouza, N. J. (1977) Structures and Stereochemistry of New Labdane Diterpenoids from *Coleus forskohlii* Briq. Tetrahedron Lett. 19: 1669-1672. (b) Saksema, A. K.; Geen, M. J.; Shue, H. J.; Wong, J. K. and McPhail, A. T. (1985) Identity of Coleonol with Forskolin: Structure Revision of a Base-catalyzed Rearrangement Product. Tetrahedron Lett. 26(5): 551-554.
39. Ammon, H. P. T. and Müller, A. B. (1985) Forskolin: From an Ayurvedic Remedy to a Modern Agent. Planta Med. 51 (6): 473-477.
40. Tatee, T.; Narita, A.; Narita, K.; Izumi, G.; Takahira, T.; Sakurai, M.; Fujita, A.; Hosono, M.; Yamashita, K. Enomoto, K. and Shiozawa, A. (1996). Forskolin Derivatives. I. Synthesis, and Cardiovascular and Adenylate Cyclase-Stimulating Activities of Water-Soluble Forskolins. Chem. Pharm. Bull. 44 (12): 2274-2279.
41. (a) Dhainaut, J. F.; Tenaillon, A.; Le Tulzo, Y.; Schlemmer, B.; Solet, J. P.; Wolff, J.; Holzapfel, L.; Zeni, F.; Dreyfus, D. and Mira, J. P. (1994) +Platelet-activating Factor Receptor Antagonist BN 52021 in the Treatment of Severe Sepsis: a Randomized, Double-blind, Placebo-controlled, Multicenter Clinical Trial. BN 52021 Sepsis Study Group. Crit. Care Med. 22(11): 1720-1728. (b) Prous, J. R. (1995) Drugs Future 20: 690-691.
42. Prous, J. R. (1995) Drugs Future 20: 524.
43. Hartwell, J. L. (1982). *Plants Used Against Cancer*. Lawrance, MA, Quaterman.
44. Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M. and Hogan, F. (1995). Antineoplastic Agents. 291. Isolation and Synthesis of Combretastatins A-4, A-5, and A-6 (1a) J. Med. Chem. 38(10): 1666-1672.
45. Philipson, J. D. (1999) New Drugs from Nature - It Could be Yew. Phytother. Res. 13: 2-8.
46. Kingston, D. G. I. (1993) Taxol, an Exciting Anticancer Drug from *Taxus brevifolia*. In: Kinghorn, A. D. and Bahandrin, M. F (eds.) *Human Medicinal Agents from Plants*. American Chemical Society, Washington, pp. 138-148.

47. Cragg, G. M.; Schepartz, S. A.; Suffness, and Grever M. R. (1993). The Taxol Supply Crisis. New NCI Policies for Handling the Large-Scale Production of Novel Natural Product Anticancer and Anti-HIV Agents. J. Nat. Prod. 56 (10): 1657-1668.
48. Cortes, J. E. and Pazdur, R. (1995) Docetaxel. J. Clin. Oncol. 13(10): 2643-2655.
49. Dev, S. and Koul, O. (1997) *Insecticides of Natural Origin*. Harwood Academic Publishers, The Nederlands.
50. Wedge, D. E. and Camper, N. D. (2000) Connections between Agrochemicals and Pharmaceuticals. In: Cutler, S. J. and Cutler, H. G. (eds.) *Biologically Active Natural Products: Pharmaceuticals*. CRC Press LLC, New York, p.2.
51. Henrick, C. A. (1995) Pyrethroids. In: Godfrey, C. R. A. (ed.) *Agrochemicals from Natural Products*. Marcel Dekker, Inc. New York, pp. 63-145.
52. Jacobson, M. (1989) Botanical Pesticides: Past, Present, and Future. In: Arnason, J. T.; Philogène, B. J. R. and Morand, P. (eds.) *Insecticides of Plant Origin*. ACS Symposium Series, American Chemical Society, Washington, D.C., p.1.
53. Casida, J. E. (ed.) (1973) *Pyrethrum, The Natural Insecticide*. Academic Press, New York.
54. Soreng, R. J. and Cope, E. A. (1991) *Baileya*, 23: 145.
55. Dev, S.; Narula, A. P. S. and Yadav, J. S. (1982) *Handbook of Terpenoids: Monoterpenoids*, Vol. II, Boca Raton: CRC Press.
56. (a) Elliot, M. (1977) Synthetic Pyretroids. In: Elliot, M. (ed.) *Synthetic Pyrethroids*. American Chemical Society. (b) Naumann, K. (1990) Chemistry of Plant Protection, Vol.4. In: Bowers, W. S.; Ebing, W.; Martin, D.; Wegler, R. (eds.) *Synthetic Pyrethroid Insecticides: Structures and Properties*. Springer-Verlag, Berlin. (c) Naumann, K. (1990) Chemistry of Plant Protection, Vol.5. In: Bowers, W. S.; Ebing, W.; Martin, D.; Wegler, R. (eds.). *Synthetic Pyrethroid Insecticides: Chemistry and Patents*. Springer-Verlag, Berlin.
57. Pruthi, H. S.; Singh, M. (1944) Stored Grain Pests and their Control. Imperial Council of Agr. Res., Misc. Bull. N° 57.
58. (a) Butterworth, J. H.; Morgan, E. D. and Percy, G. R. (1972) Structure of Azadirachtin. Functional groups. J. Chem. Soc. Perkin Trans.1 19: 2445-2450. (b) Jone, P. S.; Ley, S. V.; Morgan, E. D. and Santafianos, D. (1989) Phytochemical Pesticides, Vol. 1. In: M. Jacobson (ed.) *The Neem Tree*. CRC Press, Boca Raton, FL, p.19.

59. (a) Saxena, R. C. (1989) Insecticides from Neem. In: Arnason, J. T.; Philogène, B. J. R. and Morand, P. (eds.) *Insecticides of Plant Origin*. ACS Symposium Series, American Chemical Society, Washington, D. C, pp.110-135. (b) Addor, R. W. (1995) Insecticides. In: Godfrey, C. R. A. (ed.) *Agrochemicals from Natural Products*. Marcel Dekker, Inc. New York, pp. 1-62.

60. Casida, J. E. (1976) Prospects for New Types of Insecticides. In: Metcalf, R. L. and Mckelvey, J. J. (eds.) *The Future for Insecticides: Needs and Prospects*. Wiley-Interscience, New York, pp. 349-366.

61. (a) Saxena, R. C. and Dale, D. (1989) Plant Defense Mechanisms Against Insects. In: Swaminathan, M. S. and Kochhar, S. L. *Plants and Society*. Macmillan, London, pp. 350-366. (b) Jacobson, M. and Crosby, D. C. (eds.) (1971) *Naturally Occurring Insecticides*. Marcel Dekker, New York, p.186-224.

62. Ujvàry, I.; Eya, B. K.; Grendell, R. L.; Toia, R. F. and Casida, J. E. (1991) Insecticidal Activity of Various 3-Acyl and Other Derivatives of Veracevine Relative to *Veratum* Alkaloids Veratridine and Cevadine. J. Agr. Food Chem. 39 (10): 1875-1881.

63. Jefferies, P. R.; Toia, R. F.; Brannigan, B.; Pessah, I. and Casida, E. J. (1992) Ryania Insecticide: Analysis and Biological Activity of 10 Natural Ryanoids. J. Agr. Food Chem. (40(1): 142-146.

64. Wang, M.; Qin H.; Kong M.; Yanzi, L. (1991) Insecticidal Sesquiterpene Polyol Ester from *Celastrus angulatus*. Phytochemistry 30(12): 3931-3933.

65. (a) Wakabayashi, N.; Wu, W. J.; Waters, R. M.; Redfern, R. E.; Mills, G. D., Jr.; DeMillo, A. B.; Lusby, W. R. and Andrzejewski, D. (1988) Celangulin: a Nonalkaloidal Insect Antifeedant from Chinese Bittersweet *Celastrus angulatus*. J. Nat. Prod. 51(3): 537-542. (b) Liu, J. K.; Jia, Z.; Wu, D.; Zuou, J. and Wang, Q. C. (1990) Insect Antifeeding Agents: Sesquiterpene Alkaloids from *Celastrus angulatus*. Phytochemistry 29(8): 2503-2506.

66. Marshall, G. T.; Klocke, J. A.; Lin, L. J. and Kinghorn, A. D. (1985) Effects of Diterpene Esters of Tigliane, Daphnane, Ingenane, and Lathyrane Types on Pink Bollworm, *Pectinophora gossypiella* Sanders (*Lepidoptera*: Gelechiidae). J. Chem. Ecol. 11(2): 191-206.

67. Klocke, J. A.; Arisawa, M.; Handa, S. S.; Kinghorn, A. D.; Cordell, G. A. and Farnsworth, N. R. (1985) Growth Inhibitory, Insecticidal and Antifeedant Effects of

Some Antileukemic and Cytotoxic Quassinoids on Two Species of Agricultural Pests. Experientia 41: 379-382.

68. Robbrecht, E. (1988) *Tropical Woody Rubiaceae: Characteristic Features and Progressions, Contributions to a New Subfamilial Classification*. Nationale Plantentuin van België, Meise, pp. 13-14.
69. Verdcourt, B. (1976) Rubiaceae (Part 1). In: Polhill, R.M. (ed.), *Flora of Tropical East Africa*, Crown Agents For Oversea Governments and Administrations, Whitefriars Press Ltd, London and Tonbridge, p. 1-213.
70. Inouye, H.; Takeda, Y.; Nishimura, H.; Kanoni, A.; Okuda, T. and Puff, C. (1988) Chemotaxonomic Studies of Rubiaceous Plants Containing Iridoid Glycosides. Phytochemistry 27(8): 2591-2598.
71. Wijnsma, R. and Verpoorte, R. (1986) Anthraquinones in the Rubiaceae. In: Herz, W.; Grisebach, H.; Kirby, G. W.; and Tamm, Ch. (eds.) *Progress in the Chemistry of Organic Natural Products*. Wien, Springer-Verlag, New York, pp. 79-149.
72. Kisakuerek, M. V.; Leeuwenberg, A. J. M. and Hesse, M. (1983). A Chemotaxonomic Investigation of the Plant Families of Apocyanaceae, Loganiaceae, and Rubiaceae by the Indole Alkaloid Content. In: Pelletier, S. W. (ed.): *Alkaloids, Chemical and Biological Perspectives*. Wiley and Sons, New York.
73. Watt, J. M. and Breyer-Brandwijk, M. G. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. Second Edition, E. & S. Livingstone LTD. Edinburgh and London, pp. 900-905.
74. *Pharmacopeia of the People's Republic of China*. Guangdong Science and Technology Press. Guangzhou, People's Republic of China, 1992, p. 179.
75. Hocking, G. M. (1997). *A Dictionary of Natural Products*. Plexus Publishing. Medford, NJ, p.679.
76. (a) Itokawa, H.; Ibraheim, Z. Z.; Qiao, Y.-F. and Takeya, K. (1993) Anthraquinones, Naphthohydroquinones and Naphthohydroquinone Dimers from *Rubia cordifolia* and their Cytotoxic Activity. Chem. Pharm. Bull. 41 (10): 1869-1872. (b) Itokawa, H.; Qiao, Y. and Takeya, K. (1989) Anthraquinones and Naphthoquinones from *Rubia cordifolia*. Phytochemistry 28 (12): 3465-3468.
77. (a) Koyama, J.; Ogura, T.; Tagahara, K.; Konoshima, T. and Kozuka, M. (1992) Two Naphthoquinones from *Rubia cordifolia*. Phytochemistry 31 (8): 2907-2908. (b) Dosseh,

C.; Tessier, A. M. and Delaveau, P. (1981) Racines de *Rubia cordifolia* II: Nouvelles Quinones. Planta Med. 43: 141-147.

78. Chang, L. C.; Chavez, D.; Gills, J. J.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. (2000) Rubiasins A-C, New Anthracene Derivatives from the Roots and Stems of *Rubia cordifolia*. Tetrahedron Lett. 41: 7157-7162.
79. (a) Itokawa, H.; Takeya, K.; Mihara, K.; Mori, N.; Hamanaka, T.; Sonobe, T. and Iitaka, Y. (1983) Studies on the Antitumor Cyclic Hexapeptides Obtained from *Rubiae radix*. Chem. Pharm. Bull. 31(4): 1424-1427. (b) Itokawa, H.; Takeya, K.; Mori, N.; Hamanaka, T.; Sonobe, T. and Mihara, H. (1984) Isolation and Antitumor Activity of Cyclic Hexapeptides Isolated from *Rubiae radix*. Chem. Pharm. Bull. 32(1): 284-290.
80. (a) Hitotsuyanagi, Y.; Aihara, T. and Takeya, K. (2000) RA-dimer A, a Novel Dimeric Antitumor Bicyclic Hexapeptide from *Rubia cordifolia* L. Tetrahedron Lett. 41: 6127-6130. (b) Itokawa, H.; Morita, H.; Takeya, K.; Tomioka, N.; Itai, A. and Iitaka, Y. (1991) New Antitumor Bicyclic Hexapeptides, RA–VI and –VIII from *Rubia cordifolia*; Conformation Activity Relationship II. Tetrahedron 47 (34): 7007-7020. (c) Morita, H.; Yamaniya, T.; Takeya, K. and Itokawa, H. (1992) New Antitumor Bicyclic Hexapeptides, RA–XI, –XII, –XIII and –XIV from *Rubia Cordifolia* Chem. Pharm. Bull. 40(5): 1352-1354.
81. Boullard, B. (2001) *Plantes Médicinales du Monde: Croyances et Réalités*, Editions ESTEM, Paris, p. 356.
82. Abbiw, D. (1990) Useful Plants of Ghana, Intermediate Technologies Publications Ltd., p.154.
83. La Barre, J. and Wirtheimer, C. (1962) Etude Comparative des Effets Hypotenseurs des Extraits et Derivés du *Rauwolfia vomitoria* et du *Morinda lucida* chez le Rat Eveillé. Arch. Intern. Pharmacodyn. Ther. 139: 596-603.
84. Sandberg, F. and Cronlund, A. (1982) An Ethnopharmacological Inventory of Medicinal and Toxic Plants from Equatorial Africa. J. Ethnopharmacol. 5: 187-204.
85. Gbeassor, M.; Kossou, Y.; Amegbo, K.; De Souza, C.; Koumaglo, K. and Denke, A.(1989) Antimalarial Effects of Eight African Medicinal Plants. J. Ethnopharmacol. 25: 115-118.
86. Koumaglo, K.; Gbeassor, M.; Nikabu, O.; De Souza, C. and Werner, W. (1992) Effects of Three Compounds Extracted from *Morinda lucida* on *Plasmodium falciparum*. Planta Med. 58: 533-534.

87. Sittie, A. A.; Lemmich, E.; Olsen, C. E.; Hviid, L.; Kharazmi, A.; Nkrumah, F. K., Christensen, S. B. (1999) Structure-Activity Studies: *In vitro* Antileishmanial and Antimalarial Activities of Anthraquinones from *Morinda lucida*. Planta Med. 65: 259-261.

88. Yff, B. T. S.; Lindsey, K. L.; Taylor, M. B.; Erasmus, D. G. and Jäger, A. K. (2002) The Pharmacological Screening of *Pentanisia prunelloides* and the Isolation of the Antibacterial Compound Palmitic Acid. J. Ethnopharmacol. 79: 101-107.

89. Kaido, T. L.; Veale, D. J. H.; Havlik, I. and Rama, D. B. K. (1997) Preliminary Screening of Plants Used in South Africa as Traditional Herbal Remedies During Pregnancy and Labour. J. Ethnopharmacol. 55: 185-191.

90. (a) Troupin, G. (Ed.) (1985) *Flore du Rwanda*: Spermatophytes. Vol. III. Annales-Série IN-8°-Sciences Economiques-n°15, Musée Royale de l'Afrique Centrale. Tervuren, Belgique, pp. 195-198. (b) Dessein, S.; Scheltens, A.; Huysmans, S.; Robbrecht, E. and Smets, E. (2000) Pollen Morphological Survey of *Pentas* (Rubiaceae-Rubioideae) and Its Closest Allies. Review of Palaeobotany and Palynology 112: 189-205.

91. Kokwaro, J.O. (ed.) (1976) *Medicinal Plants of East Africa*. East African Literature Bureau, Nairobi, p. 190-191.

92. (a) Van Puyvelde, L.; Geysen, D.; Ayobangira, F.X.; Hakizamungu, E.; Nshimyimana, A. and Kalisa, A. (1985) Screening of Medicinal Plants of Rwanda for Acaricidal Activity. J. Ethnopharmacol. 13: 209-215. (b) De Kimpe, N.; Van Puyvelde, L.; Schripsema, J.; Erkelens, C. and Verpoorte, R. (1993) Complete Proton and Carbon-13 NMR Spectral Assignments of Pentalongin. Magnetic Resonance in Chemistry 31(4): 329-30.

93. Hari, L.; De Buyck, L. F. and De Pooter, H. L. (1991) Naphthoquinoid Pigments from *Pentas longiflora*. Phytochemistry 30(5): 1726-1727.

94. Schildknecht, H.; Straub, F. and Scheidel, V. (1976) Mollugin, ein Neuer Farbstoff aus Rhizomen des Wiesen-Labkrautes *Galium mollugo* L. Liebigs Ann. Chem., 1295-1306.

95. Itokawa, H.; Kazuhiko, M.; Koichi, T. (1983) Studies on a Novel Anthraquinone and Its Glycosides Isolated from *Rubia cordifolia* and *R. akane*. Chem. Pharm. Bull. 31 (7): 2353-2358.

96. El-Hady, S. (1999) Dissertation (PhD): *Isolation and Structural Elucidation of Natural Products from Pentas longiflora* Oliver *and Gossypioides kirkii* (Mast.) J.B. Hutch.,

Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium.

97. Harouna, H.; Faure, R.; Elias R.; Debrauwer, L.; Saadou, M.; Balansard, G. and Boudon, G. (1995) Harounoside, a Pentalongin Hydroquinone Diglycoside from *Mitracarpus scaber*. Phytochemistry 39(6): 1483-1484.
98. Adjanohoun, E. J.; Ahyi, A. M. R.; Dan Dicko, L.; Daouda, H.; Delmas, M.; de Souza, S.; Garba, M.; Guinko, S.; Kayonga, A.; N'Golo, D.; Raynal, J. L. and Saadou, M. (1985) Contribution aux Etudes Ethnobotaniques et Floristiques du Niger, A. C. C. T., France.
99. Naito, T.; Makita, Y.; Yazaki, S. and Kaneko, C. (1986) Cycloadditions in Synthesis. XXVI. 1,2-Dihydrocyclobuta[*b*]naphthalene-3,8-diones: Synthesis by Photochemical Means and Their Reactions *via* 2,3-Dimethylene-1,4-dioxo-1,2,3,4-tetrahydronaphthalenes. Chem. Pharm. Bull. 34(4): 1505-1517.
100. (a) Kesteleyn, B.; De Kimpe, N. and Van Puyvelde, L. (1999) Total Synthesis of Two Naphthoquinone Antibiotics, Psychorubrin and Pentalongin, and Their C(1)-Substituted Alkyl and Aryl Derivatives. J. Org. Chem. 64(4): 1173-1179. (b) Kesteleyn, B.; De Kimpe, N. and Van Puyvelde, L. (1999) Synthesis of Two Naphthoquinone Antibiotics Pentalongin and Psychorubrin. Synthesis 11: 1881-1883. (c) Van, T. N.; Kesteleyn, B. and De Kimpe, N. (2001) Synthesis of 1,3-disubstituted naphtho[2,3-*c*]pyran-5,10-diones. Tetrahedron 57: 4213-4219.
101. Hayashi, T.; Smith, F. T. and Lee, K. H. (1987) Anti-tumor Agents. 89. Psychorubrin, a New Cytotoxic Naphthoquinone from *Psychotria rubra* and Its Structure-Activity Relationships. J. Med. Chem. 30(11): 2005-2008.
102. Itokawa, H.; Qiao, Y. and Takeya, K. (1991) Anthraquinones, Naphthoquinones and Naphthohydroquinones from *Rubia onchotricha*. Phytochemistry 30(2): 637-640.
103. Heide, L. and Leistner, E. (1981) 2-Methoxycarbonyl-3-prenyl-1,4-naphthoquinone, a Metabolite Related to the Biosynthesis of Mollugin and Anthraquinones in *Galium mollugo* L. J. Chem. Soc., Chem. Commun. 7: 334-336.
104. El Hady, S.; Bukuru, J.; Kesteleyn, B.; Van Puyvelde, L.; Van, T. N. and De Kimpe, N. (2002) New Pyranonaphthoquinone and Pyranonaphthohydroquinone from the Roots of *Pentas longiflora*. J. Nat. Prod. 65(9): 1377-1379.

105. Van Puyvelde, L.; El Hady, S.; De Kimpe, N.; Feneau-Dupont, J. and Declercq, J.-P. (1998) Isagarin, a New Type of Tetracyclic Naphthohydroquinone from the Roots of *Pentas longiflora*. J. Nat. Prod. 61:1020-1021.

106. Kesteleyn, B.; Van Puyvelde, L. and De Kimpe, N. (1999) Synthesis of Isagarin, a New Type of Tetracyclic Naphthoquinone from *Pentas longiflora*. J. Org. Chem. 64(2): 438-440.

107. Sartori, G.; Bigi, F.; Tao, X.; Porta, C.; Maggi, R.; Predieri, G.; Lanfranchi, M.and Pellinghelli, M. A.(1995) An Investigation of the Reaction Mechanism of the Bis-acylation of Aromatics with *O*-Phthaloyl Dichlorides: Regioselective Synthesis of Anthraquinones. J. Org. Chem. 60(20): 6588-6591.

108. (a) Cserjesi, A. J. and Smith, R. S. (1968) Anthraquinone Production by a Fungus Causing Black Heartwood Stain in Yellow Cedar. Mycopathol. Mycol. Appl. 35(2): 91-96. (b) Van Eijk, G. W. and Roeijmans, H. J. (1984) Separation and Identification of Naturally Occurring Anthraquinones by Capillary Gas Chromatography and Gas Chromatography-Mass Spectrometry. J. Chromatogr. 295 (2): 497-502.

109. Kawasaki, Y.; Goda, Y.; Yoshihira, K. and Noguchi, H. (1990) A New Anthraquinone from *Rubia tinctorum*. Shoyakugaku Zasshi 44(2): 95-97.

110. Arisawa, M.; Kinghorn, A. D.; Cordell, G. A. and Farnsworth N. R. (1983) Plant Anticancer Agents. J. Nat. Prod. 46(2): 222-225.

111. Kojima, H.; Sato, N.; Hatano, A. and Ogura, H. (1990) Sterol Glucosides from *Prunella vulgaris*. Phytochemistry 29(7): 2351-2355.

112. Kusamba, C.; Federici, F.; De Vicente, Y. and Galeffi, C. (1993) The Anthraquinones of *Pentas zanzibarica*. Fitoterapia 64 (1): 18-22.

113. Beentje, H.J. (ed.) (1994) *Kenya Trees: Shrubs and Lianes*, National Museums of Kenya, Nairobi, Kenya, p.531.

114. Itokawa, H.; Takeya, K.; Mori, N.; Kidokoro, S. and Yamamoto, H. (1984) Studies on Antitumor Cyclic Hexapeptides RA Obtained from Rubiae Radix, Rubiaceae (IV): Quantitative Determination of RA–VII and RA–V in Commercial Rubiae Radix and Collected Plants. Planta Med. 50 (4): 313-316.

115. Marsan, M.-P.; Warnock, W.; Muller, I.; Nakatani, Y.; Ourisson, G. and Milon, A. (1996) Synthesis of Deuterium-Labeled Plant Sterols and Analysis of Their Side-chain Mobility by Solid State Deuterium NMR. J. Org. Chem. 61(13): 4252-4257.

116. Lu, Y.; Xu, P.-J.; Chen, Z.-N. and Liu, G.-M. (1998) Anthraquinones Glycosides from *Rhynchotechum vestum*. Phytochemistry 49(4): 1135-1137.

117. Bennet, G. T.; Harrisson, L. J.; Lim, M.-S.; Sim, K.-Y.; Tan, E.-C. and Connolly, J. D. (1991) Geranyl Anthraquinones from the Bark of *Ploiarium alternifolium*. Phytochemistry 30 (9): 3141-3143.

118. Huang, L.; Fullas, F.; Mc Givney R. J.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Tuker, J. C.; Beecher, C. W. W.; Pezzuto, J. M. and Kinghorn, A. D. (1996) A New Prenylated Flavonol from the Root of *Petalostemon purpureus*. J. Nat. Prod. 59 (3), 290-292.

119. Tsopmo, A.; Tene, M.; Kamnaing, P.; Ngnokam, D.; Ayafor, J. F. and Sterner, O. (1998) Geranylated Flavonoids from *Dorstenia poinsettifolia*. Phytochemistry 48 (2): 345-348.

120. Decosterd, L. A.; Parsons, I. C.; Gustafson, K. R.; Cardellina II, J. H.; McMahon, J. B.; Cragg, G. M.; Murata, M.; Pannell, L. K.; Steiner, J. R.; Clardy, J. and Boyd, M. R. (1993) Structure, Absolute Stereochemistry, and Synthesis of Conocurvone, a Potent, Novel HIV-Inhibitory Naphthoquinone Trimer from a *Conospermum* sp. J. Am. Chem. Soc. 115 (15): 6673-6679.

121. (a) Williams, D. H. and Fleming, I. (1995) *Spectroscopic Methods in Organic Chemistry*, Fifth Edition, McGraw-Hill Publishing Company, Maidenhead, Berkshire, England, pp. 17-25. (b) Silverstein, R. M.; Basler, G. C. and Morill, T. C. (1998) *Identification Spectrométrique des Composés Organiques*, 5ème Edition, De Boeck & Larcier s.a., Paris, Bruxelles, pp. 27-311.

122. Bukuru, J. F.; Tuyen, N. V.; Puyvelde, L. V.; Mathenge, S. G.; Mudida, F. P. and De Kimpe, N. (2002) A Benzochromene from the Roots of *Pentas bussei*. J. Nat. Prod. 65 (5): 783-785.

123. (a) Crombie, L.; Redshaw, S. D.; Slack, D. A. and Whiting, D. A. (1979) Synthesis and Structure of Eriobrucinol and Isomeric "Cyclol" Meroterpenes. J. Chem. Soc., Chem. Commun.: 628-630. (b) Rashid, M. A.; Armstrong, J. A.; Gray, A. I. and Waterman, P. G. (1992) Tetra- and Pentacyclic 6-C-Monoterpenyl-5,7-dioxycoumarins from *Eriostemon brucei* and *E. brucei* Subspecies *cinereus*. Phytochemistry 31 (10): 3583-3588. (c) Crombie, L. and Ponsford, R. (1971) Synthesis of Cannabinoids by Pyridine-catalysed Citral-Olivetol Condensation: Synthesis and Structure of Cannabicyclol, Cannabichromen, (Haschish Extractives), Cytrilidene-cannabis, and Related compounds. J. Chem. Soc. (*C*): 796-804.

124. (a) Montero, J. L.and Winternitz, F. (1973) Structure de la Rubranine Chalcone Isolée du Bois de Rose *Aniba Rosaeodora* Ducke-II: Synthèse d'Analogues Cannabinoides Derivant de la Phloroacétophénone. Tetrahedron 29: 1243-1252. (b) Nakagawa, N.; Saito, S.; Suzuki, A. and Itoh, M. (1967) Magnetic Anisotropy of the Cyclobutane System with a Puckered Structure. Tetrahedron Lett. (11): 1003-1007.

125. (a) Bandaranayake, W. M.; Begley, M. J.; Brown, B. O.; Clarke, D. G.; Crombie, L. and Whiting, D. A. (1974) Synthesis of Acridone and Carbazole Alkaloids involving Pyridine-catalysed Chromen Formation: Crystal and Molecular Structure of Dibromocannabicyclol and its Bearing on the Structures of the "Cyclol" Alkaloids. J. Chem. Soc. Perkin Trans. I.: 998-1007. (b) Jefferies, P. R. and Worth, G. K. (1973) The Chemistry of the Western Australian Rutaceae – VI: Two Novel Coumarins from *Eriostemon brucei*. Tetrahedron 29: 903-908. (c) Gray, A. I; Rashid, M. A.and Waterman, P. G. (1992) NMR Assignments for the Pentacyclic Coumarins Bruceol and Deoxybruceol. J. Nat. Prod. 55(5): 681-684.

126. Crombie, L.; Redshaw, S. D.; Slack, D. A. and Whiting, D. A. (1983) Synthesis of (±)-Eriobrucinol and Regioisomeric Monoterpenoid Coumarins, Using Intramolecular Cycloadditions. J. Chem. Soc. Perkin Trans. I.: 1411-1416.

127. (a) Crombie, L.; Ponsford, R.; Shani, A.; Yagnitinsky, B. and Mechoulan, R. (1968) Hashish Components: Photochemical Production of Cannabicyclol from Cannabichromene. Tetrahedron Lett. (55): 5771-5772. (b) Crombie, L. and Ponsford, R. (1971) Pyridine-Catalysed Condensation of Citral with Phloroglucinols, a Novel Reaction Leading to Tetracyclic Bis-ethers and Chromens: Two-step Synthesis of (±)-Deoxybruceol. J. Chem. Soc. (*C*): 788-795.

128. Begley, M. J.; Crombie, L.; King, R. W.; Slack, D. A. and Whiting, D. A. (1977) Chromens and Citrans Derived from Phloroacetophenone and Phloroglucinaldehyde by Citral Condensation: Regioselectivity, Mechanism, and X-Ray Crystal Structures. J. Chem. Soc., Chem. Commun.: 2393-2402.

129. (a) Kureel, S. P.; Kapil, R. S. and Popli, S. P. (1969) Terpenoid Alkaloids from *Murraya koenigii* Spreng.–II: The Constitution of Cyclomahanimbine, Bicyclomahanimbine, and Mahanimbidine. Tetrahedron Lett.: 3857-3862. (b) Begley, M. J.; Clarke, D. G.; Crombie, L. and Whiting, D. A. (1970) The X-Ray Structure of Dibromocannabicyclol: Structure of Bicyclomahanimbine. J. Chem. Soc., Chem. Commun.: 1547-1548.

130. Ghisalberti, E. L.; Jefferies, P. R.; Raston, C. L.; Skelton, B. W.; White, A. H. and Worth, G. K. (1981) Structural Studies of Some Hydroxyeriobrucinol Derivatives. J. Chem. Soc. Perkin Trans. II.: 576-582.

131. (a) Morrison, R. T. and Boyd, R. N. (1987) *Organic Chemistry*, Fifth Edition, Allyn and Bacon, Inc, Boston, pp. 1092-1093. (b) Vollhardt, C. and Schore, N. E. (1995) *Traité de Chimie Organique* (Traduit de l'anglais par Paul Depovere), 2ème Edition, De Boeck-Wesmael S.A., Bruxelles, pp. 869-870, 885-892.

132. (a) Pozharskii, A. F.; Soldatenkov, A. T. and Katritzky, A. R. (eds). (1997) *Heterocycles in Life and Society*, John Wiley & Sons, Chichester, New York, pp. 63-69. (b) Kesteleyn, B. (1999) Dissertation (Ph.D): *Synthesis of Pyranonaphthoquinones and Related Naturally Occuring Heteroanthraquinones*, Ghent University, Belgium, pp. 23-24. (c) Fukuyama, Y.; Kiriyama, Y. and Kodama, M. (1998) Naturally Occurring 5-Lipoxygenase Inhibitors. VII. Practical Synthesis of Ardisiaquinones D, E, and F. Chem. Pharm. Bull. 46(11): 1770-1775.

133. Iwaki, H.; Fukuyama, Y. and Matsui, K. (1985) 1,4-Benzoquinone Derivatives and Benzene Derivatives. Eur. Pat. Appl. (EP 0 151 995 B1), 241 pp. (Chem. Abstr., 1986, 104: 33861).

134. Haraguchi, H.; Ohmi, I. and Kubo, I. (1996) Inhibition of Aldose Reductase by Maesanin and Related *p*-Benzoquinone Derivatives and Effects on Other Enzymes. Bioorg. Med. Chem. 4(1): 49-53.

135. El-Gamal, A. A.; Takeya, K.; Itokawa, H.; Halim, A. F.; Amer, M. M.; Saad, H.-E. A. and Awad, S. A. (1996) Anthraquinones from the Polar Fractions of *Galium sinaicum*. Phytochemistry 42(4): 1149-1155.

136. Tori, K.; Hirata, T.; Koshitani, O. and Suga, T. (1976) Carbon-13 NMR Spectral Studies of Aloein and its Derivatives. Carbon-13 Signal Assignment Problem of 4-Methoxy-2-pyrones. Tetrahedron Lett. (16): 1311-1314.

137. Kasai, R.; Suzuo, M.; Asakawa, J.-I. and Tanaka, O. (1977) Carbon-13 Chemical Shifts of Isoprenoid-$\beta$-D-Glucopyranosides and -$\beta$-D-Mannopyranosides. Stereochemical Influences of Aglycone Alcohols. Tetrahedron Lett. (2): 175-178.

138. (a) El-Emary, N. A. and Backheet, E. Y. (1998) Three Hydroxymethylanthraquinone Glycosides from *Rubia tinctorum*. Phytochemistry 49 (1): 277-279. (b) Perlin, A. S.; Casu, B. and Koch, H. J. (1970) Configurational and Conformational Influences on the Carbon-13 Chemical Shifts of some Carbohydrates. Can. J. Chem. 48: 2596-2606.

139. Coskun, M.; Tanker, N.; Sakushima, A.; Kitagawa, S. and Nishibe, S. (1984) An Anthraquinone Glycoside from *Rhamnus pallasii*. Phytochemistry 23(7): 1485-1487.

140. Uniyal, G. C.; Agrawal, P. K.; Thakur, R. S. and Sati O. P. (1990) Steroidal Glycosides from *Agave cantala*. Phytochemistry 29(3): 937-940.

141. Han, Y.-S.; Van der Heijden, R.; Lefeber, A. W. M.; Erkelens, C. and Verpoorte, R. (2002) Biosynthesis of Anthraquinones in Cell Cultures of *Cinchona* "Robusta" Proceeds *via* the Methylerythritol 4-phosphate Pathway. Phytochemistry 59(1): 45-55.

142. (a) Demagos, G. P.; Baltus, W. and Höfle, G. (1981) New Anthraquinones and Anthraquinone Glycosides from *Morinda lucida*. Z. Naturforsch. 36b: 1180-1184. (b) Kawasaki, Y.; Goda, Y. and Yoshihira, K. (1992) The Mutagenic Constituents of *Rubia tinctorum*. Chem. Pharm. Bull. 40 (6): 1504-1509.

143. (a) Jagodzinska, B. M.; Trimmer, J. S.; Fenical, W. and Djerassi, C. (1985) Sterols in Marine Invertebrates: 49. Isolation and Structure Elucidation of Eight New Polyhydroxylated Sterols from the Soft Coral *Sinuralia dissecta*. J. Org. Chem. 50(9): 1435-1439. (b) Eggersdorfer, M. L.; Kokke, W. C. M. C.; Crandell, C. W.; Hochlowski; J. E.; Djerassi, C. (1982) Sterols in Marine Invertebrates: 32. Isolation of 3$\beta$-(Hydroxymethyl)-*A*-nor-5$\alpha$-cholest-15-ene, the First Naturally Occurring Sterol with a 15-16 Double Bond. J. Org. Chem. 47(27): 5304-5309. (c) Eggert, H.; VanAntwerp, C. L.; Bhacca, N. S. and Djerassi, C. (1976) Carbon-13 Nuclear Magnetic Resonance Spectra of Hydroxy Steroids. J. Org. Chem. 41(1): 71-78.

144. Agnew, A. D. Q. (1974) *Upland Kenya Wild Flowers: A flora of the Ferns & Herbaceous Flowering Plants of Upland Kenya*, Oxford University Press, London, pp. 403-404.

145. Zhao, H. and Biehl, E. (1995) Preparation of Naturally Occurring Anthraquinones Using the Aryne Reaction. J. Nat. Prod. 58(12): 1970-1974.

146. (a) Briggs, L. H.; Beachen, J. F.; Cambie, R. C.; Dudman, N. P. B.; Steggles, A. W. and Rutledge, P. S. (1976) Chemistry of the *Coprosma* Genus. Part XIV. Constituents of Five New Zealand Species. J. Chem. Soc., Perkin I: 1789-1792. (b) Brew, E. J. C. and Thomson, R. H. (1971) Naturally Occurring Quinones. Part XIX. Anthraquinones in *Hymenodictyon excelsum* and *Damnacanthus major*. J. Chem. Soc. (C): 2001-2007. (c) Adesida, G. A. and Adesogan, E K. (1972) Oruwal, a Novel Dihydroanthraquinone Pigment from *Morinda lucida* Benth. J. Chem. Soc., Chem. Comm.: 405-406.

147. Dosseh, C.; Tessier, A. M. and Delaveau, P. (1981) Nouvelles Quinones des Racines de *Rubia cordifolia* L. III. Planta Med. 43: 360-366.

148. Chang, P. and Lee, K.-H. (1984) Cytotoxic Antileukemic Anthraquinones from *Morinda parvifolia*. Phytochemistry 23(8): 1733-1736.

149. Castonguay, A. and Brassard, P. (1977) C-Alkylation of 1,3-Dihydroxyanthraquinones. Total Synthesis of (±)-Averufin and (±)-Bipolarin. Can. J. Chem. 55: 1324-1332.

150. Koyama, J.; Okatani, T.; Tagahara, K.; Kouno, I. and Irie, H. (1992) Anthraquinones of *Damnacanthus indicus*. Phytochemistry 31(2): 709-710.

151. Kuiper, J. and Labadie, R. P. (1981) Polyploid Complexes Within the Genus *Galium*. Part I: Anthraquinones of *Galium album*. Planta Med. 42: 390-399.

152. Banthorpe, D. and White, J. J. (1995) Novel Anthraquinones from Undifferentiated Cell Cultures of *Galium verum*. Phytochemistry 38(1): 107-111.

153. (a) Koyama, J.; Ogura, T. and Tagahara, K. (1993) Anthraquinones of *Galium spurium*. Phytochemistry 33(6): 1540-1542. (b) Ismail, N. H.; Ali A. M.; Aimi, N.; Kitajima, M.; Takayama, H. and Lajis, N. H. (1997) Anthraquinones from *Morinda ellipta*. Phytochemistry 45(8): 1723-1725. (c) Ferrari, F.; Monache, G. D. and Alves de Lima, R. (1985) Two Naphthopyran Derivatives from *Faramea cyanea*. Phytochemistry 24(11): 2753-2755.

154. Kitajima, M.; Fischer, U.; Nakamura, M.; Ohsawa, M.; Ueno, M. Takayama, H.; Unger, M.; Stöckigt, J. and Aimi, N. (1998) Anthraquinones from *Ophiorrhiza pumila* Tissue and Cell Cultures. Phytochemistry 48(1): 107-111.

155. Thomson, R. H. (1987) *Naturally Occurring Quinones III, Recent Advances*. Chapman and Hall, London.

156. (a) Leistner, E. (1981) Biosynthesis of Plant Quinones. In: Conn, E. E. (ed.) *The Biochemistry of plants*, Vol. 7, Academic, London, pp. 403-423. (b) Leistner, E. (1985) Biosynthesis of Chorismate-derived Quinones in Plant Cells Cultures. In: Neumann, K. H.; Barz, W. and Reinhard, E. (eds.) *Primary and Secondary Metabolism of Plant Cell Cultures*. Springer, Berlin, pp. 215-224.

157. (a) Larsen, S. D. and Monti, S. A. (1977) Total Synthesis of Racemic $\alpha$-*trans*- and $\alpha$-*cis*-Bergamotene and $\alpha$-Pinene. J. Am. Chem. Soc. 99(4): 8015-8020. (b) Ray, R. and Matteson, S. (1980) Osmium Tetroxide Catalysed Hydroxylation of Hindered Olefins. Tetrahedron Lett. 21: 449-450.

158. Roberge G. and Brassard, P. (1978) Total Synthesis of the Methyl Ether Ester Derivatives of the Coccid Anthraquinones Laccaic Acid D and Kersemic Acid. J. Chem. Soc. Perkin I 9: 1041-1046.

159. Chong, R. and Clezy, P. S. (1966) *O*-Methylation of *β*-Dicarbonyl Compounds. Tetrahedron Lett. 7: 741-742.

160. Danishefsky, S and Kitahara, T. (1974) A Useful Diene for the Diels-Alder reaction. J. Am. Chem. Soc. 96: 7807-7808.

161. VanRheenen, V.; Kelly, R. C. and Cha, D. Y. (1976) An Improved Catalytic $OsO_4$ Oxidation of Olefins to *cis*-1,2-Glycols Using Tertiary Amine Oxides as the Oxidant. Tetrahedron Lett. 23: 1973-1976.

162. Tai, D.-F.; Lin, Y.-M. and Chen, F.-C. (1979) Components of *Hedyotis diffusa* Willd. Huaxue 3: 60-61. (Chem. Abstr. 94, 1996g, 1981).

163. Brisson, C. and Brassard, P. (1981) Regiospecific Reactions of Some Vinylogous Ketene Acetals with Haloquinones and Their Regioselective Formation by Dienolization. J. Org. Chem. 46(9): 1810-1814.

164. Hirose, Y.; Kuroiwa, M.; Yamashita, H.; Tanaka, T. and Megumi, T. (1973) Chemical Studies on the Natural Anthraquinones. I. Synthesis of Munjistin, Emodin, and 3-Hydroxy-2-methylanthraquinone. Chem. Pharm. Bull. 21(12): 2790-2793.

165. Gollapudi, B. B. and Krishna, G. (2000) Practical Aspects of Mutagenicity Testing Strategy: an Industrial Perspective. Mutation Res. 455: 21-28.

166. Mortelmans, K. and Zeiger, E. (2000) The Ames *Salmonella*/Microsome Mutagenicity Assay. Mutation Res. 455: 29-60.

167. (a) Verschaeve, L.; Van Gompel, J.; Regniers, L.; Van Parijs, Ph. and Van der Lelie D. (1999) VITOTOX® Genotoxicity and Toxicity Test for the Rapid Screening of Chemicals. Environ. Molec. Mutagen. 33: 240-248. (b) Van der Lelie, D.; Regniers, L.; Borremans, B.; Provoost, A. and Verschaeve, L. (1997) The VITOTOX® Test, a SOS-bioluminescence *Salmonella typhimurium* Test to Measure Genotoxicity Kinetics. Mutation Res. 389: 279-290.

168. (a) Fenech, M. (2000) The *in vitro* Micronucleus Technique. Mutation Res. 455: 81-95. (b) Krishna, G. and Hayashi, M. (2000) *In vivo* Rodent Micronucleus Assay: Protocol, Conduct and Data Interpretation. Mutation Res. 455: 155-166.

**IX.**

# CURRICULUM VITAE

# IX. CURRICULUM VITAE

JACQUES BUKURU was born on July 6th 1965, at Kirundo-Minyago, Republic of Burundi. He obtained his "Licenciate" degree in Chemical Sciences at the Faculty of Sciences of the University of Burundi, on November 19th 1991. From July 1992 to February 1996, he worked as a research assistant at the "Centre de Recherche Universitaire sur la Pharmacopée et la Médecine Traditionnelle (CRUPHAMET)" of the University of Burundi (Faculty of Sciences). He obtained his "DEA" (Diplôme d'Etudes Approfondies) in Pharmaceutical Sciences (Chemistry) at the Catholic University of Louvain (Belgium) in September 1998. In October 1998, he was admitted to the Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, as a PhD student in natural products research under the guidance of Prof. Dr. ir. N. De Kimpe.

*Scientific Publications*

1. Bukuru, J. F.; Van T. N.; Van Puyvelde, L.; Mathenge, S. G.; Mudida, F. P. and De Kimpe, N. (2002) A Benzochromene from the Roots of *Pentas bussei*. Journal of Natural Products 65(5): 783-785.
2. El-Hady, S.; Bukuru, J.; Kestelyen, B.; Van Puyvelde, L.; Van, T. N. and De Kimpe, N. (2002) New Pyranonaphthoquinone and Pyranonaphthohydroquinone from the Roots of *Pentas longiflora*. Journal of Natural Products 65(9): 1377-1379.
3. Poupaert, J. H.; Bukuru, J. and Gozzo, A. (1999) Clay (Montmorillonite K10) Catalysis of the Michael Addition of $\alpha,\beta$-Unsaturated Carbonyl Compounds to Indoles: The Beneficial Role of Alcohols. Monatshefte für Chemie 130: 929-932.
4. Hari, L.; Bukuru, J.; De Pooter, H. L.; Demyttenaere, J. R. and Fierens, H. (1996) Essential Oil of *Plectranthus defoliatus* Hochst. Ex Benth. Journal of Essential Oil Research 8: 87-89.
5. Hari, L.; Bukuru, J. and De Pooter, H. L. (1994) The Volatile Fraction of *Aframomum sanguinum* (K. Schum) K Schum from Burundi. Journal of Essential Oil Research 6: 395-398.

*Papers Submitted for Publication*

1. Bukuru, J.; Van, N. T.; Puyvelde, L. V.; He, W. and De Kimpe, N. (2003) A New Pentacyclic Cyclol-type Naphthohydroquinone from the Roots of *Pentas bussei*. Tetrahedron (accepted).
2. Li, W.-L.; Zheng, H.-C.; Bukuru, J. and De Kimpe, N. (2003) Natural Medecines Used in the Traditional Chinese Medical System for Therapy of Diabetes Mellitus. Journal of Ethnopharmacology (submitted).

*Posters Presented at Conferences*

1. Bukuru, J. F.; Nguyen Van, T.; Van Puyvelde, L.; Mathenge, S. G.; Mudida, F. P. and De Kimpe, N. (2001) A New Benzochromene Isolated from the Roots of *Pentas bussei*. 53rd International Symposium On Crop Protection. May 8th, 2001, Ghent University, Belgium.
2. Bukuru, J. F.; He, W.; Van Puyvelde, L.; Mathenge, S. G. and De Kimpe, N. (2001) Screening of Potential Biologically Active Compounds from the Genus *Pentas*: Highly Oxygenated New Naphthohydroquinones from *P. bussei* and *P. parvifolia*. One Day Symposium on "Drug Discovery Strategies: From Leads to Drugs" by the "Société Royale De Chimie", Medicinal Chemistry Division. November 16th, 2001, Louvain-la-Neuve, Belgium.

*Participation at International Conferences*

1. "51st International Symposium On Crop Protection". Symposium Organised by the Faculty of Agricultural and Applied Biological Sciences, Ghent University. May 4th, 1999, Ghent, Belgium.
2. "Flash Chromatography in Combinatorial Chemistry". Seminary Organised by the Company Beun De Ronde SERLABO (Belgium). February 24th, 2000, Ghent, Belgium.
3. "The Screening of Plant Species for Potential Medicinal Use", Workshop Organised as a Part of the "Bilateral International Scientific and Technological Cooperation" Project Between Flanders (Belgium) and South Africa by the "Vlaamse Instelling Voor Technologisch Onderzoek (VITO)" and the University of Ghent. October 19th, 2001, Mol,

Belgium.

4. "5th SIGMA-ALDRICH Organic Synthesis Meeting". December 6th – 7th, 2001, Spa, Belgium.
5. "From Medicinal Plant to Phytomedicine: Microbial and Parasitic Infections". LOF-Symposium Organsed by the "Landelijk Overleg Farmacognosie (LOF)". February 28th, 2003, University of Antwerp (UIA), Antwerp, Belgium.