UNIVERSITY OF NAIROBI
DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF *TEPHROSIA PURPUREA* AND *ERIOSEMA PSORALEOIDES* FOR PHARMACOLOGICAL AND ANTIPLASMODIAL ACTIVITIES

by

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A THESIS SUBMITED IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF SCIENCE OF THE UNIVERSITY OF NAIROBI

July, 2010

DECLARATION

This thesis is my original work and has never been presented for a degree in any university.

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This thesis is dedicated to my beloved mother, Florah and my brothers: Tisa, Shivachi, James and my sisters: Irene and Robai. Their support and encouragement enabled me to undertake this piece of work.

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LIST OF ABBREVIATIONS

δ	Chemical shift in delta values
COSY	Correlated Spectroscopy
J	Coupling constant
d	Doublet
ddd	Doublet of a double doublet
dd	Doublet of a doublet
EIMS	Electron ionization mass spectroscopy
Hz	Hertz
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
HR-MS	High Resolution Mass spectroscopy
IFS	Isoflavonoid synthase
MS	Mass spectroscopy
m/z	Mass to charge ration
λ_{max}	Maximum wavelength of absorption
MHz	Mega Hertz
$[M]^+$	Molecular ion
m	Multiplet
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser Enhancement
s	Singlet
$[\alpha]_{589}^{25.3}$	Specific rotation measured with sodium D-line light (589 nm) at 28.8^0 C

CoA	Coenzyme A
t	Triplet
SD	Seed
brd	Broad doublet
^{13}C	Carbon-13 isotope
^{1}H	Proton
CHI	Chalcone isomerase
RT	Root
CHS	Chalcone synthase
NADPH	Nicotinamide adenine dinucleotide
IFS	Isoflavone sythase
Ap	Aerial parts
PTLC	Preparative thin layer chromatography
ax	axial
[O]	Oxidation in schemes
NOESY	Nuclear overhauser Enhancement spectroscopy
Ph	phenyl
PDE-5	Phosphodiesterase type-5
TLC	Thin layer chromatography
UV	Ultra violet
ED	Erectile Dysfunction
IC_{50}	Concentration causing 50% inhibition

ABSTRACT

The air dried and ground roots of *Eriosema psoraleoides* were exhaustively extracted with dichloromethane/methanol (1:1) by cold percolation. The extract was partitioned between water and ethyl acetate. Chromatographic separation of the ethyl acetate layer led to the isolation of four compounds. These were identified as 4',5-dihydroxy-2',7-dimethoxyisoflavone (**1**), 4',5,7-trihydroxycoumaronochromone (**2**), 4',7''-bisgenistein (**3**) and 4',5,7-trihdroxy-2'-methoxyisoflavone (**4**). Similar treatment of the stem of *Tephrosia purpurea* yielded five compounds: stigmasterol (**5**); lanceolatin B (**6**); semiglabrin (**7**); terpurinflavone (**8**); lanceolatin A (**9**). Of these, compound **8** is a novel compound. The characterization of these compounds was based on spectroscopic techniques (^{1}H NMR, ^{13}C NMR, 2D NMR, UV and MS).

The crude extacts of *E. psoraleoides* and *T. purpurea* as well as some of the pure compounds isolated from these extracts were tested for smooth muscle and blood vessel relaxant activities. The methanol extract of the roots of *E. psoraleoides* (at 88 μg/mL) had the highest relaxant effect (13 mm) on isolated aorta. The ethyl acetate fraction of the dichloromethane/methanol (1:1) extract of *T. purpurea* stem at 74 μg/mL, stigmasterol (**5**) at 12 μg/mL, lanceolatin B (**6**) at 19 μg/mL relaxed the aorta by 9 mm, 6 mm and 10 mm, respectively. This indicates that *E. psoraleoides* and *T. purpurea* may promote penile erection.

The crude extract of *E. psoraleoides* (roots) did not show a significant effect on bronchial muscle where as the ethyl acetate fraction of the dichloromethane/methanol (1:1) extract of *T. purpurea* at 22.5 μg/mL and its pure compound semiglabrin (**7**) at 7.5 μg/mL relaxed the bronchial smooth muscle by 1.5 μL and 15 μL respectively. This probably explains the traditional use of *T. purpurea* for the management of chest tightness.

The methanol extract of the roots of *E. psoraleoides* (2.5 g/Kg) was further tested for mating behavior on mice using yohimbine (360 mg/Kg) and sildenafil (600 mg/Kg) as a

reference drugs. The results indicated that *E. psoraleoides* (roots) extract may promote erection.

The crude extracts of *E. psoraleoides*, *T. purpurea* and some of the compounds isolated from *T. purpurea* were also tested for antiplasmodial activities. The crude extracts showed antiplasmodial activities with IC_{50} values of 9.33 ± 0.38 and 11.43 ± 0.47 μg/mL for *E. psoraleoides*, and 10.47 ± 2.22 μg/mL and 12.06 ± 5.53 μg/mL for *T. purpurea*, against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*, respectively. The novel compound, terpurinflavone (**8**) showed the highest antiplasmodial activity with an IC_{50} value of 2.73 ± 1.16 μg/mL and 1.36 ± 0.12 μg/mL against W2 and D6 strains respectively. Laceolatin A (**9**) also showed significant activity against the D6 and W2 strains of *P. falciparum* with IC_{50} value of 3.82 ± 1.00 and 3.82 ± 1.04μg/mL, respectively.

The crude extracts of *E. psoraleoides*, *T. purpurea* and some of the pure compounds isolated from *T. purpurea* were further tested for antimicrobial activity. The crude extract of *E. psoraleoides* showed an inhibition zone of 12 mm against *Candida albicans* at a concentration of 1.8 mg/disc while the novel compound terpurinflavone (**8**) showed an inhibition zone of 16 mm against *Microsporum gypsum* at 50 μg/disc. The crude extract of *T. purpurea* and the five pure compounds isolated from this extract were inactive against *Staphylococcus aureus*, *Pseudomonas eruginosa*, *Candida albicans*, *Cryptococcus neoformans*, *Trichphyton mentagrophytes* and *Microsporum gypsum*.

CHAPTER ONE

INTRODUCTION

ENERAL INTRODUCTION

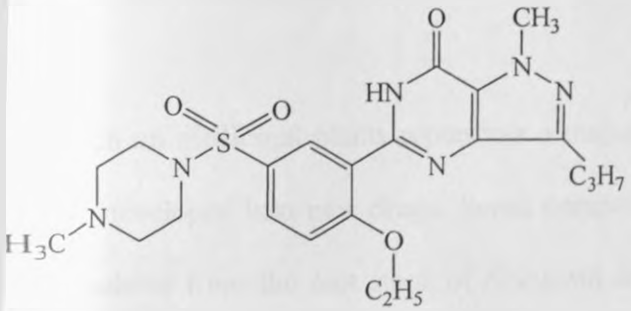
on-infectious or intrinsic diseases are those that are not caused by a pathogen and are neither ntagious nor communicable. These diseases may arise as a result of environmental conditions. r example skin cancer can be caused by radiation of the sun or dietary deficiencies. Other non-mmunicable diseases include impotency and cardiovascular diseases. One of the non-fectious disease that has received little attention is the men's reproductive health problem, ectile dysfunction.

rectile dysfunction (ED) is the repeated inability to get or keep an erection firm enough for xual intercourse. The word "impotence" has also been used to describe other problems that terfere with sexual intercourse and reproduction, such as lack of sexual desire and problems ith ejaculation or orgasm (Pamplona-Roger, 2000; Roper, 2001). Since ethnobotanical digenous knowledge (IK) cannot clearly distinguish between these two terms, the terms rectile dysfunction" and "sexual impotence" have been used interchangeably. It is therefore fficult to estimate the true prevelence of ED. The world prevalence of ED is estimated at 150 illion and is expected to increase to 322 million by the year 2025 (Feldman *et al.*, 1994; Ayta *et* ., 1999; Khalaf and Levinson, 2003).

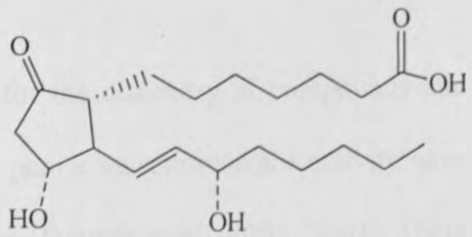
he causes of ED vary from one individual to another. These causes include: damage to nerves d tissues. Seventy percent of ED cases are as a result of other diseases (Derry *et al.*, 1998). ational Institute of Health (NIH, 2004) of the U.S.A. reported that between 35 and 50 percent men with diabetes experience ED (Melman and Christ, 2002). Many common medicines

use ED as a side effect (Melman and Christ, 2002). Psychological factors cause 10 to 20 rcent of ED cases (Melman and Christ, 2002). Smoking causes ED by varying hormonal lance and reducing blood flow in veins and arteries (Pak and Broderick, 2006).

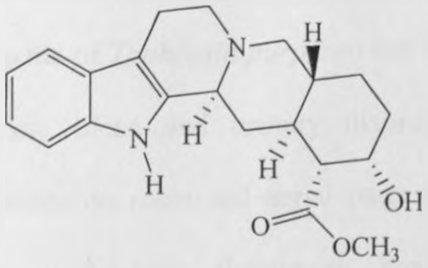
rectile dysfunction is managed using PDE-5 inhibitors such as sildenafil (**10**), PGE_1 analogs ke alprostadil (**11**), α_2 antagonists like yohimbine (**12**) (Figure 1.1). An example of α_1 and α_2 ntagonist drug is phentolamine (**13**). However sildenafil (**10**) is effective in less than 70% of atients and has side effects (Melman and Christ, 2002). Alprostadil (**11**) (Caverject ™), ohimbine (**12**), phentolamine (**13**) act by dilating blood vessels (Kametenesi-Mugisha and)ryem-Origa, 2005). Unfortunately these medications for ED are very expensive for most people n Kenya and other developing countries.



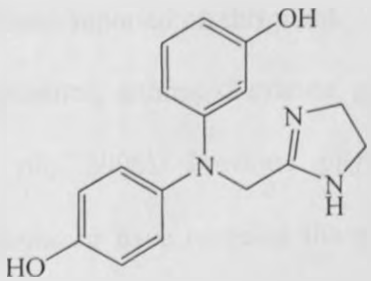
Sildenafil (**10**)



Alprostadil (**11**)



Yohimbine (**12**)



Phentolamine (**13**)

Figure 1.1: Drugs used to manage erectile dysfunction

2 PROBLEM STATEMENT

Erectile dysfunction is a major contributor to the global burden of disease and a significant impediment to socio-economic development in poor countries. Modern medication to combat ED is very expensive for most people in Kenya and other developing countries. Yet, in traditional medicinal practice there are several plants that are used to treat ED (Kokwaro, 1993). There is therefore a need for continued efforts to study such plants in the search of compounds that may be useful for the management of erectile dysfunction so that these compounds can be developed into new, cheaper and more effective drugs.

1.3 STUDY JUSTIFICATION

Research on medicinal plants represents a major strategy for the discovery of compounds that can be developed into new drugs. Some compounds with penile muscle relaxant activity have been isolated from the root stock of *Eriosema kraussianum* (Drewes *et al.*, 2002, 2003). There are several plants used traditionally to treat impotence in Kenya including *Eriosema psoraleoides*. No phytochemical and biological work has been reported on this plant.

Various parts of *Tephrosia purpurea* are used to treat impotence, asthma, diarrhoea, gonorrhoea, rheumatism, ulcer and urinary disorders (Lodhi *et al.*, 2006). Previous phytochemical investigations on roots and aerial parts of *Tephrosia purpurea* have revealed the presence of rotenoids, isoflavones, flavanones, chalcones, flavanols and sterols (Pelter *et al.*, 1981). However, phytochemicals from the stem of *T. purpurea* with fertility promoting properties have not been identified. It is therefore important that *Eriosema psoraleoides* and *Tephrosia purpurea*

ould be investigated for penile muscle relaxant activity. Previous studies have shown that avonoids and isoflavonoids have antiplasmodial activity (Yenesew *et al.*, 2003). It is worth also test the constituents of these plants for antiplasmodial activities.

.4 OBJECTIVES

.4.1 General Objective

he general objective was to isolate and characterize phytochemicals from *Eriosema soraleoides* and *Tephrosia purpurea* with pharmacological and antiplasmodial activities.

.4.2: Specific Objectives

he specific objectives were:

1. To isolate compounds from *Eriosema psoraleoides* and *Tephrosia purpurea.*
2. To determine the structures of the isolated compounds using spectroscopic techniques.
3. To establish the effects of the crude extracts and some of the isolated compounds on isolated blood vessels and trachea.
4. To establish the effect of the plant extracts on mating behavior.
5. To establish the antiplasmodial activity of the crude extracts and the isolated compounds.

CHAPTER TWO

LITERATURE REVIEW

1 ERECTILE DYSFUNCTION

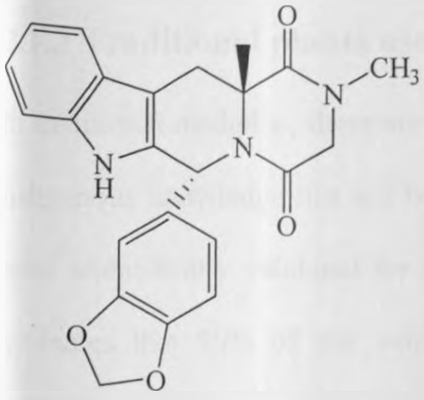
ectile dysfunction (ED), which is also referred to as impotence, is the repeated inability to get keep an erection firm enough for sexual intercourse (Moreland *et al.;* 2001, Klein and hompson, 2004). Impotence includes other problems that interfere with sexual intercourse and production, such as lack of sexual desire, problems with ejaculation or orgasm and a sfunctional organ system (Williams, 2007).

ince an erection requires a precise sequence of events, ED can occur when any of the events is isrupted. This sequence includes interruption of nerve impulses in the brain, spinal column, area round the penis, and response in muscles, fibrous tissues, veins, and arteries in and near the orpora cavernosa (Melman and Christ, 2002). Causes of ED include, damage to nerves, arteries, mooth muscles, and fibrous tissues (Williams, 2007). These are often as a result of diseases, uch as diabetes, kidney disease, chronic alcoholism, multiple sclerosis, atherosclerosis, vascular isease, and neurologic diseases that account for about 70 percent of ED cases (Rendell *et al.*, 999; Conti *et al.*, 1999; Rosen *et al.*, 2005).

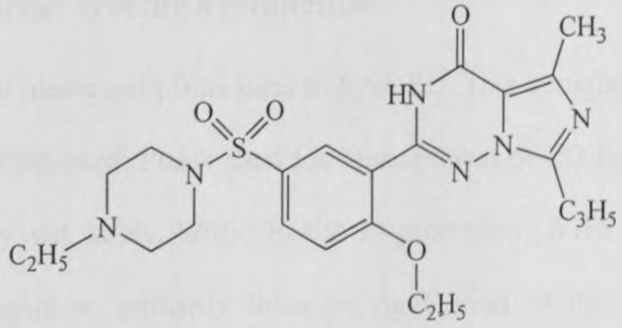
Antihypertensives, antihistamines, antidepressants, tranquilizers, appetite suppressants, and ntiulcer drugs produce ED as a side effect. Psychological factors such as stress, anxiety, guilt, epression, low self-esteem, and fear of sexual failure cause 10 to 20 percent of ED cases Melman and Christ, 2002). Other possible causes are smoking, which affects blood flow in eins and arteries, and hormonal abnormalities, such as insufficient amounts of testosterone Rosen *et al.*, 2005; Pak and Broderick, 2006).

2.1.1 Drugs used to treat erectile dysfunction

Erectile dysfunction is managed by drugs that target the peripheral organs. The drugs act by enhancing smooth muscle vasodilation or block the adrenergic or endothelin (ET)-mediated vasoconstriction associated with penile flaccidity. Peripherally acting drugs are classified as phosphodiesterase type 5 (PDE5) inhibitors. PDE5 inhibitors block the hydrolysis of cyclic guanosine monophosphate (cGMP) produced by soluble guanylate cyclase (sGC) in response to nitric oxide (NO), prolonging cGMP levels in corpus cavernosum smooth muscle causing relaxation. First generation PDE5 inhibitors include sildenafil (**10**) while tadalafil (**14**) and vardenafil (**15**) are second generation of PDE5 inhibitors (Figure 2.1). Sildenafil (**10**) is of little value in men with cord injury and lack libido. Furthermore, it potentiates cardiovascular diseases. Sildenafil (**10**) also has a negative effect on colour vision, causing difficulty in blue-green discrimination.



Tadalafil (**14**)

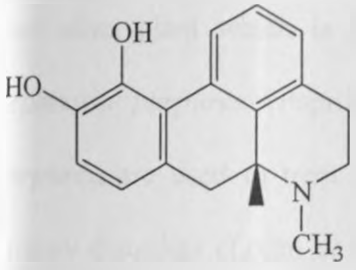


Vardenafil (**15**)

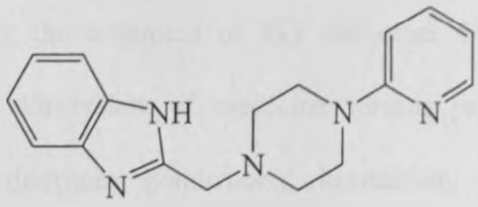
Figure 2.1: Second generation of PDE5 inhibitors

Other drugs used to manage ED are prostaglandin E_1 (PGE_1) analogs. These drugs act *via* PGE receptors EP_2 and EP_4 on smooth muscle to increase intracellular cyclic adenosine

monophosphate (cAMP) synthesis and potentiate smooth muscle relaxation. Alprostadil (**11**) that causes priapism and phentolamine (**13**), nonselective α-adrenoceptor antagonist is an example of prostaglandin E_1 (PGE_1) analogs and α-antagonist respectively. The last group of ED drugs acts on the central nervous system. The drugs include yohimbine (**12**) and apomorphine (**16**), ABT-724 (**17**) that act by interacting with dopamine receptors (DA and D4) (Figure 2.2).



Apomorphine (**16**)



2-[(4-Pyridin-2-ylpiperazin-1-yl)methyl]
-1H-benzimidazole (ABT-724) (**17**)

Figure 2.2: Drugs that act by interacting with dopamine receptors

2.1.2 Traditional plants used to treat erectile dysfunction

In traditional medicine, there are several medicinal plants used to treat ED. This ethnobotanical indigenous knowledge has not been documented. Plants used for management of ED have not been scientifically validated for efficacy and safety. World Health Organization (WHO, 2005) estimates that 80% of the world population, primarily those in rural areas of developing countries, depends on traditional medicines for their primary health care needs (Cunningham, 1993). In some cases such uses have been validated by isolation of compounds that are active against the causative agents of diseases.

Eriosema kraussianum (Leguminosae) is used for treatment of erectile dysfunction (Bryant, 1983; Hutchings, 1996a, Hutchings *et al.,* 1996b). Phytochemicals isolated from *Eriosema*

species include isoflavones with ability to promote penile erection (Drewes *et al.*, 2003). The genus *Eriosema* is represented in Kenya by *E. bogdanii*, *E. glomeratum*, *E. psoraleoides*, *E. scanderystii*, *E. nutans*, *E. buchananii*, *E. montanum* and *E. robustum* (Agnew and Agnew, 1994). Roots of *Eriosema psoraleoides* are used for the treatment of diarrhoea, dysentery and impotence (Bryant, 1983).

One other plant which is also used widely for the treatment of ED and other diseases is *Tephrosia purpurea* (Papilionaceae). In Ayurvedic system of medicine various parts of *T. purpurea* are used to treat impotence, asthma, diarrhoea, gonorrhoea, rheumatism, ulcer and urinary disorders (Lodhi *et al.*, 2006). The plant has been claimed to cure diseases of kidney, liver spleen, heart and blood (Kirtikar and Basu, 1956; Despande *et al.*, 2003). Previous phytochemical investigations of *Tephrosia purpurea* have revealed the presence of rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols (Pelter *et al.*, 1981).

Modern medication for ED is very expensive for most of the people in developing countries. Yet, in traditional medicine, there are several medicinal plants that have been relied on for the treatment of ED. Erectile dysfunction is an old problem and traditionally the indigenous knowledge had ways of treating or managing these conditions in many parts of the world. For example 33 medicinal plants both cultivated and wild-harvested are documented to be used traditionally by men to manage ED in western Uganda. Some of these plants are listed in Table 2.1. The herbal medicines used in the management of erectile dysfunction are mainly prepared by pounding, chewing and boiling and are usually orally administered. The traditional healers treat sexual impotence by prescribing some of these herbs for use in tea, local beers, fermented milk and porridge. Some herbs are roasted or smoked such as coffee before administration.

Table 2.1: Cultivated Medicinal Plants used for treatment of Sexual Impotence in Western Uganda (Kametenesi-Mugisha and Oryem-Origa, 2005)

Scientific Name	Local Name	Parts Used	Preparation	Administration
Allium cepa L.	*Katunguru* (NY, KI, RU) Onion (Engl.)	ST-BU, L, RT	Chewing, cooking	Oral in water and in food
Allium sativum L.	*Tungurusumu* (KO) Garlic (ENG)	ST, BU, L, RT	Chewing, cooking	Oral in water and in food
Cannabis sativa L.	*Njayi* (GA), *Njaga* (NY) Marijuana (ENG), *Bangi* (SW)	L	Chewing, smoking	Oral, inhaling fumes
Cleome gynandra L.	*Esobyo/Amarera* (KO) *Eshogi* (NY)	L, R, FL	Chewing, cooking	Oral or as food
Sida tenuicarpa Vollesen	*Keyoyo* (RU)	L	Pounding, boiling	Oral
Arachis hypogaea L.	*Binyebwa* (NY, RU) Ground nuts (ENG)	SE	Roasting	Oral as food
Coffee arabica L.	*Mwani* (NY) Arabica Coffee (ENG)	SE	Roasting, chewing	Oral as a beverage
Capsicum frutescens L.	*Kamurari* (GA) *Eshenda* (NY) Red pepper (ENG)	FR	Pounding, boiling, chewing	Oral in food

Key: SW (Swahili); ENG (English); KO (Rukonjo); RU (Runyaruguru); NY (Runyankore); KI (Rukiga); GA (Luganda);L (leaves); R (roots); RT (root tuber); RH (rhizome); FR (fruits); SE (seeds); ST-BU (stem-bulb).

Tephrosia purpurea has been documented to treat impotence (Lodhi *et al.*, 2006) while the use of *E. psoraleoides* to manage erectile dysfunction in Kenya has not been documented.

2 BOTANICAL INFORMATION ON *ERIOSEMA* AND *TEPHROSIA*

2.1 The genera of *Eriosema* and *Tephrosia*

The genera *Tephrosia* and *Eriosema* belong to the family Leguminosae, also known as Fabaceae. This family comprises of 657 genera and 18,000 species of trees, shrubs and herbs, which are widely distributed in the temperate as well as tropical regions of the world. The family is the second largest of the dicotyledons after the Compositae, and plants in the family are known for their ability to support nitrogen fixation through symbiosis (Heywood, 1971). The family is subdivided into three sub-families: Mimosoideae, Papilionoideae and Caesalpinioideae. The genera *Tephrosia* and *Eriosema* belongs to the Papilionoideae sub-family (Polhill, *et al.*, 1981a).

2.2.2. Botanical description of *Eriosema psoraleoides*

The genus of *Eriosema* is distributed in tropical Africa, America, Asia and Australia. Thirty-eight species are distributed in South America, primarily in Argentina, Brazil and Paraguay (Polhill, *et al.*, 1981b). The genus *Eriosema* is represented in Kenya by eight species, namely *E. bogdanii*, *E. glomeratum*, *E. psoraleoides*, *E. vanderystii*, *E. nutans*, *E. buchananii*, *E. montanum* and *E. robustum* (Agnew and Agnew, 1994).

Eriosema psoraleoides is a herb or shrublet that is rarely erect with climbing characteristics. It has branches that are strongly ribbed, brown, hairy and covered with small orange-red glands. It has three leaflets that are pale beneath, venated with buff hairs and are narrow, elliptic and 2.3-9.5 cm long and 0.8-3.5 cm wide. The apex of the leaf is round and micronulated. The rest of the leaflet surfaces are silvery. The plant has petiole of 1-5 mm long; rhachis of 1.8 mm length; petiole of 1-3 mm length. The calyx is pubescented with triangular lobes. It has deep golden-yellow corolla with a length of between 0.7-1.4 mm; and rarely with hairs outside. The

suborbicular, oval, oblique pods with length between 1.1-1.8 mm and 0.9-1.1 mm width are covered with long ferruginous hairs. The seeds are reddish-brown or pinkish with blue black mottling that is shiny with length of 4.5-5.2 mm (Gillet *et al.*, 1971a). Figure 2.3 shows a flowering plant of *E. psoraleoides.*

Figure 2.3: Flowering *E. psoraleoides* plant (photograph taken by Peter Juma)

2.2.3 Botanical information of *Tephrosia purpurea*

Tephrosia is a large tropic and sub-tropic genus of perennial woody shrubs distributed in tropical and sub-tropical regions. It is estimated to contain between 300 and 400 species, distributed all over the world as follows; 35 species occur in India, 30 are native of South America, 70 are found in South Africa, 50 in equatorial Africa of which 30 are found in Kenya (Tarus *et al.*, 2002; Beentje, 1994). Examples of some Kenyan *Tephrosia* species include; *T. aequilata*, *T. elata*, *T. hildebrandtii*, *T. holstii*, *T. interrupta*, *T. linearis*, *T. noctiflora*, *T. paucijuga*, *T. pentaphylla*, *T. pumila*, *T. purpurea*, *T. villosa* among others (Agnew and Agnew, 1994).

Tephrosia purpurea is a short lived perennial herb of up to 80-150 cm long with leaf rhachis of approximate length of 8 cm. The petiole is about 1cm and it extends beyond the lateral leaflets. It has approximately 9-17 leaflets that are elliptic with an estimated length of 2 cm and width of 6 mm. The leaflets apex is round, mucronated and pubescented at the base. The flowers are reddish-purple or bright pink. It has brown calyx with appressed strigulose and spreading pubescent. The upper filament is lightly attached, widened but not bent. The filament is 1.5 mm above the base with filament sheath of approximate length of 0.3 mm and width of 6mm. The style is glabrous, linear, gently curved with the length of 2.5 mm. The pods are gently curved towards the tip with a length of about 4-4.5 cm and width of 6.5 mm. It has 6-9 seeds that are subcylindrical with a centrally placed hillum (Gillet *et al.*, 1971b). The species is divided into the following subspecies: *purpurea*, *leptostachya*, and *dunensis*. All the subspecies are found in different parts of Kenya.

Figure 2.4: *Tephrosia purpurea* found in Kenya (photo taken by Loise Muiva)

.3. ETHNOMEDICAL INFORMATION

.3.1 Ethnomedical information of the genus *Eriosema*

Plants belonging to the genus *Eriosema* have been used traditionally in various communities for the treatment of various ailments (Pretorius *et al.*, 2002). The traditional uses of *Eriosema* species in Africa is summarized in table 2.2.

Table 2.2: Traditional uses of *Eriosema* species in Africa

	Country	Parts used	Medical use	Reference
E. burkei	Malawi	Dried roots	Water extract used orally to cure pain in adults and treat lymphoid disorders	Msonthi and Magombo, 1983
E. Cordatum	South Africa	Roots	Hot milk infusion taken as aphrodisiac	Bryant, 1966
E. glomeratum	Gabon	Petiole	Syncope, fish poisoning	Akendengue and Louis, 1994
E. krausianum	South Africa	Dried Root	Used orally to alleviate impotence in men	Ojewolw and Drawes, 2007
E. psoraleoides	Central Africa	Leeves	Water extract used orally as an oxytocic during pregnant	Sillans, 1953
	Tanganyika	Roots	Water extract combined with *Piliostigma trionningii* threaten miscarriage	Haerdi, 1964
	Tanzania	Dried twigs	Used as chewing stick	Khan *et al.*, 2000
E. salignum	South Africa	Roots	Hot milk infussion taken as aphrodisiac	Bryant, 1966
E. tisseratii	Central Africa	Roots	Aphrodisiac	Sillans, 1953
E. benthamianum	Brazil	Roots	Used as an anti-inflammatory	Hirschhorn, 1982
E. diffusum	Mexico Guatemala	Entire Plant	Unspecified female diseases	Hastings, 1990

2.3.2 Ethnomedical information on the genus *Tephrosia*

In Kenya, several plants of the genus *Tephrosia* are used for the treatment of various ailments. Table 2.3 below gives some representative Kenyan *Tephrosia* species and their respective medicinal use.

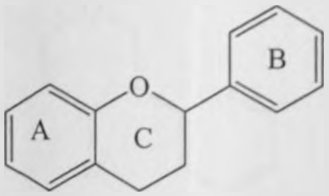
Table 2.3: Ethnomedical use of some *Tephrosia* species of Kenya

Species	Plant part and Ethno-medical use	Locality/Community	References
T. aequilata	Roots dug out, boiled and mixed with milk and drunk for pain in liver and spleen. Roots chewed with salt as a cure for venereal diseases.	Makueni (Kamba), Kajiado (Maasai), Coast (Pare, Digo)	Kokwaro, 1993; Agnew and Agnew, 1994.
T. elata	Roots chewed as a cure for stomach pains, fever and general weakness.	Machakos, (Kamba), Kajiado (Maasai)	Lwande, 1985 ; Agnew and Agnew, 1994.
T. paucijuga	Roots and leaves dried, pounded or ground into power form and applied on wounds.	Coastal region (Nyika)	Kokwaro, 1993.
T. pumila	The roots are chewed as a remedy for cold in the chest. Roots are also boiled and the infusion taken as a broth as a cure for venereal diseases.	Coastal region (Digo),Kibwezi Kamba) Kajiado (Maasai)	Kokwaro, 1993; Agnew and Agnew, 1994.
T. purpurea	Roots used as a medicine for stomach pains. Leaves used for snake bite and for headache. Aerial parts are used as laxative, deobstruent and diuretic, treatment of cough, biliary febrile attacks, obstructions of the liver, spleen and kidneys. Anthelmintic for children and chronic diarrhoea.	Kilifi (Giriama), Machakos, (Kamba)	Kokwaro, 1993; Agnew and Agnew, 1994; Ahmad *et al.*, 1999.

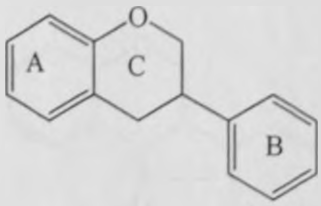
4 BIOSYNTHESIS OF FLAVONOIDS AND THEIR DERIVATIVES

avonoids *sensu lato* are natural products that have a C_6-C_3-C_6 carbon frame work or more ecifically a phenyl benzopyran functionality. They constitute one of the largest groups of turally occurring phenols (Markham, 1982). All species of terrestrial plants and the relatively vanced Algae families contain flavonoids (Induli, 2006). It is estimated that about 2% of all rbon photosynthesized by plants is converted into flavonoids or closely related compounds Markham, 1982). The growing interest in plant flavonoids is due to their use as human dietary mpounds and as pharmacological agents (Harborne *et al.*, 1986; Harbone, 1998).

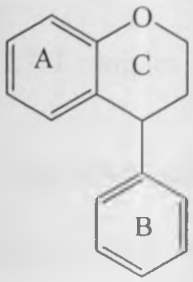
n plants, flavonoids occur in different structural forms. All flavonoid aglycones contain fifteen arbon atoms in their basic nucleus and these are arranged in a C_6-C_3-C_6 configuration. Each C_6 epresents an aromatic ring. These aromatic rings are linked by a three carbon unit which form a hird heterocyclic ring *via* cyclization with one of the aromatic ring *via* an oxygen atom. The romatic rings are labelled as ring A and B and heterocyclic ring as ring C (Figure 2.5). Depending on the position of the linkage of the aromatic ring to the benzopyrano moiety, the lavonoids *sensu lato* are divided into flavonoids *sensu stricto* (2-phenylbenzopyrans) (**18**), soflavonoids (3-benzopyrans) (**19**) and the neoflavonoids (4-benzopyrans) (**20**) (Figure 2.5). These groups usually share a common chalcone precursor, and thus are biogenetically and structurally related (Agrawal, 1989).



Flavonoids (*Sensu stricto*) (**18**)



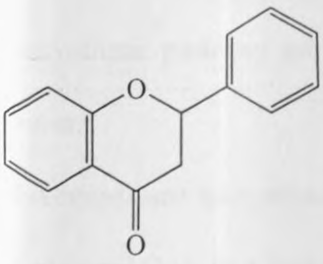
Isoflavonoids (**19**)



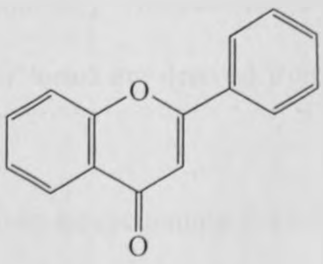
Neoflavonoids (**20**)

Figure 2.5: Skeleta of flavonoids based on the position of benzopyran ring

Oxidation and degree of saturation in the heterocyclic ring-C of the flavonoid (**18**) and isoflavonoid (**19**) results into different subclasses of flavonoids *sensu stricto* (Markham, 1982). Examples of these flavonoids subclasses are flavanones (**21**) and flavones (**22**) (Figure 2.6). Subclasses of isoflavonoids include isoflavanone (**23**) and isoflavone (**24**) (Figure 2.7) (Agrawal, 1989).

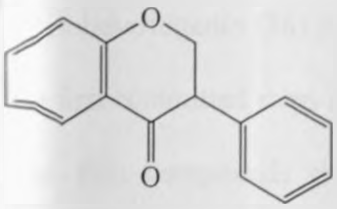


Flavanone (**21**)

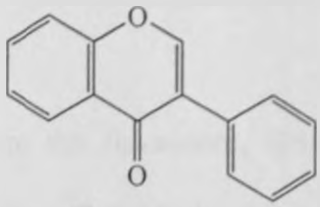


Flavone (**22**)

Figure 2.6: Examples of flavonoid (*sensu stricto*) subclasses



Isoflavanone (**23**)



Isoflavone (**24**)

Figure 2.7: Examples of isoflavonoid subclasses

Natural flavonoids and isoflavonoids are usually oxygenated and bear hydroxyl or methoxyl substituents. A large number of flavonoids occur as *O*-glycosides in which one or more of the hydroxyl groups of the flavonoid are bound to a sugar or sugars *via* an acid labile hemiacetal bond (Agrawal, 1989). In some cases, isoflavonoids skeleton may get further modified by cyclization of C-2 and C-4 of ring C with aryl ring B through an oxygen atom to generate a tetracyclic ring system called a coumaronochromone (Agrawal, 1989).

2.4.1 Biosynthesis of flavonoids and their derivatives

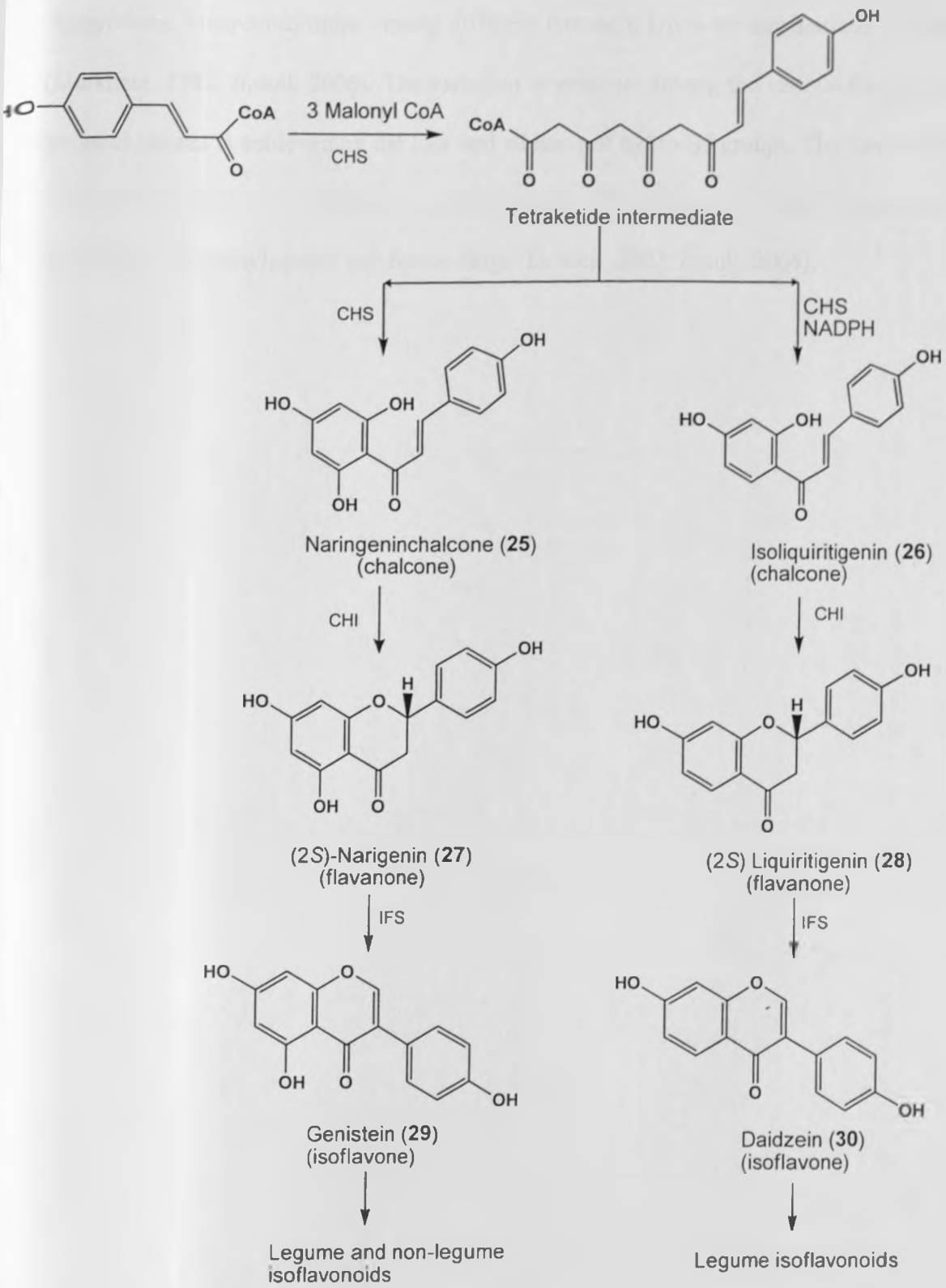
All flavonoids *sensu lato* are biosynthesized from common precursors which incorporate both shikimate and acetate malonate pathways (Scheme 2.1). The flavonoids initially formed in the biosynthetic pathway are chalcones and all other forms are derived from these by a variety of routes.

Flavonoids are biosynthesized by extension of *p*-hydroxycoumaroyl CoA with three molecules of malonyl CoA in a head-to-tail manner in order to form a tetraketide intermediate. The process is catalyzed by the enzyme chalcone synthase (CHS). The intermediate then folds and condenses further to give the chalcones naringeninchalcone (**25**) and isoliquiritigenin (**26**). The biosynthesis

of isoliquiritigenin (**26**) is catalysed by CHS with NADPH as a co-factor and these reactions are the first committed steps in flavonoid biosynthesis.

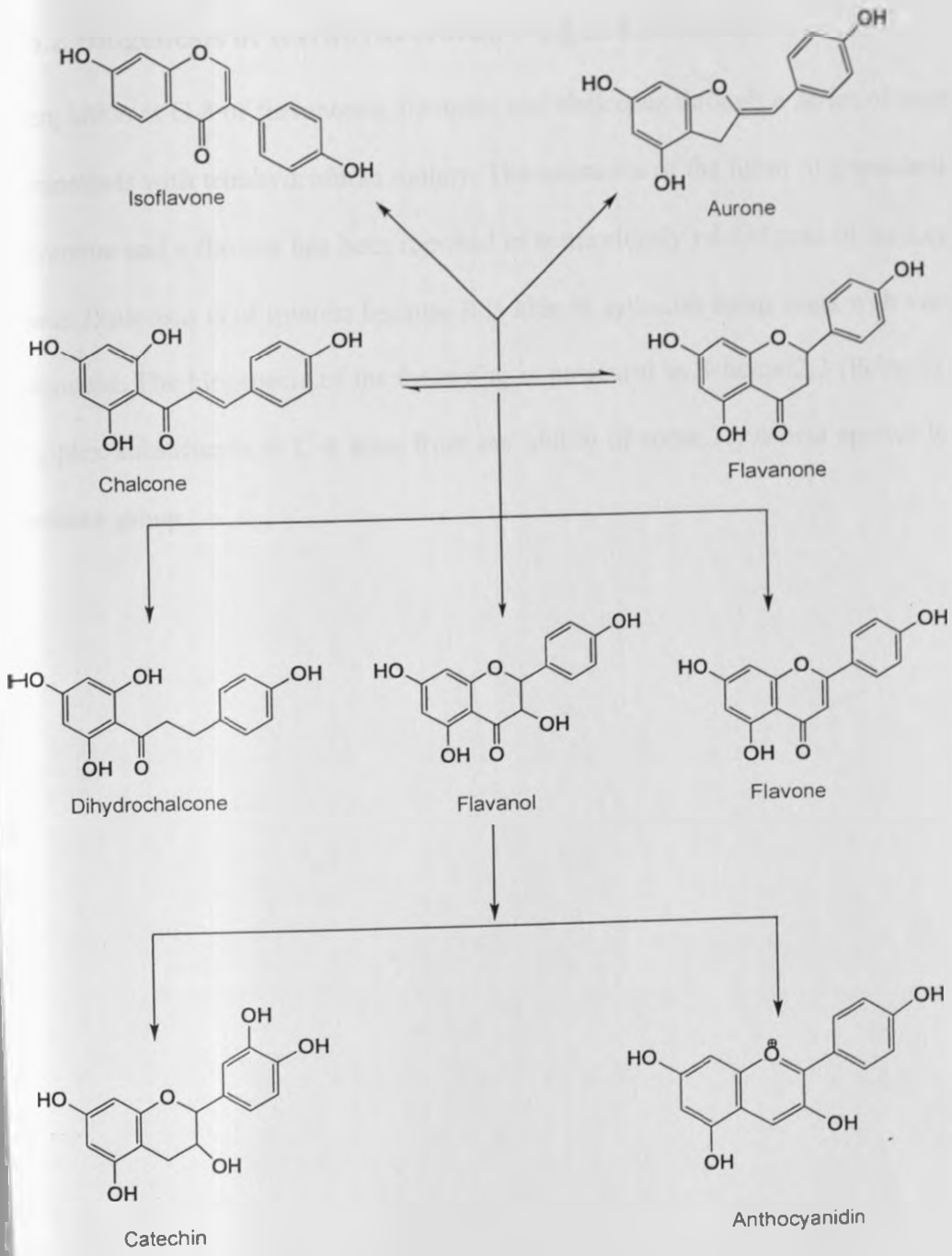
The first compounds derived from chalcones are the flavanones, (2S)-naringenin (5,7,4'-trihydroxyflavanone, **27**) and (2S)-liquiritigenin (7,4'-dihydroxyflavanone, **28**), from naringeninchalcone (**25**) and isoliquiritigenin (**26**) respectively. The biosynthesis of the two flavanones is catalysed by chalcone isomerase (CHI) (Dewick, 2002)

The enzyme isoflavone synthase (IFS) converts the flavanone substrates naringenin (**27**) and liquiritigenin (**28**) to the isoflavones genistein (**29**) and daidzein (**30**), respectively. This reaction is proposed to involve two steps: 2-hydroxylation and aryl migration from flavanone substrates to yield 2-hydroxyisoflavanone. Dehydration of the corresponding isoflavanone derivative is the last step. Genistein (**29**) and daidzein (**30**) are then further metabolised to give the various classes of isoflavonoids (Dewick, 2002).



Scheme 2.1: Biosynthesis of flavonoids and isoflavonoids (Dewick, 2002)

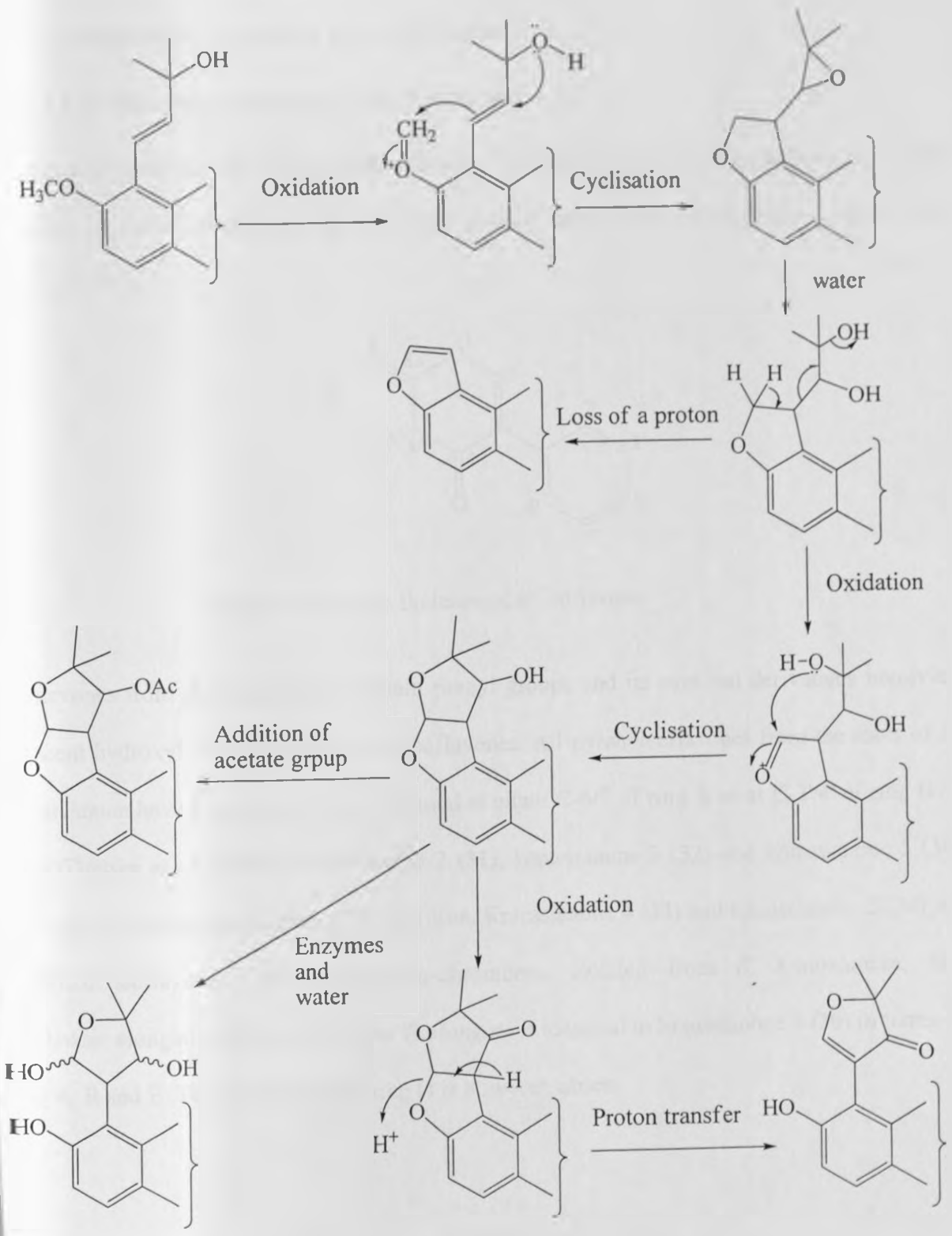
he biosynthetic interrelationships among different flavonoid types are summarized in Scheme 2 (Markham, 1982; Induli, 2006). The variation in structure among the various flavonoid and oflavonoid classes is achieved by the loss and addition of hydroxyl groups. The flavonoid and .oflavonoid can further be methylated or prenylated. The methyl and prenyl group can be odified to give dimethylpyrano and furano rings (Dewick, 2002; Induli, 2006).



Scheme 2.2: Biosynthetic interrelationships among different flavonoids (Dewick, 2002; Induli, 2006)

2.4.2 Biogenesis of tetrahydrofuran ring in flavonoids

Prenylation at C-8 of flavanones, flavones and chalcones through a series of steps forms unique compounds with tetrahydrofuran moiety. The existence of the furan ring attached on ring A of a flavanone and a flavone has been reported in some closely related taxa of the Leguminosae. The genus *Tephrosia* is of interest because it is able to sythesise furan rings with varying degrees of saturation. The biogenesis of the furan ring is presented in Scheme 2.3 (Pelter *et al.*, 1981). The complex substituents at C-8 arise from the ability of some *Tephrosia* species to oxidize the 7-methoxy group.



Scheme 2.3: Biosynthesis of furan ring in flavanones and flavones (Pelter *et al.*, 1981)

4.3 Compounds reported from *Eriosema*

4.3.1 Isoflavones reported from *Eriosema*

Isoflavones possess 3-phenylchromone skeleton. The heterocyclic ring-C in isoflavones is made up of an oxyolefinic methine (C-2), olefinic quaternary carbon (C-3) and a carbonyl carbon (C-4) (Figure 2.8).

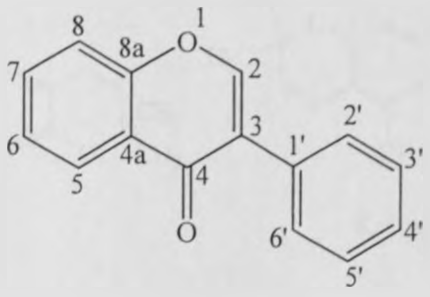
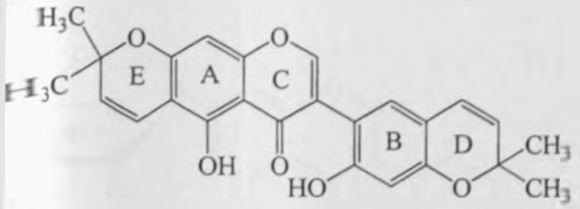
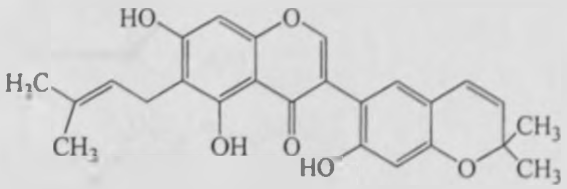


Figure 2.8: Basic skeleton of an isoflavone

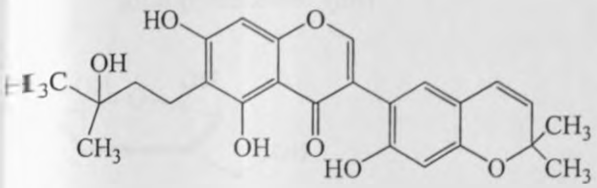
Isoflavones from *E. kraussianum* contain prenyl groups and its cyclized derivatives involving adjacent hydroxyl group to give pyranoisoflavones. All pyranoisoflavones from the roots of *E. kraussianum* have the pyrano ring substituted at either C-6/7 of ring A or at C-3'/4' of ring B or both (Drewes *et al.*, 2002). Kraussianone 2 (**31**), kraussianone 3 (**32**) and kraussianone 5 (**34**) have a prenyl group substituted at C-6 position. Kraussianone 4 (**33**) and Kraussianone 5 (**34**) are modified isoflavones, dihydrochromeno-chromones, isolated from *E. kraussianum*. The isoflavone elongatin (**35**) isolated from *T. elongata* is identical to kraussianone 1 (**30**) in terms of ring A, B and E. The dimethylpyran ring D is however, absent.



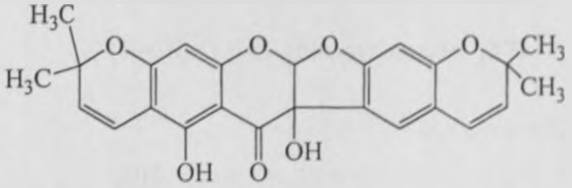
Kraussianone 1 (**30**)



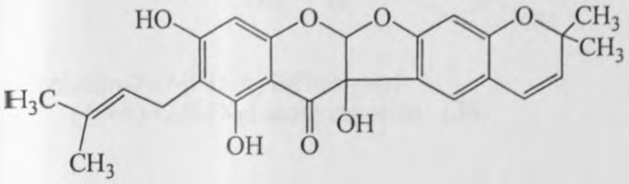
kraussianone 2 (**31**)



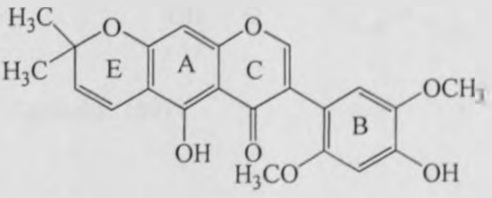
Kraussianone 3 (**32**)



Kraussianone 4 (**33**)



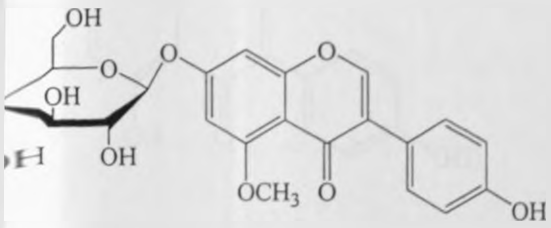
kraussianone 5 (**34**)



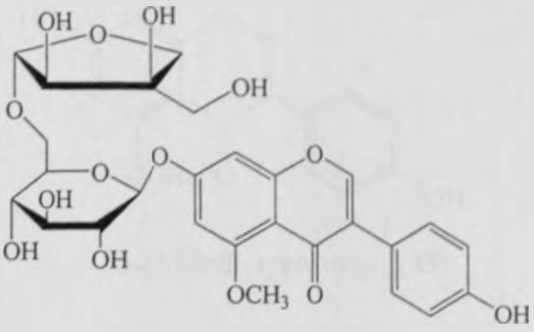
Elongatin (**35**)

Figure 2.9: Isoflavones isolated from the roots of *E. kraussianum*

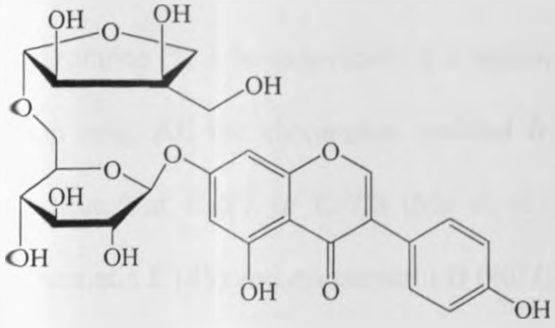
Six isoflavones isolated from the roots of *E. tuberosum* contain sugar moieties linked to oxygen (isoflavone-*O*-glycoside) through acetal linkage (Figure 2.10, Ma *et al.*, 1998). Genistein (**33**) and 5-*O*-methylgenistein (**35**) from the same plant lack a sugar moiety (Figure 2.11).



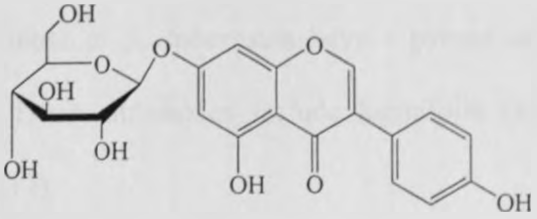
5-*O*-Methylgenistein-7-*O*-β-D-glucopyranoside (**36**)



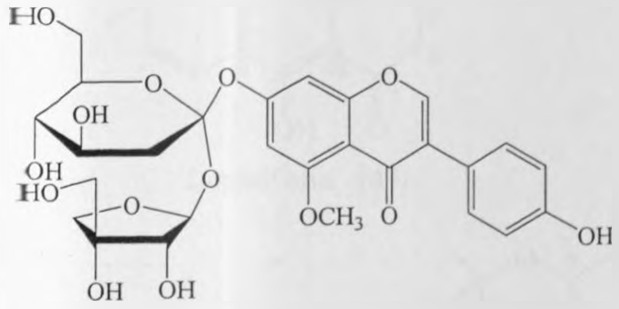
5-*O*-Methylgenistein 7-*O*-β-D apiofuranosyl-(1→ 6)-O-β-D-glucopyranoside (**37**)



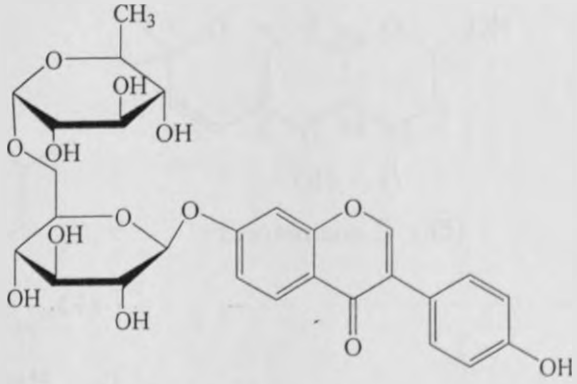
Genistein-7-*O*-β-D-Apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside (**38**)



Genistin (**39**)

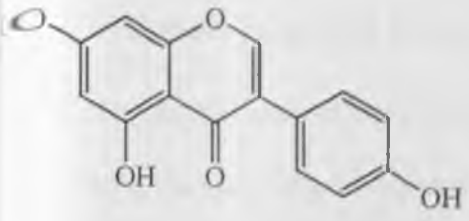


5-O-Methylgenistein 7-O-β-D-Apiofuranosyl-(1 →2)-O-β-D-Glucopyranoside (**40**)

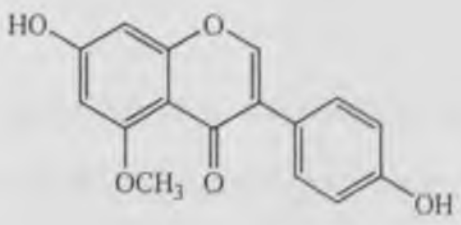


Sphaerobioside (**41**)

Figure 2.10: Isoflavone glycosides from *E. tuberosum*



Genistein (**42**)

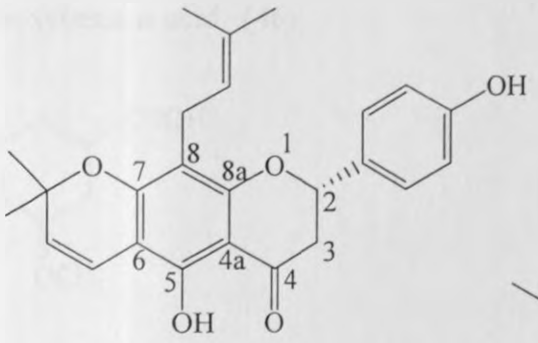


5-*O*-Methylgenistein (**43**)

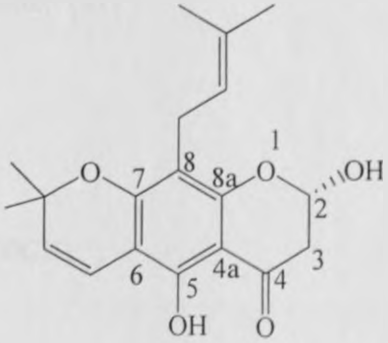
igure 2.11: Isoflavones from *E. tuberosum*

4.3.2 Chromones from *Eriosema*

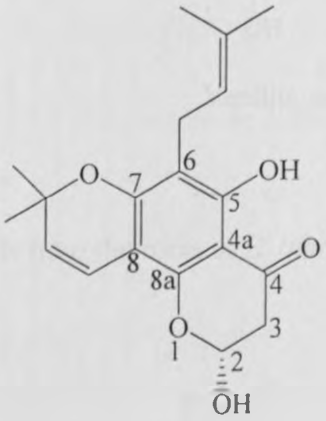
chromone (1, 4-benzopyrone) is a benzopyran derivative with a substituted keto group on the yran ring. All the chromones isolated from the roots of *E. tuberosum* have a pyrano ring ubstituted at C-6/7 or C-7/8 (Ma *et al.*, 1996). These chromones include lupinifolin (**44**), riosematin E (**45**) and eriosematin D (**46**) (Figure 2.12).



Lupinifolin (**44**)



Eriosematin E (**45**)

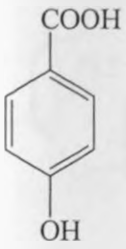


Eriosematin D (**46**)

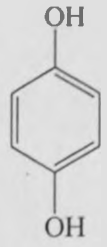
igure 2.12: Chromones isolated from the roots of *E. tuberosum*

4.3.3 Phenols reported from *Eriosema*

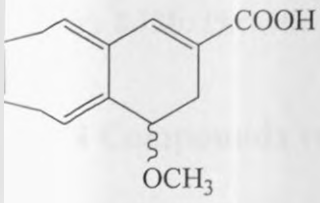
Seven phenolic derivatives have been isolated from the roots of *E. tuberosum* (Ma *et al.*, 1999). The simple phenols include 4-hydroxybenzoic acid (**46**), hydroquinone (**47**) vanillic acid (**48**), and eriosematin F (**49**) (Figure 2.13a). The other three phenols are glycosides and include arbutin (**50**), eriosemaside A (**51**) and eriosemaside B (**52**) (Figure 2.13b).



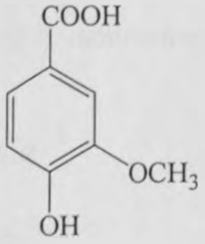
4-Hydroxybenzoic acid (**46**)



Hydroquinone (**47**)

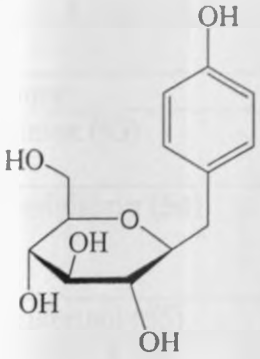


Eriosematin F (**49**)

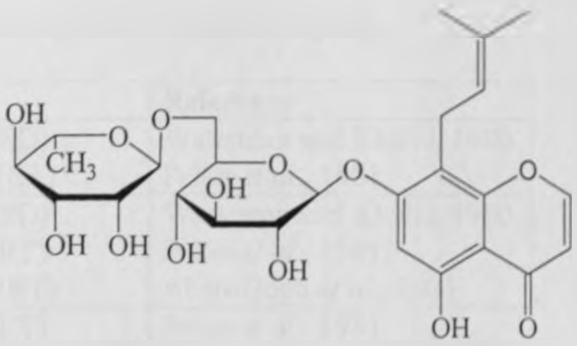


Vanillic acid (**48**)

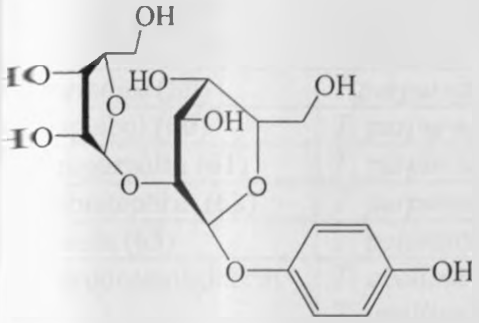
Figure 2.13a: Phenolic compounds from the roots of *E. tuberosum*



Arbutin (**50**)



Eriosemaside A (**51**)



Eriosemaside B (**52**)

Figure 2.13b: Phenolic glycosides from the roots of *E. tuberosum*

2.4.4 Compounds reported from *Tephrosia*

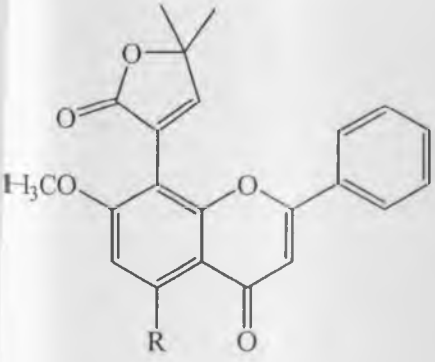
2.4.4.1 Flavones reported from *Tephrosia*

Flavones are 2,3-dehydro derivatives of the flavanones (Agrawal, 1989). C-8 prenylated flavones with extensive modifications have been reported from *T. purpurea, T. apollinea* and *T. polystachyoides* (Table 2.4). The structures of the flavones are shown in Figure 2.14a-214c. Such modification has not been observed in other *Tephrosia* species showing that the three taxa are closely related.

ble 2.4: Occurrence of C-8 prenylated flavones in *Tephrosia* species

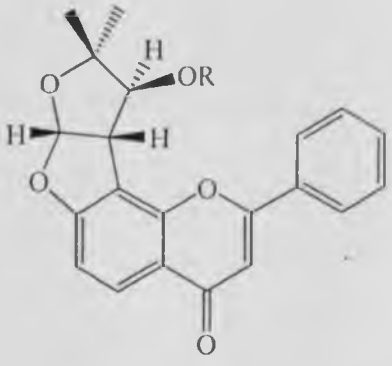
vones	Source	Reference
llinine (**53**)	*T. apollinea* (SD) *T. purpurea* (RT)	Waterman and Khalid, 1980 Pelter *et al.*, 1981
Semiglabrin (**54**)	*T. apollinea* (SD) *T. purpurea* (RT) *T. apollinea* (RT)	Waterman and Khalid, 1980 Pelter *et al.*, 1981 Abou-Douh *et al.*, 2005
niglabrinol (**55**)	*T. purpurea* (RT)	Pelter *et al.*, 1981
nceolatin A (**56**)	*T. apolline* (SD) *T. purpurea* (RT) *T. apolline* (RT)	Waterman and Khalid, 1980 Pelter *et al.*, 1981 Abou-Douh *et al.*, 2005
nceolatin B (**57**)	*T.purpurea* (RT)	Pelter *et al.*, 1981
phroglabrin (**58**)	*T.purpurea* (RT)	Pelter *et al.*, 1981
rpurindiol (**59**)	*T.purpurea* (RT)	Pelter *et al.*, 1981
ngaglabol (**60**)	*T. purpurea* (AP)	Ahmad *et al.*, 1999
phropurpulin (**61**)	*T. purpurea* (AP)	Hegazy *et al.*, 2009
glabratephrin (**62**)	*T. purpurea* (AP)	Hegazy *et al.*, 2009
chrosin (**63**)	*T. polystachyoides* (SD)	Waterman and Khalid, 1980
)- Pseudosemiglabrin 4)	*T. apolline* (SD) *T. apolline* (RT)	Waterman and Khalid, 1980 Abou-Douh *et al.*, 2005
)-Glabratephrin (**65**)	*T. apolline* (SD) *T. pupurea* (AP)	Waterman and Khalid, 1980 Hegazy *et al.*, 2009
ookerianin (**66**)	*T. hookeriana* (RT)	Prabhakar *et al.*, 1996

Key: SD-seeds; RT-Roots; AP-Aerial parts



Apollinine, R=H (**53**)

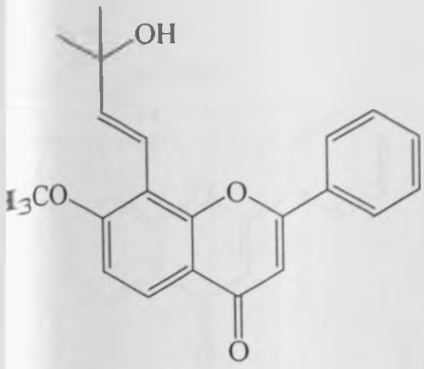
Hookerianin, R=OCH_3 (**66**)



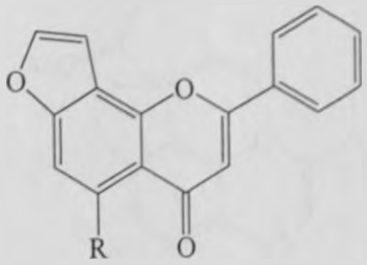
Semiglabrin, R=$COCH_3$ (**54**)

Semiglabrinol, R=H (**55**)

Figure 2.14a: C-8 prenylated flavones isolated from *Tephrosia* species

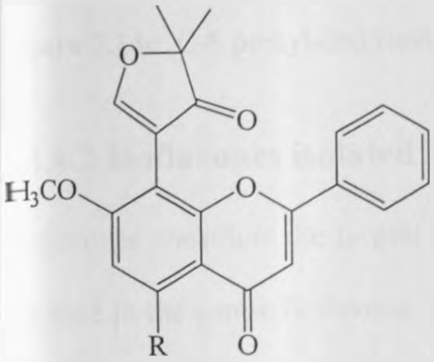


Lanceolatin A (**56**)



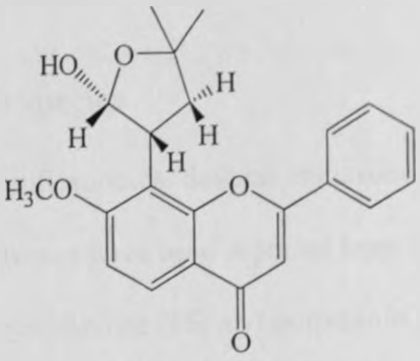
Lanceolatin B, R=H (**57**)

Pongaglabol, R=OH (**60**)

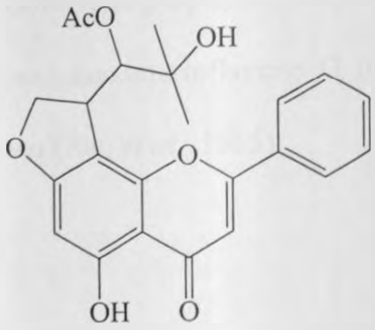


Tephroglabrin, R=H (**58**)

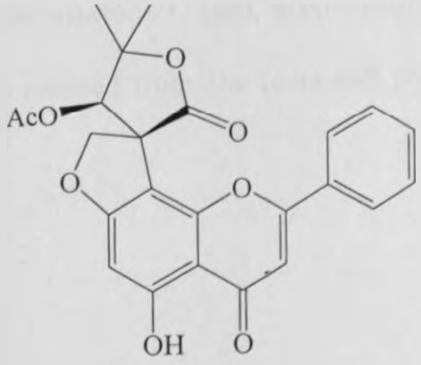
Tachrosin, R=OCH_3 (**63**)



Terpurindiol (**59**)

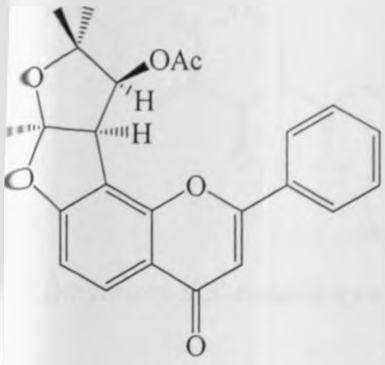


Tephropurpulin (**61**)

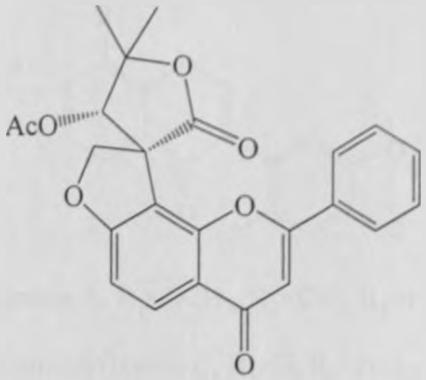


Isoglabratephrin (**62**)

Figure 2.14b: C-8 prenylated flavones isolated from *Tephrosia* species



Pseudosemiglabrin (**64**)

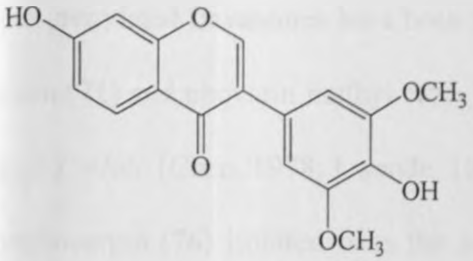


Glabratephrin (**65**)

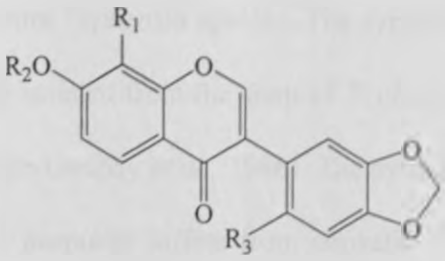
igure 2.14c: C-8 prenylated flavones isolated from *Tephrosia* species

.4.2 Isoflavones isolated from *Tephrosia* species

soflavones constitute the largest group of natural isoflavonoids. Several isoflavones have been eported in the genus *Tephrosia*. At least two isoflavones have been reported from *T. purpurea*. hese isoflavones are 4',7-dihydroxy-3',5'-dimethoxyisoflavone (**66**) and purpuranin A (**67**) from he aerial parts and pods respectively (Figure 2.13) (Rao and Raju, 1984, Chang *et al.*, 2000). he isoflavone purpuranin A (**67**) is similar to maximaisoflavone C (**68**), maximaisoflavone E **69**) and maximaisoflavone G (**69**) which have been isolated from the roots and pods of *T. naxima* (Rao *et al.*, 1985).



7-Dihydroxy-3',5'-dimethoxyisoflavone (66)



Purpuranin A, R_1=OCH_3, R_2=CH_3, R_3=H (67)

Maximmaisoflavone C, R_1=H, R_2=Prenyl

R_3=OCH_3 (68)

Maximaisoflavone E, R_1=OCH_3, R_2=H, R_3=H (69)

Maximaisoflavone G, R_1=H, R_2=H, R_3=OCH_3 (70)

igure 2.15: Isoflavones isolated from *T. purpurea* and *T. maxima*

.4.3 Flavanones of *Tephrosia*

lavanones (dihydroflavones) possess 2-phenylchromanone as the parent skeleton. Since carbon- of the flavanones molecule is a centre of asymmetry, two isomeric forms of each structure are ossible but most of the naturally occurring flavanones have a phenyl substituent at C-2 position n pseudoequatorial orientation (Agrawal, 1989). Fig. 2.16 shows the basic skeleton of lavanones and the numbering system.

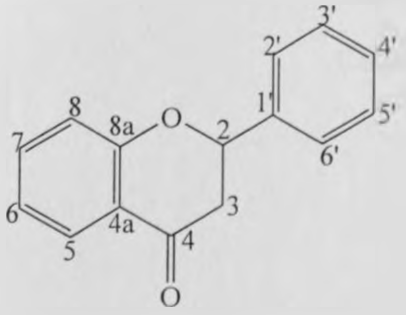
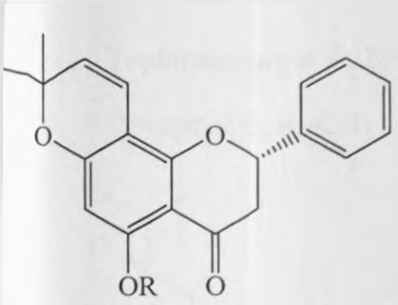


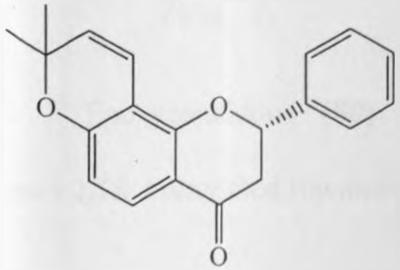
Figure 2.16: Flavanone skeleton

nple prenylated flavanones have been isolated from T*ephrosia* species. The pyranoflavanones
ovatin (**71**) and obovatin methyl ether (**72**) were isolated from the stem of *T. obovata* and the
ts of *T. elata* (Chen, 1978; Lwande, 1985; Gomez-Garibay *et al.*, 1986). The pyranoflavanone
lonchocarpin (**76**) isolated from the roots of *T. purpurea* differs from obovatin (**71**) and its
thyl ether (**72**) in substituition at C-5 carbon (Pelter *et al.*, 1981). The prenylated flavanones
abranin (**73**), methylglabranin (**74**) were isolated from the whole plant of *T. abbottiae* (Gomez-
aribay *et al.*, 1986). Methylglabranin (**74**) has also been isolated from the whole plant of *T.*
llophylla, the roots of *T. nitena* and *T. polyphylla* (Gomez-Garibay *et al.*, 2002; Dagne *et al.*,
92). The flavanone candidone (**75**) isolated from the roots of *T. elata* is a methyl ether of
abranin (**73**) (Lwande, 1985).

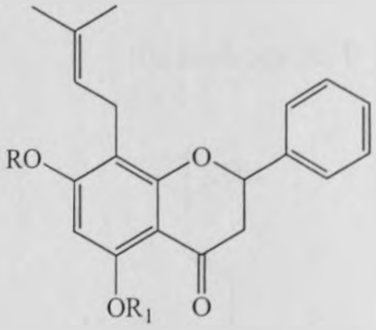


Obovatin, R=H (**71**)

Obovatin Me ether, R=CH_3 (**72**)



Isolonchocarpin (**76**)



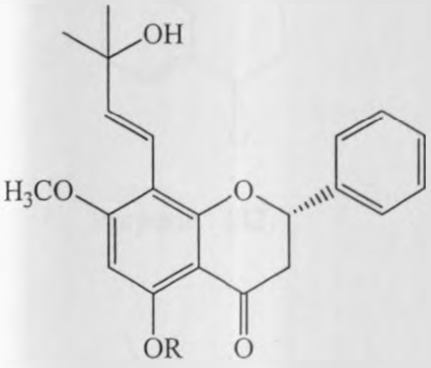
Glabranin, R=R_1=H (**73**)

Methylglabranin, R= CH_3, R_1=H (**74**)

Candidone, R=R_1=CH_3 (**75**)

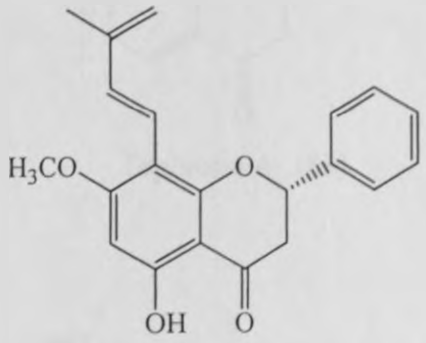
Figure 2.17: Prenylated flavanones isolated from *Tephrosia* species

er prenylated flavanones include tephroleocarpin A (77) and tephroleocarpin B (79) isolated n the aerial parts of *T. leiocarpa* (Gomez-Garibay *et al.*, 1991); quercetol (78) isolated from roots of *T. quercetorum* (Gomez-Garibay *et al.*, 1988); epoxycandidone (71) isolated from whole plant of *T. hamiltonii* (Hussaini and Shoeb, 1987) and falciformin (81) isolated from pods of *T. Falciformis* (Figure 2.18) (Khan *et al.*, 1986).

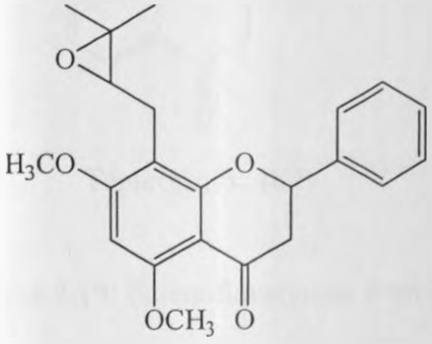


Tephroleocarpin A, R=H (77)

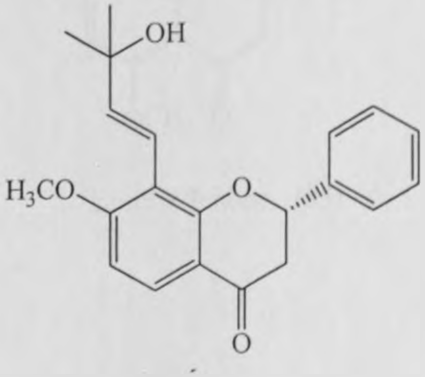
Quercetol C, R=CH_3 (78)



Tephroleocarpin B (79)



Epoxycandidone (80)

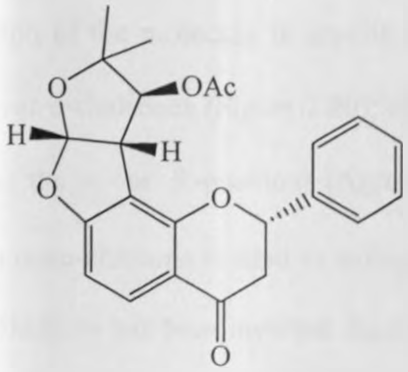


Falciformin (81)

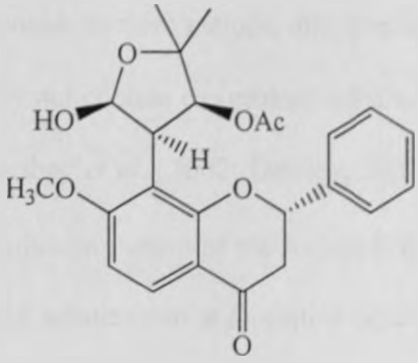
Figure 2.18: Prenylated flavanones from *Tephrosia* species

Complex 7/8-furanoflavanones have been isolated from the seeds of *T. purpurea*. These are purpurin (82), tephrorin A (83) and tephrorin B (84) (Gupta *et al.*, 1980; Chang *et al.*, 2000).

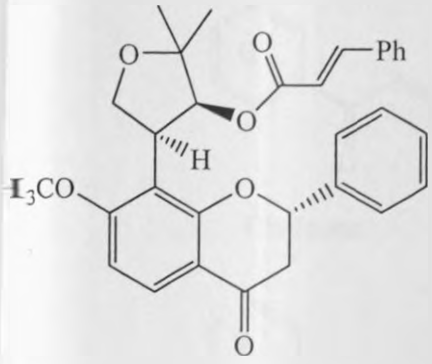
The flavanones like emoroidenone from the roots of *T. emoroide* have a single furan unlike purin which has two furan rings (Machocho *et al.*, 1995).



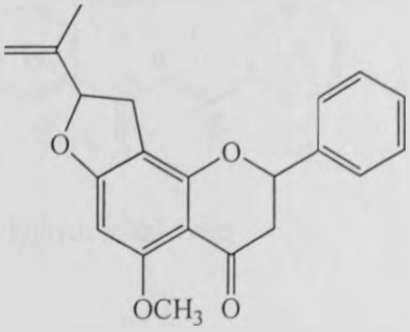
Purpurin (**82**)



Tephrorin A (**83**)



Tephrorin B (**84**)



Emoroidenone (**85**)

Figure 2.19: Furanoflavanones from the genus *Tephrosia*

4.4.4 Chalconoids of *Tephrosia*

Chalconoids are preflavonoids that exhibit the basic C_6-C_3-C_6 skeleton of flavonoids but the C_3 portion of the molecule is acyclic. Other 'Acyclic' flavonoids besides include, dihydrochalcones and *retro*-chalcones (Figure 2.20); all of which may or may not contain oxygenated substituents at either the α- or β-positions (Agrawal, 1989; Gomez-Garibay *et al.*, 2002; Dewick, 2002). The term *retro*-chalcone is used to indicate that the typical substitution pattern of the A- and B-rings of the chalcone has been inverted. Some chalcones with oxygen substituition at β-position occur in the genus *Tephrosia*.

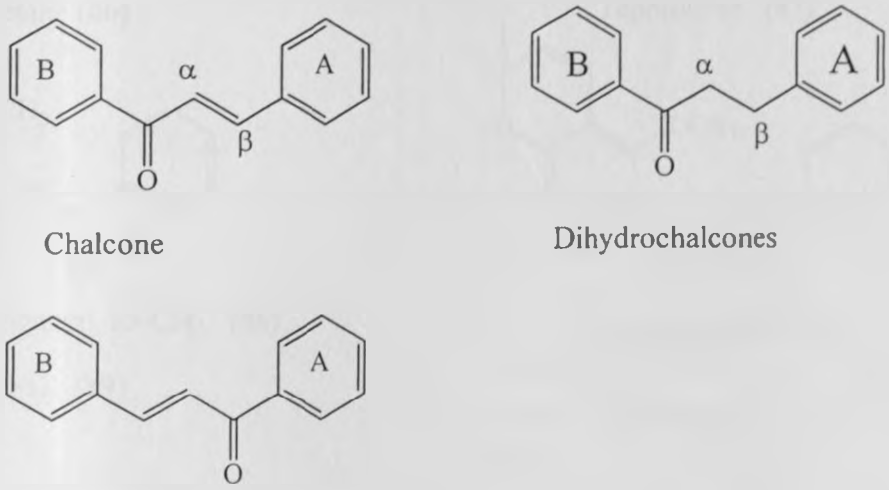
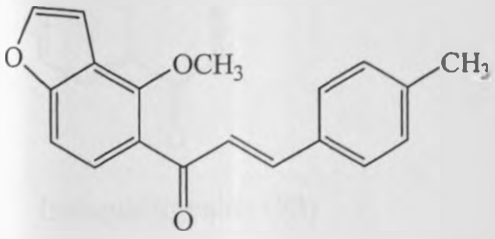


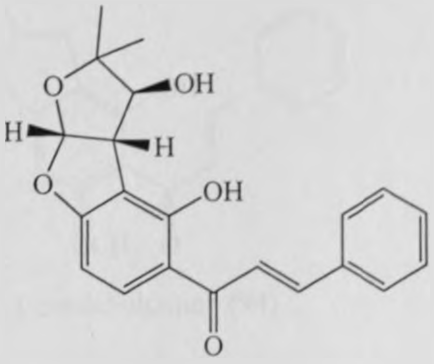
Figure 2.20: Basic skeleton of chalconoids

More than twenty chalcones have been reported from the genus *Tephrosia* (Al-Hazimi *et al.*, 2005). At least seven chalcones, purpuritenin (**86**), tephrosone (**87**), *O*-methylpongamol (**88**), pongamol (**89**), purpurenone (**90**), purpuritenin B (**91**) and (+)-tephropurpurin (**92**), have been isolated from *T. purpurea* roots, seeds and the whole plant (Pelter *et al.*, 1981; Sinha *et al.*, 1982;

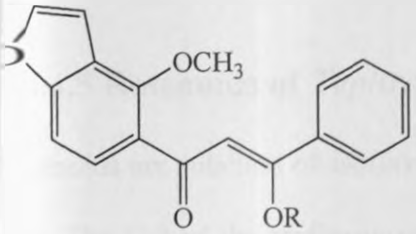
ao and Raju 1984; Saxena and Choubey 1997; Chang *et al.*, 2000). *O*-Methylpongamol (**88**), ngamol (**89**), purpurenone (**90**) are chalcones with oxygen substituition at β-position. The alcones *O*-methylpongamol (**88**) and pongamol (**89**) have also been reported in the roots of *T. miltonii* (Rao and Prasad, 1992).



Purpuritenin (**86**)

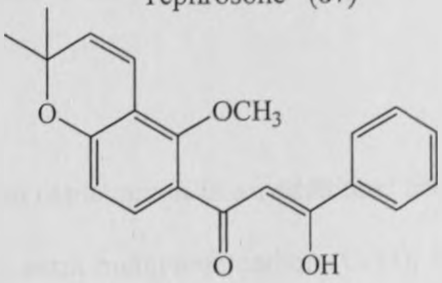


Tephrosone (**87**)

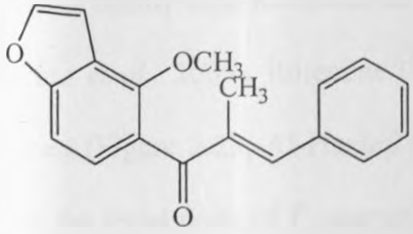


O-Methylpongamol, R=CH_3 (**88**)

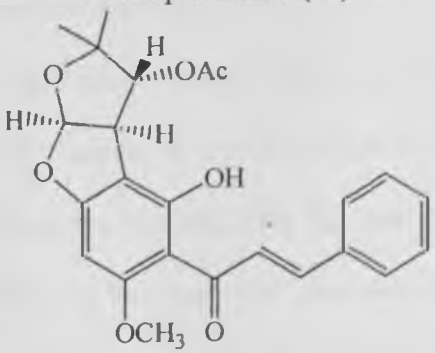
Pongamol, R=H (**89**)



Purpurenone (**90**)



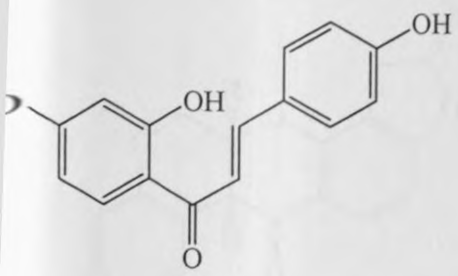
Purpuritenin B (**91**)



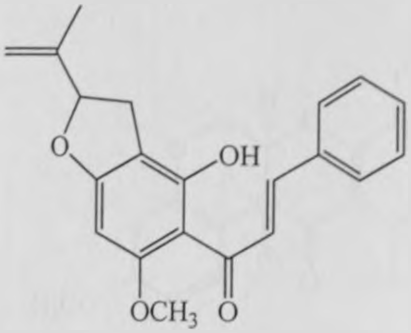
(+)-Tephropurpurin (**92**)

Figure 2.21: Chalcones isolated from *T. purpurea* and *T. hamiltonii*

simplest chalcones, isoliquiritigenin (**93**) was reported from the stem of *T. toxicaria* while ;sichalcone (**94**) occurs in the roots and aerial parts of *T. crassifolia* (Gomez-Garibay *et al.*, ›9).



Isoliquiritigenin (**93**)



Crassichalcone (**94**)

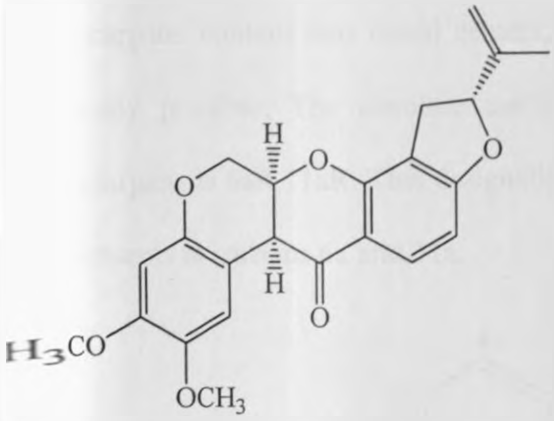
gure 2.22: Chalcones isolated from *T. toxicaria* and *T. crassifolia*

4.4.5 Rotenoids of *Tephrosia*

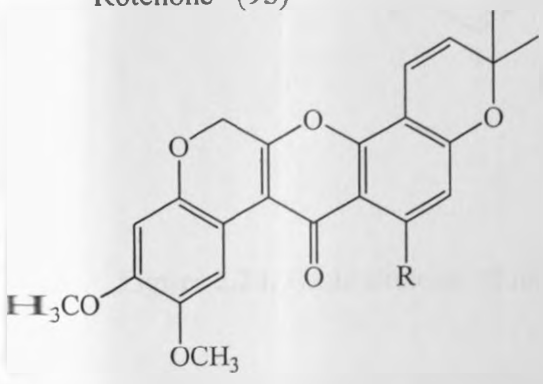
otenoids are subclass of isoflavonoids containing an extra carbon in an additional heterocyclic ng. The C-2 of the isoflavanone skeleton bears an extra methylene carbon (C-11), which get clised with C-2' of ring B to form a tetracyclic ring system (Agrawal, 1989).

t least twenty nine rotenoids have been reported by 2005 from twenty *Tephrosia* species (Al-Hazimi *et al.*, 2005). Rotenone (**95**) has been repeatedly isolated from the rotenoid containing pecies (Figure 2.23, Al-Hazimi *et al.*, 2005). The rotenoid α-toxicarol (**96**) has been reported rom the aerial parts of *T. purpurea* (Rao and Raju 1984) and the roots of *T. candida* (Andrei *et l.*, 1997). The dimethylchromene rotenoids tephrosin (**97**), deguelin (**98**) and 12a-hydroxy-α-oxicarol (**99**) have been reported in the roots of *T. candida* (Andrei *et al.*, 1997). Deguelin (**98**) as also been isolated from the whole plant of *T. abbottiae*, roots of *T. falciformis* and *T. entaphylla* (Al-Hazimi *et al.*, 2005)

The rotenoids 6a,12a-dehydrodeguelin (**100**) and 6a,12a-dehydro-α-toxicarol (**101**) have been reported from *T. candida* (Andrei *et al.*, 1997). These two rotenoids are unsaturated at C-6a and C-12a (Figure 2.23)

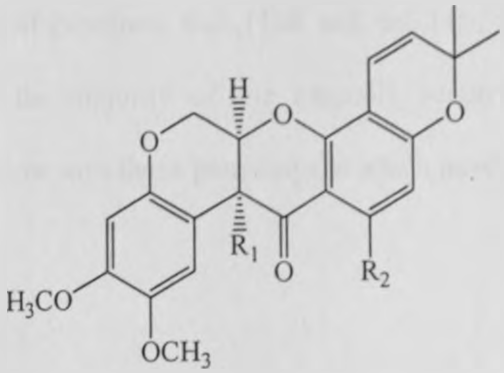


Rotenone (**95**)



6a,12a-dehydrodeguelin, R=H (**100**)

6a,12a-dehydro-α-toxicarol, R=OH (**101**)



α-Toxicarol, R_1=H, R_2=OH (**96**)

Tephrosin, R_1=OH, R_2=H (**97**)

Deguelin, R_1=R_2=H (**98**)

12a-Hydroxy-α-toxocarol

R_1=R_2=OH (**99**)

Figure 2.23: Rotenoids of the genus *Tephrosia*

2.4.6 Pterocarpanoids of the genus *Tephrosia*

Pterocarpanoids are a group of naturally occurring heterocycles containing a 6a,11a-dihydrofurobenzopyran nucleus which has two asymmetric centers (Figure 2.24). Although pterocarpans contain two chiral centers, only the configurations, 6aR,11aR and 6aS,11S, are sterically possible. The absolute configuration of the majority of the naturally occurring pterocarpans is 6aR,11aR. This designation is constistent with those pterocarpans which have no substituents at carbons 6a and 11a.

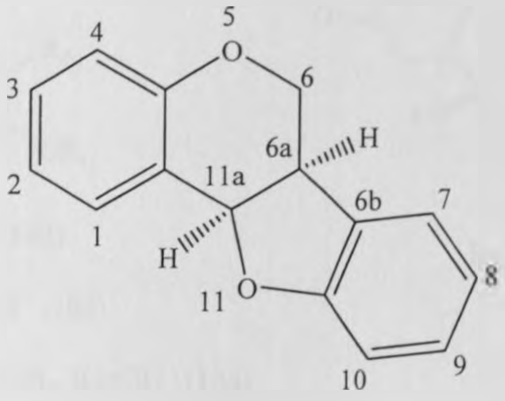
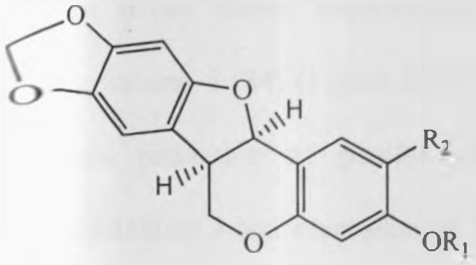


Figure 2.24: Basic skeleton of most commonly occurring pterocarpan

Many of the pterocarpans are phytoalexins that are produced in plants during infection by fungi, bacteria or viruses. A total of forty-four nonprenylated pterocarpans have been obtained from different leguminous plants (Al-Hazimi *et al.,* 2005). Among these, the compounds maackiain (**102**), pterocarpin (**103**) and medicarpin (**107**) (Figure 2.25) widely occur in plants belonging to various genera (Al-Hazimi and Alkhathlan 2000). Both prenylated and non-prenylated pterocarpans have been reported in the genus *Tephrosia*. Maackiain (**102**) has been isolated from the roots of *T. purpurea*, *T. maxima T. elata*, *T. fulvinervis*, *T. hamiltoni* and *T. bidwilli* (Al-Hazimi *et al.,* 2005). A derivative of pterocarpin, 2-hydroxypterocarpin (**104**) has been reported

the pods of *T. pentaphylla* (Al-Hazimi *et al.*, 2005) while 2-methoxymaackiain (**105**), a derivative of maackiain has bee reported in aerial parts of *T. bidwilli* (Maximo and Lourenco 1998). Pterocarpans reported in the roots of *T. hilderbrandtii* and pods of *T. pentaphylla* are hildercarpin and 2-hydroxypterocarpin respectively (Lwande *et al.*, 1987). The pterocarpin 3,4:8,9-dimethylenedioxypterocarpan (**108**) isolated from the roots of *T. aequilata* is the only pterocarpan with 6aR and 11aS configuration (Tarus *et al.*, 2002).

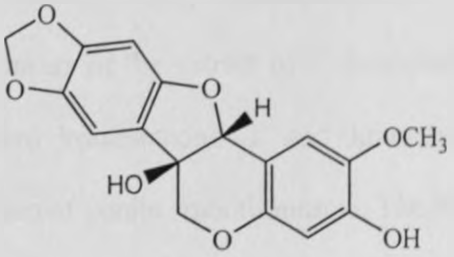


Maackiain, R_1=H, R_2=H (**102**)

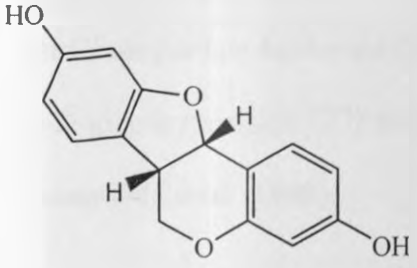
Pterocarpin, R_1=CH_3, R_2=H (**103**)

2-hydroxypterocarpin, R_1=CH_3, R_2=OH (**104**)

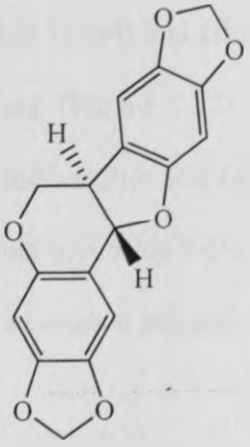
2- Methoxymaackiain, R_1=H, R_2=OCH_3 (**105**)



Hildercarpin (**106**)



Medicarpin (**107**)



3,4:8,9-Dimethylenedioxypterocarpan (**108**)

Figure 2.25: Pterocarpans from *Tephrosia* species

5 BIOLOGICAL ACTIVITIES

5.1 Biological activities of the genus *Eriosema*

vestigations of *Eriosema* species has resulted to the isolation of bioactive compounds. xamples of such bioactive compounds are five pyrano – isoflavones isolated from the roots of *iosema kraussianum* with penile relaxant activity (Drewes *et al.*, 2002). The compounds were ven trivial names kraussianone 1 (**30**), kraussianone 2 (**31**), kraussianone 3 (**32**) and aussianone 5 (**34**) (Figure 2.9). The major components of the extract of *E. kraussianum* that used relaxation of penile smooth muscle were kraussianone 1 and kraussianone 2. raussianone 3 and kraussianone 5 caused contraction of penile smooth muscle. The biological tivity of the roots of this plant was mainly due to kraussianone 1 and kraussianone 2.

hromones and phenolic compounds with antifungal activity have been isolated from the roots f *E. tuberosum* (Ma *et al.*, 1996). The chromones, eriosematin D (**44**) and eriosematin E (**45**) ere active against *Candida cucumerinum* and *C. albicans* (Figure 2.12). The phenolic ompounds, 4-hydroxybenzoic acid (**46**), hydroquinone (**47**) and vanillic acid (**48**), were active gainst *Cladosporium herbarum* (Figure 2.13a). An isoflavonoid glycoside 5-*O*-methylgenistein -*O*-β-D-glucopyranoside (**37**) isolated from the roots of *E. tuberosum* has antiviral activity *in itro* assay (Ma *et al.*, 1998).

5.2 Biological activities of the genus *Tephrosia*

hemical investigations of plants of the genus *Tephrosia* has resulted to the isolation of active mpounds with anticancer, insecticidal and anitumour activities. In the case of *T. purpurea* the tivities are attributed to lipophilic flavonoid aglycones such as flavanones, flavonols, flavones nd chalcones present in the extracts (Santram *et al.*, 2006). Rotenoids which have insecticidal ctivity also exhibit strong ictiotoxic activity (Andrei *et al.*, 1997). Rotenone (**95**, Figure 2.23) as insecticidal properties (Ramen *et al.*, 1992). Tephrosin (**97**) is active against tumours ncluding skin cancer (Andrei *et al.*, 1997). The roots of *T. emoroides* yielded emoroidenone (**85**, igure 2.19) which has insect anti-feedant activity against the larvae of stalk borer, *Chillo artellus* (Machocho *et al.*, 1995). The roots of *T. hildebrandtii* yielded hildecarpin (**106**, Figure .25) which has insect anti-feedant activity against the legume pod-borer, *Maruca testulalis* as well as anti-fungal properties (Tarus *et al.*, 2002; Lwande *et al.*, 1986).

Pongamol (**89**, Figure 2.21), isolated from *T. lanceolata* and *T. purpurea*, is used in insecticide and pesticide manufacture (Parmar *et al.*, 1989). Pseudosemiglabrin (**64,** Figure 2.14), isolated from *T. apollinea* is a platelet aggregation inhibitor (Waterman and Khalid, 1980). The pterocarpan 2-methoxymaackiain (**105**, Figure 2.25), which is a constituent of *T. bidwilli*, has antifungal activity (Tarus *et al.*, 2002). α-toxicarol (**96,** Figure 2.23) obtained from the stem of *T. odorata*, *T. toxicaria* and aerial parts of *T. purpurea* is used as a fish poison and its biological activities are comparable to rotenone (**95**) (Jang *et al.*, 2003).

CHAPTER THREE

MATERIALS AND METHODS

.1 GENERAL

.1.1 Reagents

echnical grade organic solvents which included *n*-hexane, dichroromethane, ethyl acetate, ethanol and acetone were used.

alts used to prepare physiological solutions were of analytic grade. Sodium hydrogen carbonate, alcium chloride and sodium chloride were sourced from Loba Chemie, Mumbai, India. otasium chloride was obtained from S. D. Fine-Chem, Mumbai India; glucose was obtained rom Fisher Scientific UK limited; sodium dihydrogen orthophosphate was sourced from BDH hemicals Ltd. Poole England and magnesium sulphate was sourced from Howse & McGeorge imited, Nairobi, Kenya. Oxygen and carbon dioxide was obtained from BOC, Kenya.

3.1.2 Instrumentation

The ^{1}H NMR (600, 500 or 200 MHz) and ^{13}C NMR (150, 125 or 50 MHz) spectra were recorded on Bruker or Varian-Mercury spectrometers using tetramethylsilane (TMS) as the internal standard. Homonuclear Correlation spectroscopy (COSY), Nuclear Overhauser Enhancement Spectroscopy (NOESY), Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple bond Correlation (HMBC) spectra were acquired using the standard Bruker software. EI-MS spectra were recorded on a direct inlet, 70 eV, on SSq 710, Finnigan MAT mass spectrometer. Melting points were determined using a Gallenkamp melting point

apparatus with capillary tubes. UV/VIS spectra were recorded using a Pye-Unican SPS-150 Spectrophotometer. The plant materials were ground using a Willymill.

3.1.3 Collection of plant materials

Eriosema psoraleoides and *Tephrosia purpurea* were collected from the wild. *E. psoraleoides* from Kilungu hills in Makueni district in January, 2009; while *T. purpurea* was collected from Kilifi district in August, 2007. The plant species were identified by Mr. Patrick C. Mutiso of the University Herbarium, School of Biological Sciences, University of Nairobi. The voucher specimen (Mutiso- 015 / January 2009) for *E. psoraleoides* and (Mutiso - 520/ August 2007) for *T. purpurea* were deposited in the University Herbarium. The plant parts were dried under a shade before being milled.

3.1.4 Chromatographic conditions

Column chromatography was carried out using silica gel 60 (70-230 mesh) and Sephadex LH 20. The fractions were monitored by TLC using Merck pre-coated silica gel 60 F_{254} plates, with UV (254, 366 nm) and iodine vapour used for detection. Preparative thin layer chromatography (PTLC) was done on silica gel (Merck). PTLC plates were prepared by adding 200 ml of water to 80 g of silica gel. Slurry that formed was allowed to stand for 45 min. The slurry was then poured and spread evenly on clean 20 cm^2 glass plates and was left to dry at room temperature. Activation of the silica gel was done in an oven for 30 min at 383 K, removed and allowed to cool to room temperature before use.

1.5 Animals

Mice weighing about 25 g and a Guinea pig weighing 300-400 g were obtained from the Animal House of the Department of Pharmacology and Pharmacognosy, School of Pharmacy and the Department of Public Health Pharmacology and Toxicology, Kabete Campus. The animals were housed in plastic cages and maintained at standard conditions (12 hour light/dark cycle, 25 ± 5 °C; 35 – 60% relative humidity). The mice and guinea pig were fed on commercial pelleted feed and tap water *ad libitum*. The feeds were obtained from Unga Feeds, Limited Nairobi, Kenya. The aorta and vas deferens of the sheep was obtained from slaughter house in Dagoretti, Kiambu, Nairobi.

3.1.6 Sources of microorganisms for antiplasmodial test

The D6 and W2 strains of *P. falciparum* were obtained from the United States Army Medical Research Unit-Kenya, Walter Reed Project, Kisumu (courtesy of Mr Hoseah M. Akala), all preserved at -20^{0}C.

3.1.7 Sources of bacteria and fungi test strains

Antibacteria activity was tested against standard strains of bacteria: *Staphylococcus aureus* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 25923. The antifungal activity was tested against standard strains, *Candida albicans* ATCC 90028, *Cryptococcus neoformans*, *Trichphyton mentagrophytes* and *Microsporum gypsum* fungi. The standard drugs used were chloramphenicol and fluconazole for antibacterial and antifungal activities, respectively.

2 EXTRACTION AND ISOLATION OF COMPOUNDS

2.1 Extraction and isolation of compounds from *Eriosema psoraleoides* (roots)

The air dried and ground roots (164 g) of *E. psoraleodes* were extracted with dichloromethane/methanol (1:1) by cold percolation at room temperature (4 x 800 ml). The solvent was removed under vacuum by use of a rotary evaporator at 35°C. This afforded a dark oily extract that was partitioned between water and ethyl acetate. The organic layer (4 g) was subjected to CC on silica gel (61 g) eluting with hexane containing increasing amounts (1%, 2%, 3%, 5%, 7%, 10%, 14%, 16%, 20%, 30%, 40% and 50%) of EtOAc.

The fraction eluted with 10% EtOAc in n-hexane was separated on Sephadex LH-20 column using CH_2Cl_2/MeOH (1:1) to yield yellow solid of **1** (4.0 mg). The fractions eluted with 14% and 16% EtOAc in hexane were combined and separated on Sephadex LH-20 column using CH_2Cl_2/MeOH (1:1) and gave an orange solid of **2** (2.6 mg). The fraction eluted with 20% EtOAc in hexane gave seven fractions after CC on Sephadex. Further separation of fraction one gave amorphous brown powder of **3** (5.6 mg). The remaining six fractions were combined and purified by PTLC (dichloromethane/ethyl acetate/hexane (30:50:20) to yield compound **4** (1.4 mg).

3.2.2 Extraction and isolation of compounds from *Tephrosia purpurea* (stem)

Air dried ground stems of *Tephrosia. purpurea* (2 Kg) were extracted with dichloromethane/methanol (1:1) by cold percolation at room temperature (3 x 1.5 L). The extract was filtered and the solvent removed under vacuum using a rotary evaporator at 35°C. This gave

rk oily extract that was partitioned between water and ethyl acetate. The organic layer (36 g) as subjected to CC on silica gel (400 g) eluting with hexane containing increasing amounts of hyl acetate (2%, 4%, 6%, 8%, 10%, 12.5%, 15%, 20%, 25%, 30%, 40%, and 50% ethyl acetate hexane).

ompound **5** (108.6 mg) crystallized from the fraction eluted with 4% ethyl acetate in hexane. he fraction eluted with 6% Ethyl acetate in hexane was crystallized from xane/dichloromethane to yield **6** (317.1 mg).

he fractions eluted with 10% and 12.5 % ethyl acetate in hexane were mixed, and subjected to olumn chromatography on Sephadex LH-20 from which **7** (46.3 mg) crystallized from one of ie fraction. The second fraction (1.12 g) was further purified by CC [on silica gel (92 g) eluting ith hexane/dichloromethane (40:60, 30:70, 10:90) followed by dichloromethane containing ncreasing amounts of ethyl acetate (2% and 10%)] to give Solid **7** (41.2 mg).

ractions eluted using 15% ethyl acetate was crystallized in hexane/dichloromethane to give a rown stick solid and colourless filtrate. The filtrate was separated on Sephadex to give a white owder of **8** (33.5 mg). The fraction that was eluted with 25% ethyl acetate was crystallized from exane/dichloromethane to yield a pure amorphous powder of **9** (106.1 mg).

3.3 PHYSICAL AND SPECTROSCOPIC DATA OF ISOLATED COMPOUNDS

Compound 1

Yellow solid. R_f = 0.64 (CH_2Cl_2/$(CH_3)_2CO$, 9:1). UV λ_{max} (MeOH) nm: 208, 286 nm. ^{1}H NMR (200 MHz, acetone-d_6): δ_H 12.34 (*s*, 5-OH), 6.96 (*d*, *J*=8.4 Hz, H-6'), 6.60 (*d*, *J*=2.4 Hz, H-3'),

2 (*dd*, *J*=8.2, 2.2 Hz, H-5'), 6.05 (*d*, *J*=2.4 Hz, H-6), 6.02 (*d*, *J*=1.6 Hz, H-8), 4.55 (*dd*, *J*=10.6, 0 Hz, H-2_{ax}), 4.41 (*dd*, *J*=5.2, 10.6 Hz, H-2_{eq}), 4.26 (*dd*, *J*=5.4, 11.0 Hz, H-3), 3.86 (*s*, H_3-7), 3.76 (*s*, OCH_3-2''). ^{13}C NMR (150 MHz, acetone-d_6): δ_C 199.3 (C-4), 165.2 (C-5), 5.0 (C-8a), 162.4 (C-7), 160.1, (C-4'), 160.0 (C-2'), 132.5 (C-6'), 115.7 (C-1'), 108.7 (C-5'), 4.3 (C-4a), 101.1 (C-3'), 96.1 (C-6), 95.0 (C-8), 71.9 (C-2), 56.9 (OCH_3-7), 56.6 (OCH_3-2'), .1 (C-3). EI-MS *m/z* (rel. int.): 316 (37, $[M]^+$), 167 (100), 149 (26).

ompound 2

rown-yellow powder. R_f = 0.4 (n-C_6H_{14}/CH_2Cl_2/EtoAc, 2:4:4). UV λ_{max} (MeOH) nm: 208, 257 m. ^{1}H NMR (200 MHz, acetone-d_6): δ_H 12.98 (*s*, 5-OH), 7.82 (*d*, *J*=8.6 Hz, H-6'), 7.14 (*d*, =1.8 Hz, H-3'), 7.02 (*dd*, *J*=2.2, 8.6 Hz, H-5'), 6.60 (*d*, *J*=2.2 Hz, H-6), 6.37 (*d*, *J*=2.0 Hz, H-8). ^{3}C NMR (50 MHz, acetone d_6): δ_H 183.2 (C-4), 168.4 (C-2), 163.6 (C-5), 163.0 (C-7), 158.7 (C-'), 156.2 (C-8a), 152.9 (C-4'), 121.8 (C-6'), 115.5 (C-1'), 113.8 (C-5'), 110.5 (C-30, 105.0 (C-a), 100.2 (C-3'), 98.9 (C-6), 95.0 (C-8). EI-MS *m/z* (rel. int.): 285 (38, $[M+H]^+$), 153 (22), 125 14).

Compound 3

Amorphous light brown powder. Rf = 0.6 (n-C_6H_{14}/CH_2Cl_2/EtOAc, 8:7:5). UV λ_{max} (MeOH) nm: 207, 262 nm. ^{1}H NMR (600 MHz, acetone-d_6): δ_H 13.04 (*s*, 5-OH), 8.16 (*s*, H-2), 7.46 (*d*, *J*=8.4 Hz, H-6'), 7.46 (*d*, *J*=8.4 Hz, H-2'), 6.91 (*d*, *J*=8.4 Hz, H-5'), 6.91 (*d*, *J*=8.4 Hz, H-3'), 6.42 (*d*, *J*=1.8 Hz, H-8), 6.29 (*d*, *J*=1.8 Hz, H-6). ^{13}C NMR (150 MHz, acetone-d_6): δ_c 182.22/182.30 (C-4, 4''), 165.58/165.63 (C-5, 5''), 164.27/164.57 (C-7, 7''), 159.32/159.70 (C-8a, 8a''), 159.04/159.70 (C-4', 4'''), 154.91/154.95 (C-2, 2''), 131.83/131.83 (C-2', 2'''),124.68/124.73 (C-3,

123.70/123.73 (C-1', 1'''), 116.58 (C-3', 3'''), 106.78/106.81 (C-4a, 4a''), 100.41/100.46 (C-6, . 95.10/95.17 (C-8, 8''). EI-MS *m/z* (rel. int.): 270 (100), 153 (75), 118 (22).

mpound 4

lourless oily substance. R_f = 0.6 (n-C_6H_{14}/CH_2Cl_2/EtOAc, 1:4:5). ^{1}H NMR (600 MHz, tone-d_6): δ_H 12.38 (*s*, 5-OH), 6.97 (*d*, *J*=8.4 Hz, H-6'), 6.52 (*d*, *J*=2.4 Hz, H-3'), 6.42 (*dd*, 2.4, 8.4 Hz, H-5'), 5.97 (*d*, *J*=2.4 Hz, H-6), 5.95 (*d*, *J*=1.8 Hz, H-8), 4.52 (*dd*, *J*=10.8, 11.4 Hz, -2_{ax}), 4.42 (*dd*, *J*=5.4, 11.4 Hz, H-2_{eq}), 4.27 (*dd*, *J*=5.4, 10.8 Hz, H-3), 3.77 (*s*, OCH_3-2'). ^{13}C MR (150 MHz, acetone-d_6): δ_c 198.0 (C-4), 167.9 (C-5), 166.3 (C-7), 165.3 (C-8a), 160.2 (C-), 159.9 (C-2'), 132.4 (C-6'), 115.9 (C-1'), 108.7 (C-5'), 104.0 (C-4), 101.0 (C-3'), 97.6 (C-6), 6.3 (C-8), 71.9 (C-2), 56.5 (OCH_3-2'), 48.0 (C-3).

ompound 5

olourless amorphous powder. R_f = 0.69 (CH_2Cl_2/EtOAc, 9:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ_H .34 (*d*, 5.2 Hz, H-6), 5.17 (*dd*, *J*=8.2, 15.2 Hz, H-22), 4.98 (*dd*, *J*=7.8, 15.4 Hz, H-23), 3.49 (*m*, -3), 1.02 (*d*, *J*=5.6 Hz, H-21), 1.00 (*s*, H-19), 0.91 (*d*, *J*=6.2, H-27), 0.81 (*t*, *J*=5.6, H-29), 0.69 (*s*, H-18). ^{13}C NMR (50 MHz, $CDCl_3$): δ_c 141.0 (C-5), 138.6 (C-22), 129.5 (C-23), 121.9 (C-6), 72.0 (C-3), 57.1 (C-14), 56.2 (C-17), 51.5 (C-24), 50.4 (C-9), 42.5 (C-130), 42.4 (C-4), 39.9 (C- 12), 37.5 (C-1), 36.7 (C-10), 32.1 (C-7, 8, 24), 31.9 (C-2), 29.2 (C-15), 25.6 (C-28), 24.5 (C-15), 21.3 (C-11, 21), 19.6 (C-27), 19.2 (C-26), 12.5 (C-29).

Compound 6

Amorphous powder. R_f = 0.42 (n-C_6H_{14}/EtOAc, 9:1). UV λ_{max} (MeOH) nm: 280, 310 nm. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.18 (*d*, *J*=9.0 Hz, H-5), 7.97 (*m*, H-2', 6'), 7.78 (*d*, *J*=2.5 Hz, H-2"), 7.56 (*m*, H-3', 4', 5', 6), 7.22 (*d*, *J*=2.5 Hz, H-3"), 6.94 (*s*, H-3). ^{13}C NMR (50 MHz, $CDCl_3$): δ_C 178.8 (C-4), 163.3 (C-2), 158.6 (C-7), 151.1 (C-8a), 146.1 (C-2"), 131.9 (C-1', 4'), 129.4 (C-3', 5'), 126.5 (C-2', 6'), 122.0 (C-5), 119.3 (C-4a), 117.7 (C-8), 110.6 (C-6), 108.0 (C-3), 104.4 (C-3"). EI-MS *m/z* (rel. int.): 263 (15, $[M+H]^+$), 161 (10), 132 (20).

Compound 7

Amorphous white powder. R_f = 0.47 (CH_2Cl_2/EtOAc, 9:1). UV λ_{max} (MeOH) nm: 295, 310 nm. ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 8.16 (*d*, *J*=8.4 Hz, H-5), 7.91 (*m*, H-2', 6'), 7.54 (*m*, H-3', 5', 4'), 7.94 (*d*, *J*=8.4 Hz, H-6), 6.83 (*s*, H-3), 6.64 (*d*, *J*=6.4 Hz, H-2"), 5.64 (*s*, H-4"), 4.30 (*d*, *J*=6.4 Hz, H-3"), 2.22 (*s*, $COCH_3$-4"), 1.32 (*s*, CH_3-5"), 1.09 (*s*, CH_3-5). ^{13}C NMR (50 MHz, $CDCl_3$): δ_C 178.3 (C-4), 170.4 ($COCH_3$-4"), 164.7 (C-7), 164.0 (C-2), 154.1 (C-8a), 132.6 (C-5), 132.2 (C-1'), 129.9 (C-4'), 129.7 (C-3', 5'), 127.3 (C-2', 6'), 119.9 (C-4a), 113.4 (C-8), 113.2 (C-2"), 110.0 (C-6), 108.3 (C-3), 88.7 (C-5"), 80.9 (C-4"), 53.6 (C-3"), 28.3 (CH_3-5"), 23.9 (CH_3-5").

Compound 8

White amorphous powder. R_f = 0.45 (n-C_6H_{14}/EtOAc, 3:2), melting point 144-145°c. $[\alpha]_D^{25.3}$ +58.2399. UV λ_{max} (MeOH) nm: 295, 325 nm. ^{1}H NMR (500 MHz, acetone-d_6): δ_H 8.18 (*m*, H-2', 6'), 8.00 (*d*, J=8.5 Hz, H-5), 7.63 (*m*, H-3', 4', 5'), 6.94 (*d*, *J*=8.5 Hz, H-6), 6.79 (*s*, H-3), 5.35 (*d*, *J*=8.5, H-4"), 5.02 (*dd*, *J*=2.0, 9.5 Hz, H-2"), 4.84 (*dd*, *J*=8.0, 9.5 Hz, H-2"), 4.44 (*ddd*, *J*=2.0,

8.0, 8.5 Hz, H-3"), 2.00 (*s*, $COCH_3$-7), 1.76 (*s*, CH_3-5"), 1.61 (*s*, CH_3-5"), 1.61 (*s*, $COCH_3$-4") ^{13}C NMR (75 MHz, acetone-d_6): δ_c 177.6 (C-4), 170.8 ($COCH_3$-7), 170.3 ($COCH_3$-4"), 167.9 (C-7), 163.9 (C-2), 155.9 (C-8a), 133.6 (C-1'), 133.1 (C-4'), 130.7 (C-3', 5'), 129.3 (C-5), 127.9 (C-2', 6'), 119.9 (C-4a), 116.0 (C-8), 110.1 (C-6), 108.5 (C-3), 84.0 (C-5"), 79.1 (C-2"), 78.7 (C-4"), 42.3 (C-3"), 24.4 (CH_3-5"), 23.0 ($COCH_3$-7), 22.4 (CH_3-5"), 20.9 ($COCH_3$-4"). EI-MS *m/z* (rel. int.): 437 (7, $[M+H]^+$), 319 (6), 317 (60), 316 (100), 263 (76), 161 (14), 102 (5).

Compound 9

Amorphous powder. R_f = 0.54 (CH_2Cl_2/$(CH_3)_2CO$, 8:2). UV λ_{max} (MeOH) nm: 295 nm. ^1H NMR (200 MHz, acetone-d_6): δ_H 8.12 (*m*, H-2', 6'), 8.02 (*d*, *J*=9.0 Hz, H-5), 7.61 (*m*, H-3', 4', 5'), 7.25 (*d*, *J*=9.0 Hz, H-6), 7.09 (*d*, *J*=16.6 Hz, H-1"), 6.93 (*d*, *J*=16.2 Hz, H-2"), 6.81 (*s*, H-3), 4.05 (*s*, OCH_3-7), 1.46 (s, $2CH_3$-3"). ^{13}C NMR (50 MHz, acetone-d_6): δ_c 177.0 (C-4), 163.1 (C-2), 161.9 (C-7), 161.5 (C-8a), 145.6 (C-1"), 132.4 (C-1'), 131.7 (C-4'), 129.3 (C-3', 5'), 126.7 (C-2', 6'), 124.9 (C-5), 115.0 (C-4a, 8), 114.6 (C-2"), 110.0 (C-6), 106.8 (C-3), 70.5 (C-3"), 56.1 (OCH_3-7), 29.5 (CH_3-3"), 29.2 (CH_3-3"). EI-MS *m/z* (rel. int.): 336 (14, $[M]^+$), 318 (51 ([M-H_2O), 102 (100).

3.4 BIOLOGICAL ASSAY

3.4.1 Effects of plant extracts and isolated compounds on isolated Guinea pig Trachea

The method used is described in literature (Leal *et al.*, 2006). A Guinea pig was killed by cervical dislocation. The whole isolated trachea of Guinea pig with the nerve supply was

removed and attached to capillary tube in an organ bath containing Kreb's solution of the following composition (mM): NaCl 118.0; KCl 4.4; $CaCl_2$ 2.5; $MgSO_4$ 1.1; KH_2PO_4 1.2; $NaHCO_3$ 25.0 and glucose 11.1. The organ bath was maintained at a temperature of 37°C and Kreb's solution was continuously aerated with oxygen – carbon dioxide mixture (95:5).

Each of the plant extract and pure compound was dissolved in water and introduced into the organ bath. The displacement of Krebs solution in the capillary tube was recorded after adding each of the extract and pure compound. The organ bath was drained and the tissue rinsed twice. It was let to rest for two to three minutes before adding another drug.

3.4.2 Effects of plant extracts and isolated compounds on isolated aorta of a Sheep

A sheep was killed by cervical dislocation. The whole isolated aorta of a sheep with the nerve supply was removed. The aorta was cut transversely between the segments so as to give rings which were tied together with cotton to form chains. The chains were mounted in an organ bath containing Krebs solution at a tension of 1 g maintained at 37°C.

Each of the plant extract and pure compound was dissolved in water and introduced into the organ bath. The isomeric muscle twitch-tension after adding each of the extract and pure compound was recorded using a force displacement transducer coupled to a physiograph. The organ bath was drained and the tissue rinsed twice. It was let to rest for two to three minutes before adding another drug.

3.4.3 Effects of plant extracts and isolated compounds on vas deferens of a rat and sheep

A rat was killed by cervical dislocation. The whole isolated vas deferens with the nerve supply was removed. Each of the vas deferens was cut transversely between the segments so as to give rings which were tied together with cotton to form chains. Each of the chains was set up in an organ bath containing Krebs solution.

Each of the plant extract and pure compound was dissolved in water and introduced into the organ bath. The isomeric muscle twitch-tension after adding each of the extract and pure compound was recorded using a force displacement transducer coupled to a physiograph. The organ bath was drained and the tissue rinsed twice. It was let to rest for two to three minutes before adding another drug.

3.4.4 Effect of *E. psoraleoides* root extract on mating behavior

The method used is described in literature (Carro-Juarez *et al.*, 2004). The male mice were devided into four groups of five each. Group one was treated with the roots of *E. psoraleoides* (2.5 g/kg of body weight) extract orally, group two was treated with sildenafil (600 mg/kg of body weight) orally, group three was treated with yohimbine (360 mg/kg of body weight) orally and group five acted as control. Twelve females were treated with stilboestrol (40 mg/ kg of body weight) intraperitoneally 24 hours before experiment.

About 30 minutes later three females were introduced into each of the male group. The time that elapsed from the introduction of the female into a cage until the first mount, the frequency of mounts in a period of 3 hours, the intromission frequency and latency, the number of mounts and

intromission preceding ejaculation, frequency of penile erection (PE) and the number of times the mouse bends down to lick the penis were observed and recorded.

3.5 *IN-VITRO* ANTIPLASMODIAL ACTIVITY ASSAY

Antiplasmodial activity was tested against chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) strains of *Plasmodium falciparum*. They were cultured as described in literature (Johnson *et al.,* 2007). The crude extract and pure compounds were assayed using a non-radioactive assay technique with modifications to determine 50% growth inhibition of cultured parasites (Smilkstein *et al.,* 2004). The *in vitro* drug susceptibility method that uses the fluorochrome called "SYBR Green I", a non-radioactive intercalating DNA marker that accurately depicts *in vitro* parasite replication was applied. Concurrently, twofold serial dilutions of the drugs chloroquine (1.953 to 1,000 ng/ml), mefloquine (0.488 to 250 ng/ml) and test sample (97.7 – 50,000 $ngml^{-1}$) were prepared on a 96 well plate. The culture-adapted *P. falciparum* were added on to the plate containing dose range of drugs and incubated in gas mixture (5% CO_2, 5% O_2, and 90% N_2) at 37°C. The assay was terminated 72 hrs later by freezing at -80°C. After thawing, lysis buffer containing SYBR Green I (1x final concentration) were added directly to the plates and gently mixed by using the Beckman Coulter Biomek 2000 automated laboratory workstation (Beckman Coulter, Inc., Fullerton, CA). The plates were incubated for 5 - 15 minutes at room temperature in the dark. Parasite growth inhibition was quantified by measuring the per-well relative fluorescence units (RFU) of SYBR green 1 dye using the Tecan Genios Plus (Tecan US, Inc., Durham, NC) with excitation and emission wavelengths of 485 nm and 535 nm, respectively, and with the gain set at 60. Differential counts of relative fluorescence units (RFUs) were used in calculating IC_{50}'s for each drug using Prism

4.0 software for Windows (Graphpad Software, San Diego, CA). A minimum of three separate determinations was carried out for each sample. Replicates had narrow data ranges hence presented as mean ± SD. The antiplasmodial tests were done in collaboration with Mr. Hosea M. Akala of Kenya Medical Research Institute and United States Medical Research Unit, Kenya.

3.6 ANTIMICROBIAL TEST USING DISC DIFFUSION TECHNIQUE

All glassware was sterilized by dry heat at 200 °C for one hour. Ten milliliters of water was sterilized autoclaving at 121 °C, 2 bar pressure for 15 minutes. All work was carried out using aseptic techniques. All bacteria were grown on Mueller Hinton medium while. All fungi were grown on Sabouraud's Dextrose Agar medium.

The crude extracts of *E. psoraleoides*, *T. purpurea* and pure compounds from *T. purpurea* were tested for activity against *Candida albicans, Microsporum gypsum, Staphylococcus aureus, Pseudomonas aeruginosa, Cryptococcus neoformans, Trichophyton mentagrophytes* and *Microsporum gypsum.*

Working cultures were grown using the slope technique. A microbial suspension of each organism was prepared by gently suspending the organism in the slope culture in about 10 ml of sterile water. Three milliliters of the microbial suspension was added to one liter of sterile molten medium at a temperature of about 45 °C. Twenty milliliters of seeded molten medium was added to petri dishes and medium and was left to set for about one hour. Whatman filter paper no. 1 disks of 6-mm diameter were used to screen the antimicrobial activity. Each sterile disc was impregnated with 20 µL of the extract and pure compound. Sterile water was used as the negative control. Chloramphenicol and fluconazole were used as reference drugs for antibacterial activity and antifungal activity respectively. All bacteria were incubated at 37.4 °C for 24 hours and the fungi were cultured at 24 °C for three days. After incubation, the diameter of zones of inhibition of the control and test was measured (mm) using an automated zone reader.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 PHYTOCHEMICAL INVESTIGATION ON *ERIOSEMA PSORALEOIDES*

4.1.1 Preliminary test results

The root extract of *E. psoraleoides* was tested for anti-microbial, anti-plasmodial, vasorelaxant, anti-asthmatic and penile relaxant activities and showed significant activities. TLC analysis of the crude extract showed the presence of several UV (254, 366 nm) sensitive components. From biogenetic point of view most of these compounds were assumed to be flavonoids and isoflavanoids. The extract was subjected to a combination of chromatographic separations that led to the isolation of four compounds. The identity of the isolated compounds was established by the use of a combination of spectroscopic methods. The characterization of the compounds is discussed below.

4.1.2 Characterization of compounds from *Eriosema psoraleoides* (roots)

4.1.2.1 4', 5-Dihydroxy-2', 7-dimethoxyisoflavanone (1)

EI-MS of compound **1** showed $[M]^+$ at *m/z* 316, corresponding to the molecular formula $C_{17}H_{16}O_6$. The presence of an isoflavanone skeleton was deduced from UV (λ_{max} 286 nm), ^1H (δ 4.55, *dd*, *J*=10.6, 11.0 Hz, for H-2_{ax}; δ 4.41, *dd*, *J*=5.2, 10.6 Hz for H-2_{eq}; δ 4.26, *dd*, *J*=5.4, 11.0 Hz for H-3) and ^{13}C (δ 71.9 for C-2; 48.1 for C-3 and 199.3 for C-4) NMR spectroscopic data (Yenesew *et al.*, 2000; Tanaka *et al.*, 2003; Zhao *et al.*, 2007). The ^1H NMR (Table 4.1) further

revealed the presence of a chelated hydroxyl (δ_H 12.34) and two methoxyl (δ_H 3.86 and δ 3.76) substituents.

In the ^{1}H NMR spectrum, the *meta*-coupled protons at δ 6.02 and 6.05 were assigned to H-8 and H-6 with C-5 and C-7 of ring-A being oxygenated, as expected from biogenetic considerations. In the EI-MS, the fragment at *m/z* 167 (**1a**, Figure 4.2) allowed the placement of one of the two methoxyl groups in ring-A (at C-7). In agreement with this, the HMBC spectrum showed correlation of the methoxyl protons (δ 3.86) with C-7 and the hydroxy proton (δ 12.34) with C-5. An ABX spin system (δ 6.42 *dd*, *J*= 8.2, 2.2 Hz for H-5'; δ 6.60 *d*, *J*=2.4 Hz for H-3'; δ 6.96 *d*, J=8.4 Hz for H-6') was attributed to 2', 4'-dioxygenation ring B. The placement of methoxyl group at C-2' rather than C-4' was established from the HMBC spectrum which showed correlation of methoxyl protons (δ 3.76) with C-2'. The (rel) *R*- configuration at C-3 was deduced from the presence of a *trans*-diaxial relationship between H-2_{ax} and H-3 (*J*=11.0 Hz) in the ^{1}H NMR spectrum (Yenesew *et al.*, 2000). This compound was therefore identified as 4',5-dihydroxy-2',7-dimethoxyisoflavanone (**1**), whose trivial name is cajanol.

This compound has earlier been reported from the roots and leaves of *Cajanus cajan* (Duker-Eshun *et al.*, 2004). However this is the first report of its occurrence in the genus *Eriosema*.

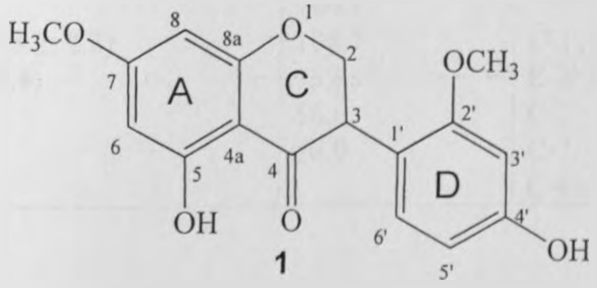
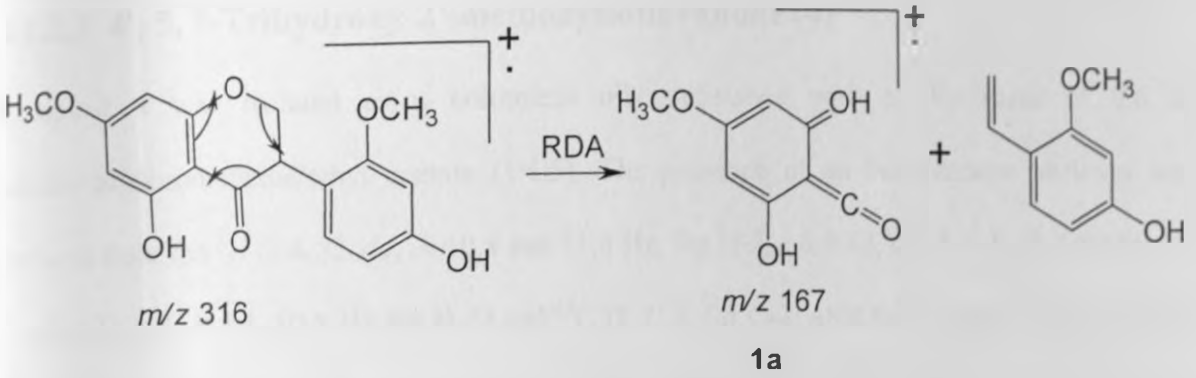


Figure 4.1: Structure of compound **1**



1a

Figure 4.2: *Retro*-Diels Alder fragmentation of compound **1**

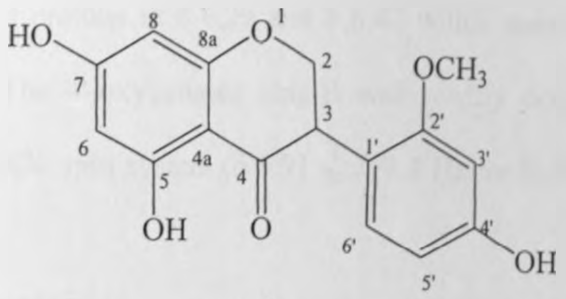
Table 4.1: ^{1}H and ^{13}C NMR data of compound **1** in acetone-d_6.

Position	^{1}H (200 MHz) δ_H (*J* in Hz)	^{13}C (150 MHz) δ_C	HMBC (^{2}J, ^{3}J)
2 *ax*	4.55 *dd* (10.6, 11.0)	71.9	C-1', 3 , 4 , 8a
2 *eq*	4.41 *dd* (5.2, 10.6)		
3	4.26 *dd* (5.4, 11.0)	48.1	C-1', 2', 4, 6'
4		199.3	
4a		104.3	
5		165.2	
6	6.05 *d* (2.4)	96.1	C-4a, 5, 8
7		162.4	
8	6.02 *d* (1.6)	95.0	C-6, 8a
8a		165.0	
1'		115.7	
2'		160.0	
3'	6.60 *d* (2.4)	101.1	C-1', 5'
4'		160.1	
5'	6.42 *dd* (8.2, 2.2)	108.7	C-1', 3', 4'
6'	6.96 *d* (8.4)	132.5	C-2', 3
2'-OCH_3	3.76 *s*	56.6	C-2'
7-OCH_3	3.86 *s*	56.9	C-7
5- OH	12.34 *s*		C-4a , 5, 6

4.1.2.2 4', 5, 7-Trihydroxy-2'-methoxyisoflavanone (4)

Compund **4** was isolated as a colourless oily substance with an R_f value of 0.6 in hexane/dichloromethane/ethyl acetate (1:4:5). The presence of an isoflavanone skeleton was deduced from the ^1H (δ 4.52, *dd*, *J*=10.8 and 11.4 Hz, for H-2_{ax}; δ 4.42, *dd*, *J*=5.4, 11.4 Hz for H-2_{eq}; δ 4.27, *dd*, *J*=5.4, 10.8 Hz for H-3) and ^{13}C (δ 71.9 for C-2; 48.0 for C-3 and 198.0 for C-4) NMR spectroscopic data (Yenesew *et al.*, 2000; Tanaka *et al.*, 2003; Zhao *et al.*, 2007). The NMR spectrum (Table 4.2) further revealed the presence of a chelated hydroxy (δ_H 12.38) and one methoxy (δ_H 3.77) substituents. In the ^1H NMR spectrum, the presence of *meta*-coupled protons at δ 5.95 and 5.97 was consistent with oxygenation at C-5 and C-7 of ring-A, which is expected from biogenetic considerations.

The HMBC spectrum showed correlation of methoxy (δ 3.77) with the carbon at δ 159.9, suggesting that the methoxy is attached on either C-2' or C-4'. This was resolved from NOESY spectrum which showed spatial interaction of methoxy protons at δ_H 3.77 with the proton at δ_H 6.52 but not proton at δ_H 6.42. This allowed the placement of the methoxy at C-2' leaving the hydroxyl to be attached on C-4' because of the existence of an ABX system (δ 6.97 *d*, *J*=8.4 Hz; δ 6.52 *d*, *J*=2.4 Hz; δ 6.42 *dd*, *J*=2.4 and 8.4 Hz) on ring-B. The presence of *trans*-diaxial relationship between H-2_{ax} and H-3 (*J*=10.8 Hz) was consistent with (rel) *R*- configuration at C-3 (Yenesew *et al.*, 2000). The compound was identified as 4',5,7-trihydroxy-2'-methoxy isoflavanone. This is the first report of the occurrence of this compound in the genus *Eriosema*.



4

Figure 4.3: Structure of compound **4**

Table 4.2: ^{1}H and ^{13}C NMR data of compound **4** in acetone-d_6

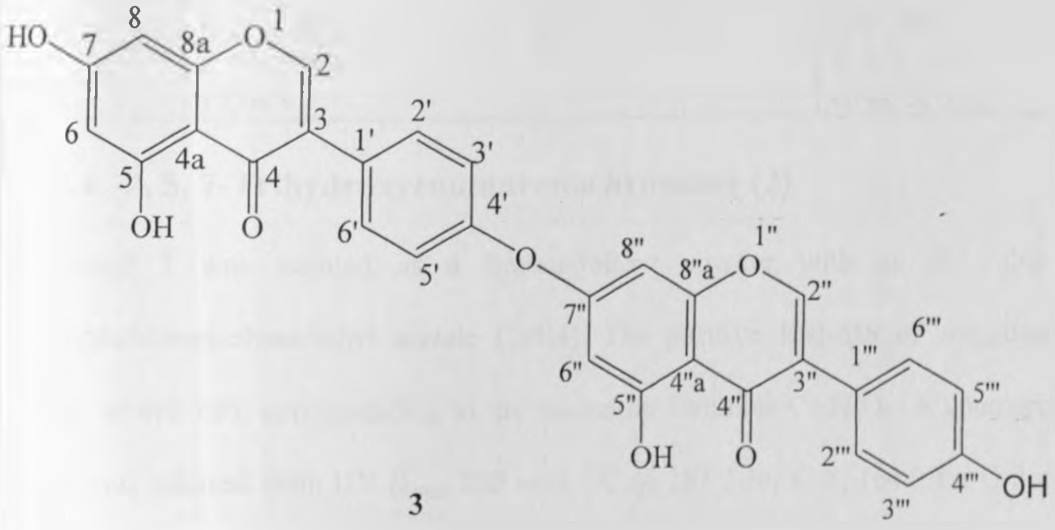
Position	^{1}H (600 MHz) δ_H (*J* in Hz)	^{13}C (150 MHz) δ_C	HMBC (^{2}J, ^{3}J
2 *ax*	4.52 *dd* (10.8, 11.4)	71.9	C-1', 4 , 8a,
2 *eq*	4.42 *dd* (5.4, 11.4)		
3	4.27 *dd* (5.4, 10.8)	48.0	
4		198.0	
4a		104.0	
5		167.9	
6	5.97 *d* (2.4)	97.6	C-4a
7		166.3	
8	5.95 *d* (1.8)	96.3	C-6, 4a
8a		165.3	
1'		115.9	
2'		159.9	
3'	6.52 *d* (2.4)	101.0	C-1', 5', 4
4'		160.2	
5'	6.42 *dd* (8.4, 2.4)	108.7	C-1', 3', 4'
6'	6.97 *d* (8.4)	132.4	C-2', 4
2'-OCH_3	3.77 *s*	56.5	C-2'
5-OH	12.38 *s*		

4.1.2.3 4',7''-Bisgenistein (3)

Compound **3** was isolated as an amorphous light brown powder with an R_f value of 0.6 in hexane/dichloromethane/ethyl acetate (8:7:5). The presence of an isoflavone skeleton was deduced from UV (λ_{max} 207. and 262 nm), ^{1}H (δ 8.16 for H-2) and ^{13}C (δ 154.91/154.95 for C-2/2'', 124.68/124.73 for C-3/3'' and 182.22/182.30 for C-4/4''). The ^{1}H NMR spectrum (Table

4.3) showed *meta*-coupled protons at δ 6.29 and δ 6.42 which were assigned to H-6 and H-8 respectively on ring-A. The 4'-oxygenated ring-B was readily deduced from the ^{1}H NMR spectrum forming an AA'XX' spin system (δ 6.91 *d*, *J*=8.4 Hz for H-3'/H-5'; δ 7.46 *d*, *J*=8.4 Hz for H-2'/H-6') (Table 4.3).

In agreement with this, the NOESY spectrum showed the protons at δ 6.91 (H-3'/H-5') correlated with protons at δ 7.46 (H-2'/H-6') that also correlated with proton at δ 8.16 (H-2'). These data are in agreement with 4',5,7-trihydroxyisoflavone with trivial name genistein. However the doubling of ^{13}C NMR signals were observed which suggested that this compound could be a non-symmetrical dimer such as **3**. The EI-MS (70 eV) did not show the molecular ion peak, but rather showed a peak at *m/z* 270 (Figure 4.5), which is due to the monomeric genistein (**3a**). Compound **3** was provisionally identified as 4',7"-bisgenistein. The identity of this compound needs to be confirmed through further experiments involving determination of the molecular mass through chemical ionization (CI) method.



3

Figure 4.4: Structure of compound **3**

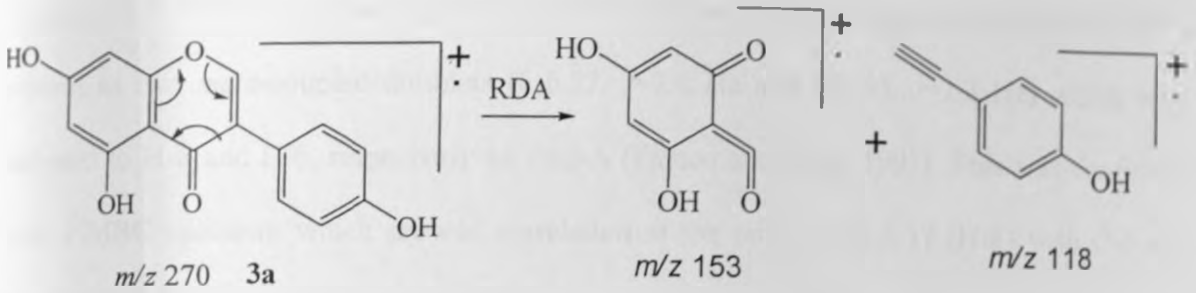


Figure 4.5: *Retro*-Diels Alder fragmentation of compound **3a**

Table 4.3: ^{1}H and ^{13}C NMR data of compound **3** in acetone-d_6

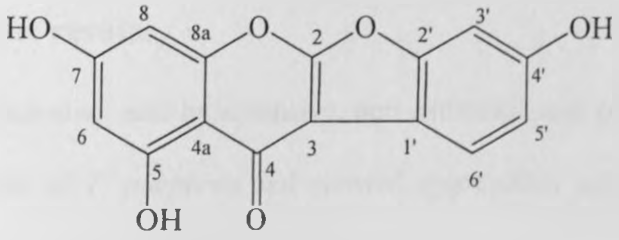
Position	^{1}H (600 MHz) δ_H (*J* in Hz)	^{13}C (150 MHz) δ_C	HMBC (^{2}J, ^{3}J)
2/2"	8.16 *s*	154.91/154.95	C-3, 4, 8a
3/3"		124.68/124.73	
4/4"		182.22/182.30	
4a/4"a		106.78/106.81	
5/5"		165.58/165.63	
6/6"	6.29 *d* (1.8)	100.41/100.46	C-4a, 5, 7, 8
7/7"		164.27/164.57	
8/8"	6.42 *d* (1.8)	95.10/95.17	C-4a, 6, 8a
8a/8"a		159.32/159.70	
1'/1"'		123.70/123.73	
2'/2"'	7.46 *d* (8.4)	131.83/131.83	C-3, 3', 4'
3'/3"'	6.91 *d* (8.4)	116.58/116.58	C-1', 4'
4'/4"'		159.04/159.70	
5'/5"'	6.91 *d* (8.4)		C-1', 4'
6'/6"'	7.46 *d* (8.4)		C-3, 3', 4'
5-OH	13.04 s		C-4a, 5, 6

4.1.2.4 4', 5, 7-Trihydroxycoumaronochromone (2)

Compound **2** was isolated as a brown-yellow powder with an R_f value of 0.6 in hexane/dichloromethane/ethyl acetate (2:4:4). The positive ESI-MS of compound **2** showed $[M+H]^+$ at *m/z* 285, corresponding to the molecular formular $C_{15}H_8O_6$. A coumaronochromone system was inferred from UV (λ_{max} 257 nm), ^{13}C (δ 183.2 for C-4, 164.0 for C-2 and 110.5 for C-3) NMR spectrum (Franco and Irene, 1991). Lack of a characteristic isoflavone H-2 singlet in the ^{1}H NMR spectrum further proved the existence of a coumaronochromone ring system

(Tahara *et al.*, 1985). Aromatic signals in the ^{1}H NMR spectrum of compound **2** (Table 4.4) were evident as two *meta*-coupled doublets (δ 6.37, *J*=2.0 Hz and δ 6.60, *J*=2.2 Hz) which were assigned to H-8 and H-6, respecively on ring-A (Franco and Irene, 1991). This was confirmed from HMBC spectrum which showed correlation of the proton at δ 6.37 (H-8) with C-6 and proton at δ 6.60 (H-6) with C-4a, 5 and C-8.

An ABX spin system (δ 7.02 *dd*, *J*=8.6 Hz and *J*=2.2 Hz for H-5'; δ 7.14 *d*, *J*=1.8 Hz for H-3'; δ7.82 *d*, *J*=8.6 Hz for H-6') were asigned to the ring-B protons of a coumaronochromone system with oxygenation at C-2' (δ_C 158.7) and C-4' (δ_C 152.9). Compound **2** was therefore identified as 4',5,7-trihydroxycoumaronochromone. This compound has earlier been reported from the roots of *Lupinus albus* (Tahara *et al.*, 1985). However this is the first report of its occurrence in the genus *Eriosema*.



2

Figure 4.6: Structure of compound **2**

Table 4.4: ^{1}H and ^{13}C NMR data of compound **2** in acetone-d_6

Position	^{1}H (200 MHz) δ_H (J in Hz)	^{13}C (50 MHz) δ_C	HMBC (^{2}J, ^{3}J)
2		164.0	
3		110.5	
4		183.2	
4a		105.0	
5		163.6	
6	6.60 *d* (2.2)	98.9	C-4a, 5, 8
7		163.0	
8	6.37 *d* (2.0)	95.0	C-6
8a		156.2	
1'		115.5	
2'		158.7	
3'	7.14 *d* (1.8)	100.2	C-4'
4'		152.9	
5'	7.02 *dd* (2.2, 8.6)	113.8	C-1', 3'
6'	7.82 *d* (8.6)	121.8	C-2', 4'
5-OH	12.98 *s*		C- 4a, 5, 6

4.2 PHYTOCHEMICAL INVESTIGATION ON *TEPHROSIA PURPUREA*

4.2.1 Preliminary test results

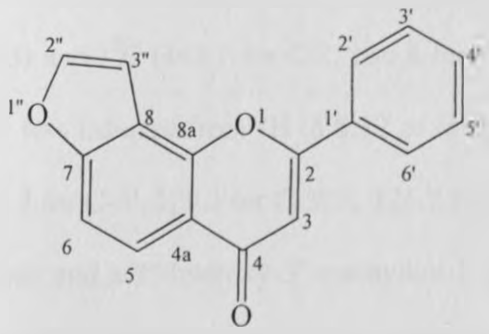
Anti-microbial, anti-plasmodial, anti-hypertensive, anti-asthmatic and penile relaxant tests were done on the stem extract of *T. purpurea* and showed appreciable activities. The presence of several UV (254, 366 nm) sensitive components of the crude extract was detected on TLC. From biogenesis, these compounds were assumed to be flavonoids and isoflavanoids. The extract was subjected to a combination of chromatographic separation that led to the isolation of five compounds. Spectroscopic methods were used to identify the isolated compounds. The characterization of the compounds is presented below.

4.2.2 Characterization of compounds from *Tephrosia purpurea* (stem)

4.2.2.1 Lanceolatin B (6)

Compound **6** was isolated as an amorphous powder with an R_f value of 0.42 in hexane/ethyl acetate (9:1). The positive ESI-MS of compound **6** showed $[M+H]^+$ at *m/z* 263, corresponding to the molecular formular $C_{17}H_{10}O_3$. The UV (λ_{max} 310 and 280 nm), ^1H (δ 6.94 s for H-3) and ^{13}C (163.3 for C-2, 108.0 for C-3 and 178.8 for C-4) NMR provided evidence of a flavone skeleton (Aneja *et al.*, 1963; Talapatra *et al.*, 1980; Huang and Chen, 2003). Unsubstituted aromatic ring-B was deduced from ^1H (δ 7.97 *m* for H-2'/6'; 7.56 *m* for H-3'/4'/5' and ^{13}C (δ 131.9 for C-1'/4'; 129.4 for C-3'/5'; 126.5 for C-2'/6') NMR spectrum (Table 4.5). The presence of *ortho*-coupled protons at δ 8.18 and δ 7.56 (*J*=9.0 Hz) in ring-A suggested that C-7 and C-8 are substituted. In the ^1H NMR spectrum, an AX doublets at δ 7.22 and δ 7.78 (*J* = 3.0 Hz) revealed that the substituent at C-7/8 is a furano ring.

The UV, ^1H, ^{13}C NMR and MS data for compound **6** was identical with those previously published (Table 4.5) for lanceolatin B (Tanaka *et al.*, 1992; Lee *et al.*, 1995; Huang and Chen, 2003). Therefore compound **6** was identified as lanceolatin B.



6

Figure 4.7: Structure of compound **6**

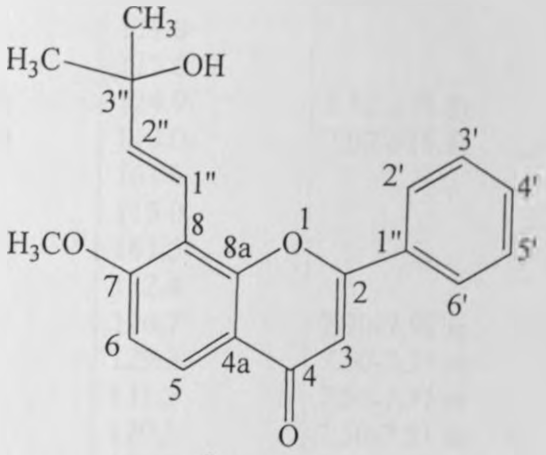
Table 4.5: ^{1}H and ^{13}C NMR data of compound **6** and lanceolatin B (**6**)

Compound **6** ($CDCl_3$)			Lanceolatin B (**6**, $CDCl_3$)(Huang and Chen 2003)	
Position	^{1}H (500 MHz) δ_H(*J* in Hz)	^{13}C (50 MHz)	^{1}H (300 MHz) δ_H(*J* in Hz)	^{13}C (75 MHz)
2		163.3		161.7
3	6.94 *s*	108.0	6.89 *s*	107.6
4		178.8		178.3
4a		119.3		118.9
5	8.18 *d* (9.0)	122.0	8.17 *d* (8.6)	121.7
6	7.56 *m*	110.6	7.52 *m*	110.5
7		158.6		158.4
8		117.7		117.2
8a		151.1		151.0
1'		131.9		131.8
2'	7.97*m*	126.5	7.97 *m*	126.3
3'	7.56 *m*	129.4	7.52 *m*	129.2
4'	7.56 *m*	131.9	7.52 *m*	131.8
5'	7.56 *m*	129.4	7.52 *m*	129.2
6'	7.97 *m*	126.5	7.97 *m*	126.3
2"	7.78 *d* (3.0)	146.1	7.78 *d* (1.8)	145.9
3"	7.22 *d* (3.0)	104.4	7.21 *d* (1.8)	104.2

4.2.2.2 Lanceolatin A (9)

Compound **9** was isolated as an amorphous powder with an R_f value of 0.54 in 20% acetone in dichloromethane. The positive-ion EI-MS of compound **9** showed a molecular ion peak at *m/z* 336 indicating a molecular formula of $C_{21}H_{20}O_4$. A flavone skeleton was deduced from UV (λ_{max} 295 nm), ^{1}H (δ 6.81 s for H-3) and ^{13}C (163.1 for C-2, 106.8 for C-3 and 177.0 for C-4) NMR spectra. Unsubstituted ring-B was inferred from ^{1}H (δ 8.12 *m* for H-2'/6'; 7.61 *m* for H-3'/4'/5') and ^{13}C (δ 132.4 for C-1', 131.7 for C-4', 129.3 for C-3'/5', 126.7 for C-2'/6') NMR spectra (Table 4.6). The presence of a methoxy and a 3"-hydroxy-3"-methylbut-1"-enyl substituent was evident from NMR (Table 4.6). The appearance of a pair of *ortho*-coupled protons (δ 7.25 and δ 8.02 J=9.0 Hz) requires the placement of the methoxy (δ 4.05 *s*) at C-7 and the 3"-hydroxy-3"-methylbut-1"-enyl substituent at C-8.

In the EI-MS, the appearance of a fragment ion at *m/z* 102 (**9a**) resulting from *retro*-Diels-Alder (RDA) cleavage of the ring-C (Figure 4.9) was in agreement with the placement of these groups in ring-A. This compound was therefore identified as 7-methoxy-8-(3"-hydroxy-3"-methylbut-1"-enyl)-flavone, trivial name lanceolatin A (**9**) (Pelter *et al.*, 1981, Abou-Douh *et al.*, 2005).



9

Figure 4.8: Structure of compound **9**

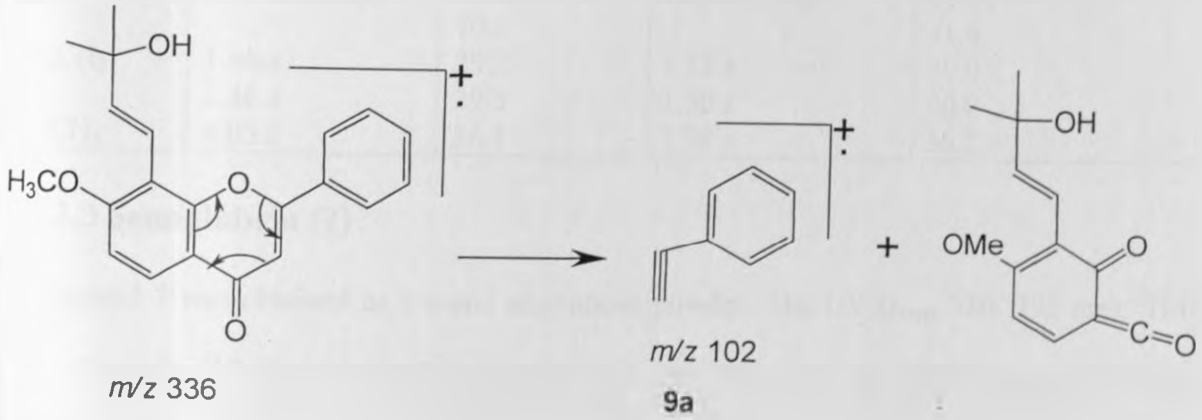


Figure 4.9: *Retro*-Diels Alder fragmentation of compound **9**

Table 4.6: ^{1}H and ^{13}C NMR data of compound **9** and lanceolatin A (**9**)

	9 (acetone-d_6)		Lanceolatin A (**9**, $CDCl_3$)(Abou-Douh *et al.*, 2005)	
Position	^{1}H (600 MHz) δ_H(*J* in Hz)	^{13}C (50 MHz)	^{1}H (500 MHz) δ_H(*J* in Hz)	^{13}C (125 MHz)
2		163.1		163.3
3	6.81 *s*	106.8	6.76 *s*	107.1
4		177.0		178.3
4a		115.0		118.1
5	8.02 *d* (9.0)	124.9	8.12 *d* (8.8)	125.5
6	7.25 *d* (9.0)	110.0	7.02 *d* (8.8)	109.0
7		161.9		162.7
8		115.0		114.3
8a		161.5		154.6
1'		132.4		132.1
2'	8.12 *m*	126.7	7.90-7.92 *m*	126.4
3'	7.61 *m*	129.3	7.50-7.51 *m*	129.0
4'	7.61 *m*	131.7	7.50-7.51 *m*	131.5
5'	7.61 *m*	129.3	7.50-7.51 *m*	129.0
6'	8.12 *m*	126.7	7.90-7.92 *m*	126.4
1"	7.09 *d* (16.6)	145.6	6.97 *d* (16.5)	144.1
2"	6.93 *d* (16.2)	114.6	6.82 *d* (16.5)	115.2
3"		70.5		71.6
3"-2CH_3	1.46 *s*	29.2	1.55 *s*	30.0
	1.46 *s*	29.5	1.50 *s*	30.0
7-OCH_3	4.05 *s*	56.1	3.98 *s*	56.2

4.2.2.3 Semiglabrin (7)

Compound 7 was obtained as a white amorphous powder. The UV (λ_{max} 310, 295 nm), ^{1}H (δ 6.83 *s* for H-3), ^{13}C (δ 164.0 for C-2, 108.3 for C-3 and 178.3 for C-4) NMR indicated a flavone skeleton (Waterman and Khalid, 1980; Huang and Chen, 2003; Abou-Douh *et al.*, 2005). The presence of an unsubstituted ring-B was again deduced from ^{1}H (δ 7.91 *m* for H-2'/6'; 7.54 *m* for H-3'/ 4'/ 5') and ^{13}C (δ 132.2 for C-1', 127.3 for C-2'/ 6', 129.7 for C-3'/ 5' and 129.9 for C-4') NMR spectra.

The ^{1}H NMR spectrum further revealed the presence of a pair of *ortho*-coupled protons (δ 6.94, and δ 8.16, *J*=8.4 Hz) on ring-A, indicating the presence of substituents at C-7 and C-8. The

substituent at these positions was found to be two fused furan rings (Table 4.7) derived from a prenyl group at C-8 and a methoxy at C-7 as in lanceolatin A. Thus the presence of a gem-dimethyl group at C-5" was evident from two singlets (δ 1.09 and δ 1.32) attached to oxygenated sp^3 carbon atom (δ_c 88.7). The presence of an acetoxy group at C-4" was also evident from NMR (Table 4.7). The ^{1}H NMR spectrum further showed a pair of mutually coupled doublets at δ 4.30 and 6.64 (*J*=6.4 Hz) were assigned to H-3" and H-2" respectively. The coupling constant (*J*=6.4 Hz) was constistent with *cis* orientation between these hydrogen atoms (Abou-Douh *et al.*, 2005). Furthermore a singlet at δ 5.64 was assigned to the acetoxymethine proton at C-4". This proton appeared as a singlet despite the presence of a vicinal proton (H-3"). This indicated that the relative stereochemical orientation of the former to the latter was *trans* as in semiglabrin (Abou-Douh *et al.*, 2005).

Comparison of the NMR data of this compound with literature report indeed confirmed that 7 was 2"',2"'-dimethyl-3"'-acetoxy-tetrahydrofurano-[3",2"-b]-dihydrofurano-[5",4"-h]-flavone, trivial name semiglabrin (Table 4.7) (Smalberger *et al.*, 1973; Waterman and Khalid, 1980; Pelter *et al.*, 1981; Ahmad *et al.*, 1999; Abou-Douh *et al.*, 2005).

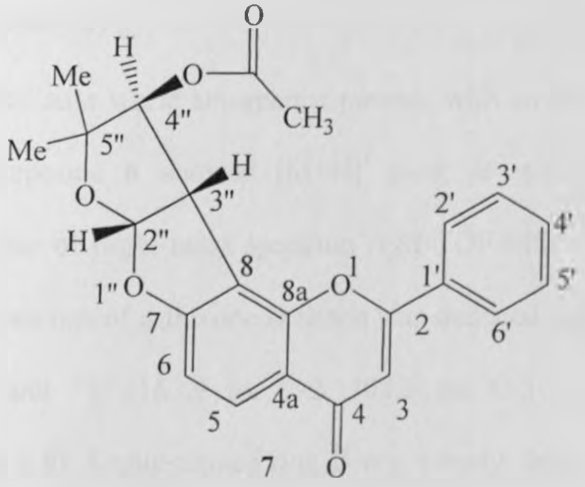


Figure 4.10: Structure of compound 7

Table 4.7: ^{1}H and ^{13}C NMR data of compound 7 and semiglabrin (7)

7 ($CDCl_3$)			Semiglabrin (7, $CDCl_3$)(Abou-Douh *et al.*, 2005)	
Position	^{1}H (200 MHz) δ_H(*J* in Hz)	^{13}C (50 MHz)	^{1}H (500 MHz) δ_H(*J* in Hz)	^{13}C (125 MHz)
2		164.0		162.9
3	6.83 *s*	108.3	6.76 *s*	108.8
4		178.3		177.4
4a		119.9		118.7
5	8.16 *d* (8.4)	132.6	8.15 *d* (8.5)	128.9
6	6.94 *d* (8.4)	110.0	6.93 *d* (8.5)	109.0
7		164.7		163.7
8		113.4		112.4
8a		154.1		153.1
1'		132.2		131.6
2'/6'	7.91 *m*	127.3	7.90 *m*	126.4
3'/5'	7.54 *m*	129.7	7.52 *m*	129.0
4'	7.54 *m*	129.9	7.51 *m*	131.6
2"	6.64 *d* (6.4)	113.2	6.62 *d* (6.5)	112.4
3"	4.30 *d* (6.4)	53.6	4.30 *d* (6.5)	52.8
4"	5.64 *s*	80.9	5.63 *s*	80.2
4"-$COCH_3$		170.4		169.6
4"-$COCH_3$	2.22 *s*	21.6	2.22 *s*	20.8
5"		88.7		87.8
5"-CH_3	1.09 *s*	28.3	1.08 *s*	27.5
5"-CH_3	1.32 *s*	23.9	1.30 *s*	23.1

4.2.2.4 Terpurinflavone (8)

Compound **8** was obtained as a white amorphous powder with an R_f value of 0.45 in hexane/ ethyl acetate (3:2). Compound **8** showed $[M+H]^+$ peak at *m/z* 437.1593 in its positive electrospray ionization time of flight mass spectrum (ESI-TOF-MS) constituting the molecular formula $C_{25}H_{24}O_7$. The presence of a flavone skeleton was deduced from the UV (λ_{max} 295 nm), ^{1}H (δ 6.79 s for H-3) and ^{13}C (163.9 for C-2, 108.5 for C-3 and 177.6 for C-4) NMR spectroscopic data (Table 4.8). Unsubstituted ring-B was clearly shown in ^{1}H (δ 7.63 *m* for H-3'/4'/5', δ 8.18 *m* for H-2'/6') and ^{13}C (δ 127.9 for C-2'/6', δ 130.7 for C-3'/5', δ 133.1 for C-4' and

133.6 for C-1') NMR spectra. In ring-A, an AX protons which are *ortho*-coupled at δ 6.94 and 8.00 (J=8.5 Hz) were assigned to H-6 and H-5 respectively, with C-7 and C-8 being substituted with an acetoxy group (at C-7) and a furan ring (at C-8) derived from modified prenyl group as in tephrorin B (**84**, Chang *et al.*, 2000) (Table 4.8). The presence of a second acetate group was also evident from the NMR spectrum (Table 4.8) and placed at C-4" of the furan group.

The ^{1}H and ^{13}C NMR chemical shift values of the furan ring of **8** were quite similar to those of tephrorin B (**84**, Table 4.9). The coupling constant (J= 8.5 Hz) between H-3" and H-4" indicated that the relative orientation of H-3" and H-4" is *cis* as in tephrorin B (**84**). NOE interaction of H-3" with H-4" from NOESY spectrum supported the *cis* orientation.

The HMBC spectrum showed correlation of H-3" (δ 4.44) with C-2" (79.1), C-4" (78.7), C-5" (84.0), C-7 (167.9), C-8 (116.0) and C-8a (155.9) confirming that the furan ring is attached at C-8. The HMBC spectrum further showed that H-4" (δ 5.35) correlated with acetoxy carbon (170.3) and the two methyl carbon (22.4 and 24.4) which confirmed the placement of one of the acetoxy groups at C-4" and the two methyl groups at C-5".

The new compound was therefore characterized as 7-acetoxy-8-[3-acetoxy-2,2-dimethyl tetrahydro-4-furanyl] flavone (**8**) for which the trivial name terpurinflavone was assigned.

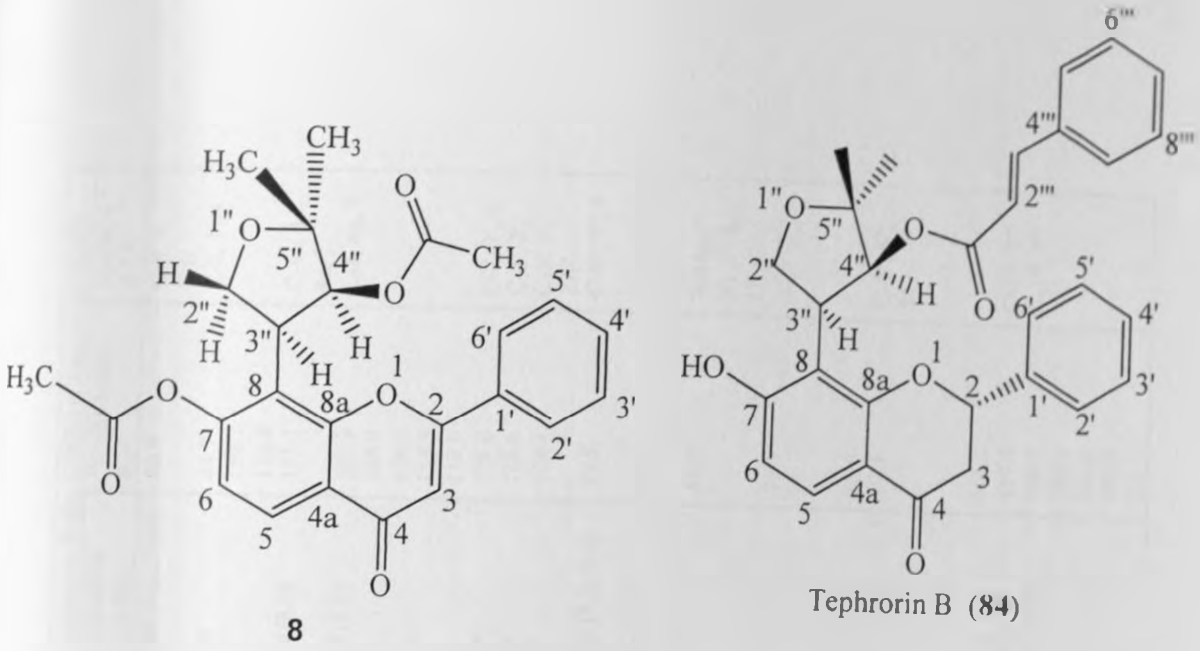
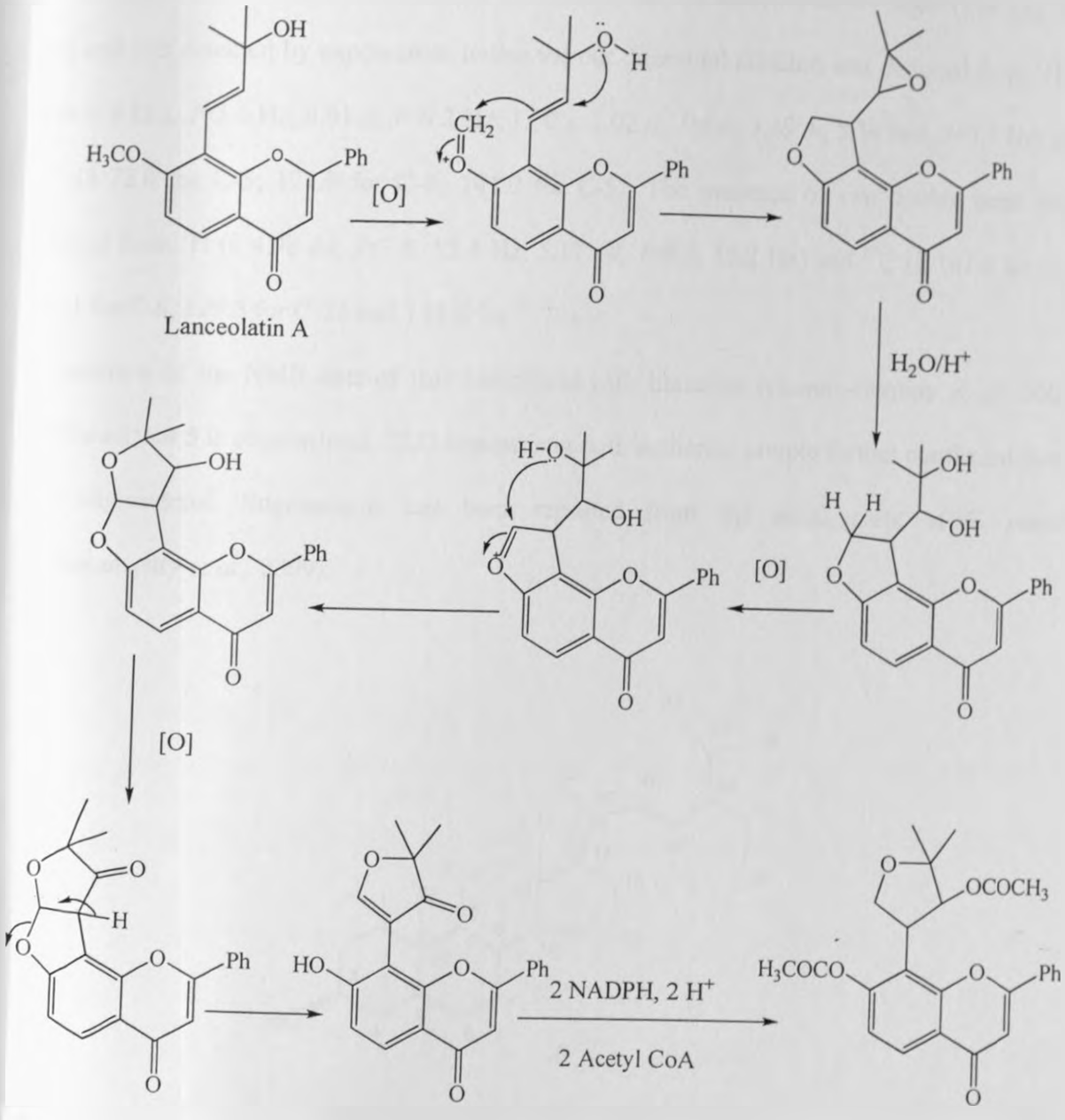


Figure 4.11: Structure of compound **8** and tephrorin B

Table 4.8: ^{1}H and ^{13}C NMR data and HMBC correlation for compound **8** and tephrorin B (**84**).

	8 (acetone-d_6)			Tephrorin B (**84**, $CDCl_3$) (Chang *et al.*, 2000)		
Position	^{1}H (500 MHz) δ_H (*J* in Hz)	^{13}C (75 MHz) δ_C	HMBC (^{2}J, ^{3}J)	^{1}H (300 MHz) δ_H (*J* in Hz)	^{13}C (75 MHz) δ_C	HMBC (^{2}J, ^{3}J)
2		163.9		5.42 *dd* (3.2, 12.6)	80.8	C-1', 4, 2'/6'
3	6.79 *s*	108.5	C-1', 2, 4, 4a	2.83 *m*	44.7	C-2, 1', 4
4		177.6			190.7	
4a		119.9			115.9	
5	8.00 *d* (8.5)	129.3	C-4, 7, 8a	7.83 *d* (8.6)	131.1	C-4a, 4, 8a, 7
6	6.94 *d* (8.5)	110.1	C-4a, 8	6.57 *d* (8.6)	105.9	C-8, 4a, 7
7		167.9			168.9	
8		116.0			114.0	
8a		155.9			159.5	
1'		133.6			138.9	
2'/6'	8.18 *m*	127.9	C-1', 2	7.46 *m*	126.0	C-2, 3', 4'
3'/5'	7.63 *m*	130.7	C-2'/6', 4'	7.36 *m*	128.6	C-1', 2',
4'	7.63 *m*	133.1		7.36 *m*	130.7	C-3', 5', 2'/6'
2"	4.84 *dd* (8.0, 9.5) 5.02 *dd* (2.0, 9.5)	79.1	C-4" C-3", 8	4.66 *dd* (9.3, 8.5)	78.5	C-4", 3", 8
3"	4.44 *ddd* (2.0, 8.0, 8.5)	42.3	C-2", 4", 5",7, 8, 8a	4.07 *m*	40.6	C-2", 4", 5",7, 8
4"	5.35 *d* (8.5)	78.7	$COCH_3$-4", 2", 5"	5.10 *d* (6.3)	80.1	$2CH_3$-5", 3", 5", 8, 1'"
5"		84.0			72.9	
4"-$COCH_3$		170.3				
4"-$COCH_3$	1.61 *s*	20.9	$COCH_3$-4"			
5"-CH_3	1.76 *s*	22.4	CH_3-5", 4", 5"	1.24 *s*	26.6	
5"-CH_3	1.61 *s*	24.4	Me-5", 4", 5"	1.24 *s*	27.1	C-5", 6", 7"
7-$COCH_3$		170.8				
7-$COCH_3$	2.00 *s*	23.0	$COCH_3$-7			
1'"					166.3	
2'"				6.24 *d* (16.0)	117.4	C-1'", 3'"
3'"				7.45 *d* (16.0)	146.0	C-1'", 2'"
4'"					134.2	
5'"/9'"				7.36 *m*	129.1	
6'"/8'"				7.46 *m*	129.3	
7'"				7.36 *m*	131.1	

The biogenesis of terpurinflavone (**8**) appears to be derived from lanceolatin A as shown in Scheme 4.1



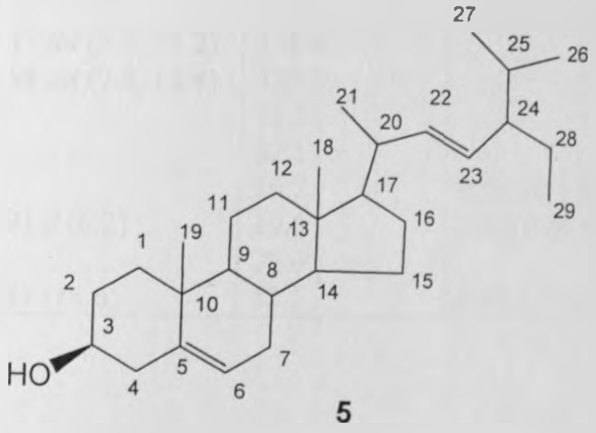
Terpurinflavone (**8**)

Scheme 4.1: Proposed biogenesis of terpurinflavone **8**

4.2.2.5 Stigmasterol (5)

Compound **5** was isolated as a colourless amorphous powder with an R_f value of 0.69 in CH_2Cl_2/EtOAc (9:1). The compound on TLC plate was not sensitive to UV light (254 and 366 nm) and was detected by exposure to iodine vapour. Steroidal skeleton was deduced from ^1H (δ 0.69 *s*; 0.81 *t*, *J*=5.6 Hz; 0.91 *d*, *J*=6.2 Hz; 1.00 *s*; 1.02 *d*; *J*=5.6, 3.49 *m*; 5.34 *brd*, *J*=5.2 Hz) and ^{13}C (δ 72.0 for C-3; 121.9 for C-6; 141.0 for C-5). The presence of two double bond were inferred from ^1H (δ 4.98 *dd*, *J*=7.8, 15.4 Hz; 5.17 *dd*, *J*=8.2, 15.2 Hz) and ^{13}C (δ 141.0 for C-5, 121.1 for C-6, 129.5 for C-23 and 138.6 for C-22).

Comparison of the NMR data of this compound with literature (Gomez-Garibay *et al.*, 2002) confirmed that **5** is stigmasterol. TLC comparison with authentic sample further confirmed that **5** was stigmasterol. Stigmasterol has been reported from the aerial parts of *T. pumila* (Pethakamsetty *et al.*, 2009).



5

Figure 4.12: Structure of compound **5**

Table 4.9: ^{1}H and ^{13}C NMR data of compound **5** and stigmasterol (**5**)

5 ($CDCl_3$)			Stigmasterol (**5**, $CDCl_3$) (Patch *et al.*, 2009)	
Position	^{1}H (200 MHz) δ_H (*J* in Hz)	^{13}C (50 MHz) δ_C	^{1}H (400 MHz) δ_H (*J* in Hz)	^{13}C (100 MHz) δ_C
1		37.5		37.3
2		31.9		31.6
3	3.49 *m*	72.0	3.52 *m*	71.8
4		42.4		42.3
5		141.0		140.8
6	5.34 *d* (5.2)	121.9	5.36 *br s*	121.7
7		32.1		31.9
8		32.1		31.9
9		50.4		51.2
10		36.7		36.5
11		21.3		21.1
12		39.9		39.7
13		42.5		42.3
14		57.1		56.9
15		24.5		24.4
16		29.2		28.4
17		56.2		56.1
18	0.69 *s*	12.2	0.69 *s*	11.0
19	1.00 *s*	21.4	1.01 *s*	21.2
20		40.7		40.5
21	1.02 *d* (5.6)	21.3	1.02 *d* (7.5)	21.2
22	5.17 *dd* (8.2, 15.2)	138.6		138.3
23	4.98 *dd* (7.8, 15.4)	129.5		129.3
24		51.5		51.2
25		32.1		31.9
26		19.2	0.80 *d* (6.5)	21.2
27	0.91 *d* (6.2)	19.6	0.85 *d* (6.5)	19
28		25.6		25.4
29	0.81 *t* (5.6)	12.5	0.80 *t* (7.5)	12.1

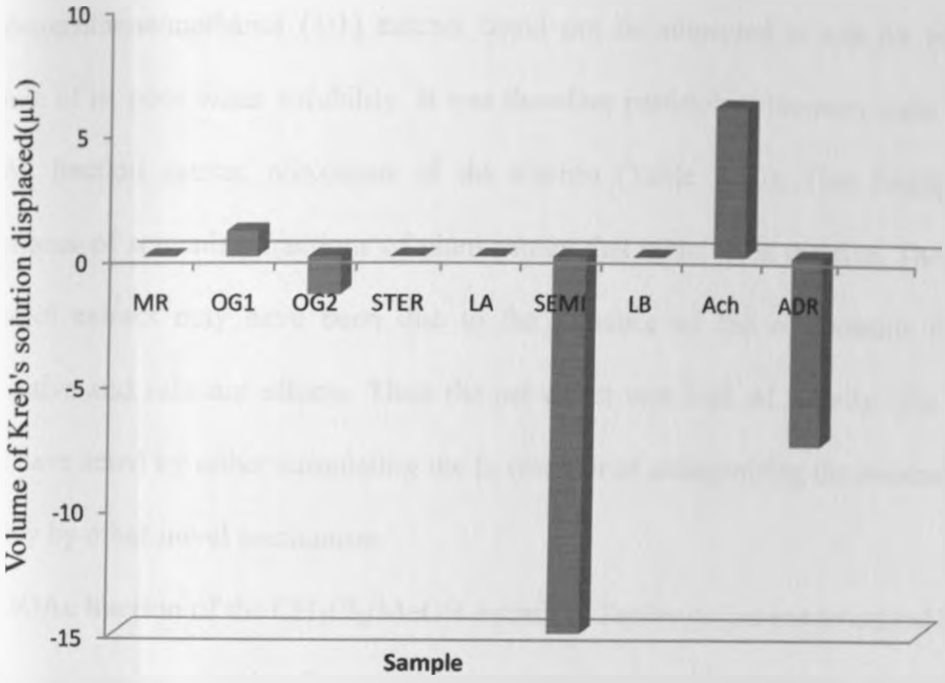
4.3 RESULTS OF THE BIOLOGICAL ASSAYS

4.3.1 Effects of plant extracts and isolated compounds on the Guinea pig trachea

The effects of *Eriosema psoraleoides* (roots), *Tephrosia purpurea* (stem) extracts and pure compounds from *Tephrosia purpurea* (stem) on isolated trachea of a Guinea pig were tested and the results are presented in table 4.10 and figure 4.13.

Table 4.10: Effects of plant extracts and pure compounds on isolated trachea of a Guinea pig

	Test sample	Organ bath concentration (µg/mL)	Volume of Krebs solution displaced(µL)	Physiological effect
Eriosema psoraleoides (Root)	MeOH extract	31.5	0	No effect
	EtOAc fraction of CH_2Cl_2/MeOH extract	20.3	-1	Relaxation
Tephrosia purpurea (stem)	EtOAc fraction from CH_2Cl_2/MeOH extract	22.5	-1.5	Relaxation
	Stigmasterol (**5**)	12	0	No effect
	Lanceolatin B (**6**)	9	0	No effect
	Semiglabrin (**7**)	7.5	-15	Relaxation
	Lanceolatin A (**9**)	21.8	0	No effect
Reference contractile drug	Acetylcholine	0.5	+6	Contraction
Reference relaxant drug	Adrenaline	0.25	-7.5	Relaxation



Key

MR-Methanol extracts of *E.psoraleoides* roots.
OG1-EtOAc layer of *E.psoraleoides* roots
OG2-EtOAc layer of *T.purpurea* stems
STER-Stigmasterol (**5**)
LB-Lanceolatin B (**6**)
SEMI-Semiglabrin (**7**)
LA-Lanceolatin A (**9**)
Ach-Acetylcholine
ADR-Adrenaline

Figure 4.13: The effect of the pure compounds and plant extracts on isolated trachea-volume of Krebs' solution displaced

The trachea has β_2 adrenoreceptors as well as M_3 muscarinic receptors (Katzung, 2007). Stimulation of the β_2 adrenoreceptors by adrenaline resulted in relaxation of the bronchial smooth muscle (Katzung, 2007). This is observed experimentally as a decrease in the volume of Krebs solution in the capillary tube attached to the trachea (Table 4.10). On the other hand stimulation of M_3 receptors resulted in contraction of the bronchial smooth muscle which is observed as an increase in the volume of Krebs solution in the capillary tube attached to the trachea (Katzung, 2007).

The root powder of *Eriosema psoraleoides* was extracted with CH_2Cl_2/MeOH (1:1) and methanol separately. The methanol extract showed no activity on the isolated trachea. The dichloromethane/methanol (1:1) extract could not be subjected to test for biological activity because of its poor water solubility. It was therefore partitioned between water and EtOAc. The EtOAc fraction caused relaxation of the trachea (Table 4.10). This finding highlights the importance of screening fractions of plant extract that seem to be inactive. The inactivity of the methanol extract may have been due to the presence of the components that have both a contractive and relaxant effects. Thus the net effect was lack of activity. The EtOAc fraction could have acted by either stimulating the β_2 receptor or antagonizing the muscarinic receptors or possibly by other novel mechanism.

The EtOAc fraction of the CH_2Cl_2/MeOH extract of *Tephrosia purpurea* caused relaxation of the Guinea pig trachea. Of the five compounds isolated from *T. purpurea*, only four were tested. Semiglabrin (7) showed a potent relaxant effect. This compound could therefore be responsible for the relaxant effect of the EtOAc fraction of the extract of *T. purpurea*. This compound has two structural features required for β_2 receptor stimulation. These features are a bulk aromatic group and a free OH group that is likely to arise from the hydrolysis of the ester group. The free OH group is required for interaction with β_2 receptors. However receptor binding assays are required to confirm whether semiglabrin (7) interact with the β-receptor or not. The relaxant effect of the extract and semiglabrin (7) provides scientific justification for the traditional use of *T. purpurea* for the management of tightness of the chest (Ahmad *et al.*, 1999). Thymoquinone from *Nigella sativa* and isokaempferide from *Amburana cearensis* have been reported to have relaxant effect on the trachea (Boskabady and Aslani, 2005; Leal *et al.*, 2006).

4.3.2 The effect of plant extracts and isolated compounds on isolated aorta

The effects of *Eriosema psoraleoides* (roots), *Tephrosia purpurea* (stem) extracts and isolated compounds on isolated aorta of sheep were tested and the results are presented in table 4.11.

Table 4.11: Effect of plant extracts and isolated compounds on isolated aorta of sheep

	Sample	Organ bath concentration (μg/mL)	Displacement (mm) from baseline	Physiological effect
Eriosema psoraleoides (Root)	MeOH extract	88	-13	Relaxation
	EtOAc fraction of CH_2Cl_2/MeOH extract	60	+7.5	Contraction
Tephrosia purpurea (stem)	EtOAc fraction from CH_2Cl_2/MeOH extract	74	-9	Relaxation
	Stigmasterol (**5**)	12	-6	Relaxation
	Lanceolatin B (**6**)	19	-10	Relaxation
	Lanceolatin A (**9**)	21	+2	Contraction
Referrence Contractile drug	Adrenaline	0.5	+54	Contraction
Referrence Relaxant drug	Acetylcholine	0.25	-5	Relaxation

Compounds that cause vasorelaxation initiates penile erection by dilating the penile carvenosa. The methanol extract of *E. psoraleoides* (roots) caused relaxation (Table 4.11). This may indicate that it can cause relaxation of the penile carvenosa vessels. The EtOAc fraction of the

CH_2Cl_2/MeOH extract of *E. psoraleoides* caused vasoconstriction. This may indicate that compounds in *E. psoraleoides* (roots) responsible for penile erection may reside in the methanol fraction. Relaxation may have been due to stimulation of muscarinic receptors found in the endothelium of vascular smooth muscle of the aorta.

The EtOAc fraction of CH_2Cl_2/MeOH of *Teprosia purpurea* caused relaxation which indicates that it may contain vasoactive component that may be useful for the management of ED. Stigmasterol (**5**) and lanceolatin B (**6**) isolated from this fraction caused vasodilation (Table 4.11). The leaves extracts of *Brillantaisia nitens* and *Epimedium brevicornum Maxima* have been reported to have relaxant effect on isolated aorta of sheep, rat and a rabbit (Chien *et al.*, 2006; Dimo *et al.*, 2007).

4.3.3 Effects of plant extracts and pure compounds on the isolated vas deferens of sheep and rat

The crude extracts of *E. psoraleoides*, *T. purpurea* and isolated compounds did not have an effect on isolated vas deferens of a rat and sheep. This shows that neither the crude extract nor the isolated compounds can promote ejaculation. This indicates lack of α-adrenoceptor stimulant activity.

4.3.4 Effect of *Eriosema psoraleoides* root extract on the mating behavior

The male mice were divided into four groups of six each. The groups were treated with methanol extract of *Eriosema psoraleoides* (roots) (2.5 g/kg), sildenafil (600 mg/kg), yohimbine (360 mg/kg) orally and the remaining group acted as a control. Three female injected with stilboestrol (40 mg/kg) were introduced into each group. Cumulative frequencies of male mice smelling the vagina, licking their penis and number of times erection present was represented on graphs.

Cumulative frequency of male mice smelling the vagina after being given the *Eriosema psoraleoides* root extract (2.5 g/kg) and yohimbine (360 mg/kg) was represented in Figure 4.14.

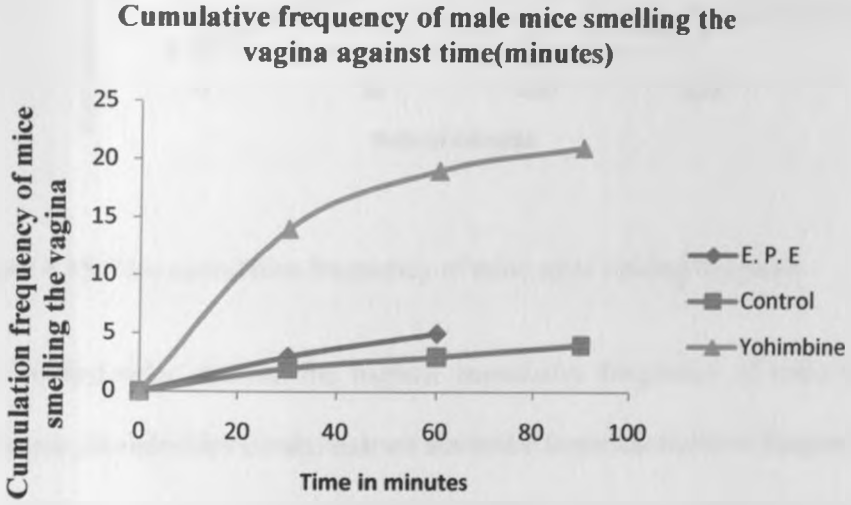


Figure 4.14: A graph showing cumulative frequency of male mice smelling the vagina.

Yohimbine showed the highest cumulative frequency of male mice smelling the vagina. *Eriosema psoraleoides* (roots) extract showed a lower cumulative frequency of male mice smelling the vagina as compared to yohimbine but slightly higher than control. This shows that

E. psoraleoides (roots) extract may promote sexual desire by probably acting on central nervous system.

Cumulative frequency of male mice licking the penis after being given the *Eriosema psoraleoides* extract and yohimbine is shown in Figure 4.15.

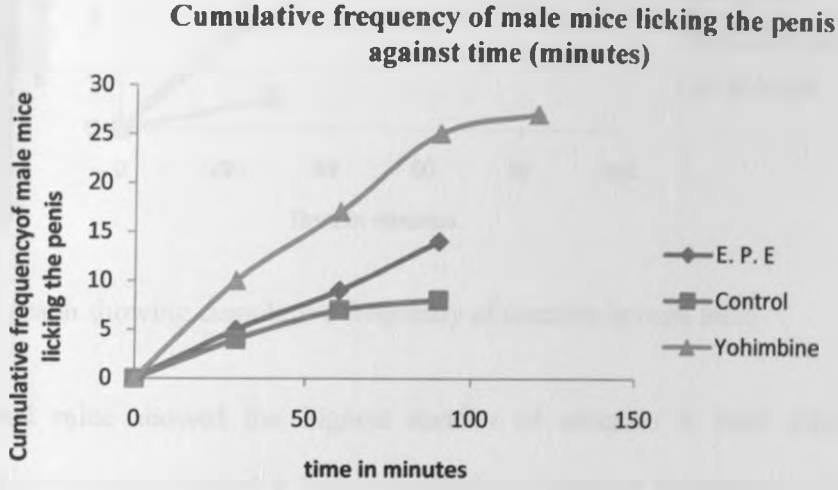


Figure 4.15: The cumulative frequency of male mice licking the penis

Yohimbine treated mice showed the highest cumulative frequency of male mice licking the penis. *Eriosema psoraleoides* (roots) extract showed a lower cumulative frequency of male mice licking the penis as compared to yohimbine but higher than the control. Licking the penis was used as an indicator of erection.

Cumulative frequency of erection in male mice after being given *Eriosema psoraleoides* roots extract, yohimbine and sildenafil is presented in Figure 4.16.

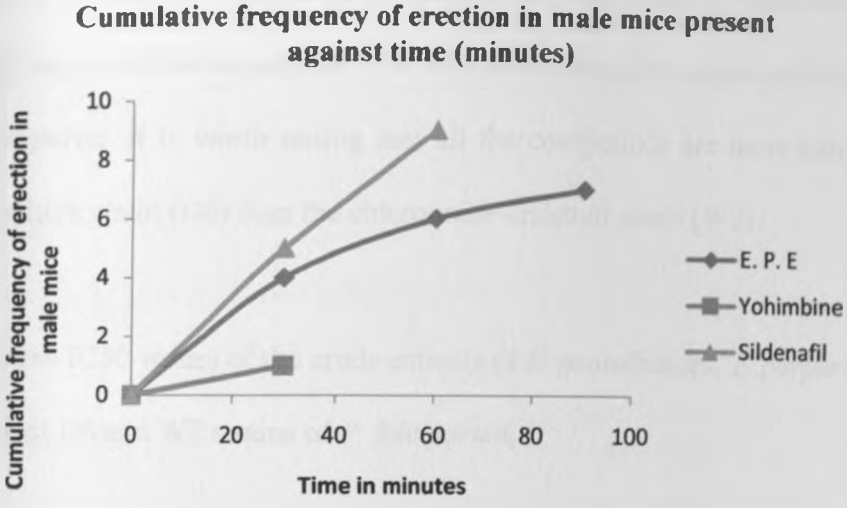


Figure 4.16: A graph showing cumulative frequency of erection in male mice

Sildenafil treated mice showed the highest number of erection in male mice. *Eriosema psoraleoides* (roots) extract showed a lower cumulative frequency of erection in male mice as compared to sildenafil but higher than yohimbine. *E. psoraleoides* root extract may have penile erectile activity. This supports its traditional use as an aphrodisiac.

4.4.1 Antiplasmodial activities of the plant extracts and pure compounds

The EtOAc fraction of the CH_2Cl_2/MeOH (1:1) extracts of *E. psoraleoides*, *T. purpurea* and the pure compounds were screened for antiplasmodial activities chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains, of *Plasmodium falciparum*. The results obtained indicated good antiplasmodial activities against both D6 and W2 strains of *P. falciparum*. Table 4.12 summarizes the *in vitro* antiplasmodial activity for the crude extracts and pure compounds.

The new compound terpurinflavone (**8**) exhibited the highest antiplasmodial activity with IC_{50} values of 1.36 $\pm$ 0.12 and 2.73 $\pm$ 1.16 µg/ml against D6 and W2 strains of *P. falciparum*,

respectively. Lanceolatin A (**9**) also showed good activity with IC_{50} values of 3.82±1.00 and 6.03±1.04 against D6 and W2 strains of *P. falciparum*, respectively. Terpurinflavone (**8**) and lanceolatin A (**9**) appears to be responsible for the antiplasmodial activities observed in the crude extract of *T. purpurea*. It is worth noting that all the compounds are more active against the chloroquine-sensitive strain (D6) than the chloroquine-resistant strain (W2).

Table 4.12: *In vitro* IC50 values of the crude extracts of *E. psoraleoides*, *T. purpurea* and pure compounds against D6 and W2 strains of *P. falciparum*.

Sample	IC_{50} (µg/ml+ SD)		Resistance index (RI)
	D6	W2	
E. psoraleoides root extract	9.33 ± 0.38	11.43 ± 0.47	1.23
T. purpurea stem extract	10.47 ± 2.22	12.06 ±5.54	1.15
Lanceolatin B (**6**)	7.08 ± 0.59	9.43 ± 1.11	1.33
Semiglabrin (**7**)	9.33 ± 2.20	12.88 ± 1.96	1.38
Terpurinflavone (**8**)	1.36 ± 0.12	2.73 ± 1.16	2.00
Lanceolatin A (**9**)	3.82 ± 1.00	6.03 ± 1.04	1.58
Mefloquine	-	0.0036 ± 0.0006	-
Chloroquine	0.011 ± 0.001	-	-

There was reduced activity against chloroquine-resistant (W2) strain with resistance indices of 1.10-2.00. Activities were inferior as compared to mefloquine and structure modification are required to improve the acivity. Terpurinflavone (**8**) had the highest resistance value which may indicate high risk of future cross resistance development.

4.4.2 Antimicrobial activities of plant extracts and pure compounds

The crude extracts of *E. psoraleoides*, *T. purpurea* and pure compounds from *T. purpurea* were tested for antimicrobial activity. The crude extract of *E. psoraleoides* showed an inhibition zone of 12 mm against *Candida albicans* at a concentration of 1.8 mg/disc while the new compound terpurinflavone (**8**) showed an inhibition zone of 16 mm against *Microsporum gypsum* at a concentration of 50 µg/disc. The crude extract of *T. purpurea*, lanceolatin B (**6**), semiglabrin (**7**) and lanceolatin A (**9**) were inactive against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* and *Microsporum gypsum*.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

1. The phytochemical study on the *E. psoraleoides* (roots) led to the isolation of four compounds and these were characterized as the isoflavanone derivatives, 4',5-dihydroxy-2',7-dimethoxyisoflavanone (**1**), 4',5,7-trihdroxy-2'-methoxyisoflavanone (**4**). an isoflavone dimer, 4',7"-bisgenistein (**3**), and the coumaronochromone 4',5,7-trihydroxycoumaronochromone (**2**). 4',7"-Bisgenistein (**3**) appear to be new.
2. The study on *T. purpurea* (stem) led to the isolation of five compounds which included a steroid, stigmasterol (**5**), four prenylated flavone derivatives, lanceolatin B (**6**), semiglabrin (7), terpurinflavone (**8**) and lanceolatin A (**9**). Of these, compound **8** is novel.
3. *E. psoraleoides* (roots) and *T. purpurea* (stem) showed significant relaxant effect on the aorta and therefore they may be used for the management of erectile dysfunction (ED).
4. Semiglabrin (7) had the highest relaxant effect on the bronchial smooth muscle of an isolated trachea. This provides scientific justification for the use of *T. purpurea* for the management of tightness of the chest.
5. The crude extracts and the prenylated flavones from *T. purpurea* (stem) showed antiplasmodial activities against chloroquine-sensitive strain (D6) and the chloroquine-resistant strain (W2) of *P. falciparum* parasite for malaria. Among the prenylated flavones tested, the terpurinflavone (**8**) had the highest activity.
6. The crude extract of *E. psoraleoides* (roots) was active against *Candida albicans* while terpurinflavone (**8**) was active against *Microsporum gypsum.*

5.2 RECOMMENDATIONS

1. Further phytochemical investigation on *E. psoraleoides* (roots) and *T. purpurea* (stem) should be carried out in order to establish the chemical profiles of these medicinal plants.

2. *In vivo* penile relaxant activity of *E. psoraleoides* (roots) extract should be carried out in order to establish its potency and efficacy.

3. *In vivo* anti-asthmatic activity should be carried out on *T. purpurea* (stem) extract and semiglabrin (7) in order to establish the potency and efficacy.

4. *In vivo* antiplasmodial activity tests should be carried out on the extracts and isolated compounds from these medicinal plants in order to establish their potency and efficacy.

5. Toxicity study of the extracts and compounds from these medicinal plants should be done in order to establish their safety in long term use, as might be required for long term infection management.

6. The mechanism of action of the compounds needs to be fully examined so that targeted proteins by these compounds can be clearly demonstrated for differentiation with those of already established drugs. This information is important for developing the compounds as therapies for management of infections arising from resistant strains of *P. falciparum* malaria parasite.

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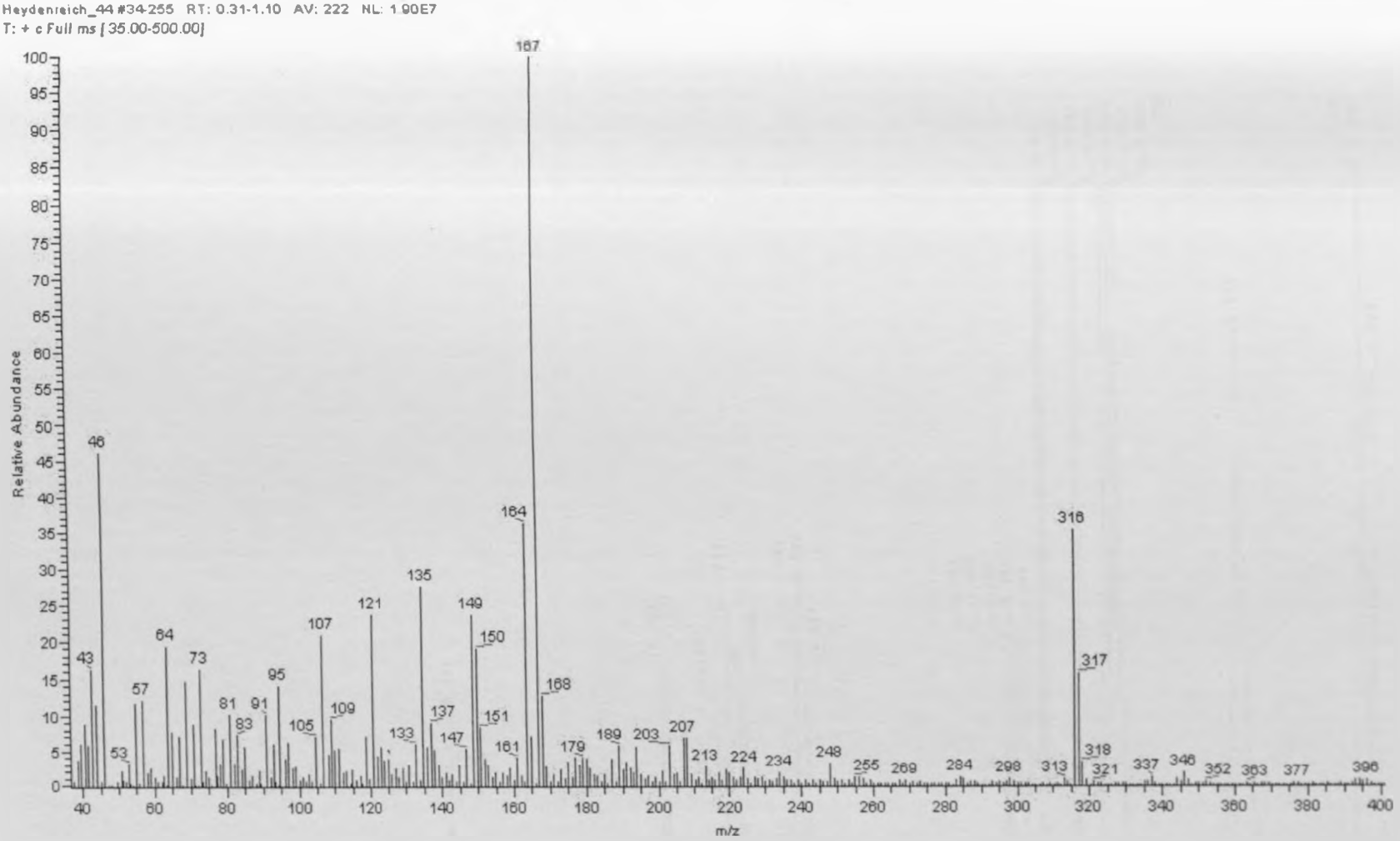
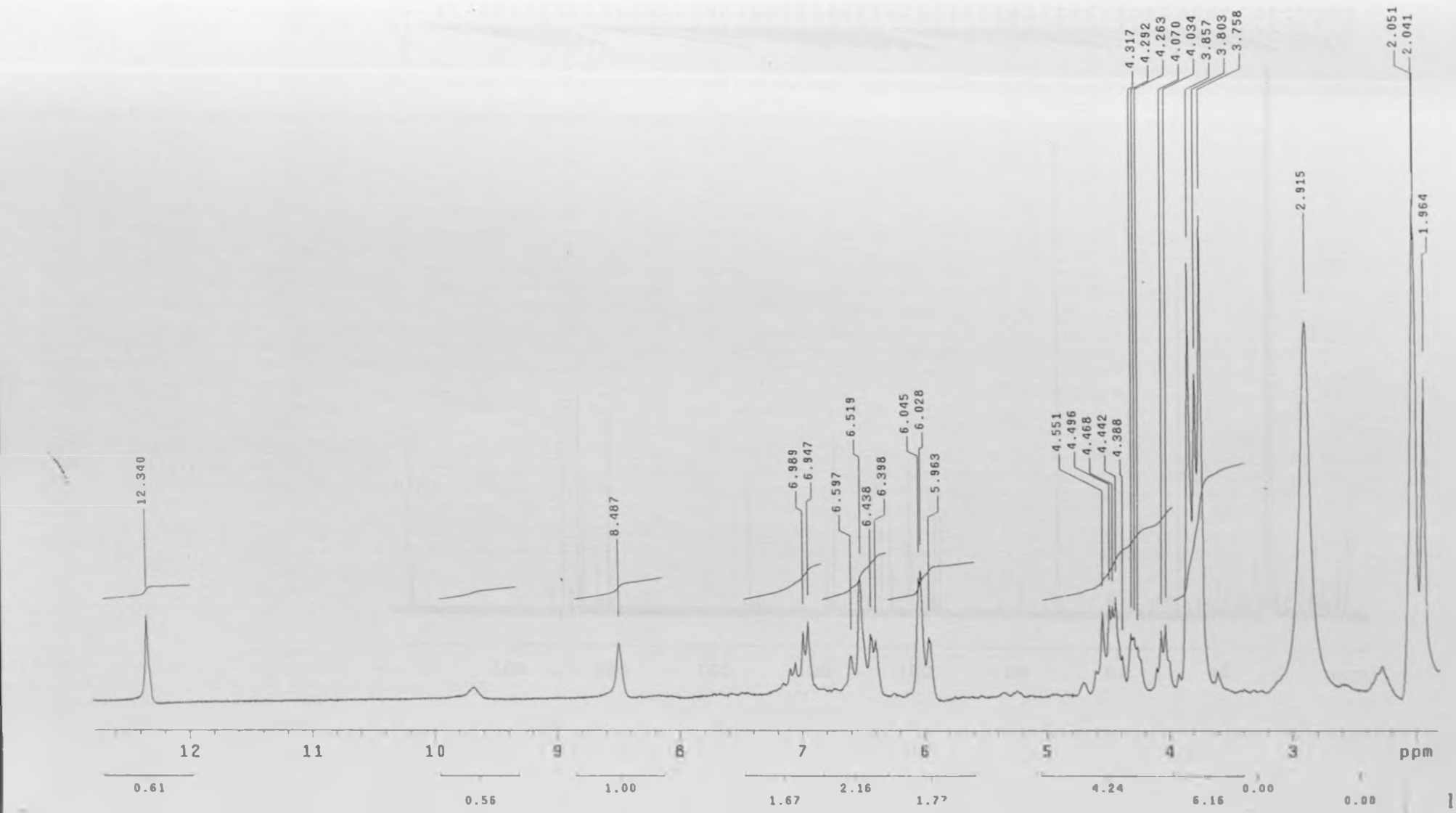
APPENDICES

Appendix A: Spectra for compound 1

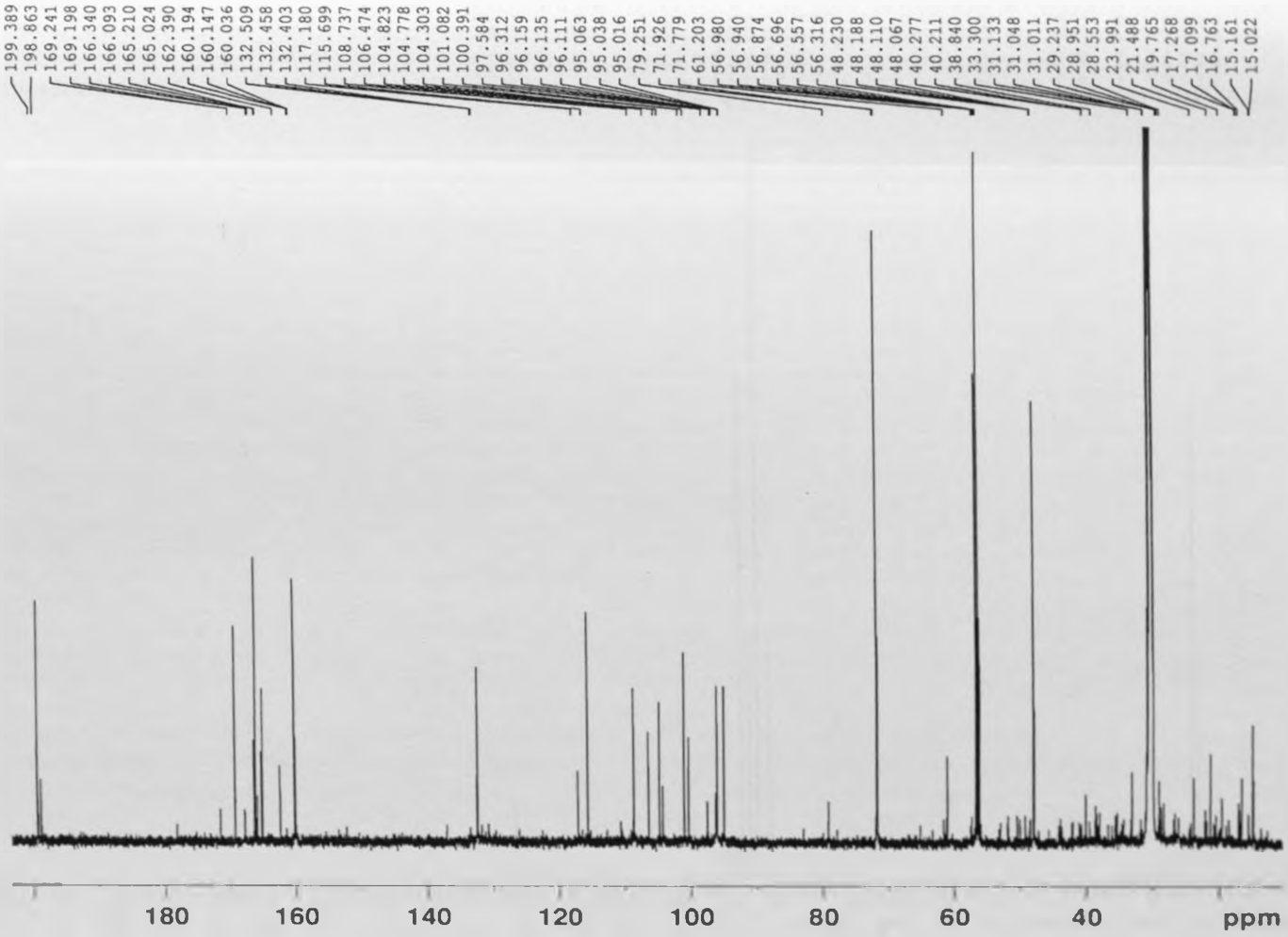
EI-MS SPECTRUM FOR COMPOUND 1



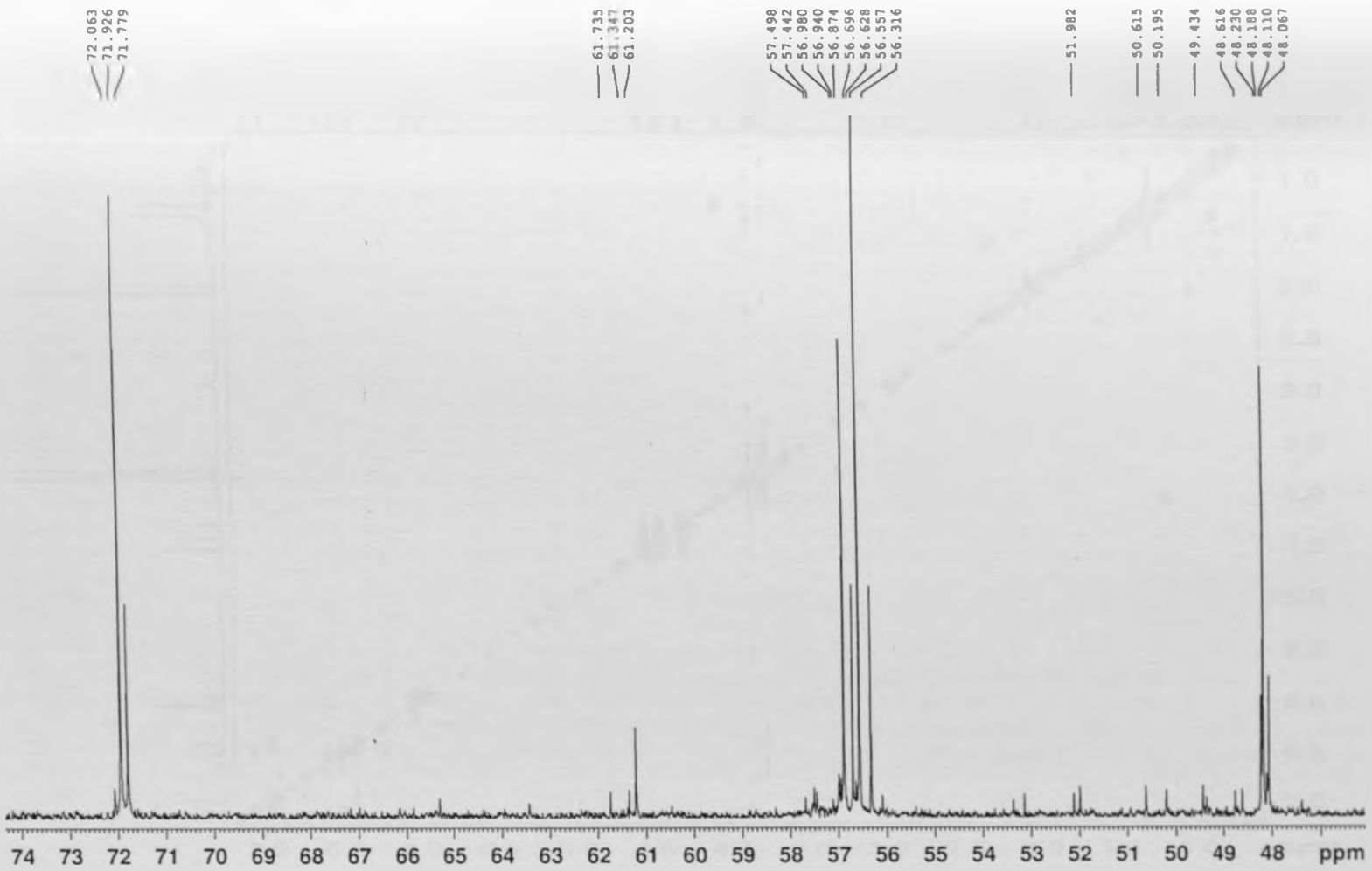
^{1}H NMR SPECTRUM FOR COMPOUND 1 (200 MHz, ACETONE-d_6)



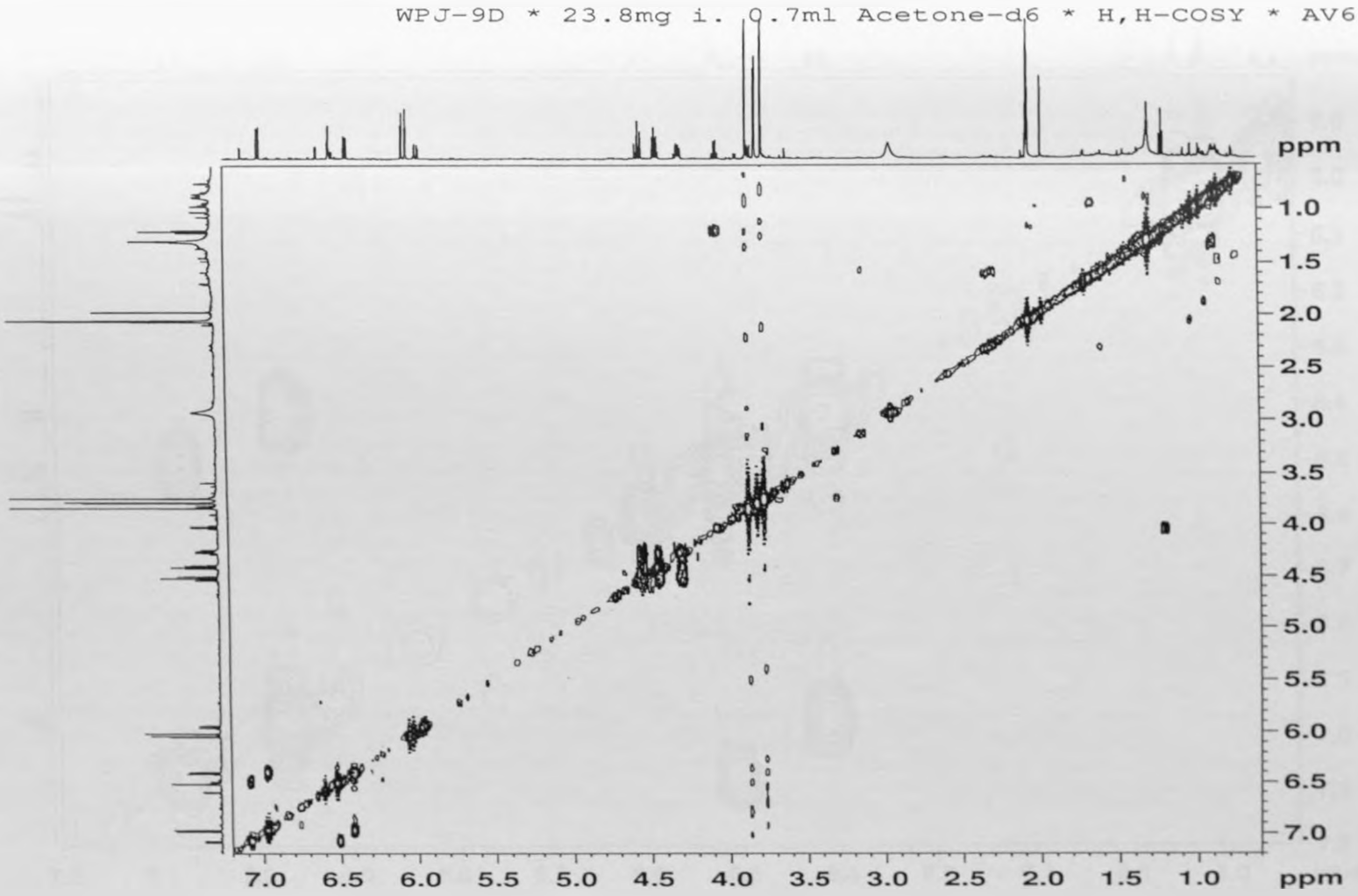
^{13}C NMR SPECTRUM FOR COMPOUND 1 (125 MHz, ACETONE-d_6)



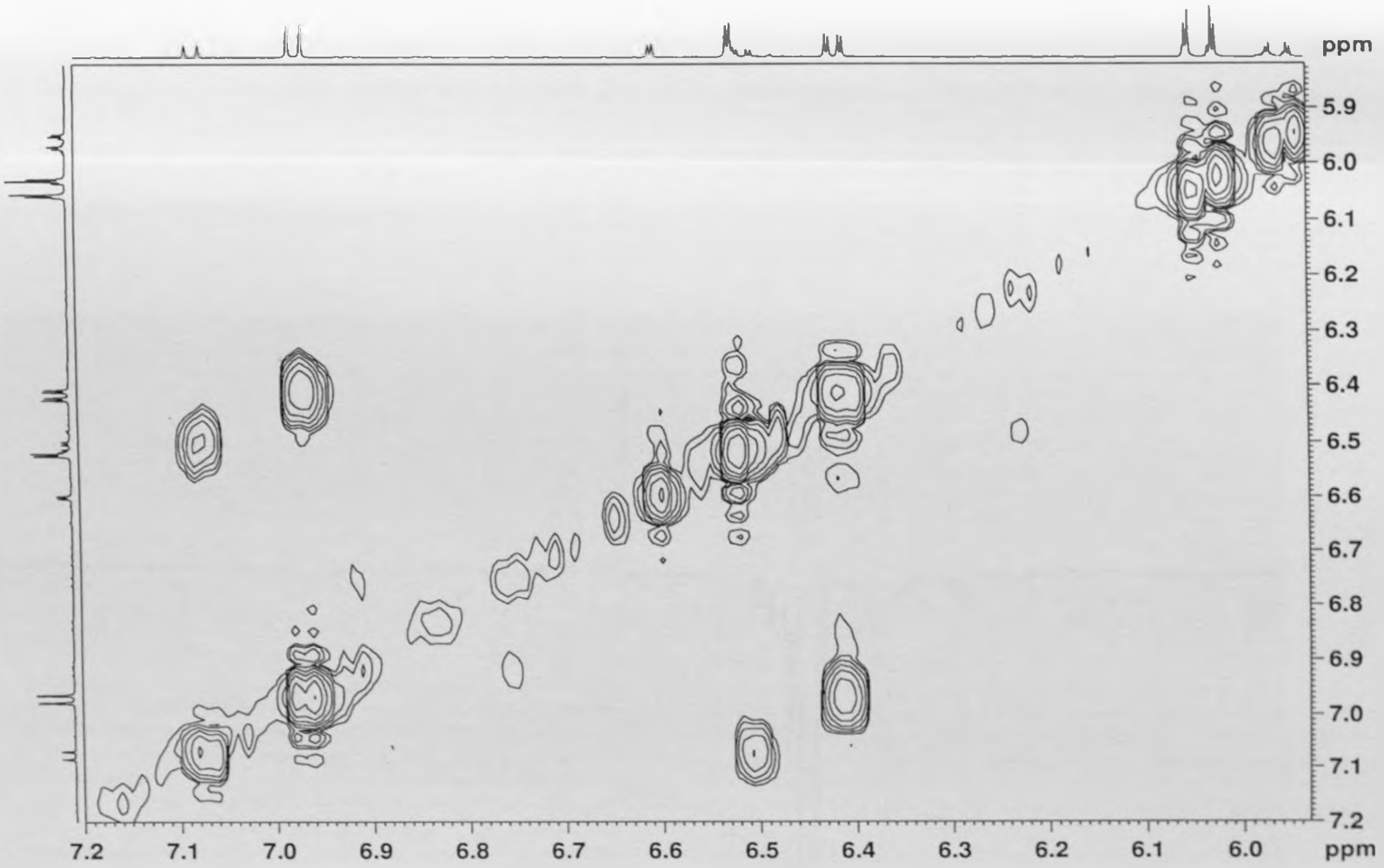
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 1



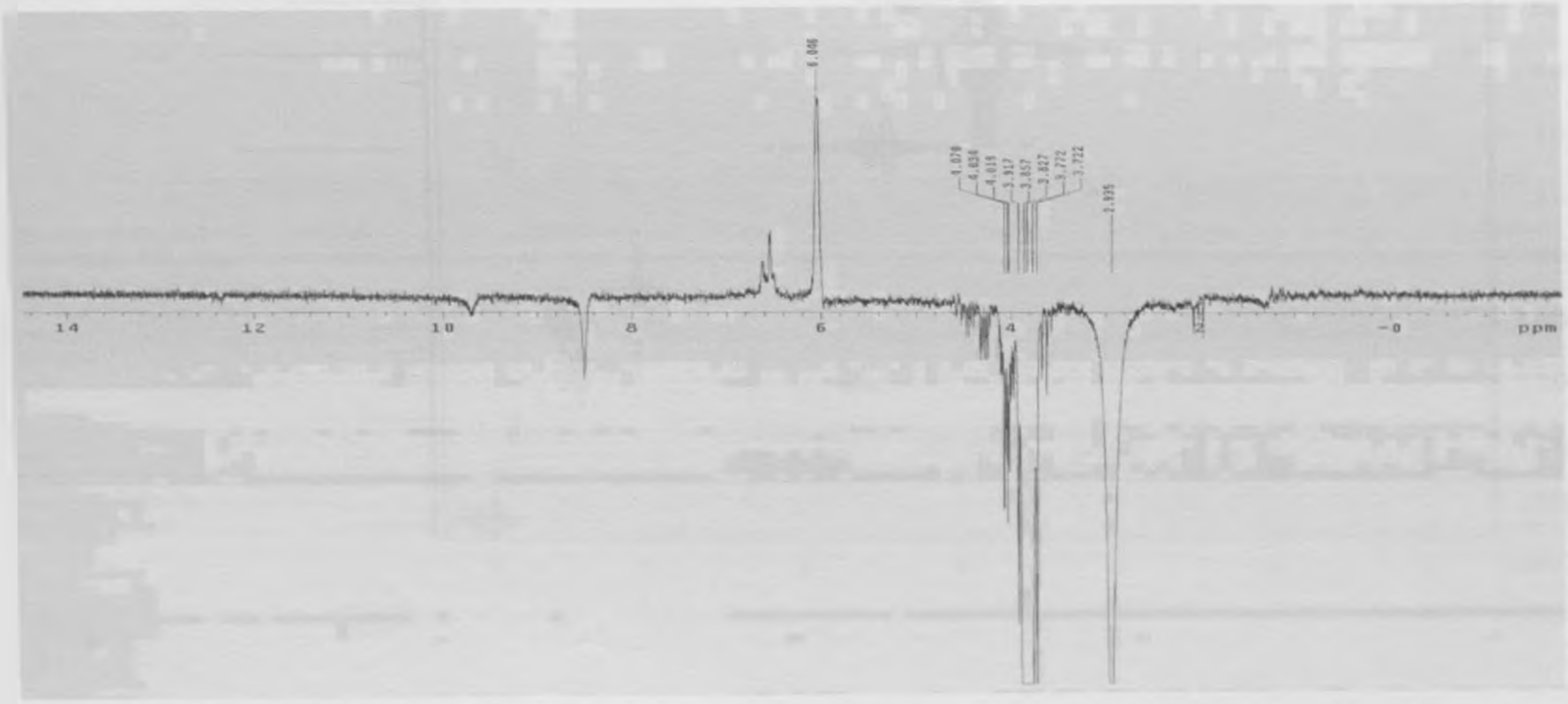
^{1}H-^{1}H COSY SPECTRUM FOR COMPOUND 1



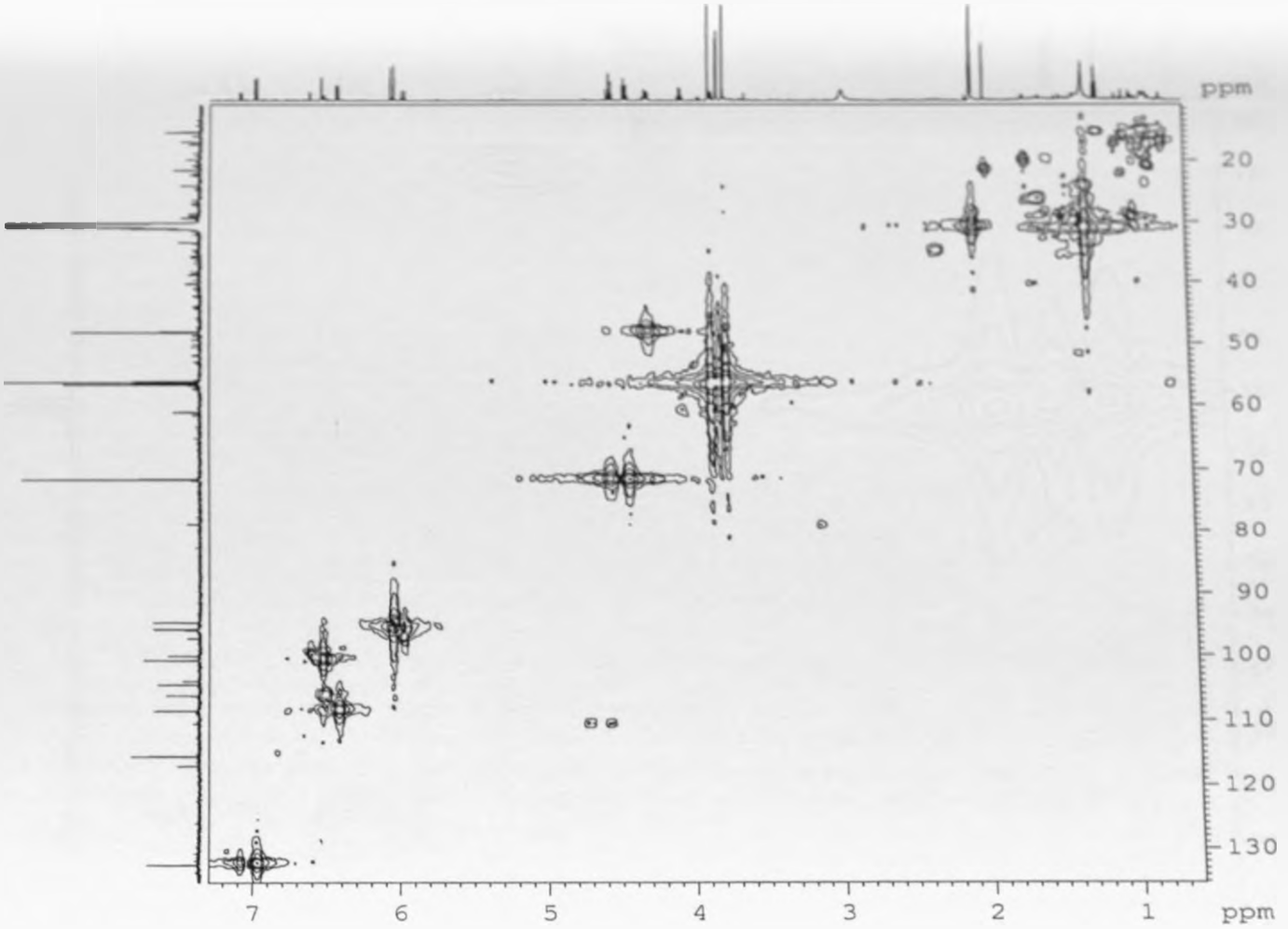
^{1}H-^{1}H COSY SPECTRUM EXPANSION FOR COMPOUND 1



NOE DIFF SPECTRUM FOR COMPOUND I



HMQC SPECTRUM FOR COMPOUND I



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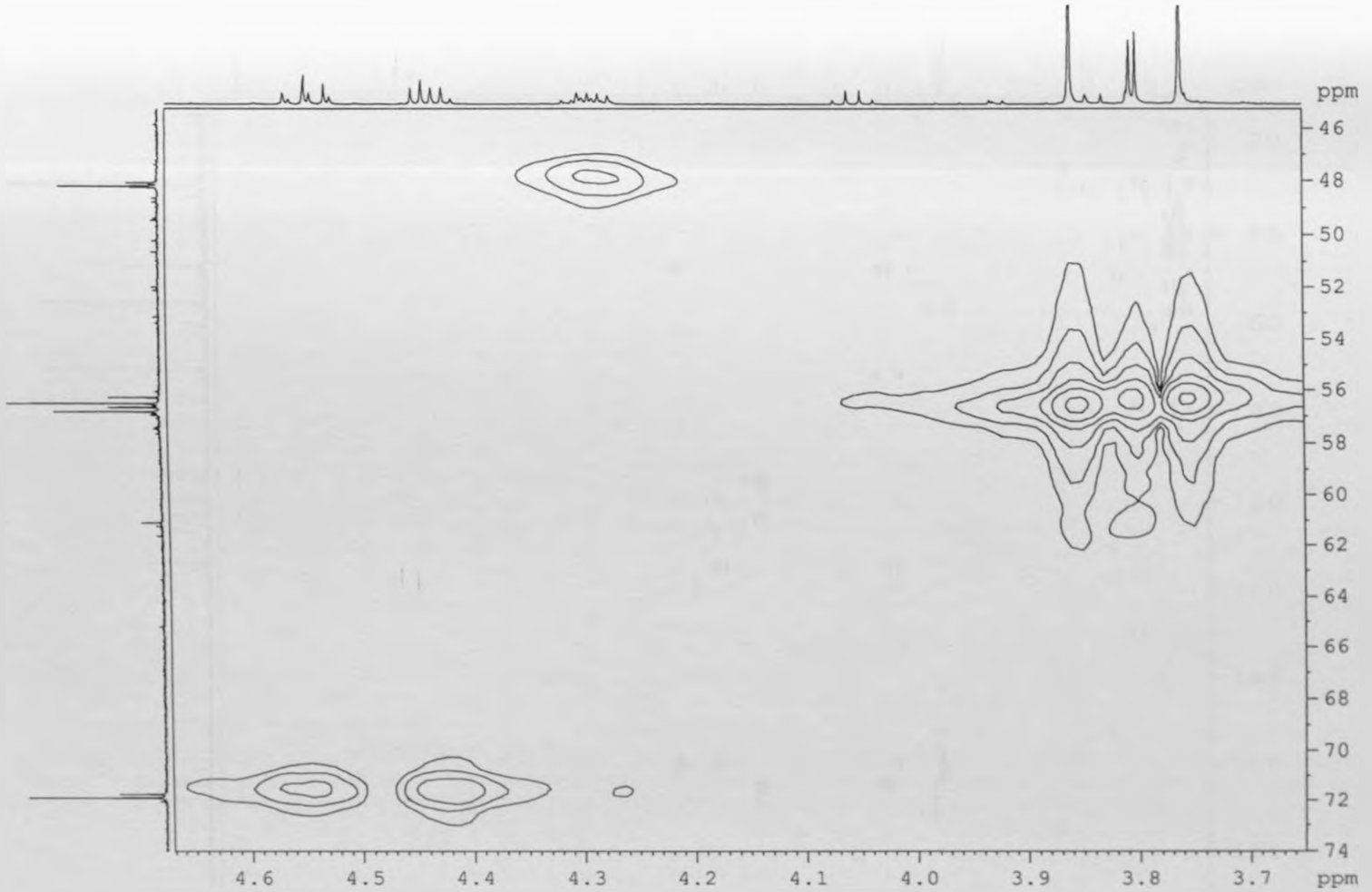

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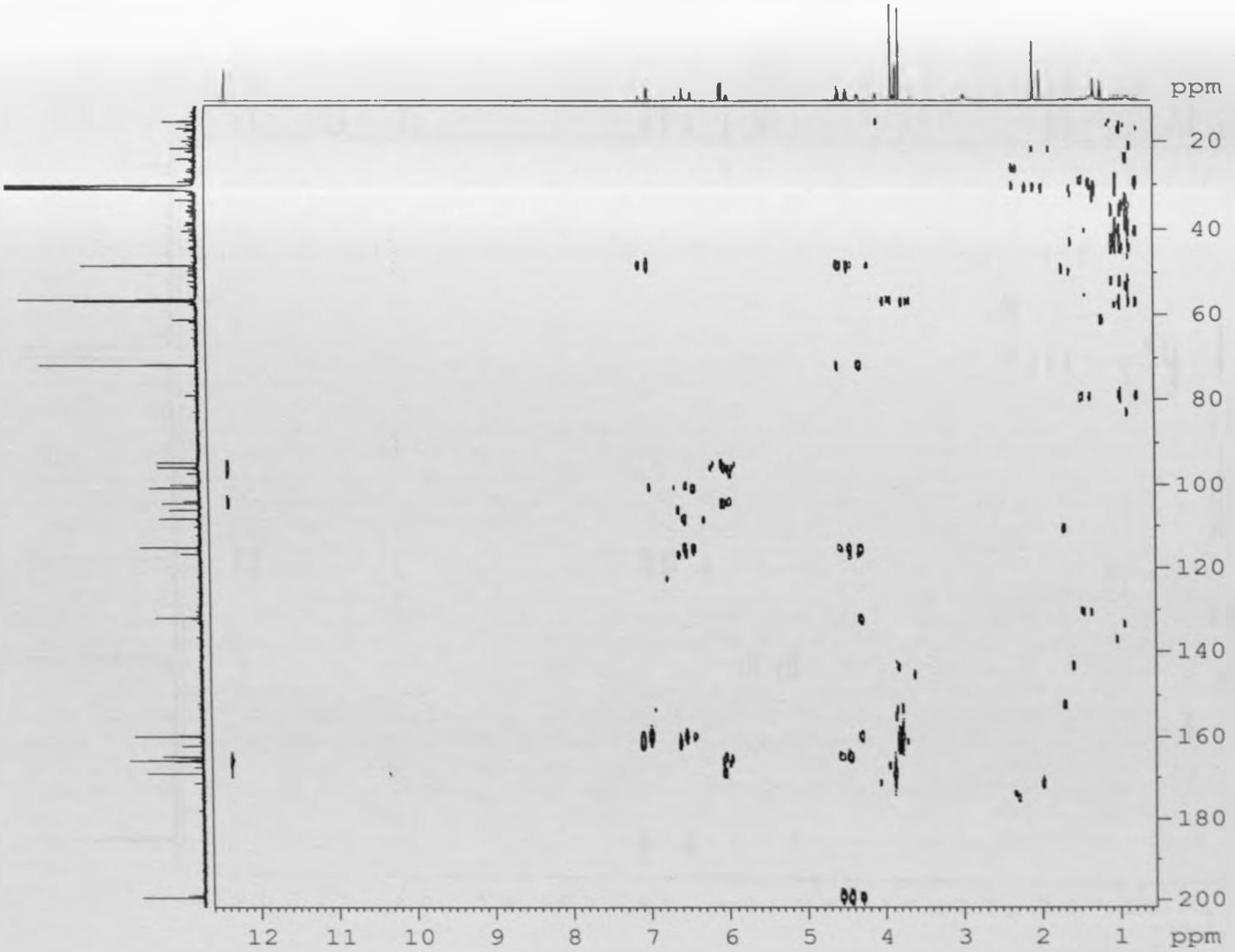

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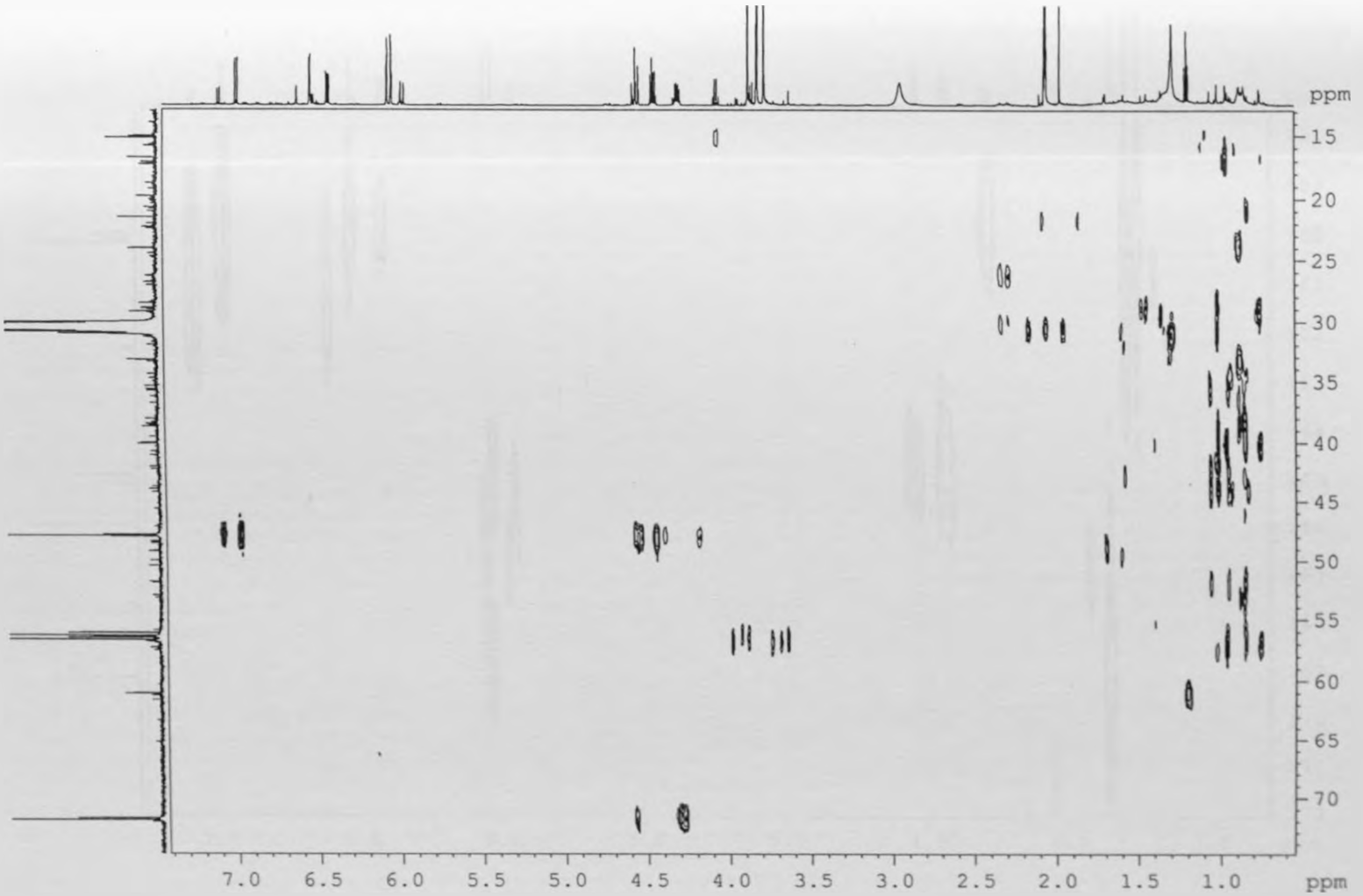

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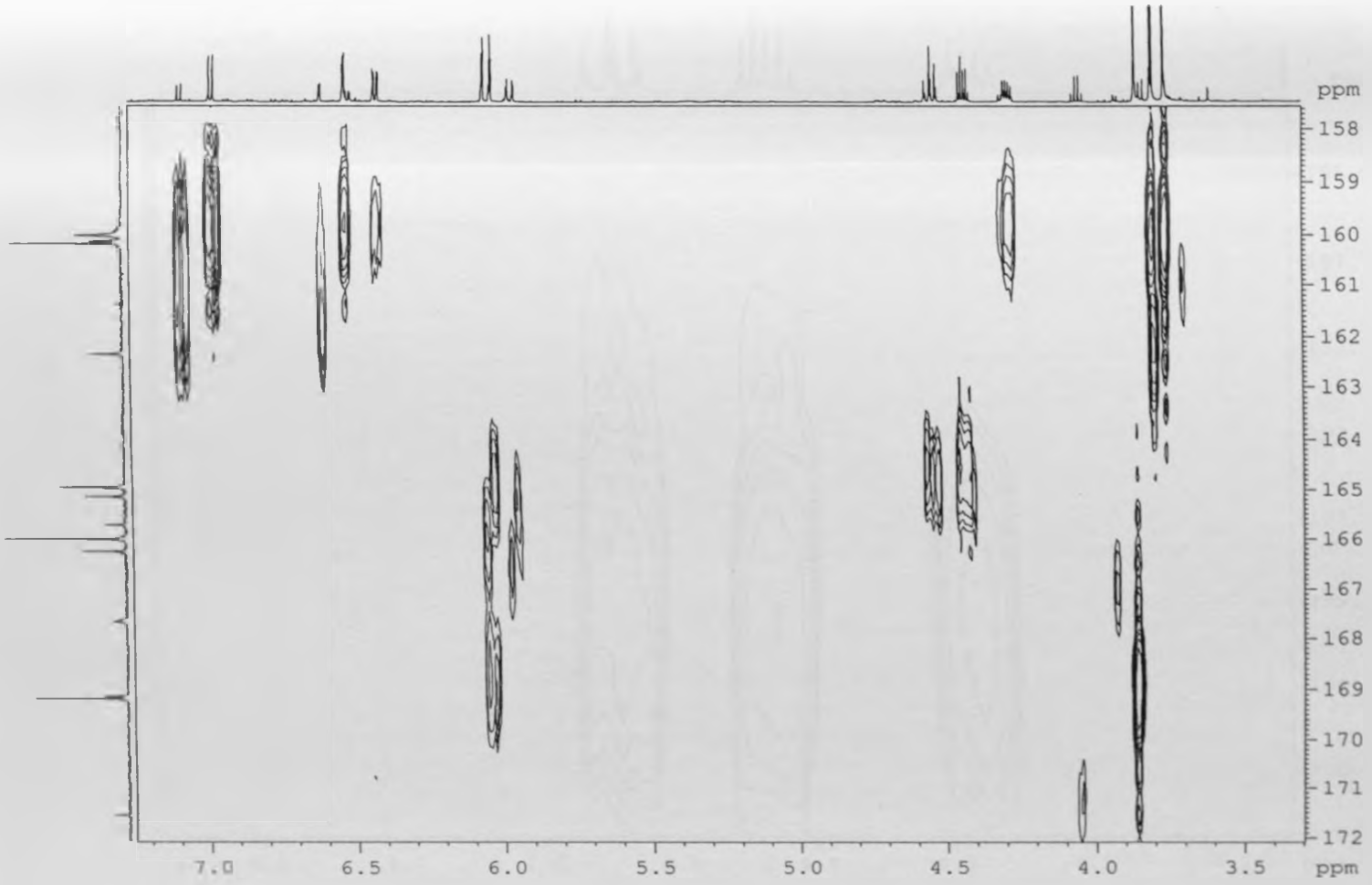

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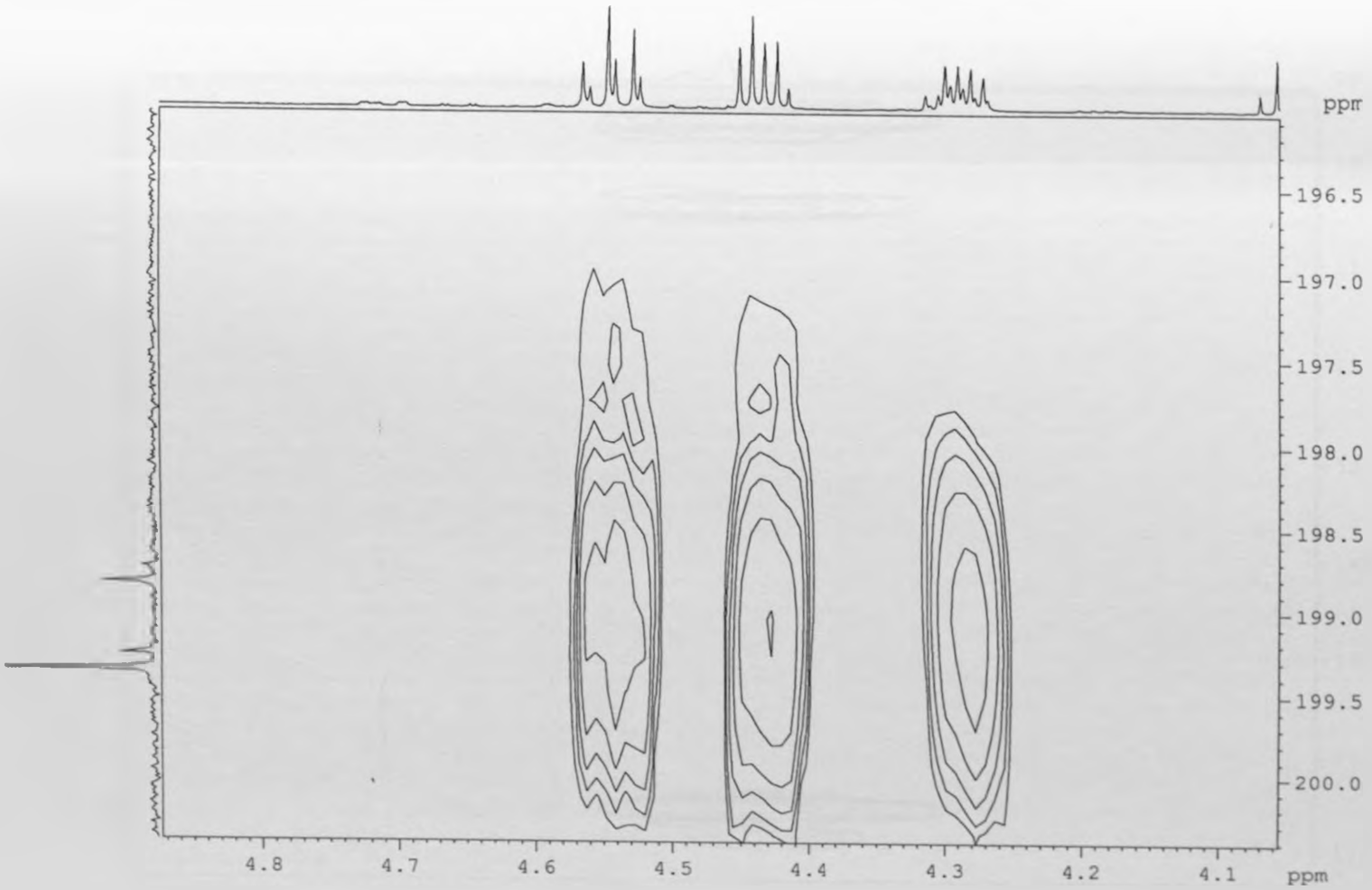

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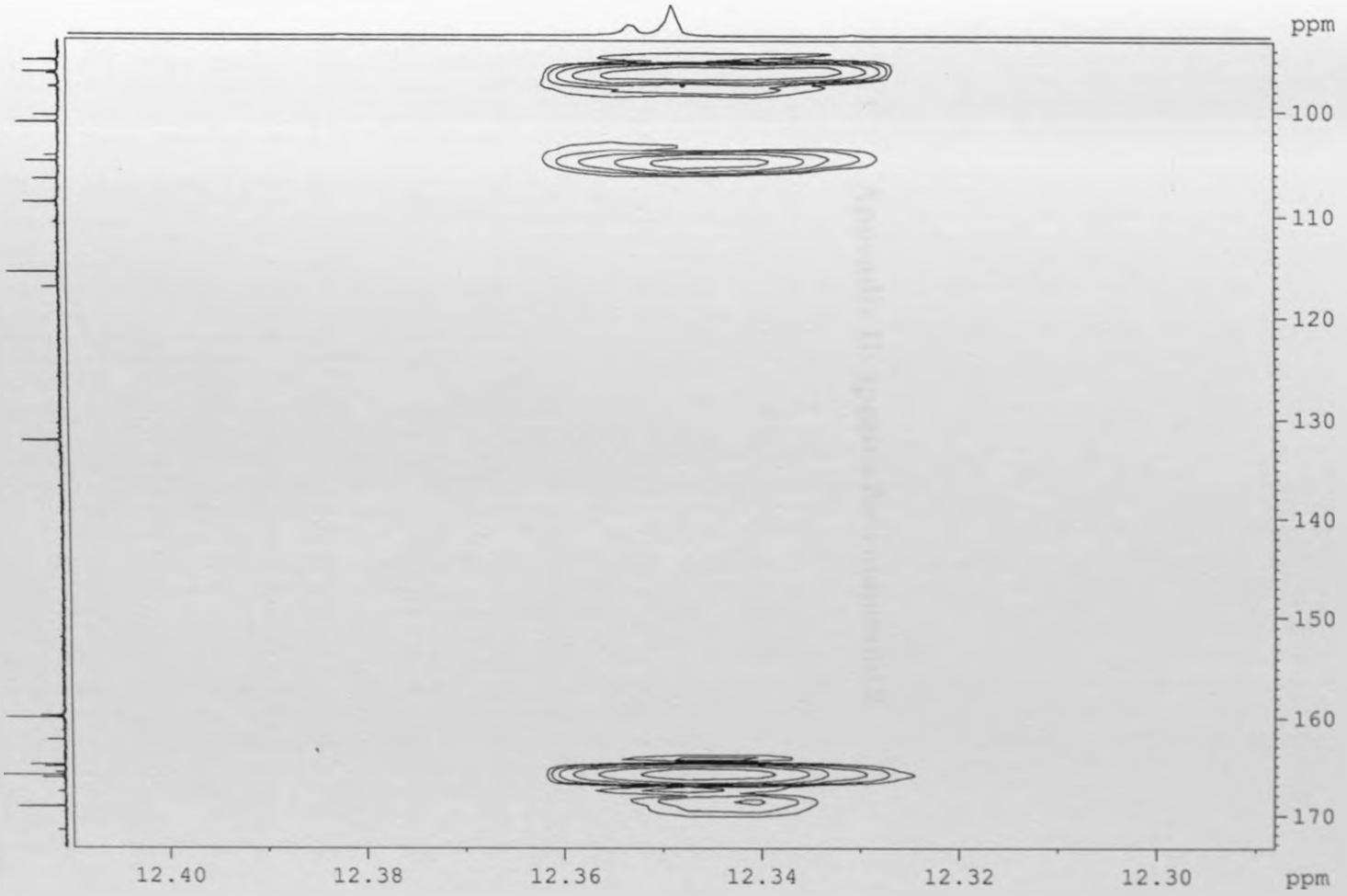
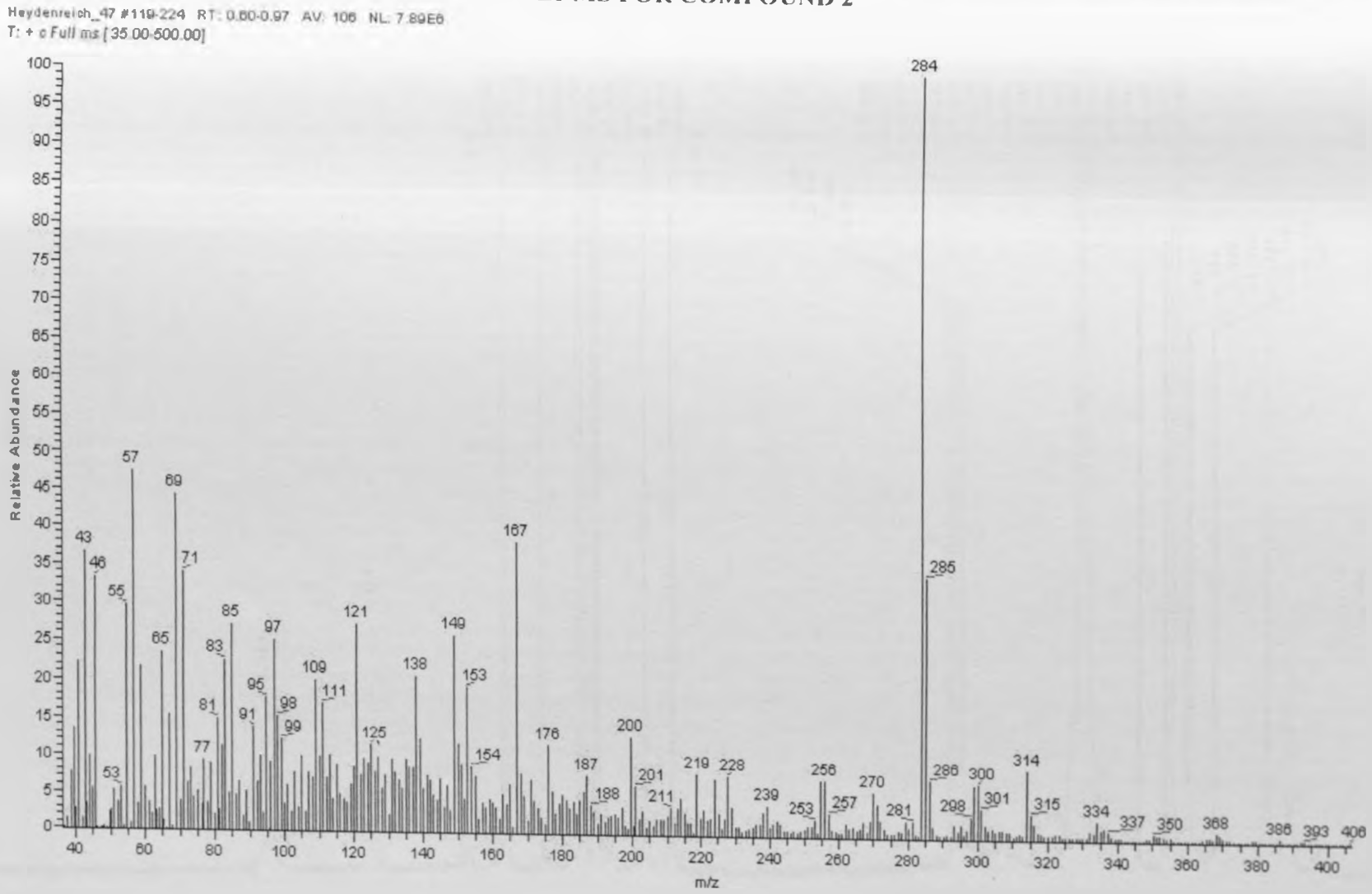
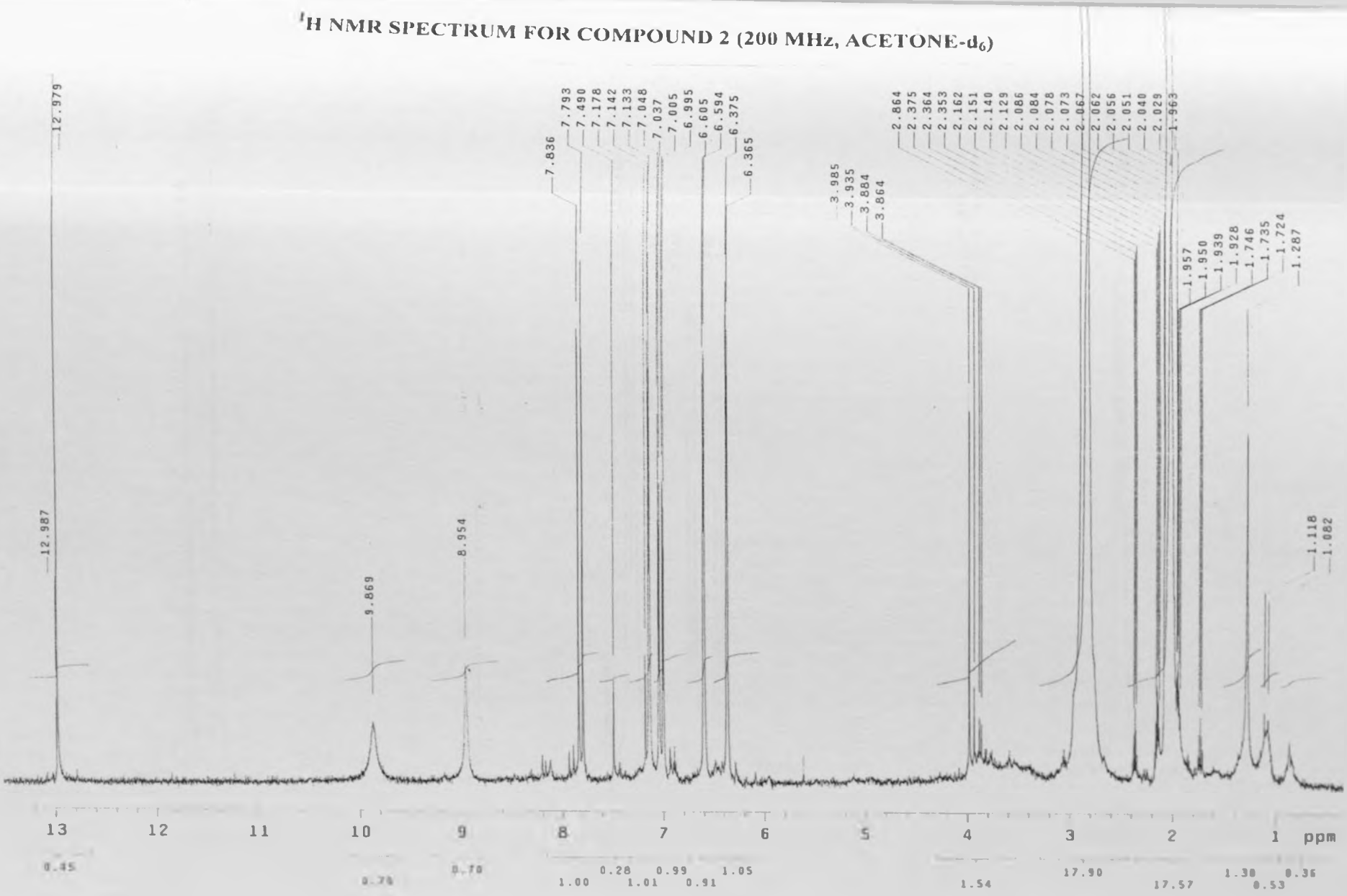


Appendix B: spectra for compound 2

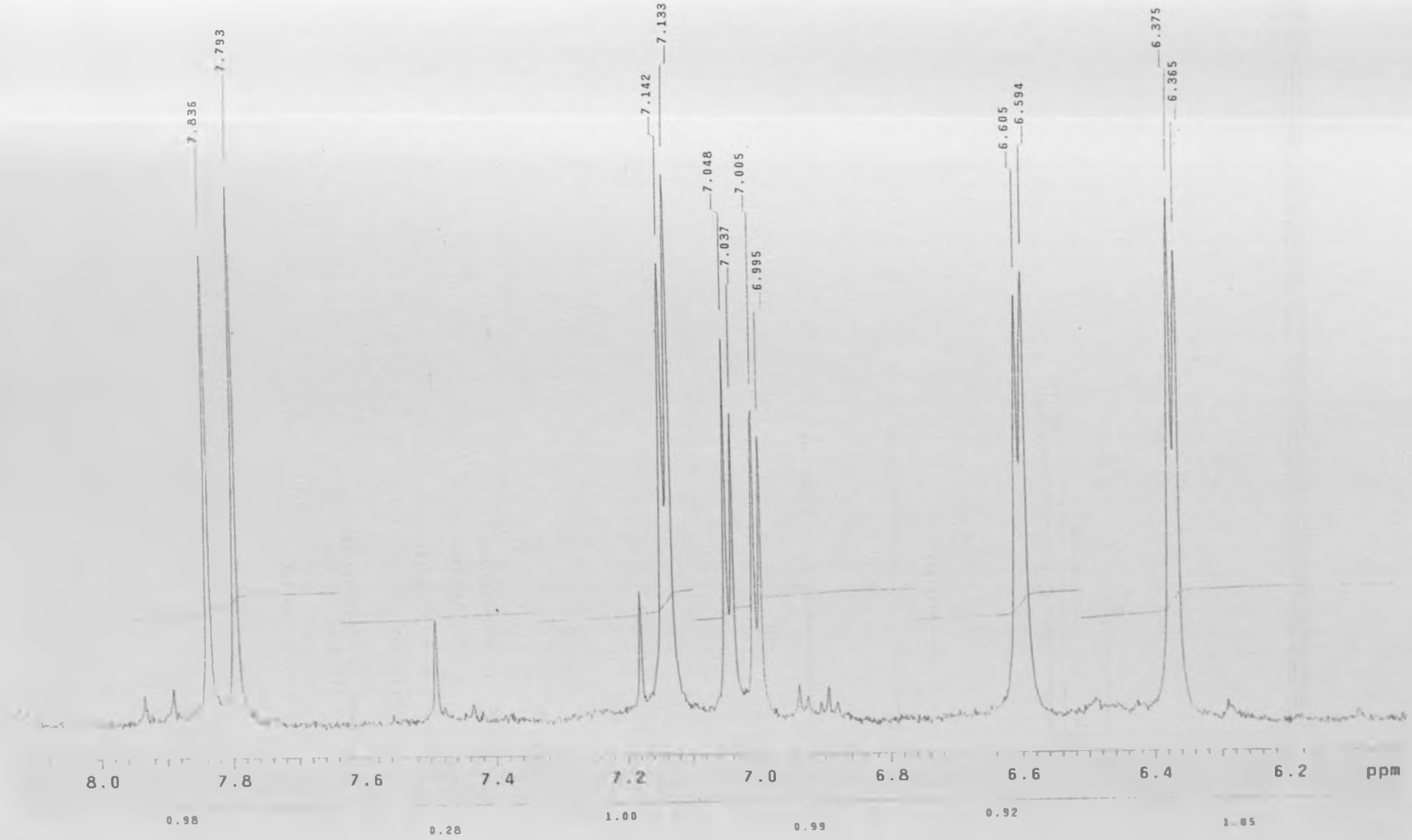
EI-MS FOR COMPOUND 2



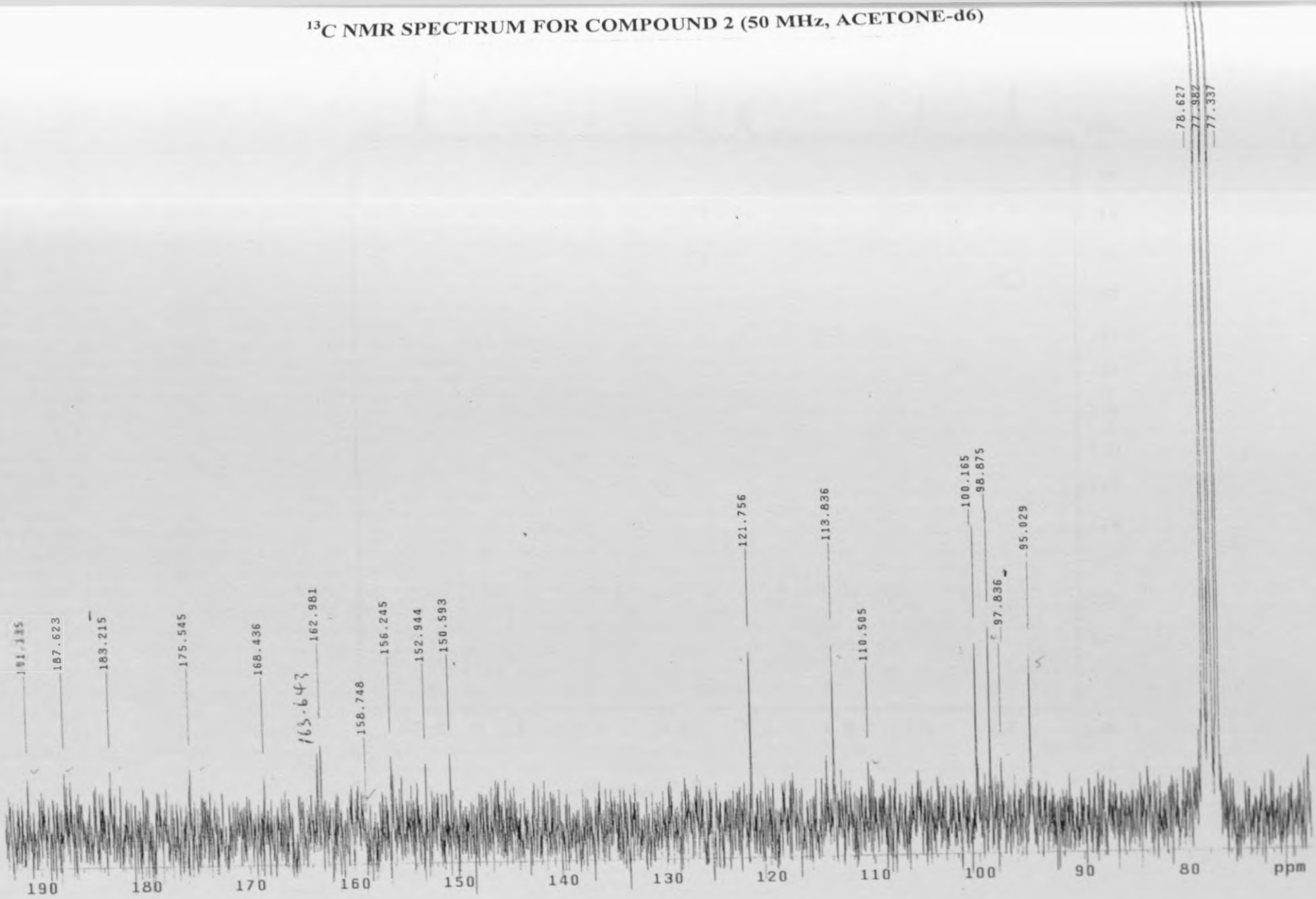
^{1}H NMR SPECTRUM FOR COMPOUND 2 (200 MHz, ACETONE-d_6)



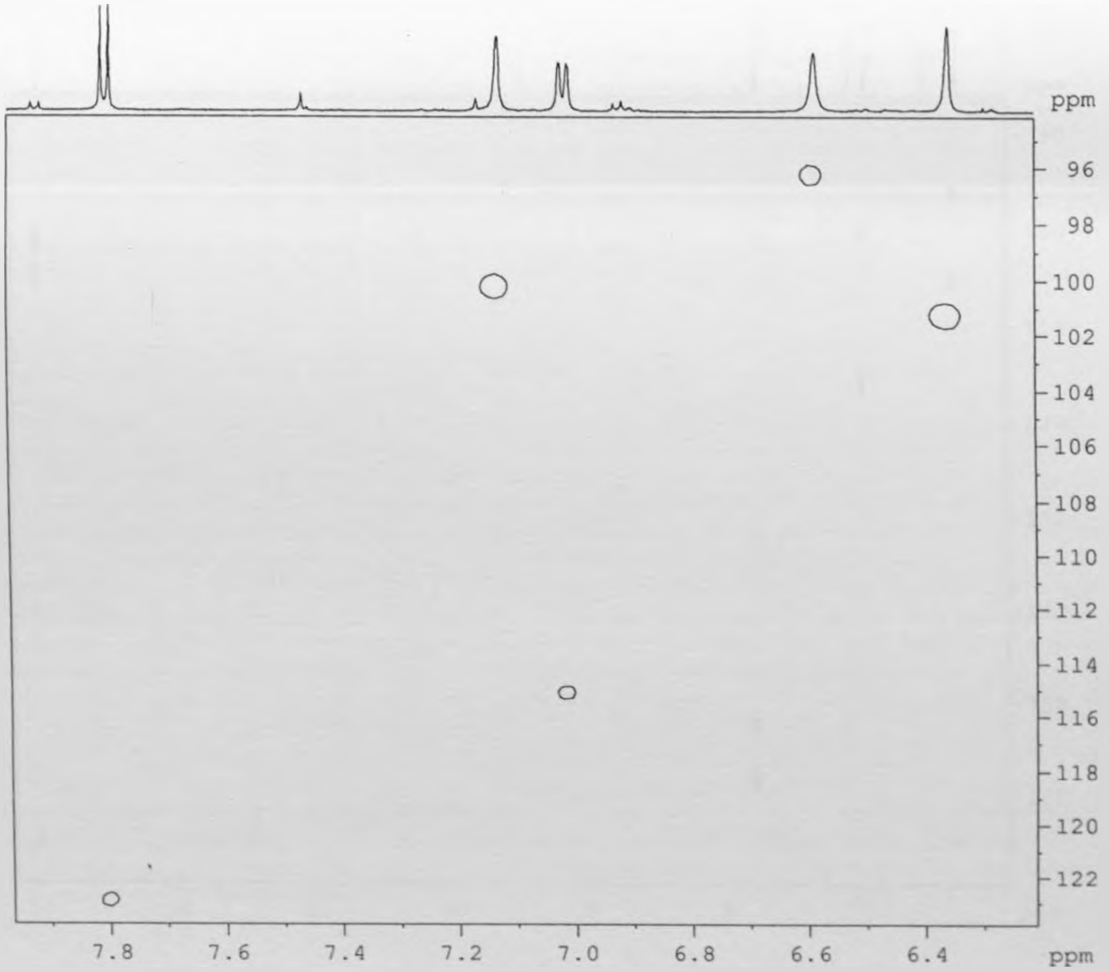
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 2



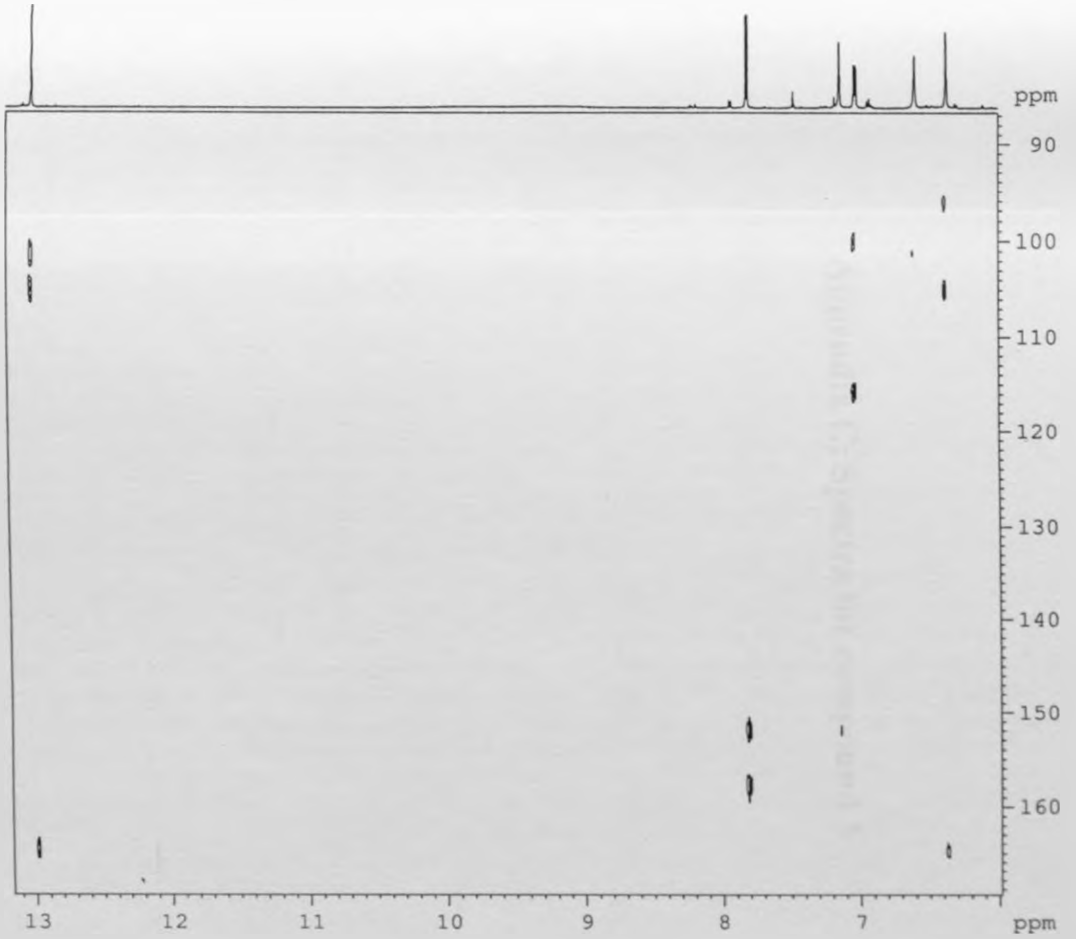
^{13}C NMR SPECTRUM FOR COMPOUND 2 (50 MHz, ACETONE-d6)



HMQC SPECTRUM FOR COMPOUND 2

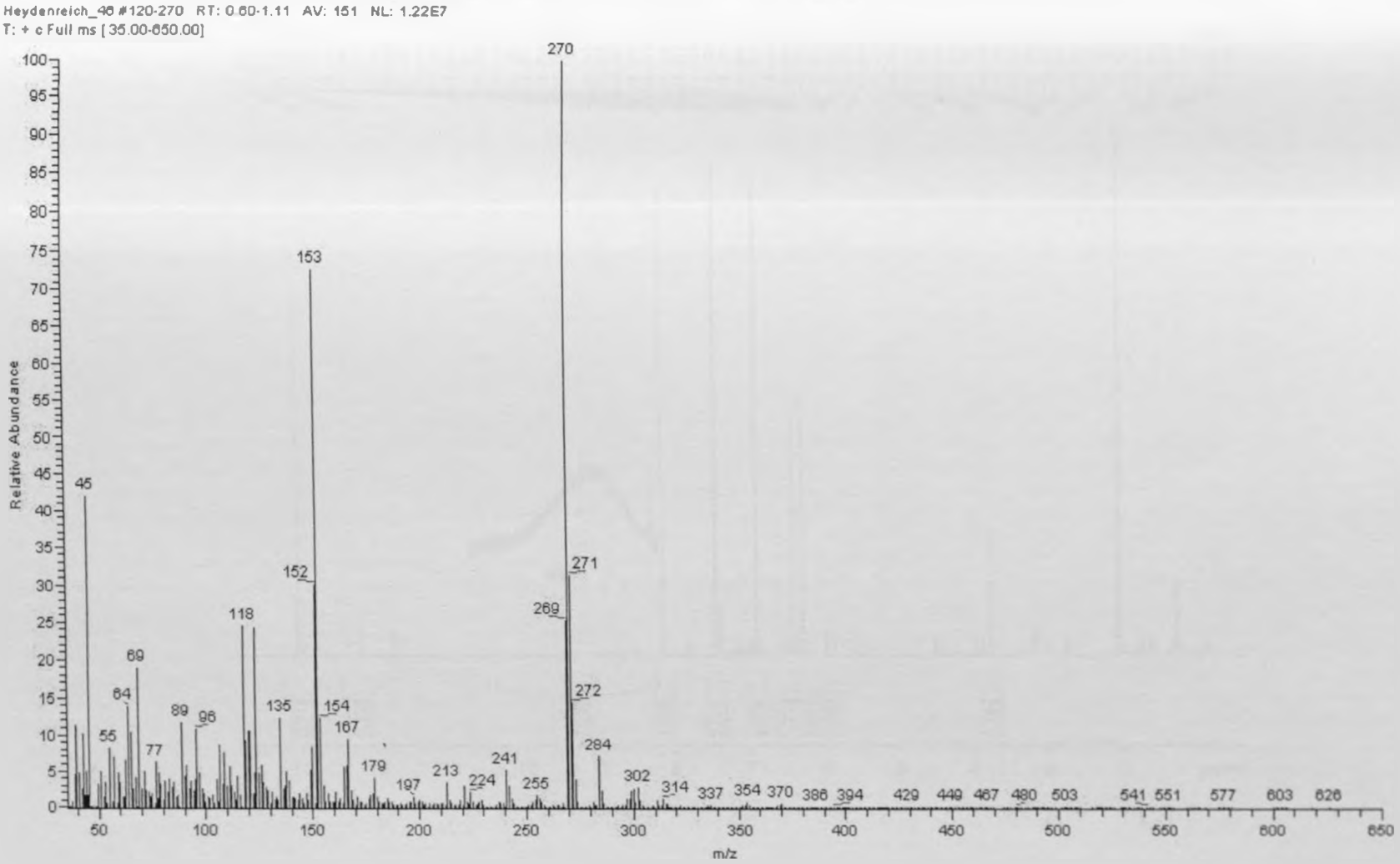


HMBC SPECTRUM FOR COMPOUND 2

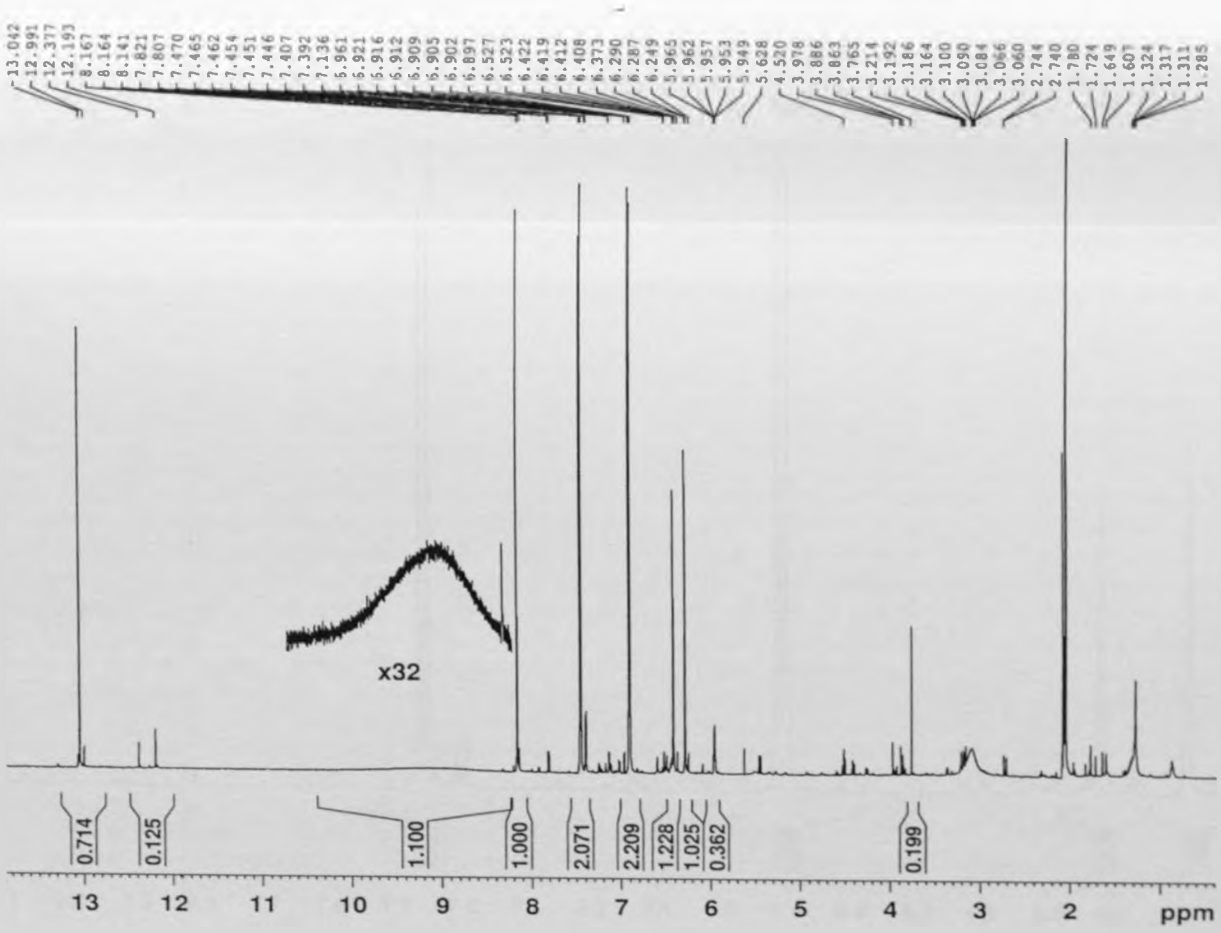


Appendix C: Spectra for compound 3

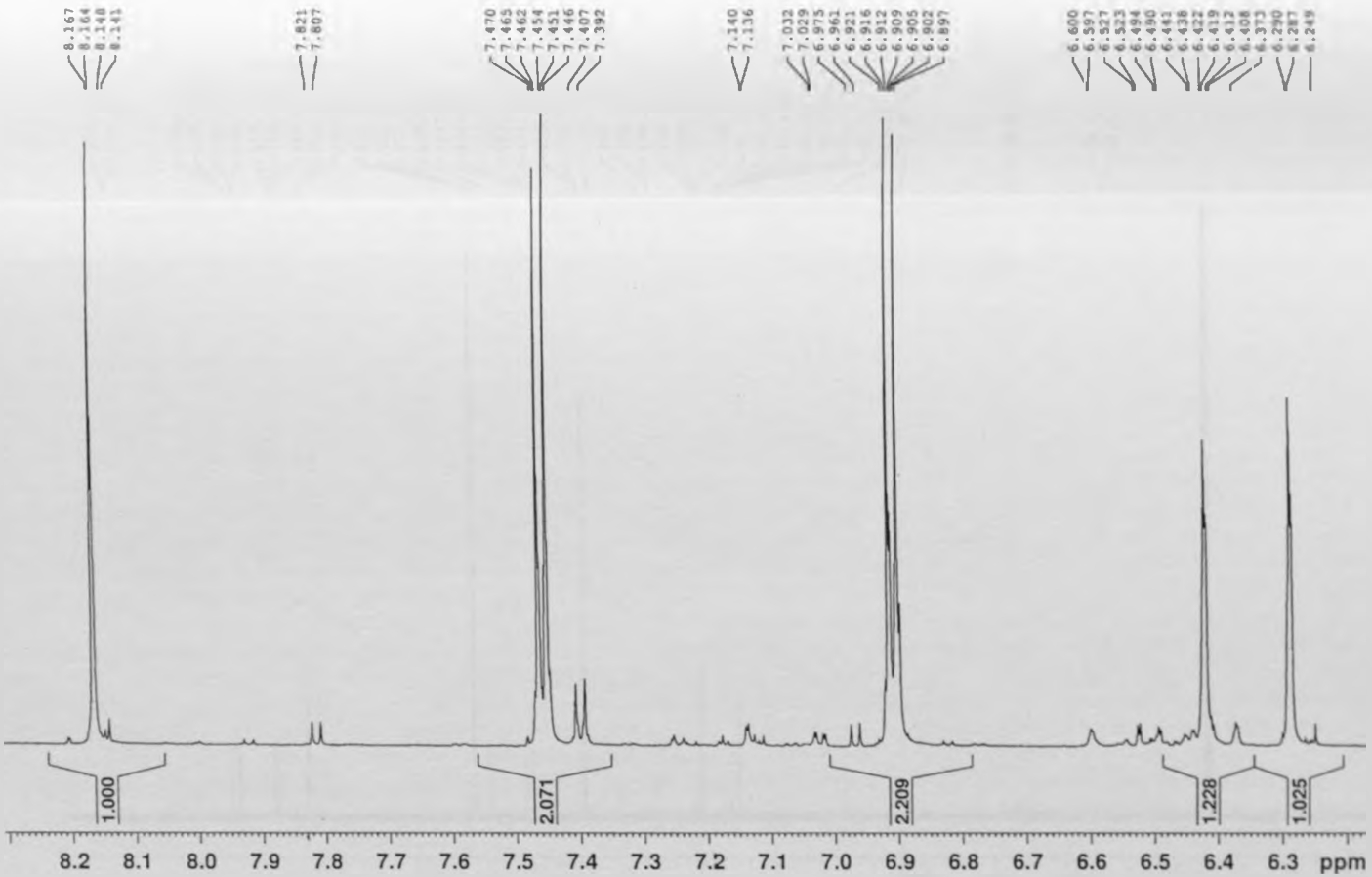
EI-MS SPECTRUM FOR COMPOUND 3



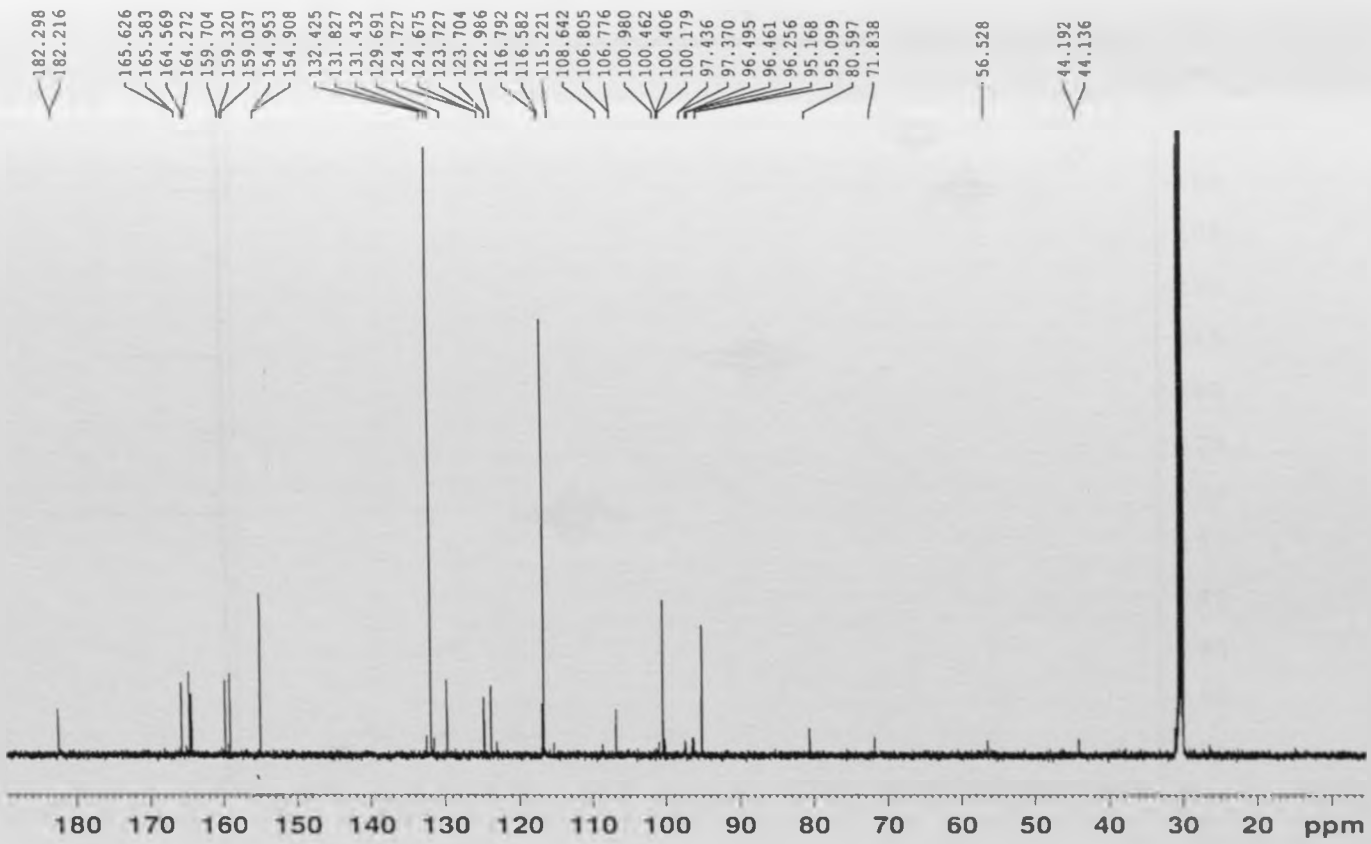
^1H NMR SPECTRUM FOR COMPOUND 3 (600 MHz, ACETONE-d_6)



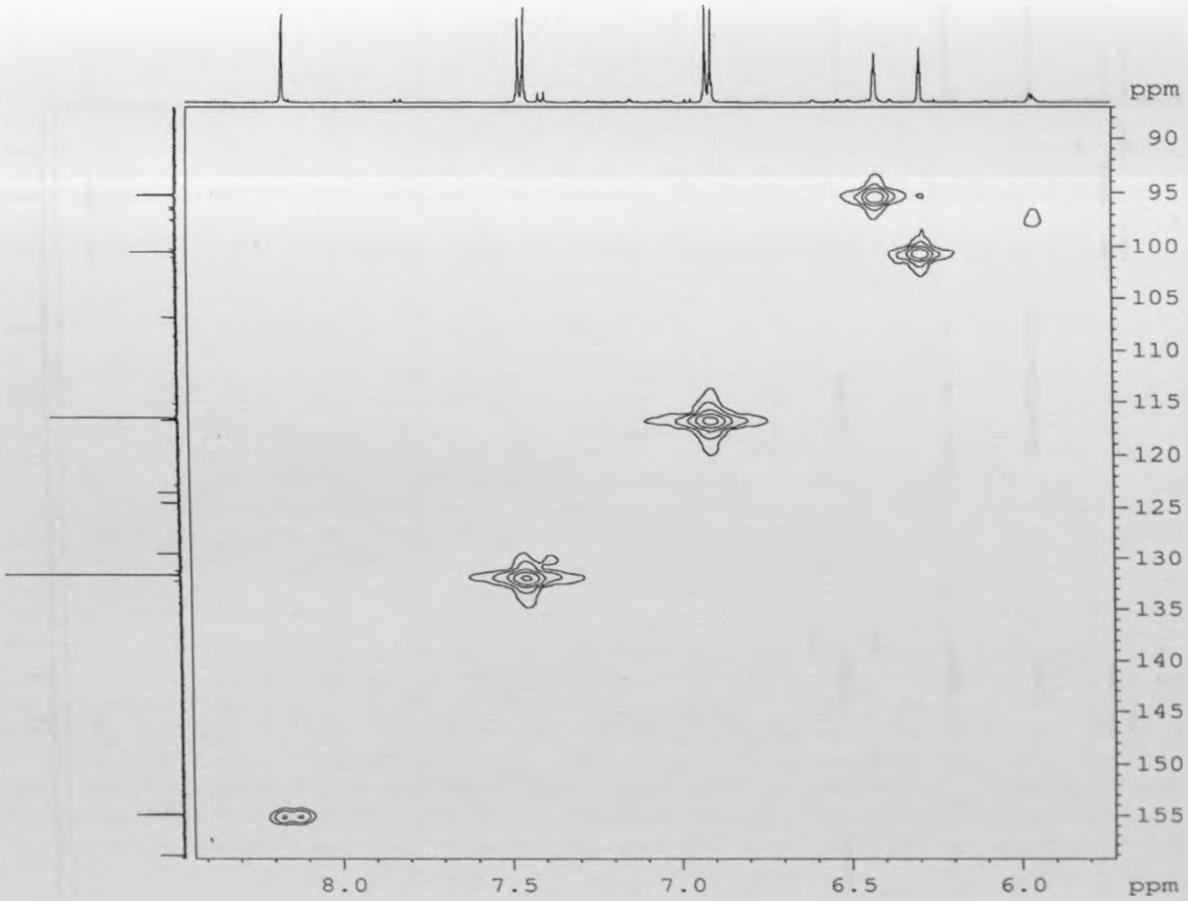
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 3



^{13}C NMR SPECTRUM FOR COMPOUND 3 (150 MHz, ACETONE-d_6)



HMQC SPECTRUM FOR COMPOUND 3



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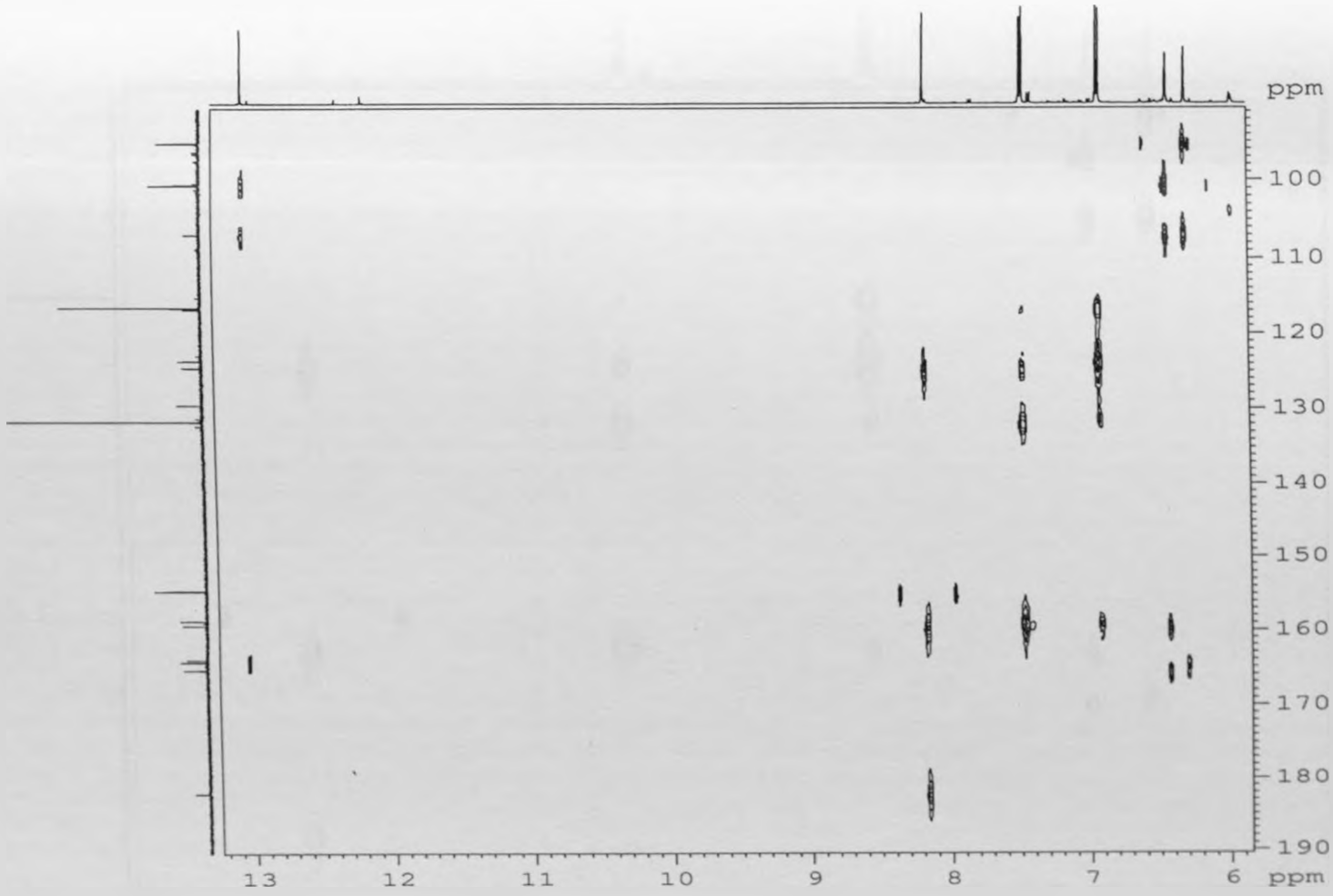
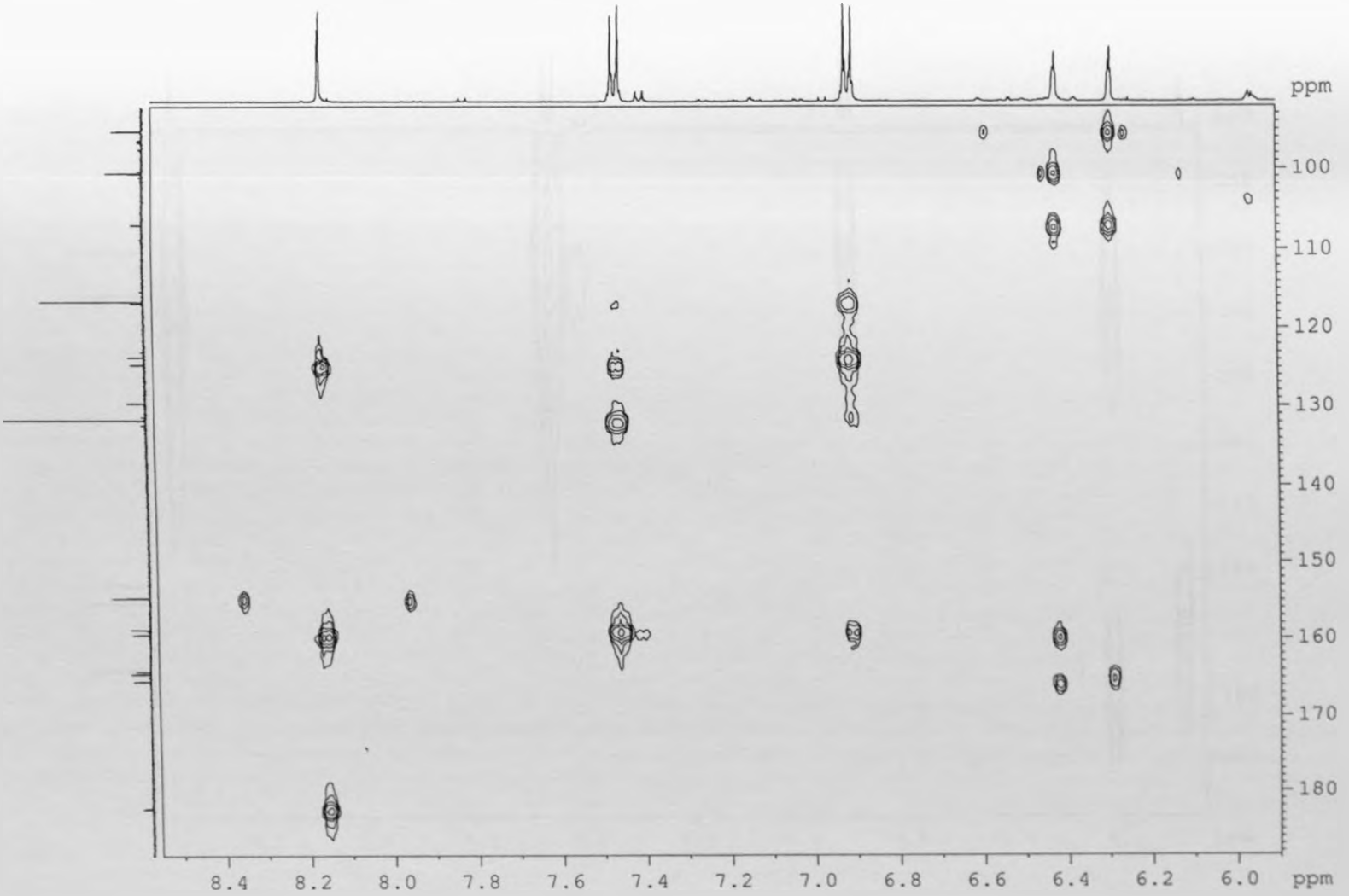


HMBC SPECTRUM EXPANSION FOR COMPOUND 3



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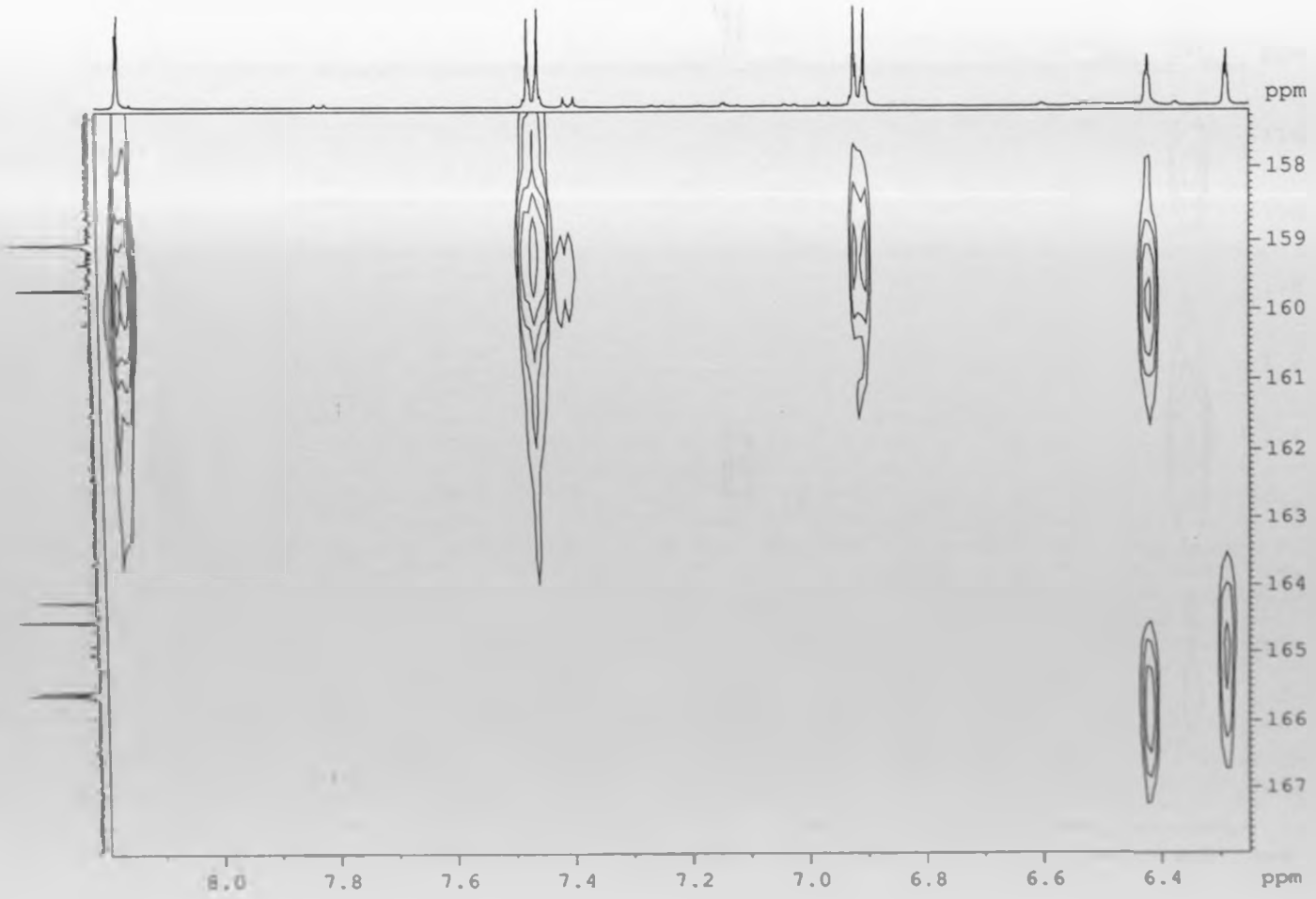

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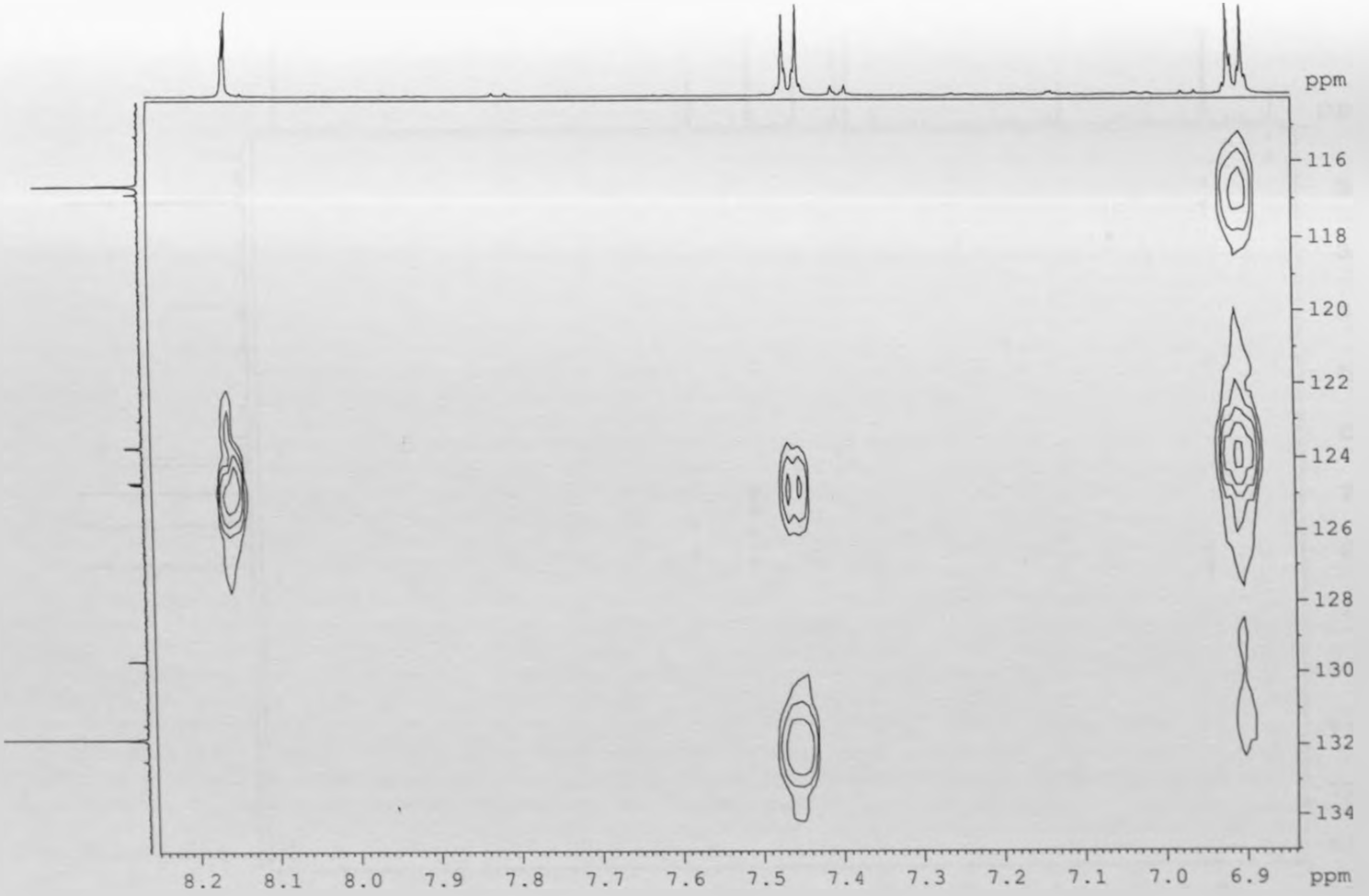

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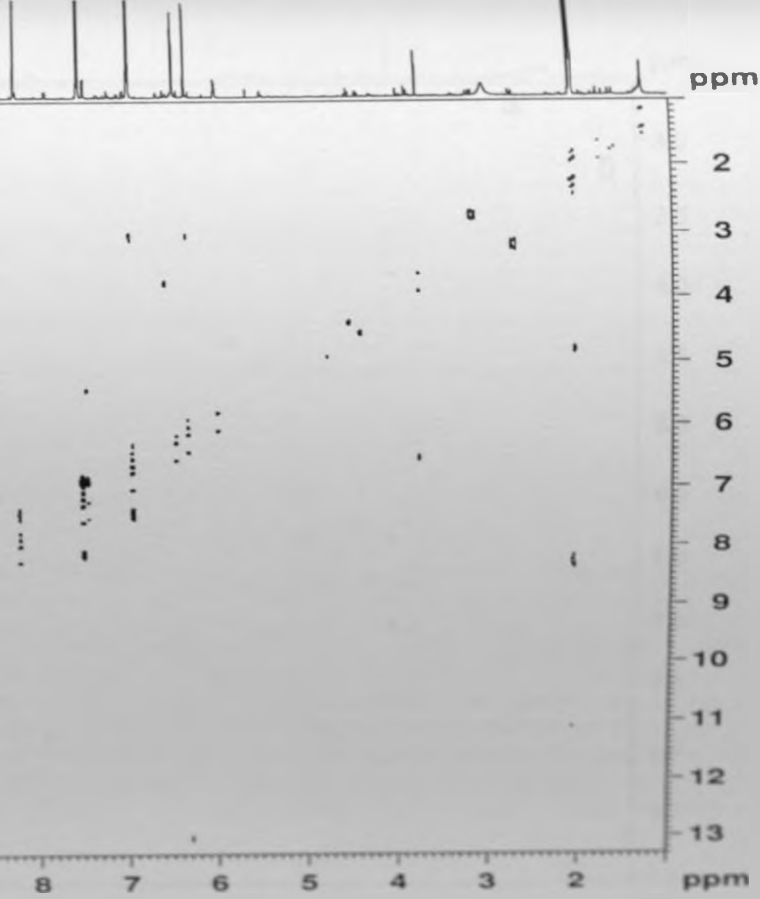
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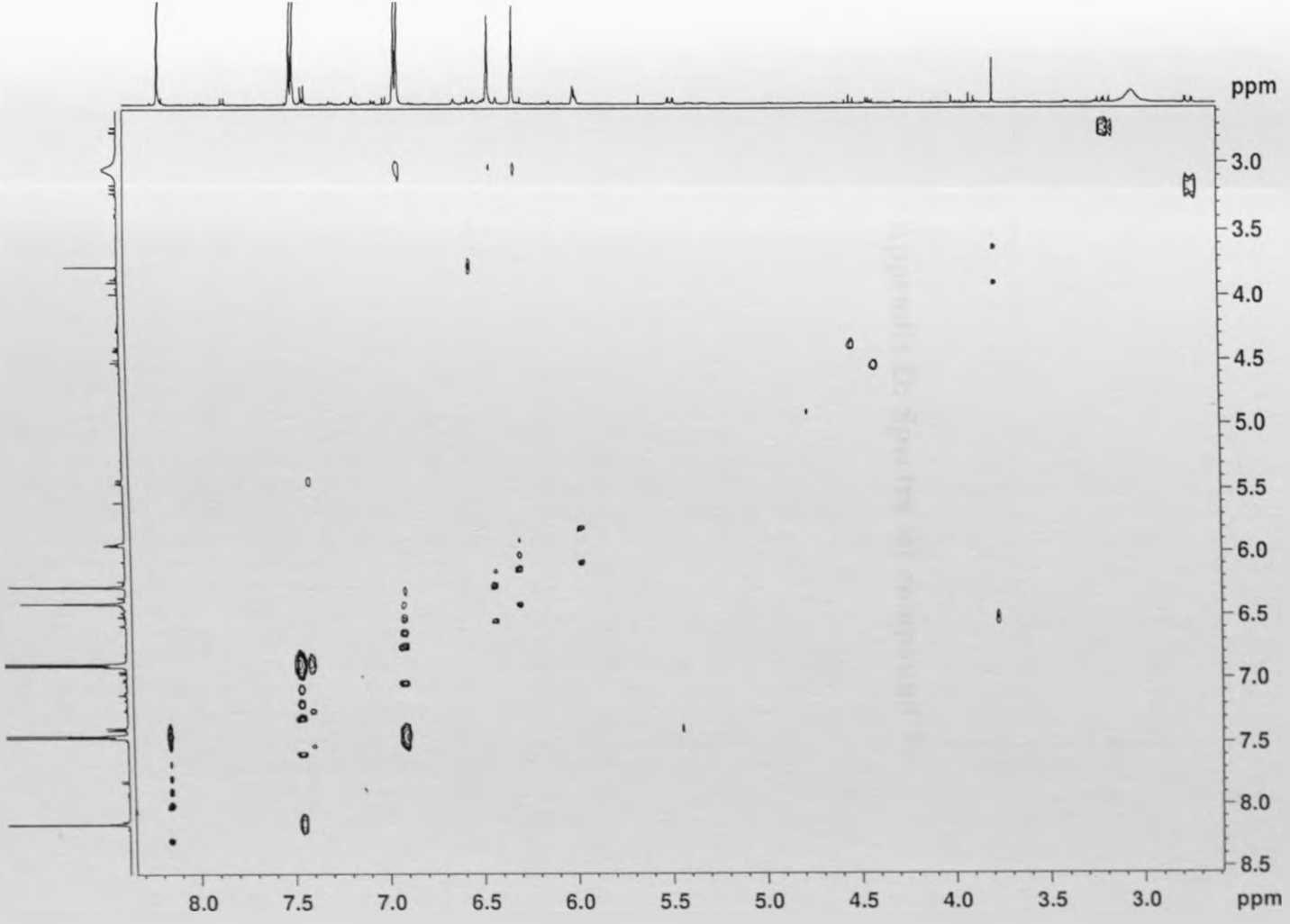
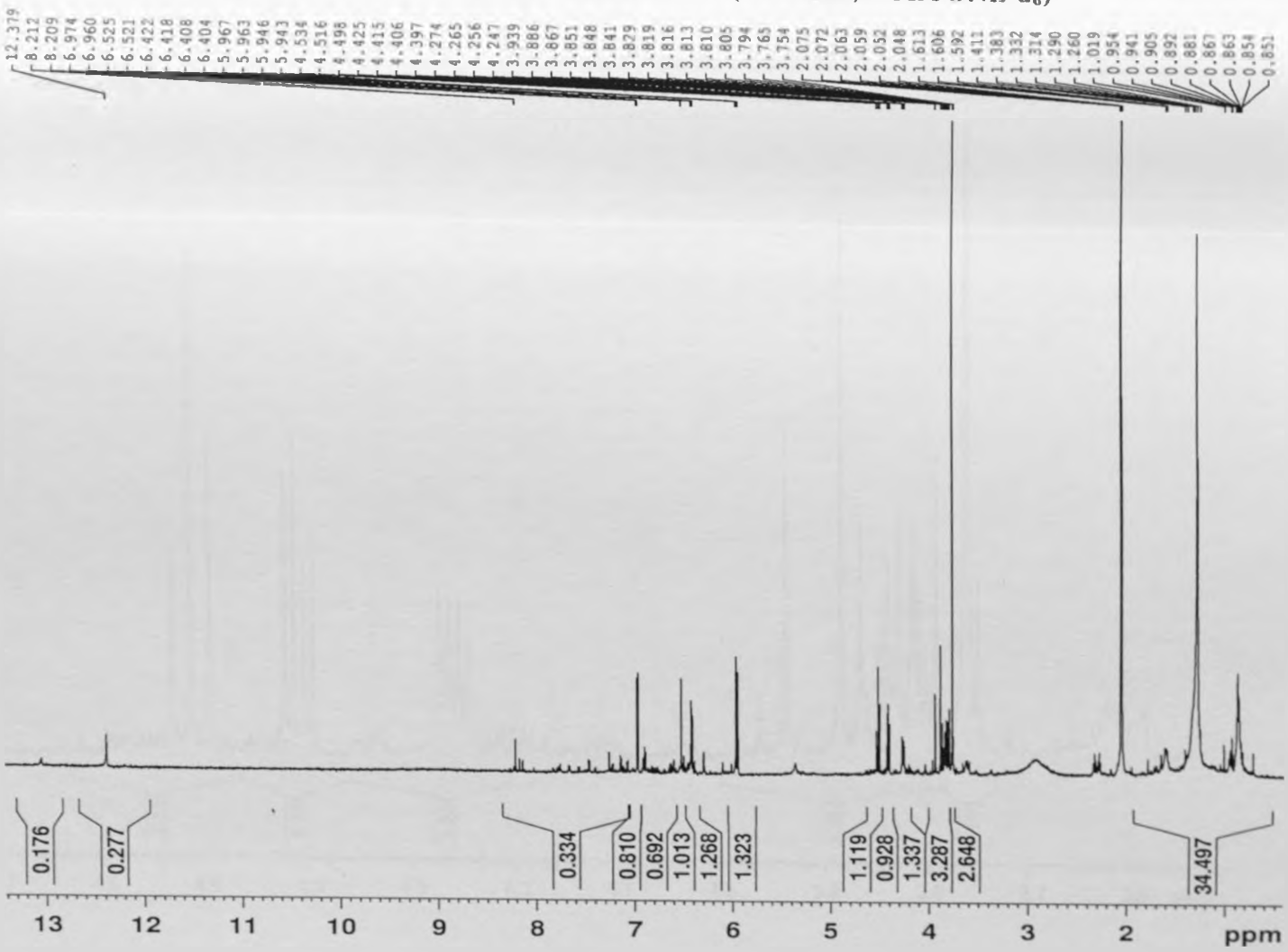
NOESY SPECTRUM EXPANSION FOR COMPOUND 3

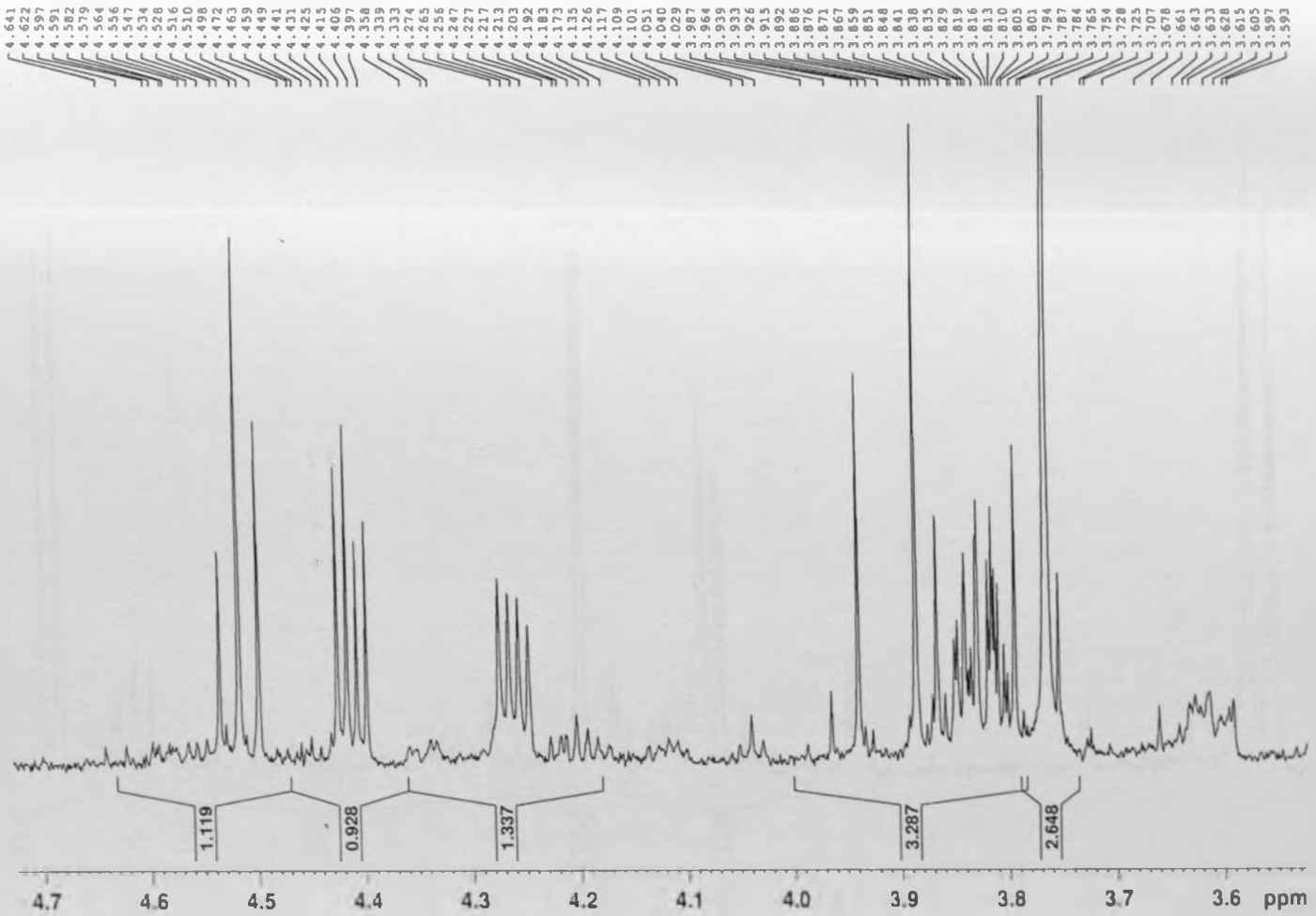


Appendix D: Spectra for compound 4

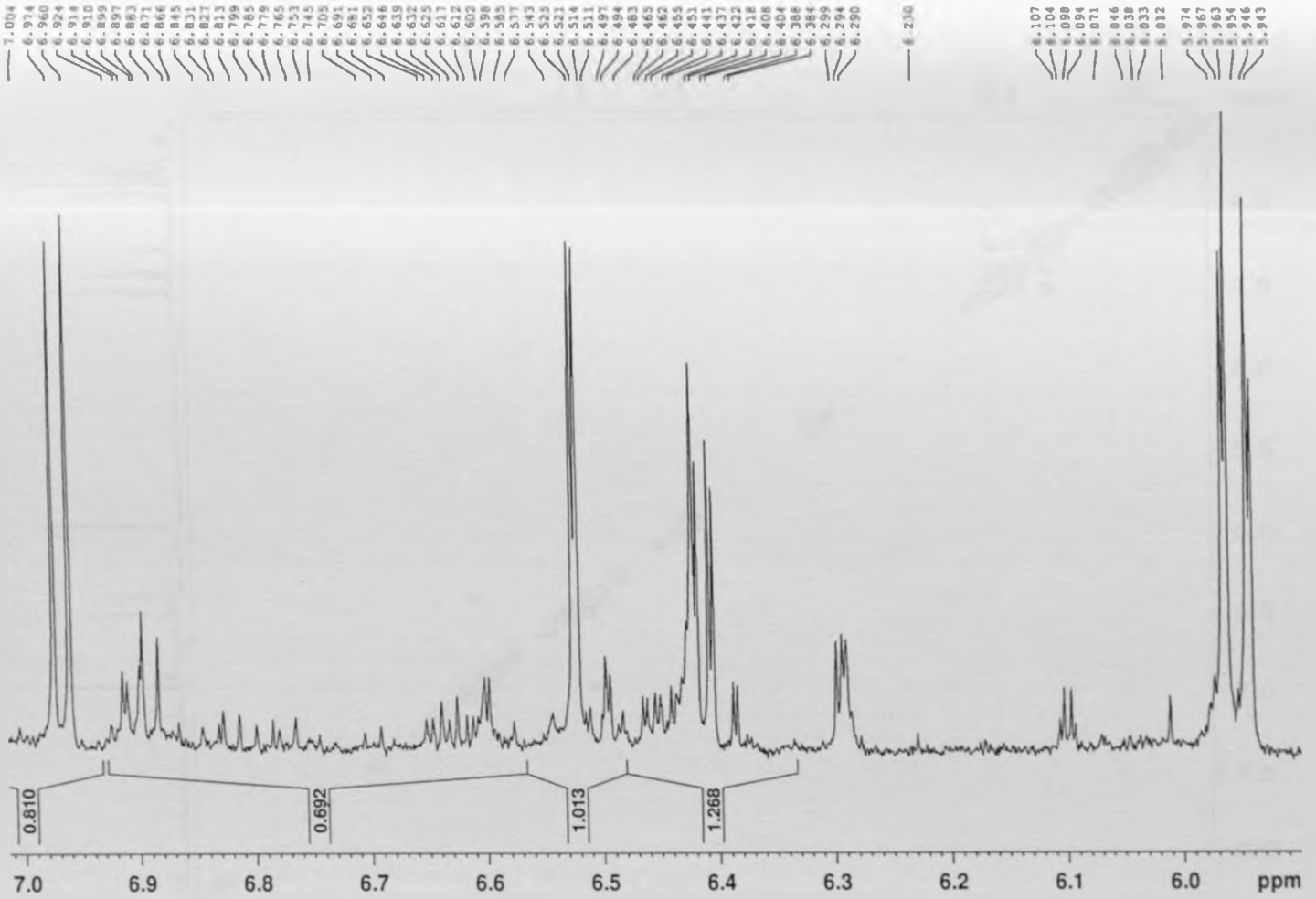
^{1}H NMR SPECTRUM FOR COMPOUND 4 (600 MHz, ACETONE-d_6)



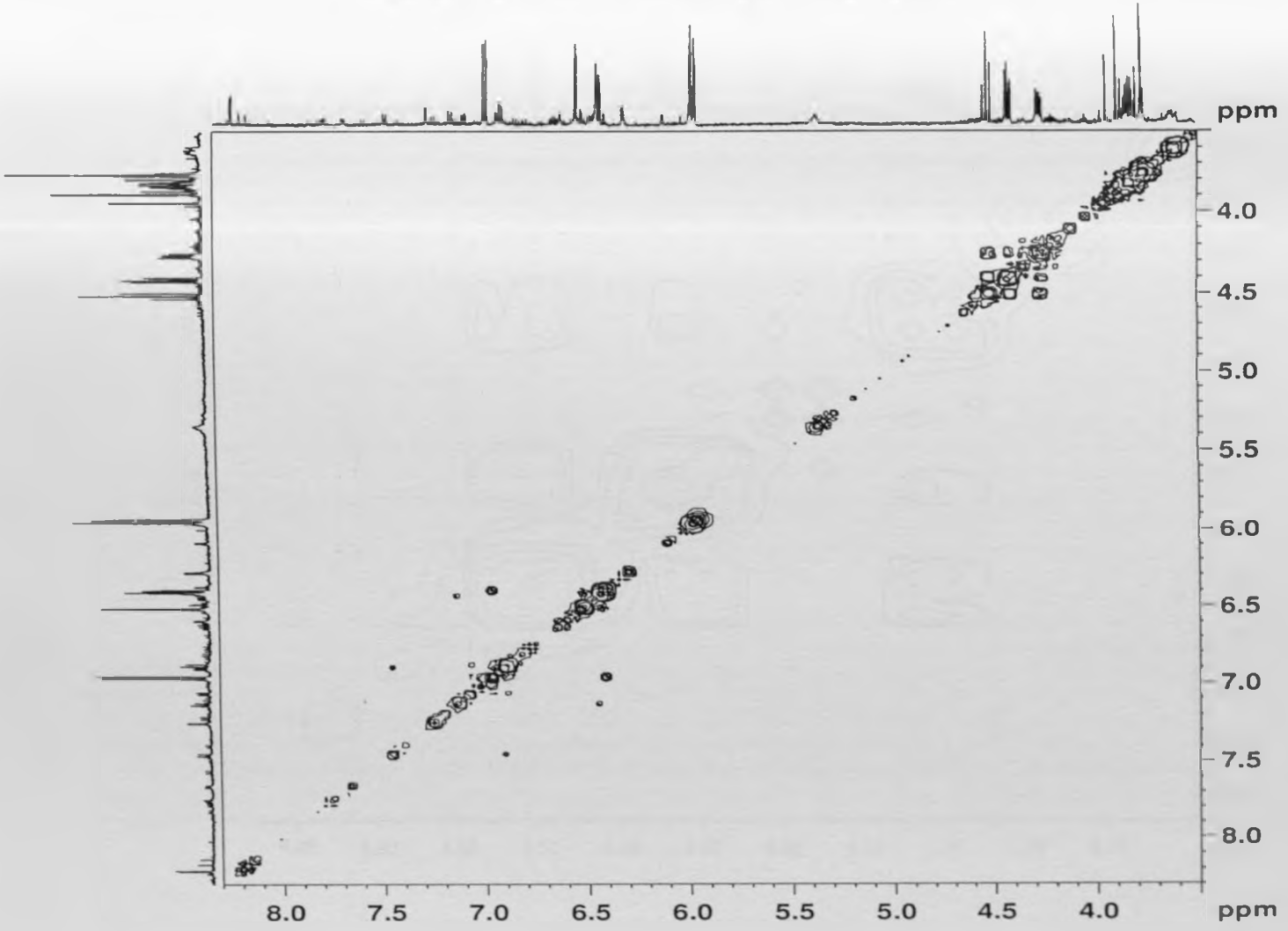
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 4



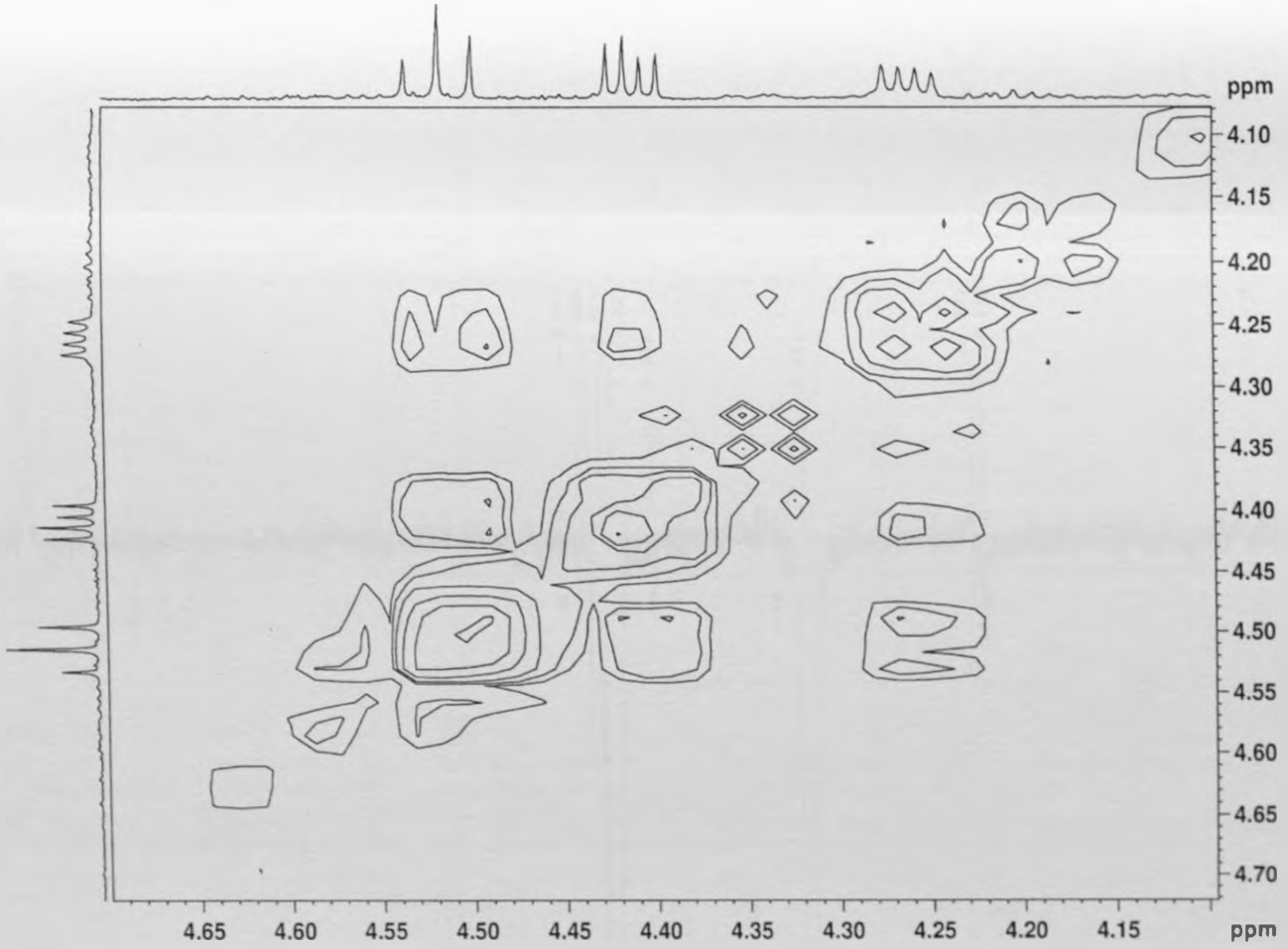
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 4



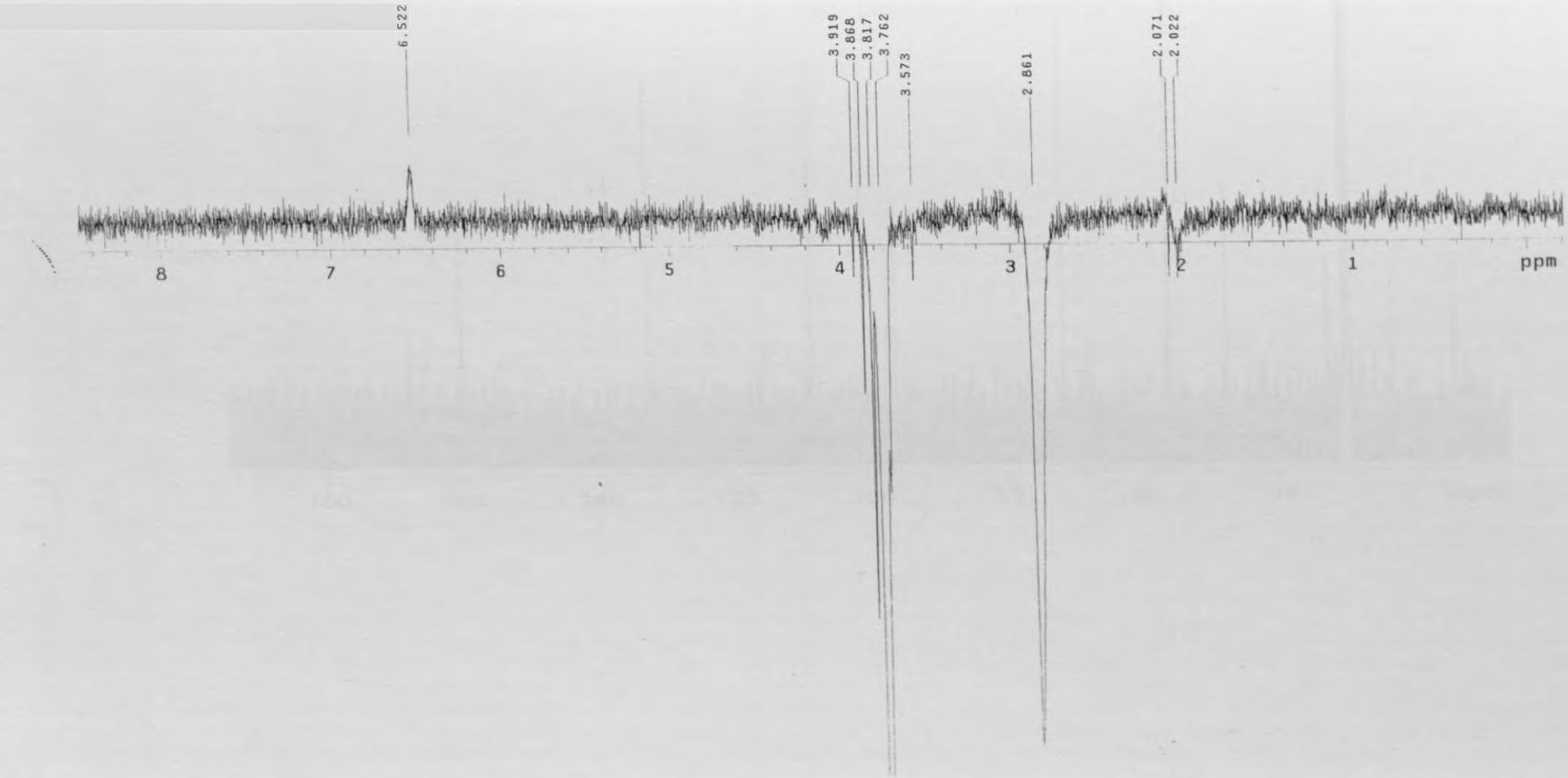
^{1}H-^{1}H COSY SPECTRUM FOR COMPOUND 4



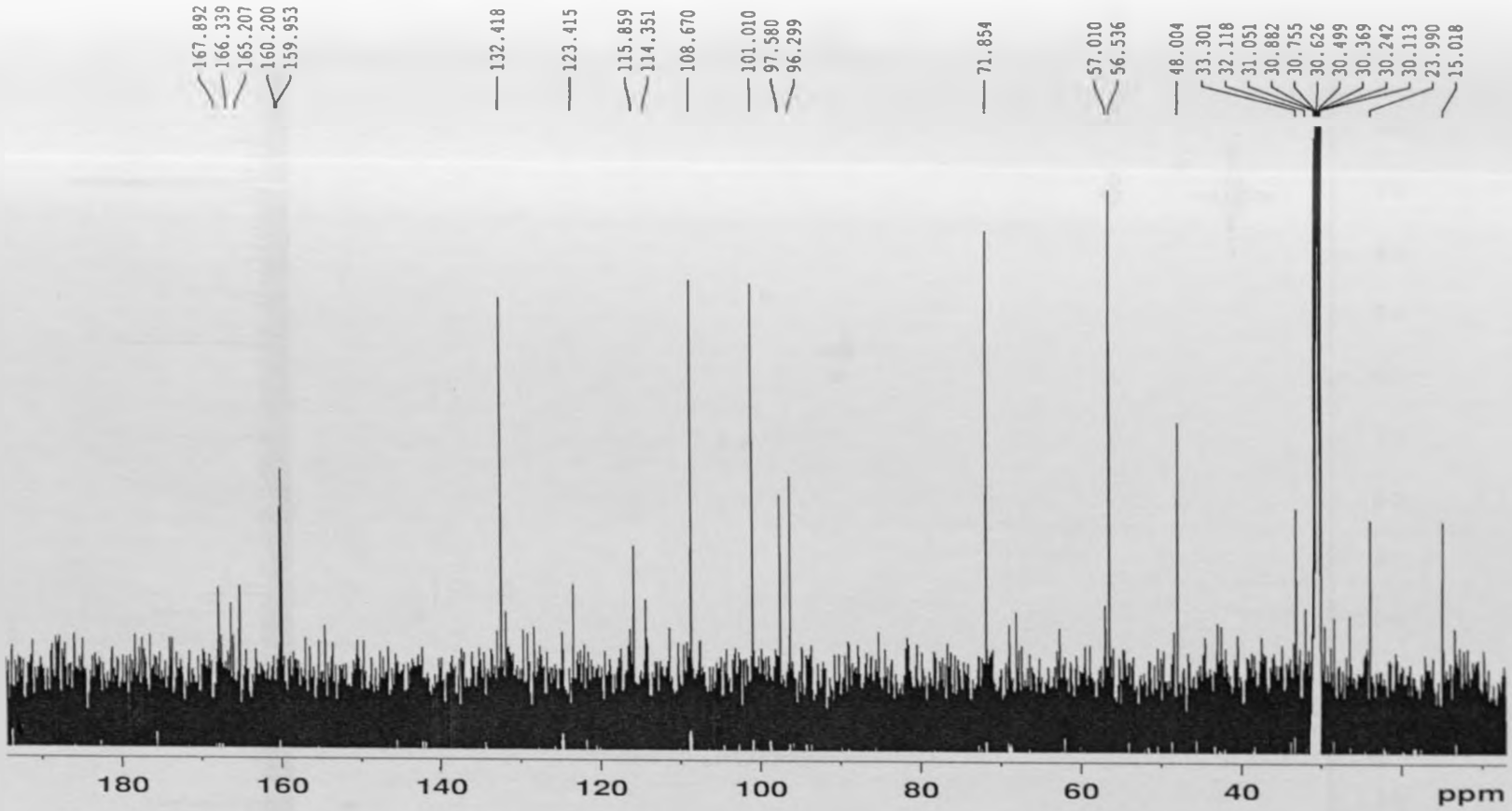
^1H-^1H COSY SPECTRUM EXPANSION FOR COMPOUND 4



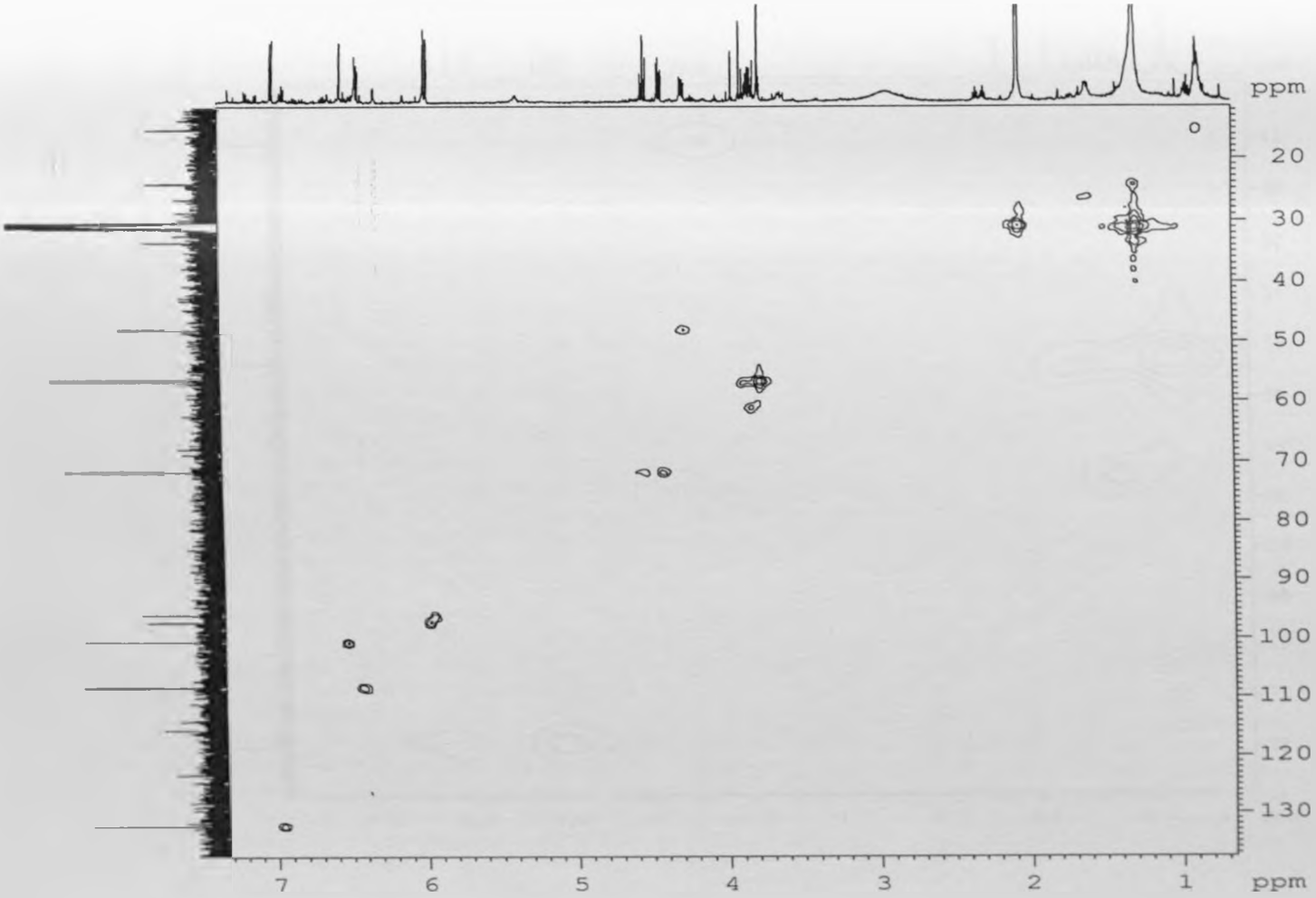
NOE DIFF SPECTRUM FOR COMPOUND 4



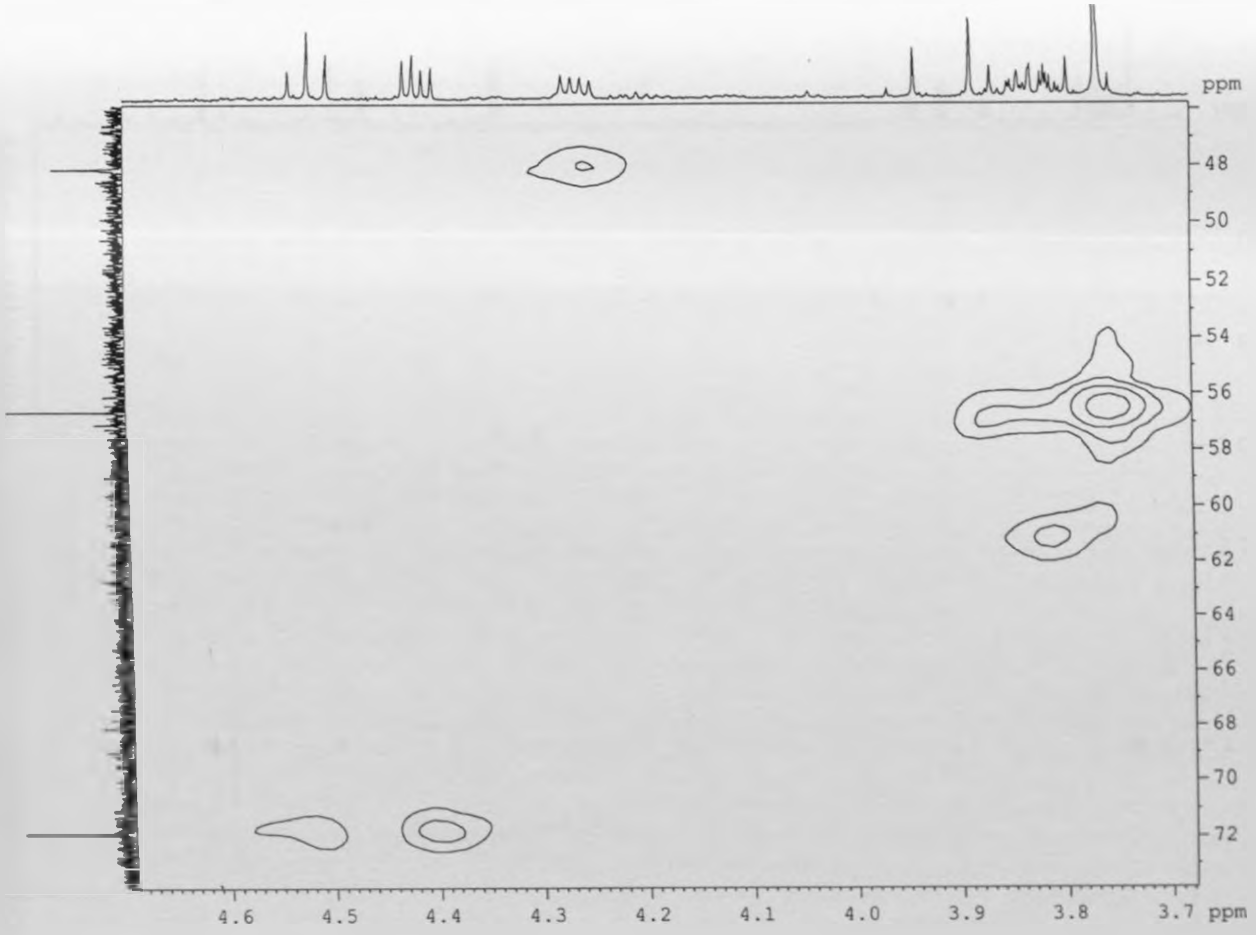
^{13}C NMR SPECTRUM FOR COMPOUND 4 (150 MHz, ACETONE-d_6)



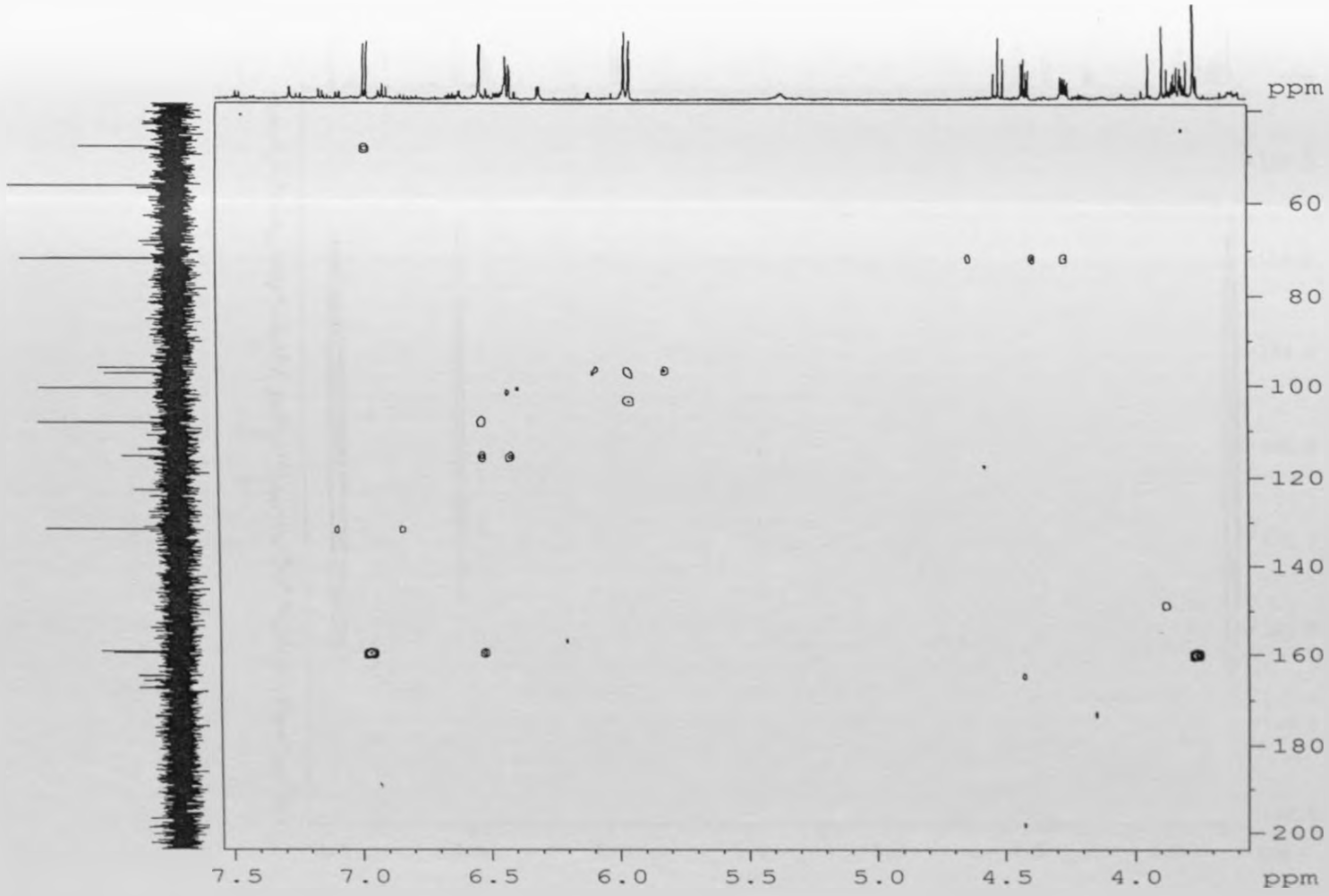
HMQC SPECTRUM FOR COMPOUND 4



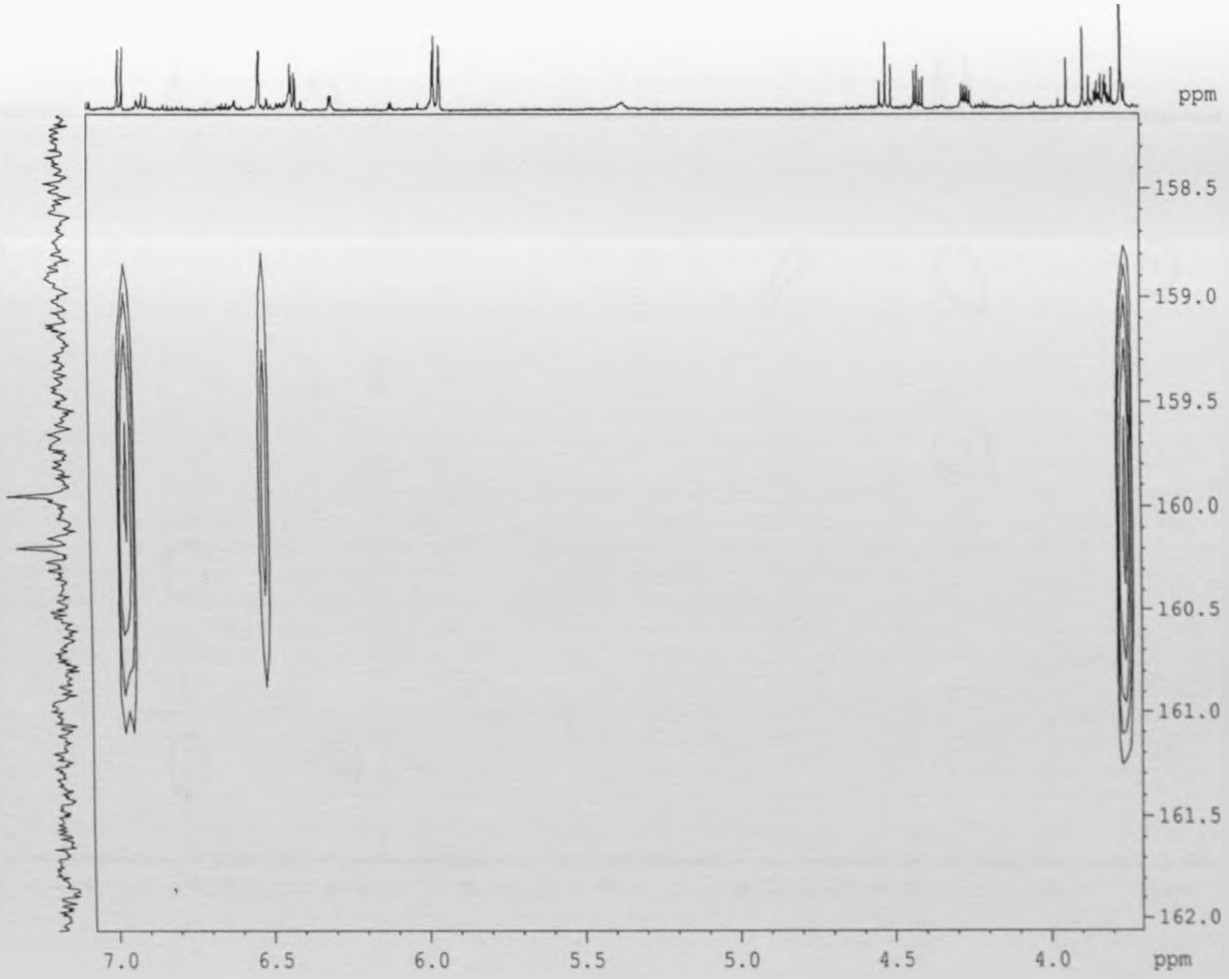
HMQC SPECTRUM EXPANSION FOR COMPOUND 4



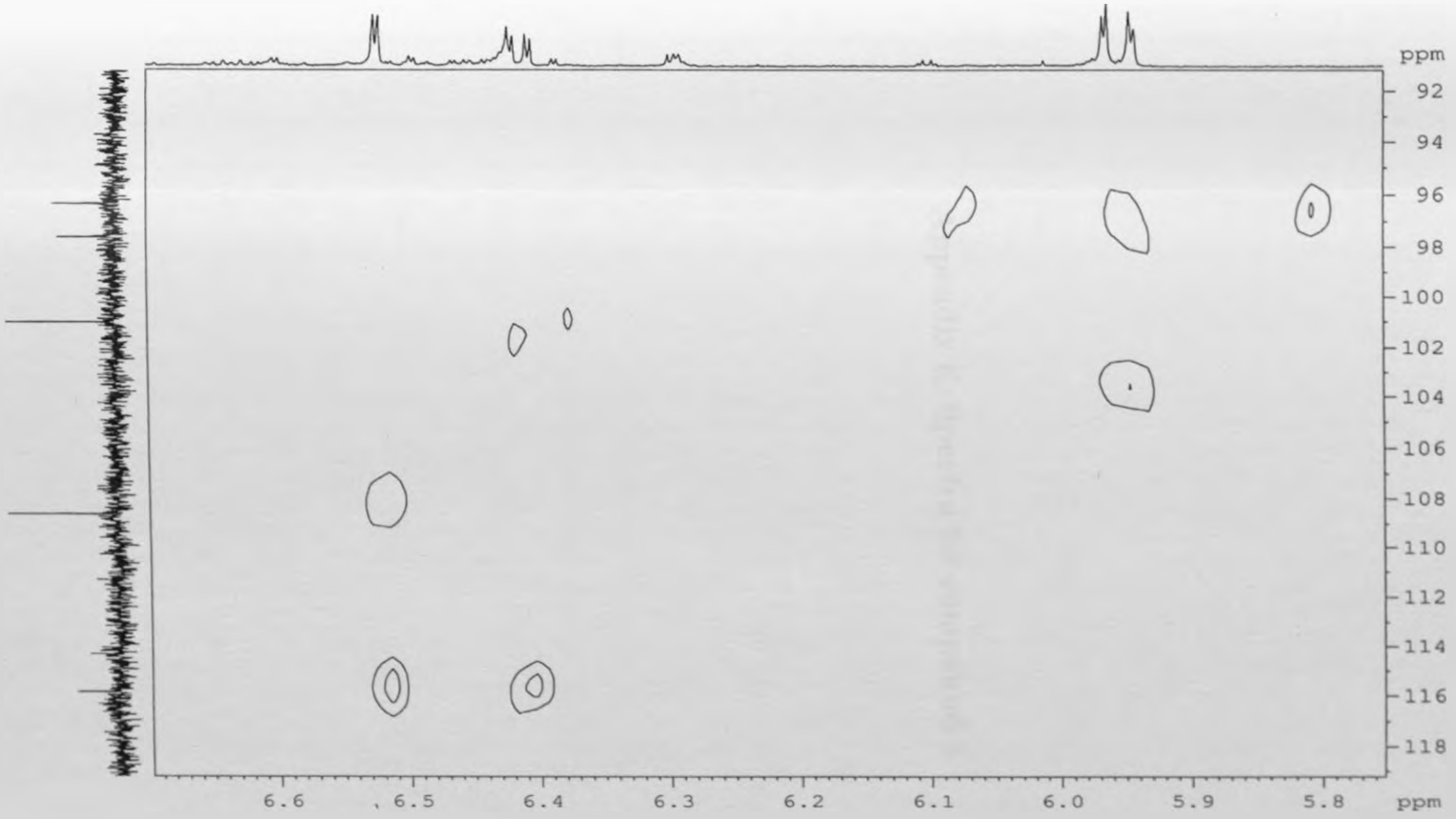
HMBC SPECTRUM FOR COMPOUND 4



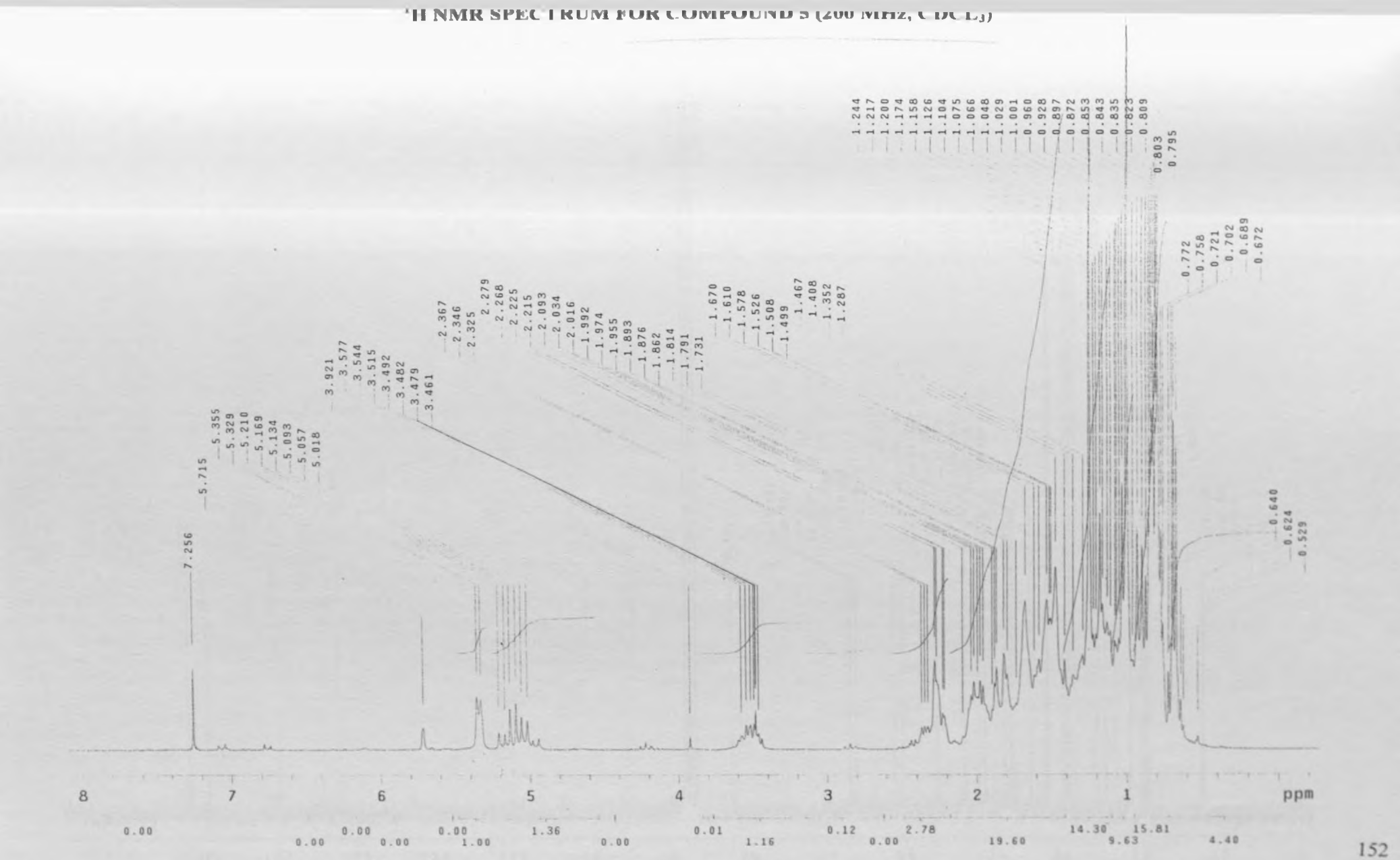
HMBC SPECTRUM EXPANSIONFOR COMPOUND 4



HMBC SPECTRUM EXPANSIONFOR COMPOUND 4

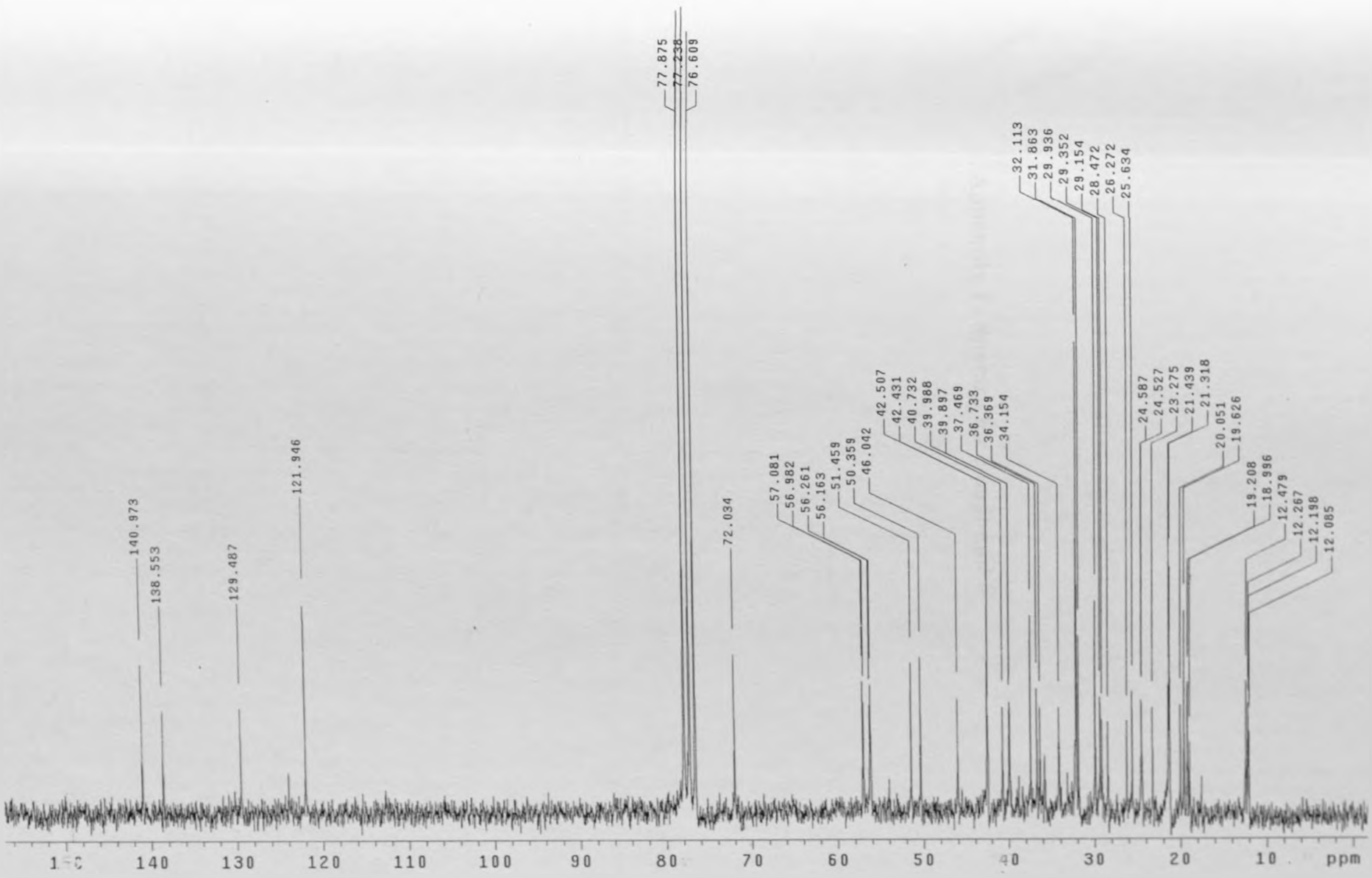


Appendix E: Spectra for compound 5



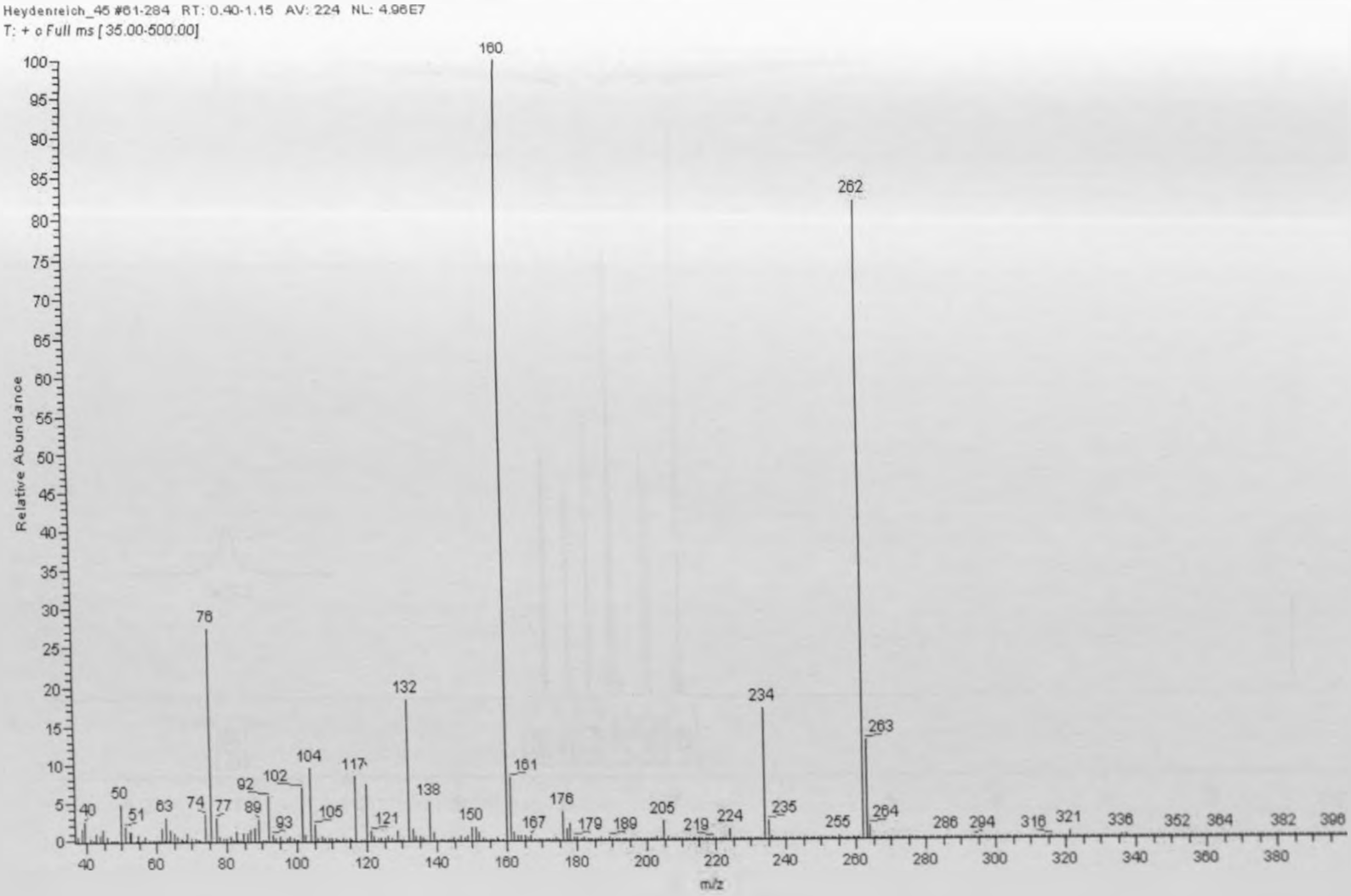
'H NMR SPECTRUM FOR COMPOUND 5 (200 MHz, CDCl3)

^{13}C NMR SPECTRUM FOR COMPOUND 5 (50 MHz, $CDCL_3$)

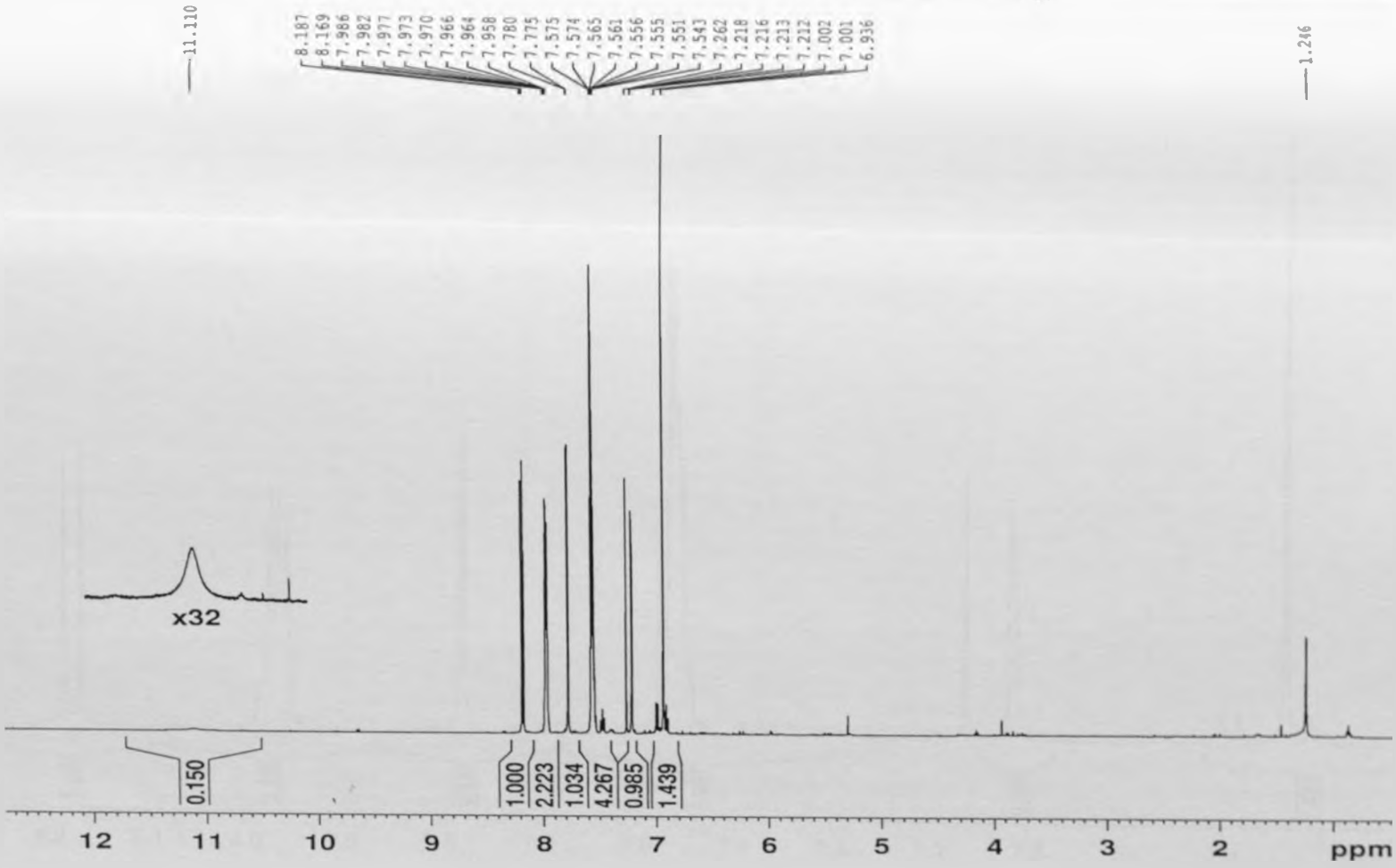


Appendix F: Spectra for compound 6

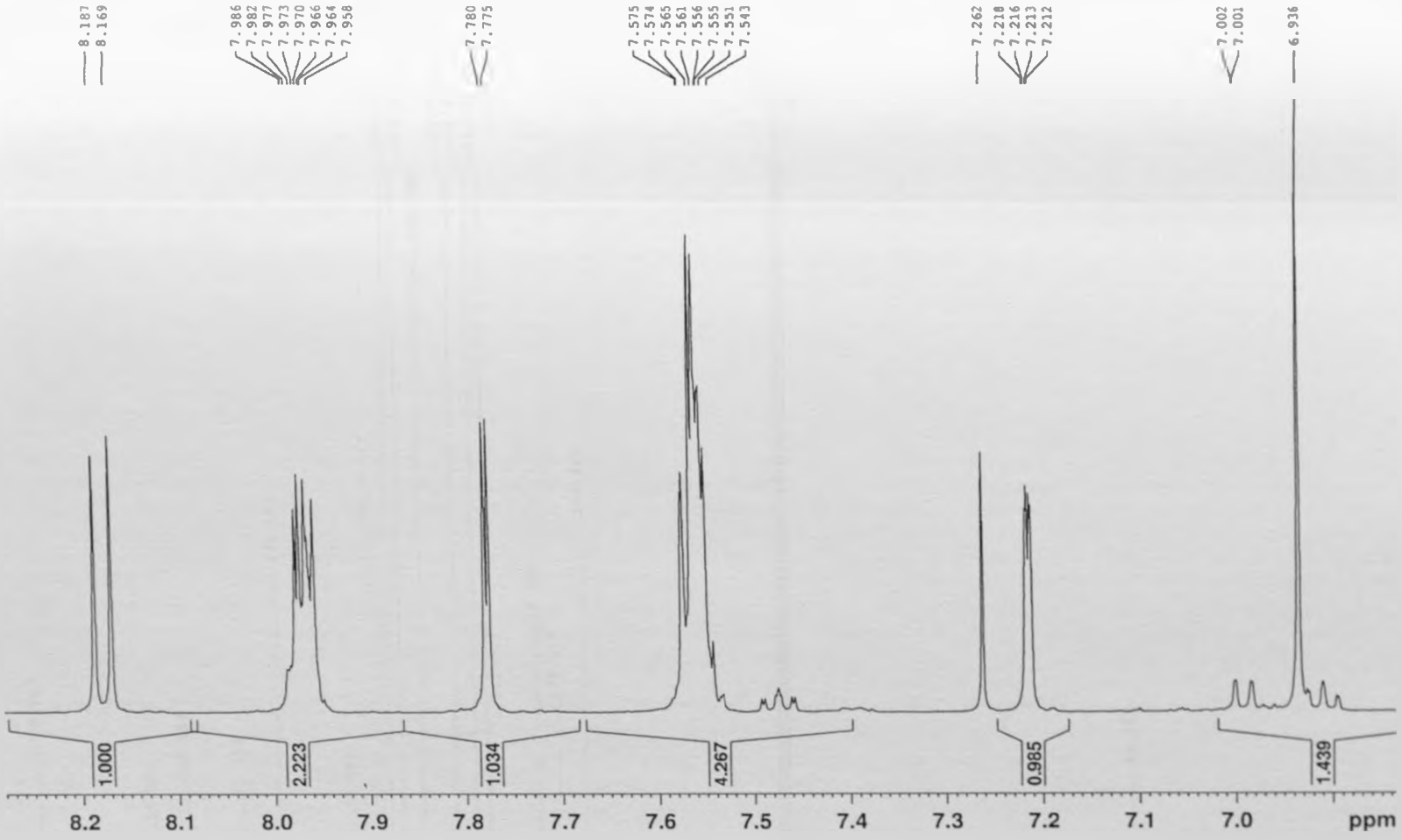
EI-MS SPECTRUM FOR COMPOUND 6



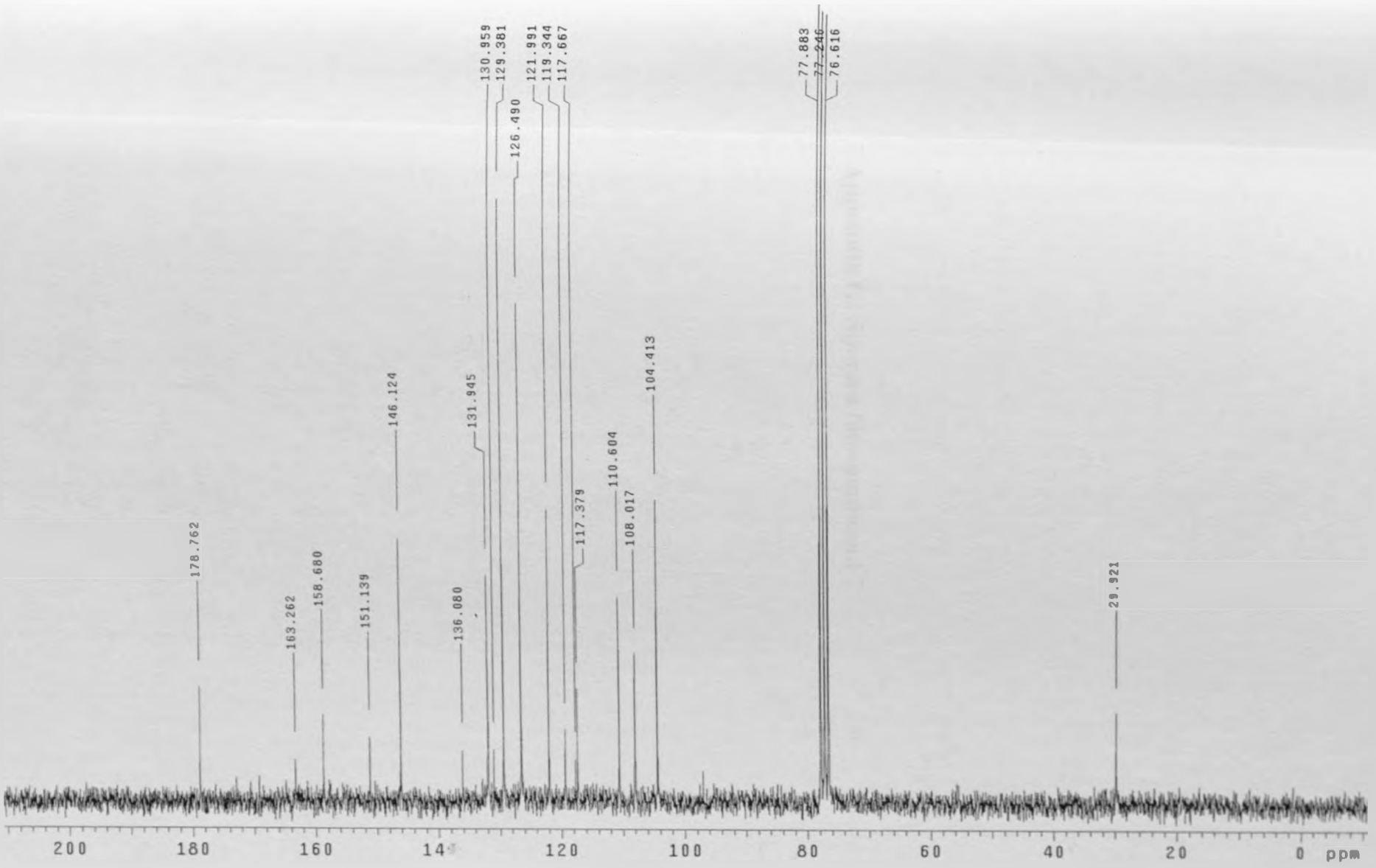
^{1}H NMR SPECTRUM FOR COMPOUND 6 (500 MHz, $CDCL_3$)



^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 6

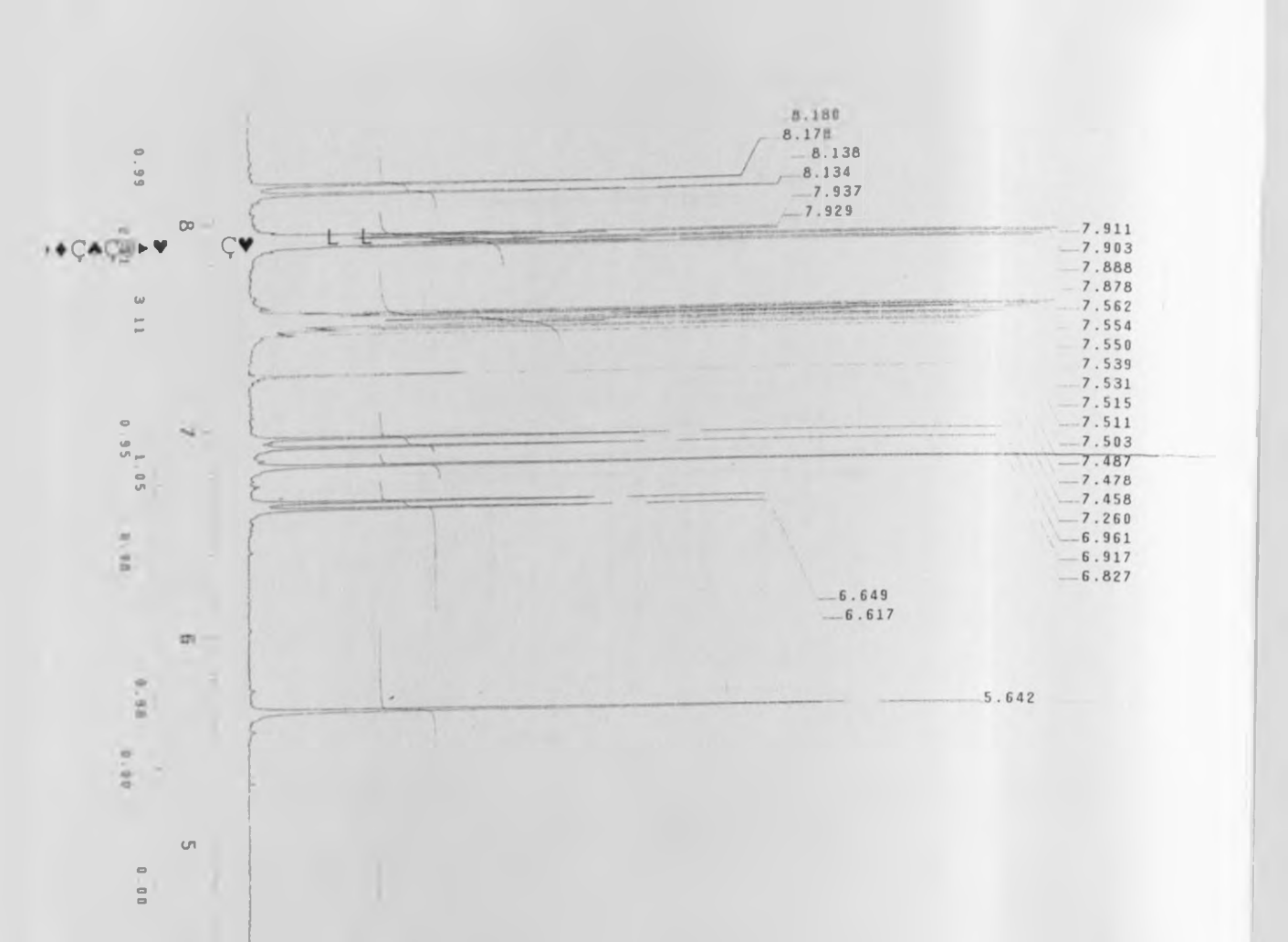


^{13}C NMR SPECTRUM FOR COMPOUND 6 (50 MHz, $CDCL_3$)

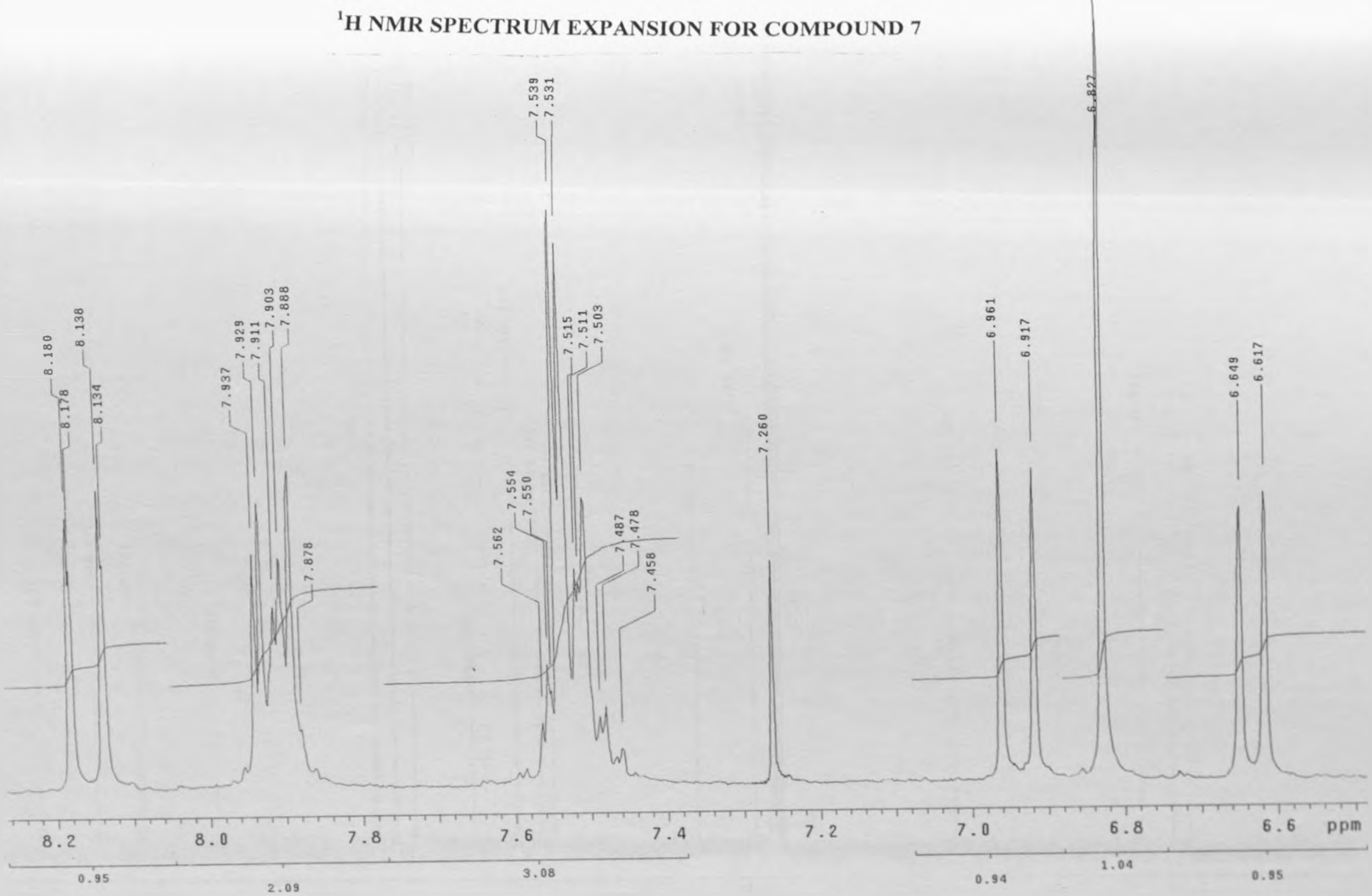


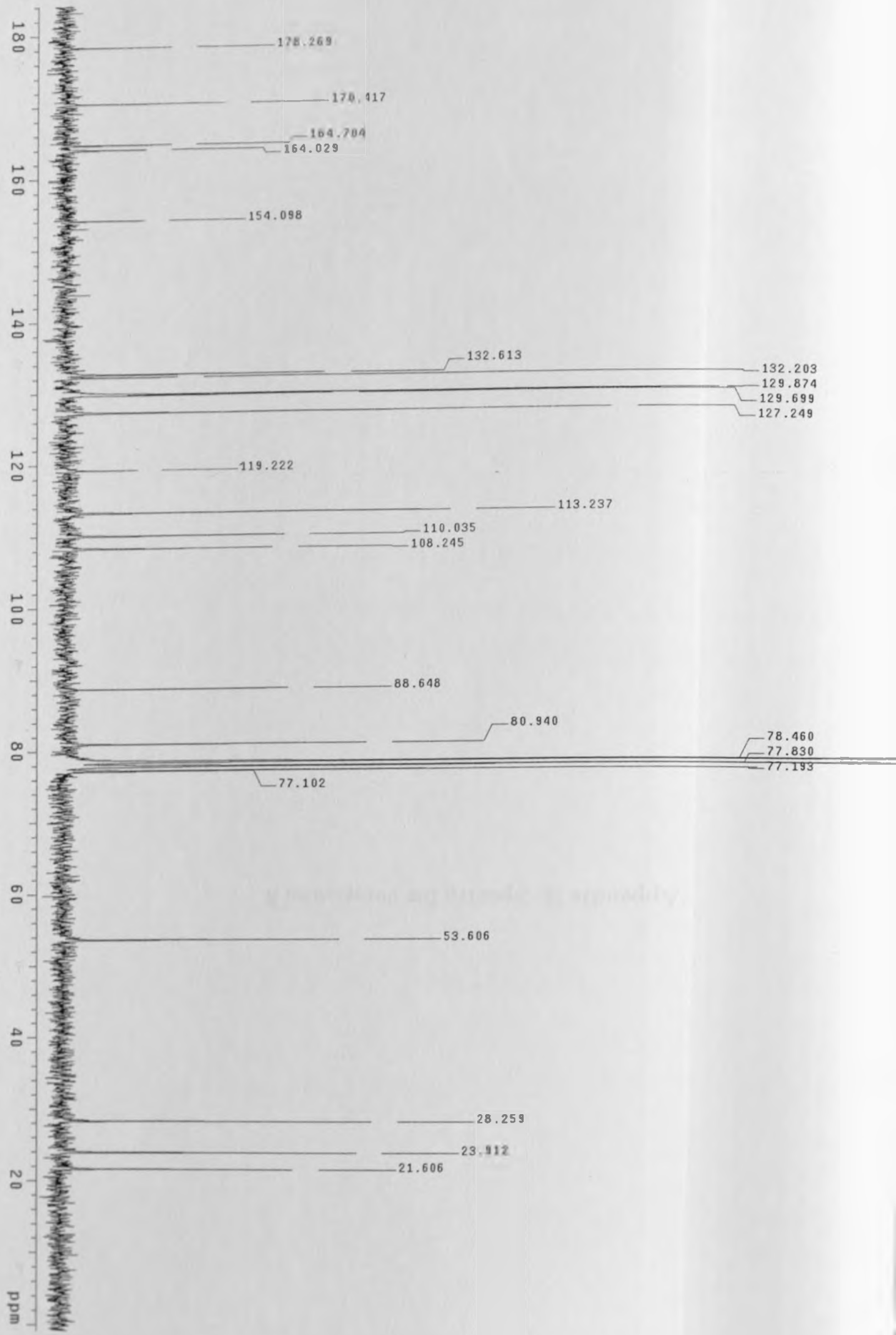
Appendix G: Spectra for compound 7

4.312
4.281
2.339
2.253
2.251
2.219
2.209
2.207
2.236
2.222
1.322
1.094
4
3
2
1
ppm


8.180
8.178
8.138
8.134
7.937
7.929
7.911
7.903
7.888
7.878
7.562
7.554
7.550
7.539
7.531
7.515
7.511
7.503
7.487
7.478
7.458
7.260
6.961
6.917
6.827
6.649
6.617
5.642

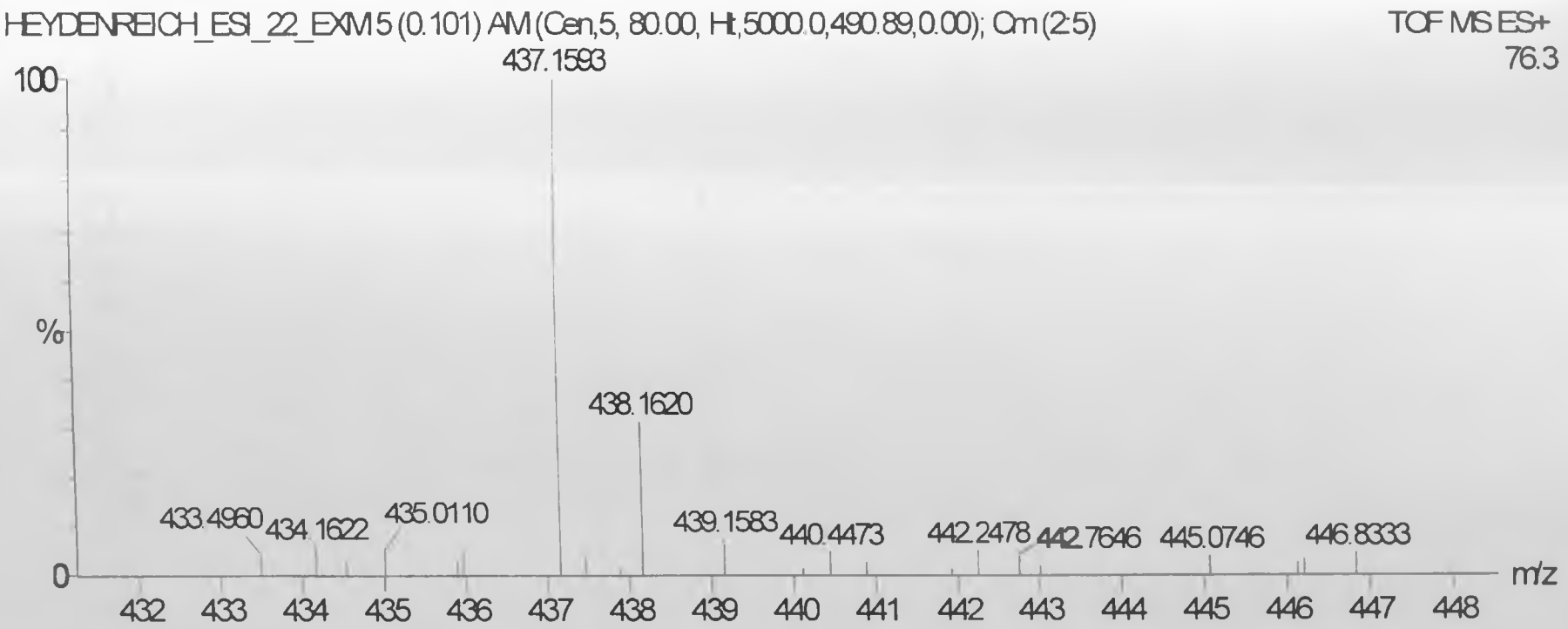
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 7



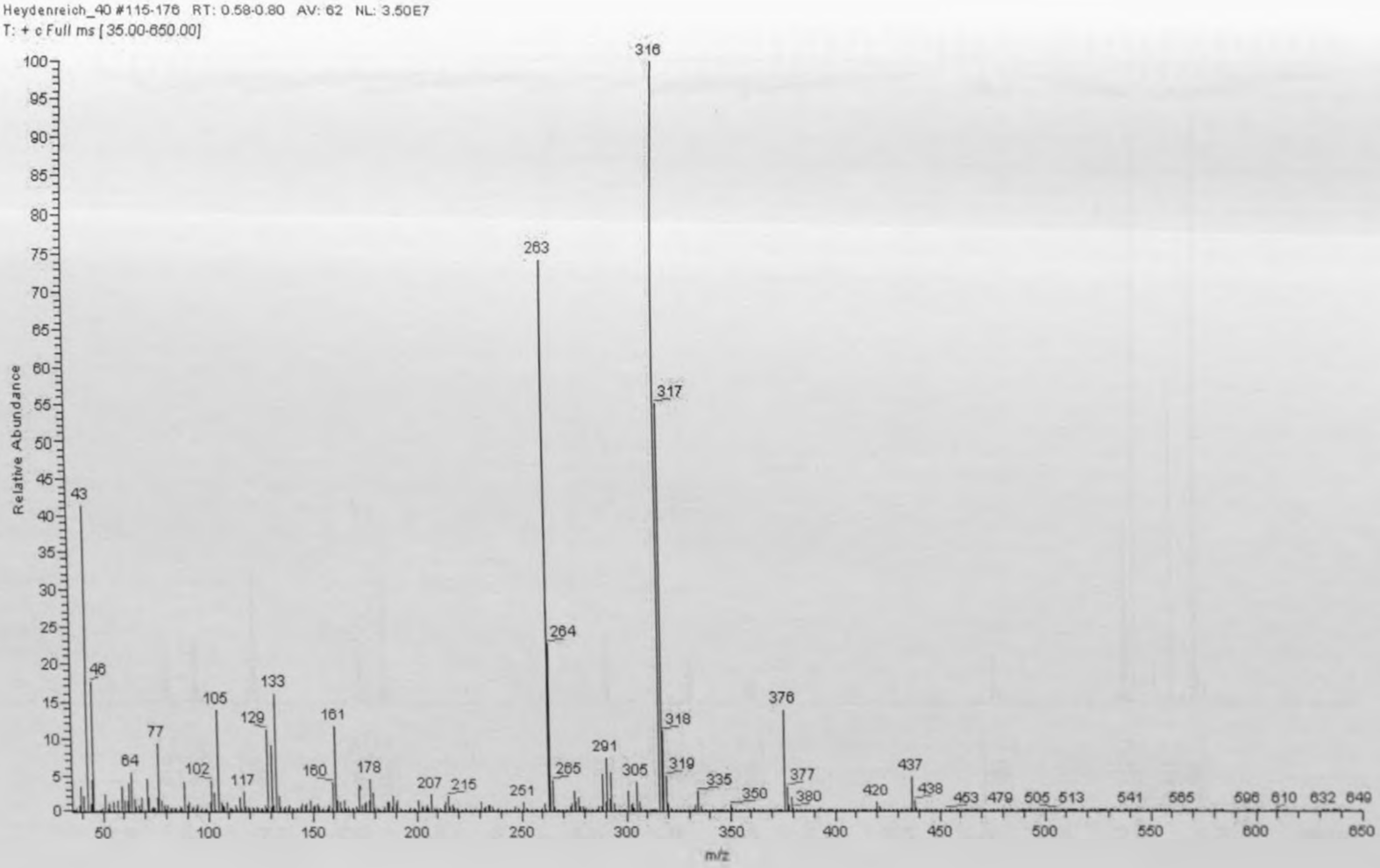

178.269
170.417
164.704
164.029
154.098
132.613
132.203
129.874
129.699
127.249
119.222
113.237
110.035
108.245
88.648
80.940
78.460
77.830
77.193
77.102
53.606
28.259
23.912
21.606
180
160
140
120
100
80
60
40
20
ppm

Appendix H: Spectra for compound **8**

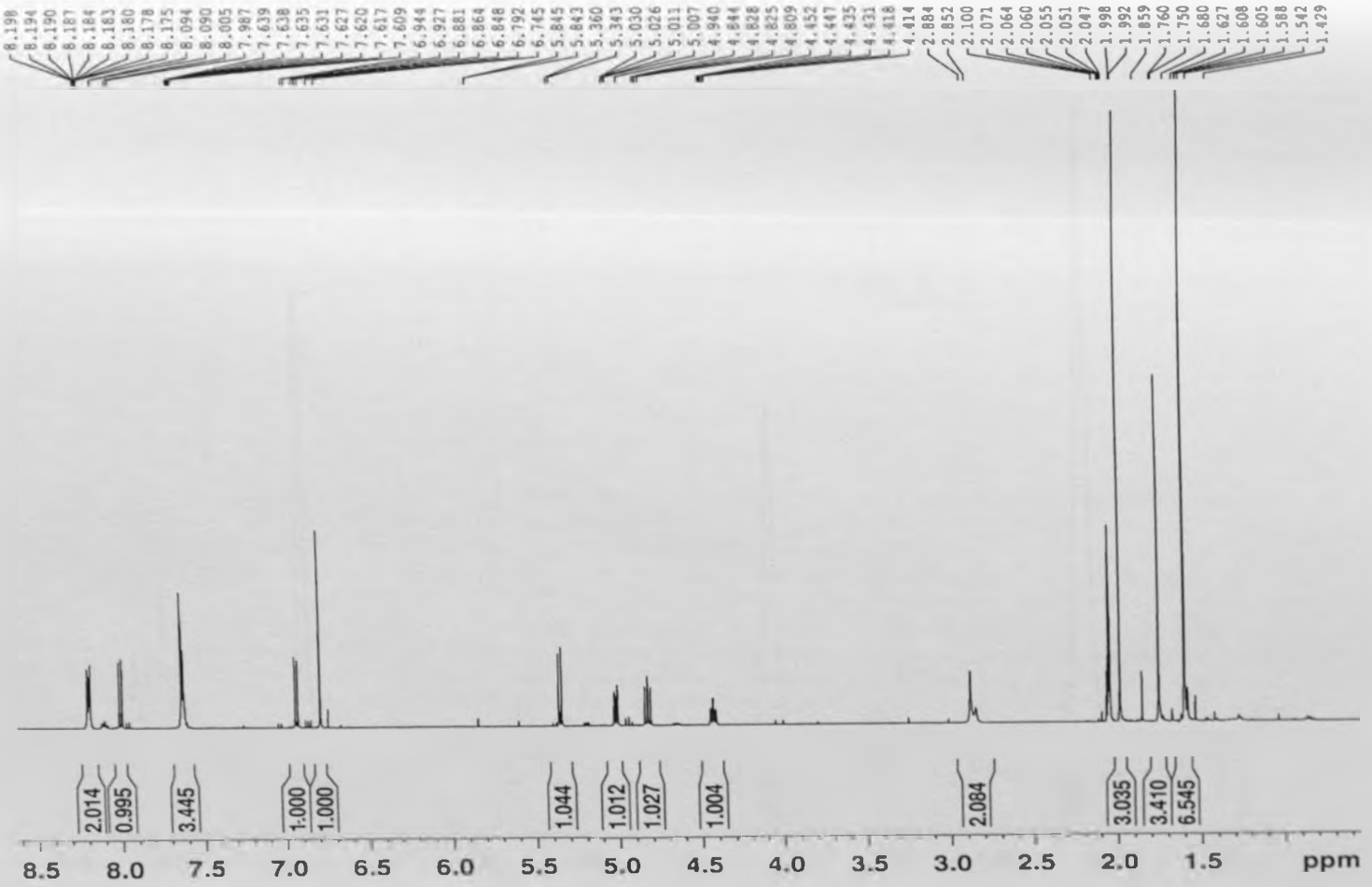
TOF-MASS SPECTRUM FOR COMPOUND 8



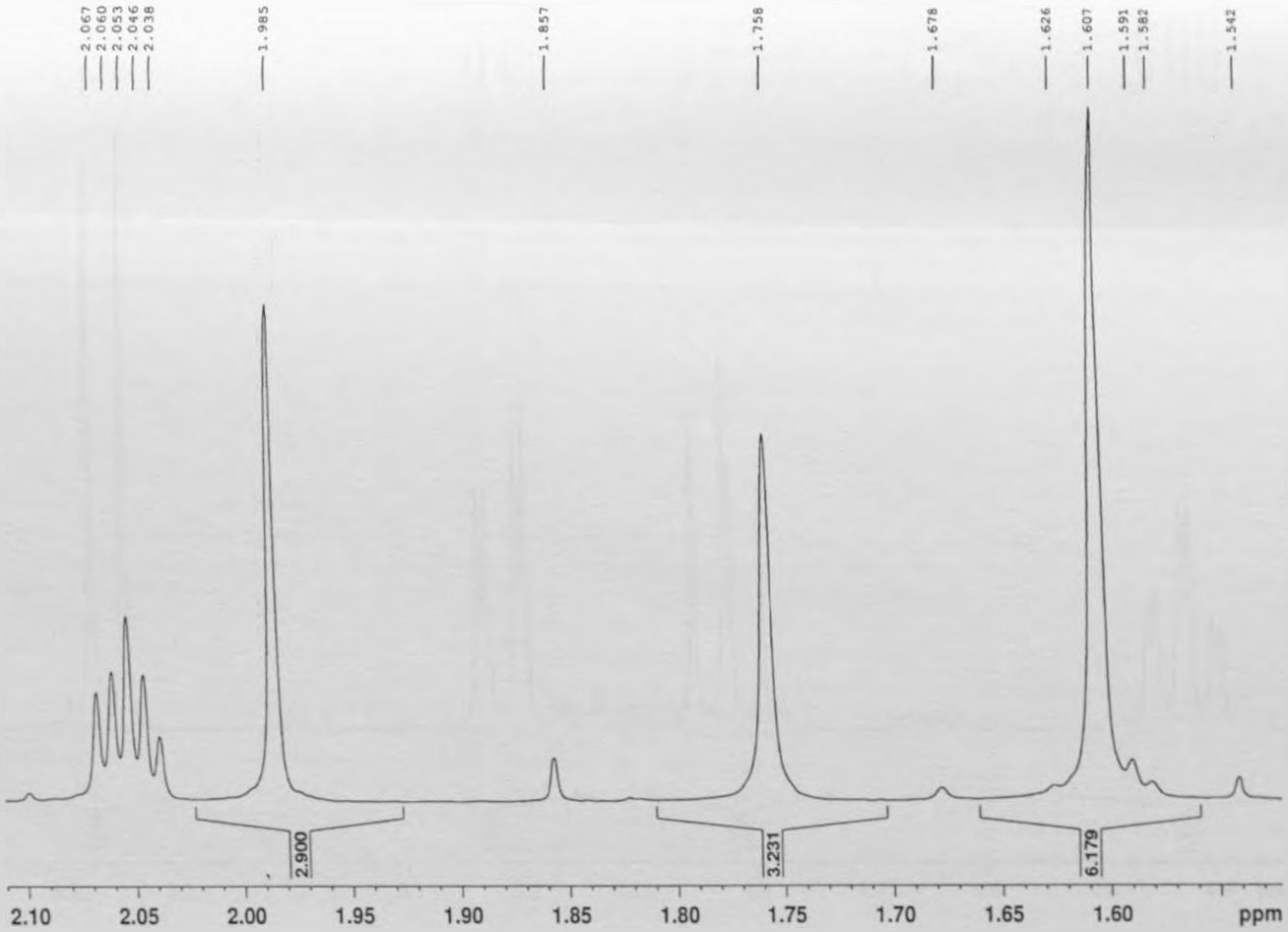
EI-MS SPECTRUM FOR COMPOUND 8



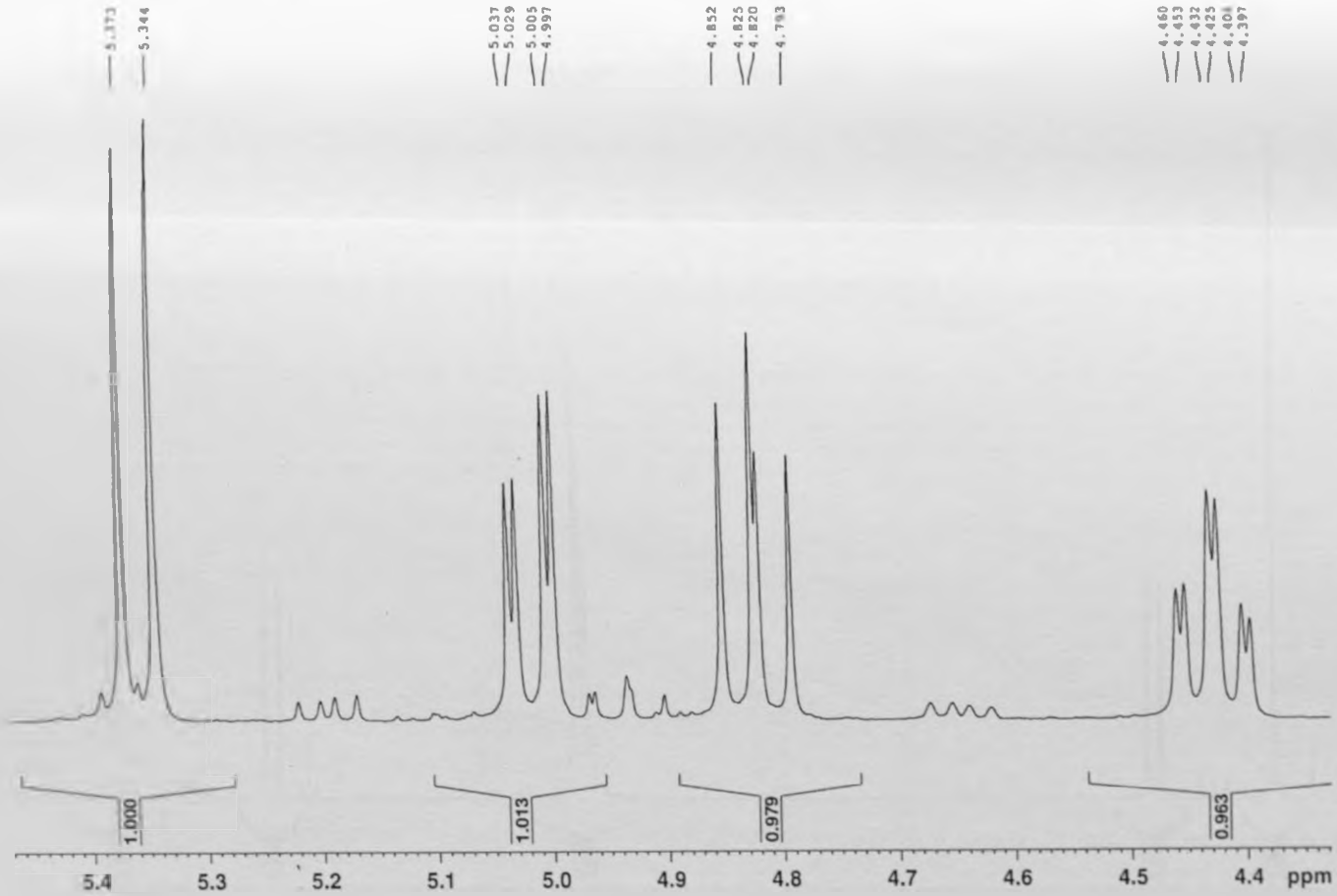
^1H NMR SPECTRUM FOR COMPOUND 8 (500 MHz, ACETONE-d_6)



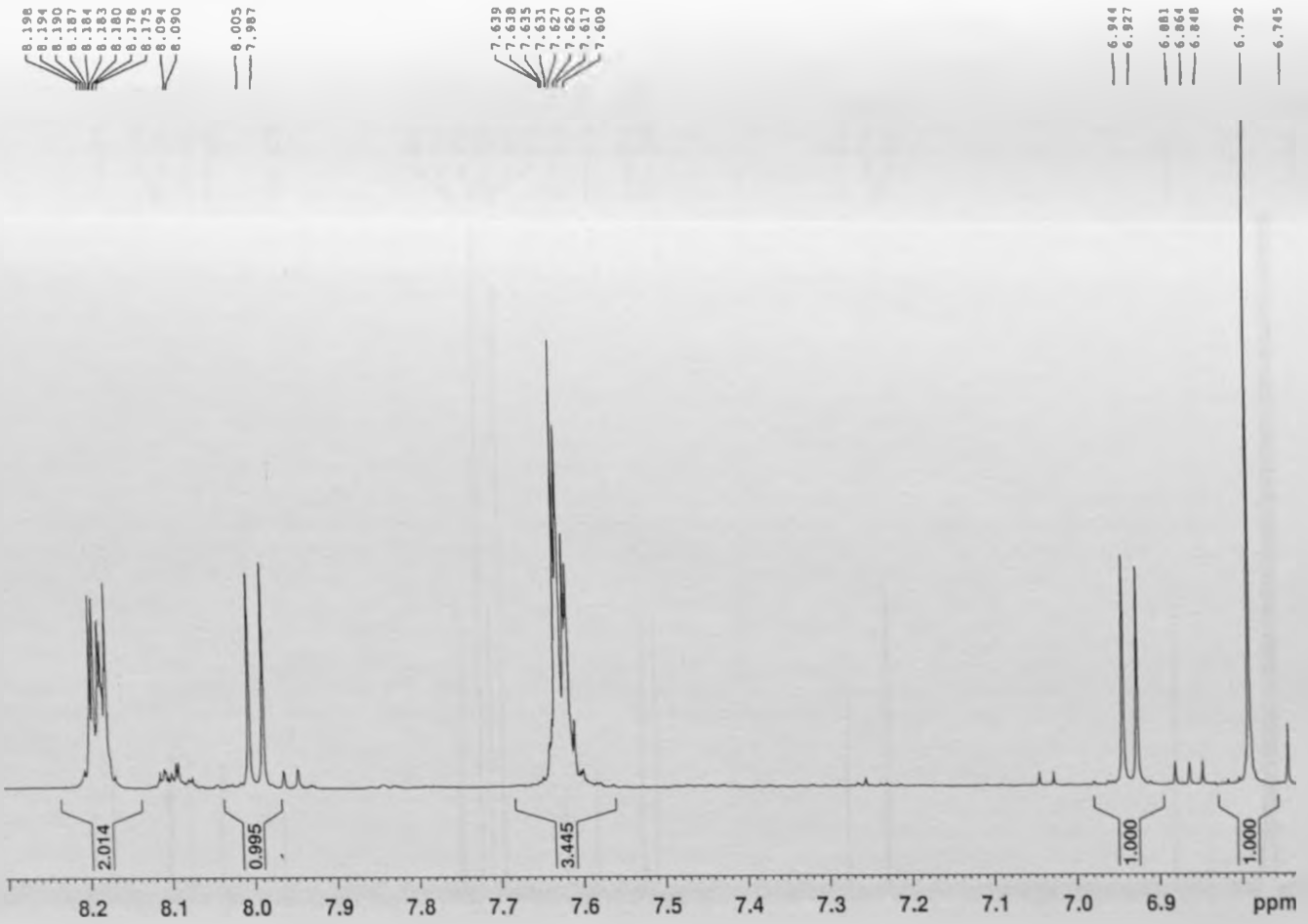
^1H NMR SPECTRUM EXPANSION FOR COMPOUND 8



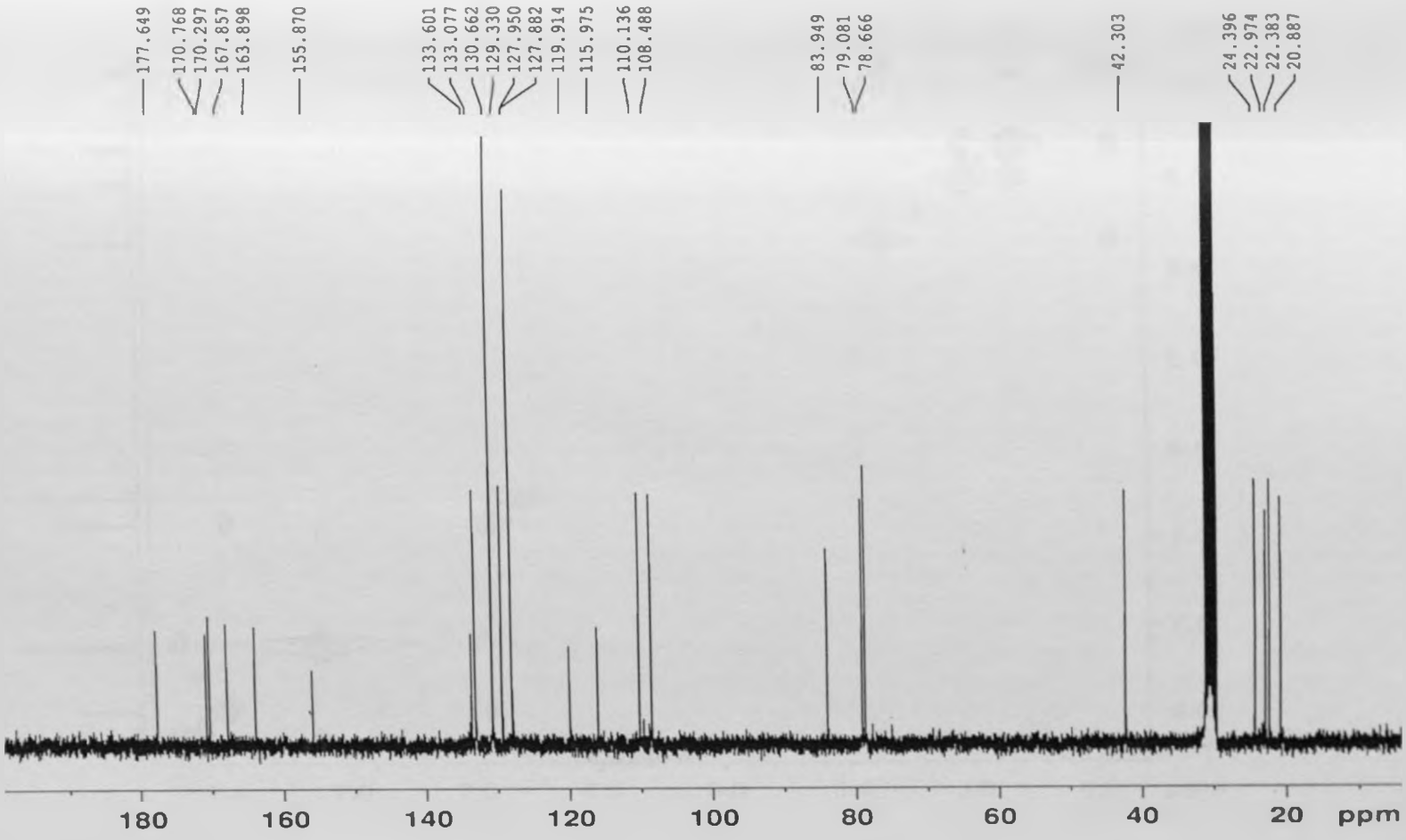
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 8



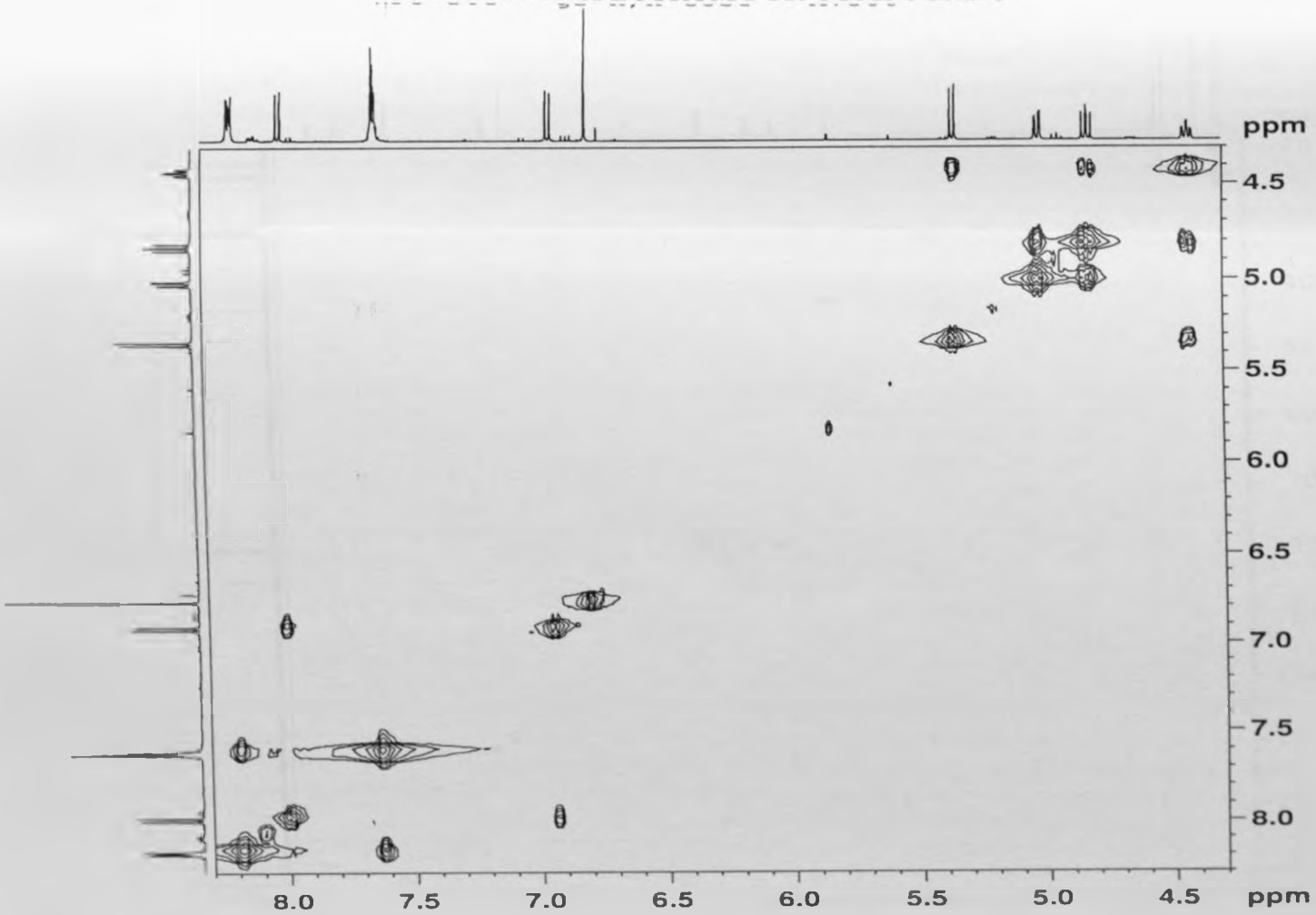
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 8



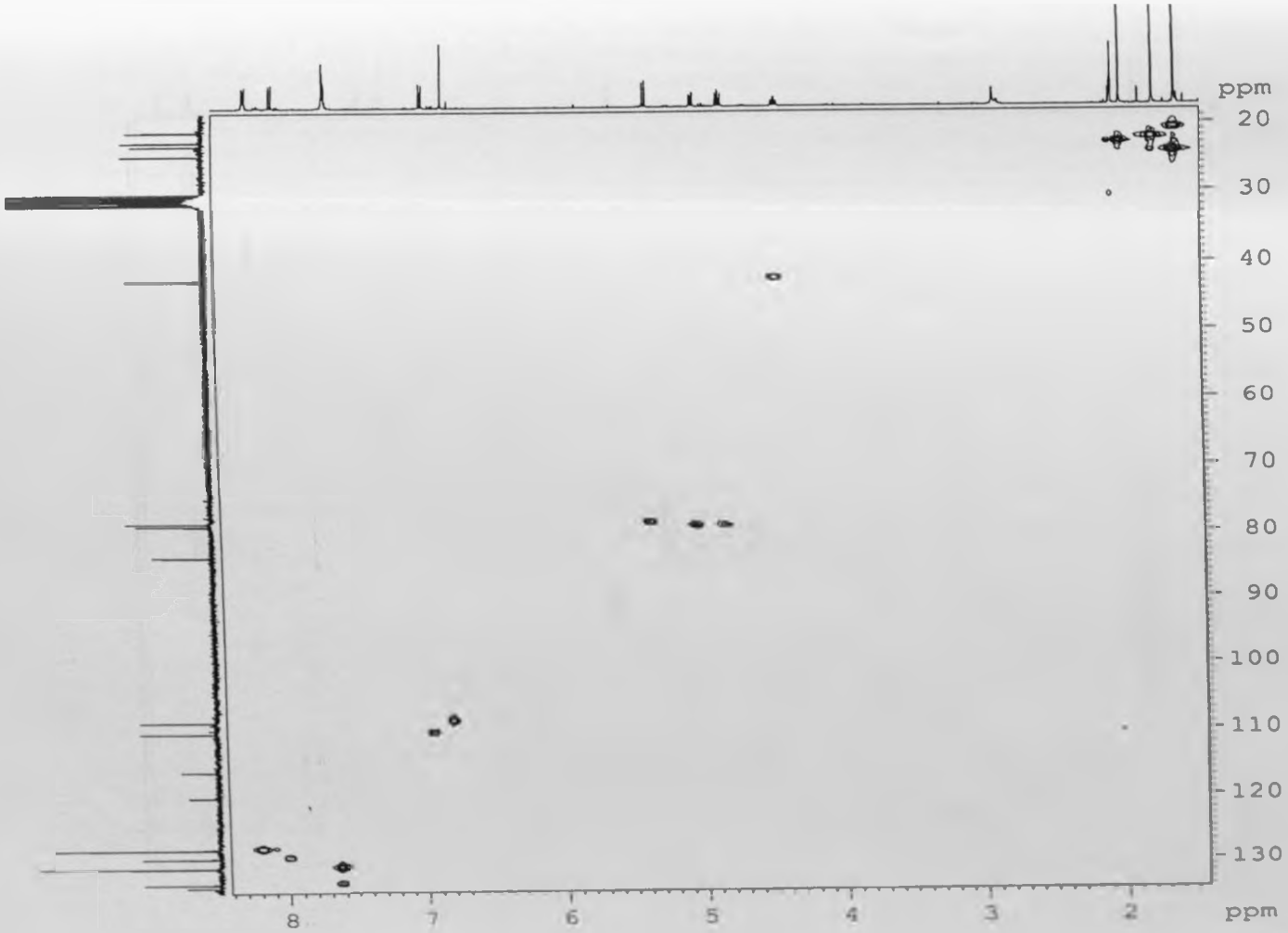
^{13}C NMR SPECTRUM FOR COMPOUND 8 (75 MHz, ACETONE-d_6)



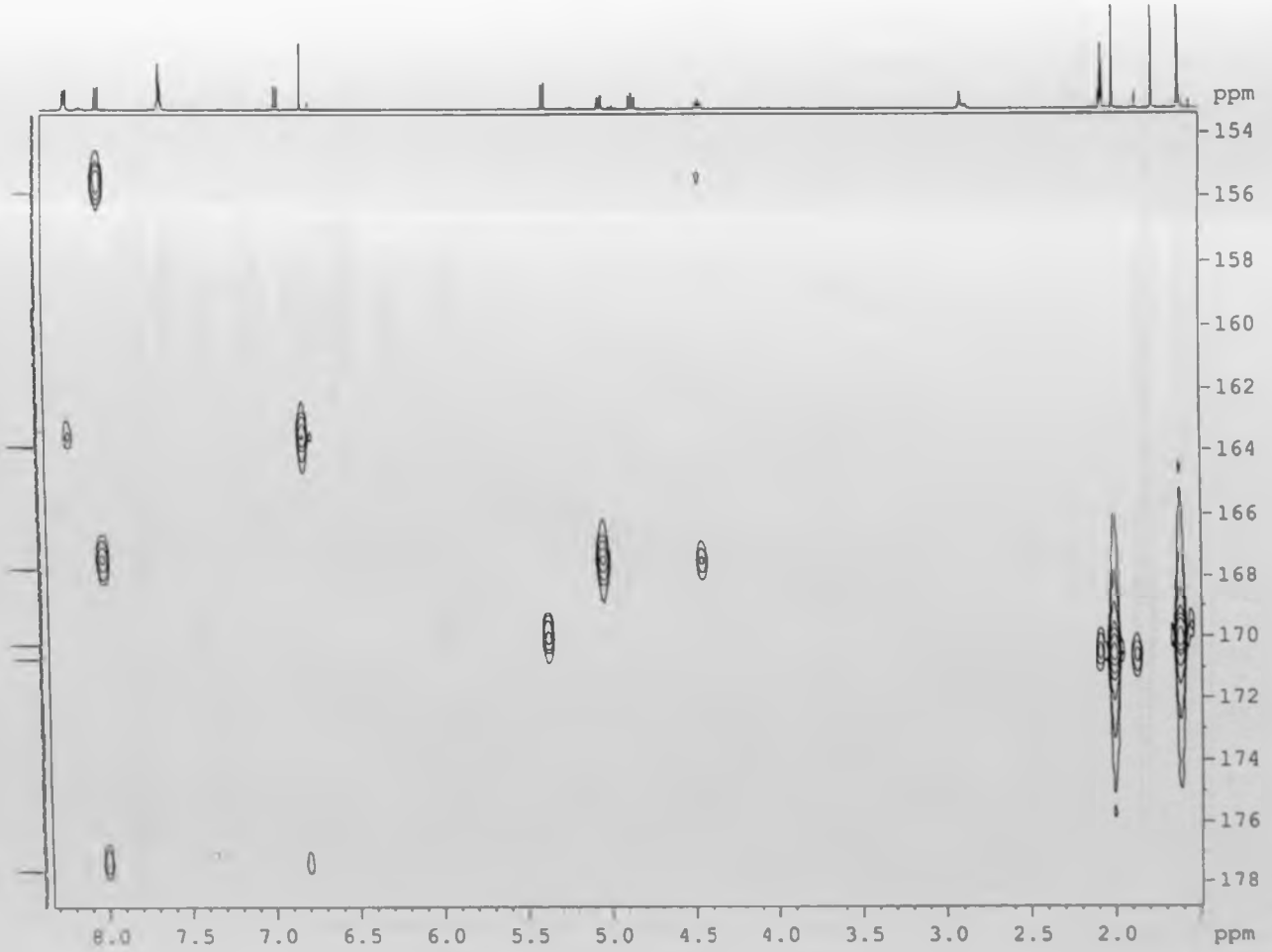
^{1}H-^{1}H COSY SPECTRUM FOR COMPOUND 8



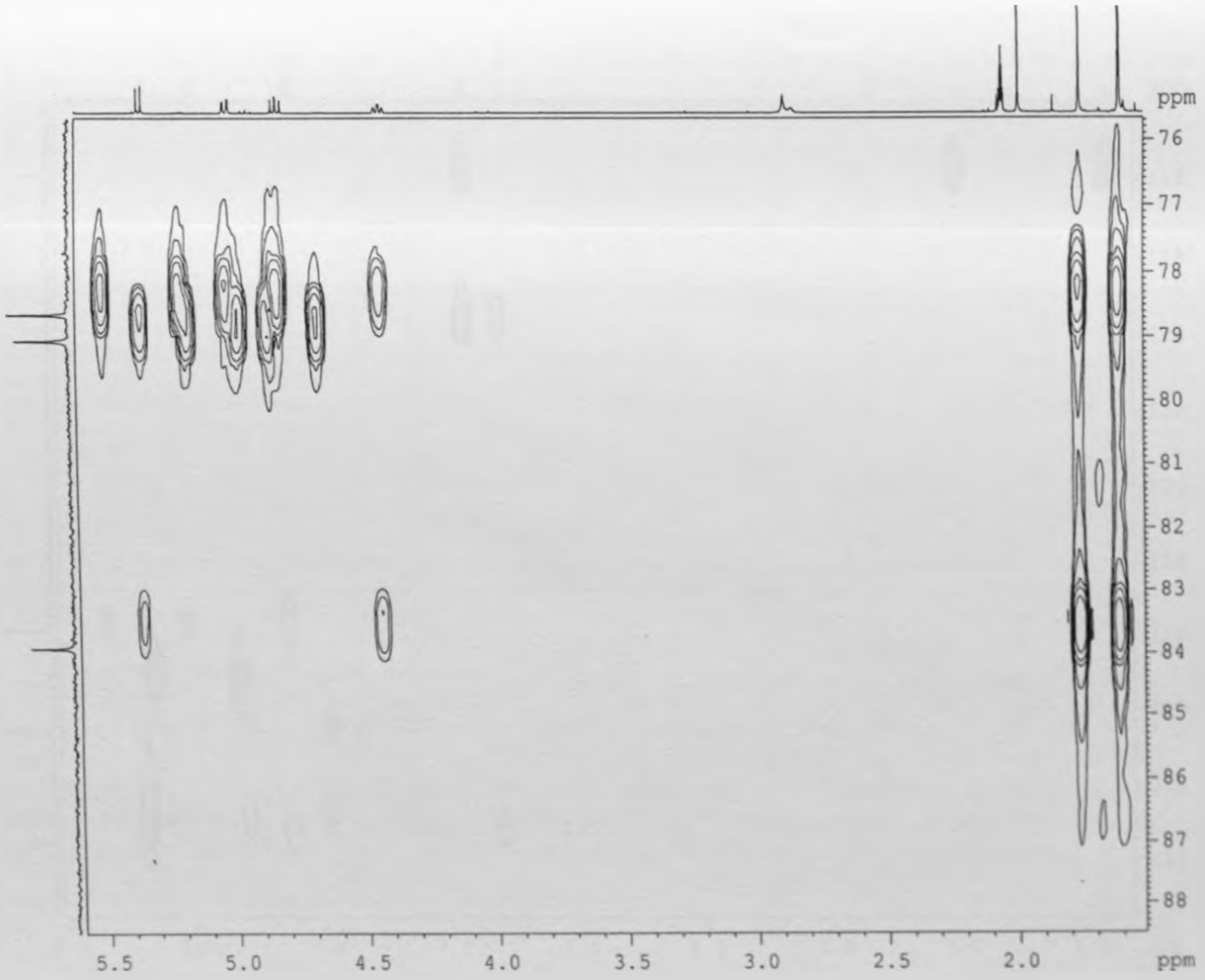
HMQC SPECTRUM FOR COMPOUND 8



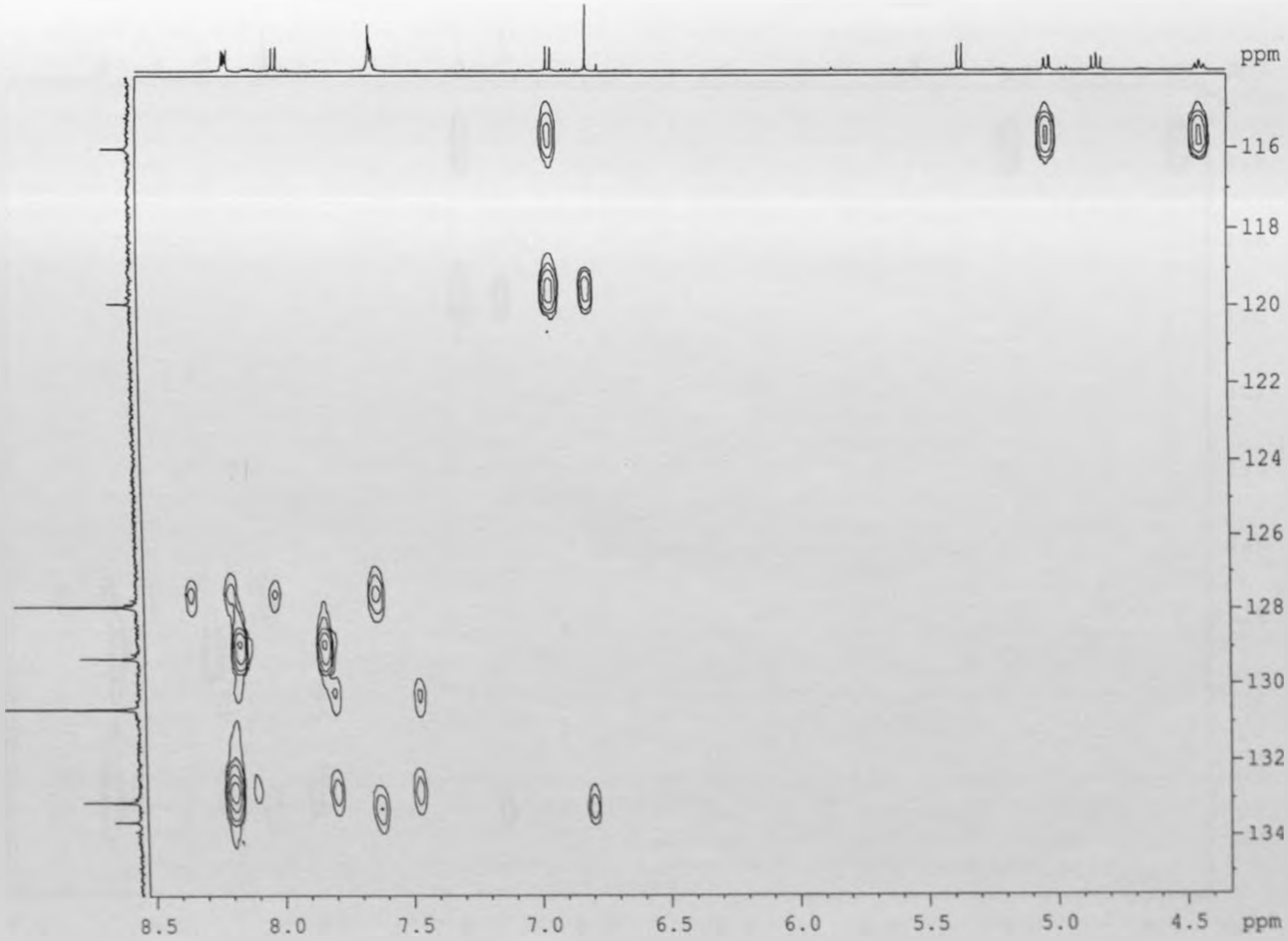
HMBC SPECTRUM FOR COMPOUND 8



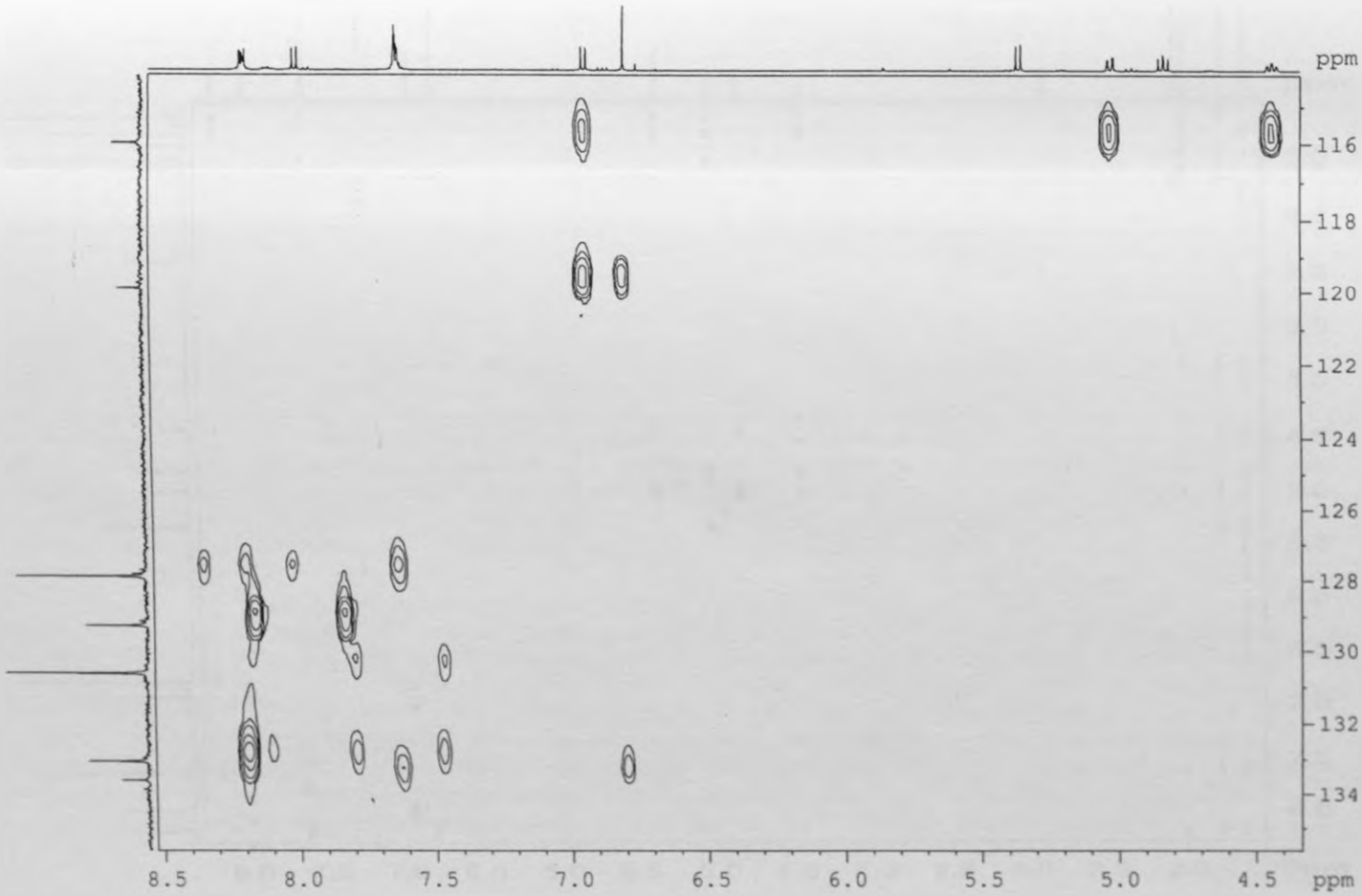
HMBC SPECTRUM EXPANSION FOR COMPOUND 8



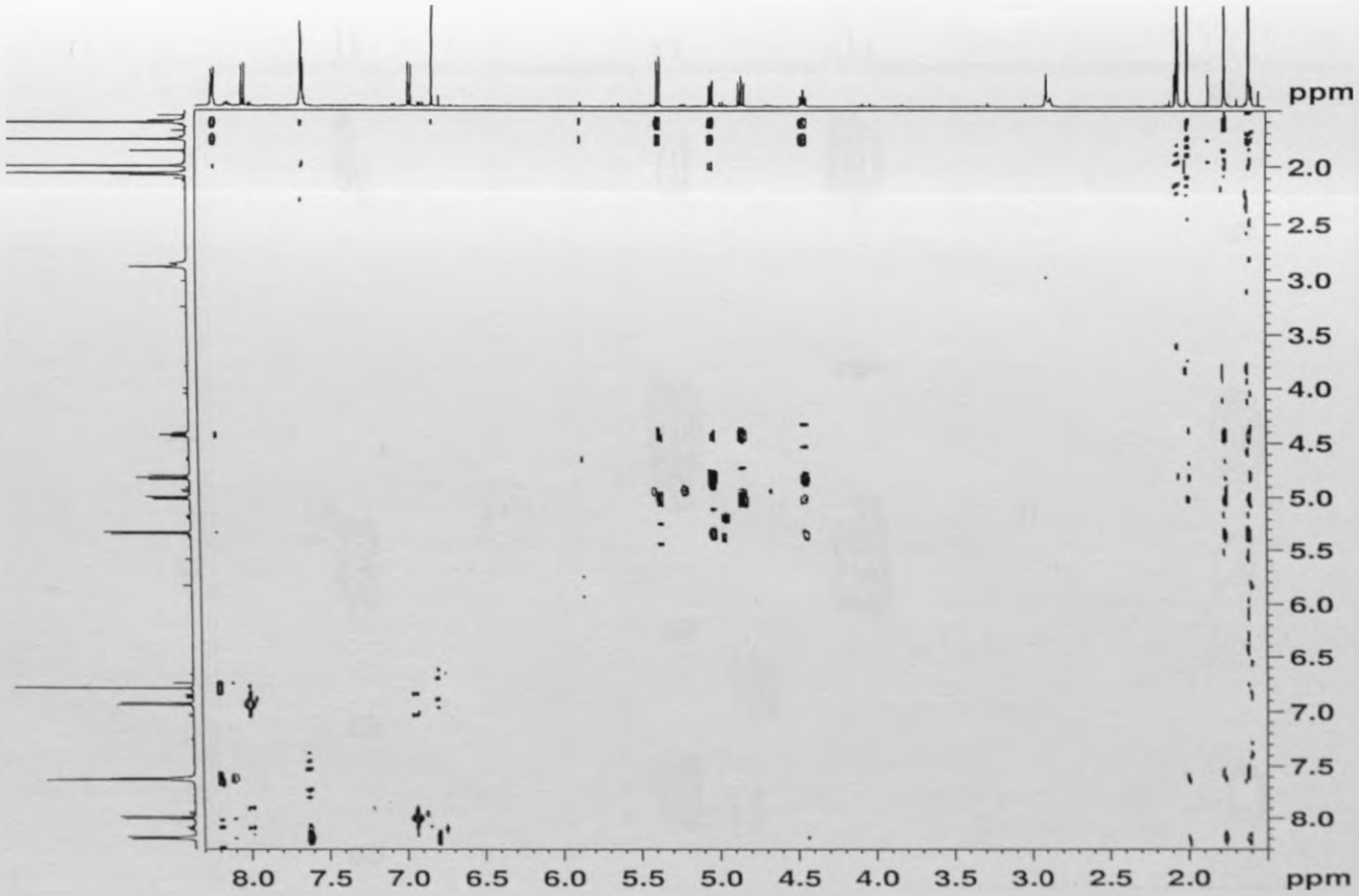
HMBC SPECTRUM EXPANSION FOR COMPOUND 8



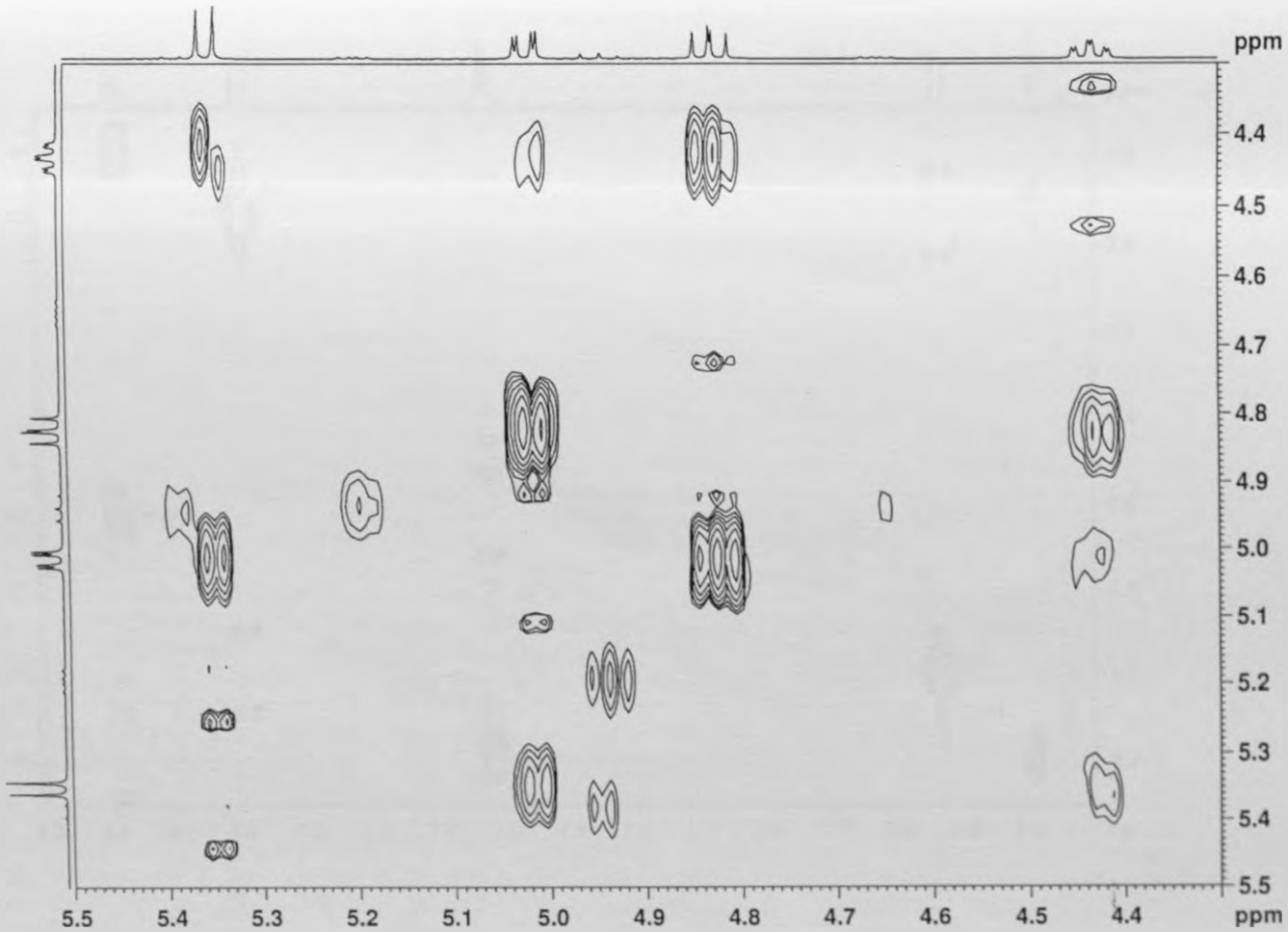
HMBC SPECTRUM EXPANSION FOR COMPOUND 8



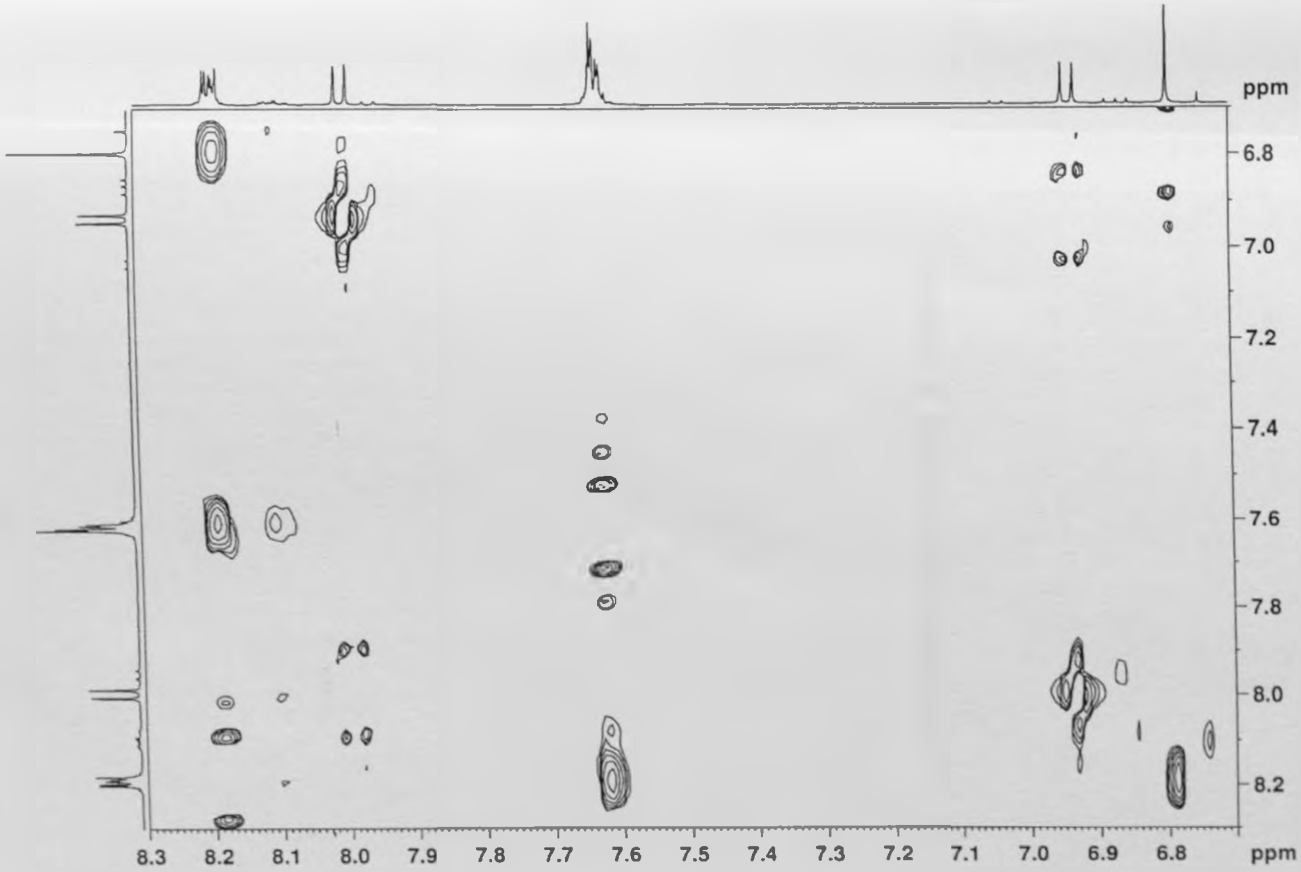
NOESY SPECTRUM FOR COMPOUND 8



NOESY SPECTRUM EXPANSION FOR COMPOUND 8

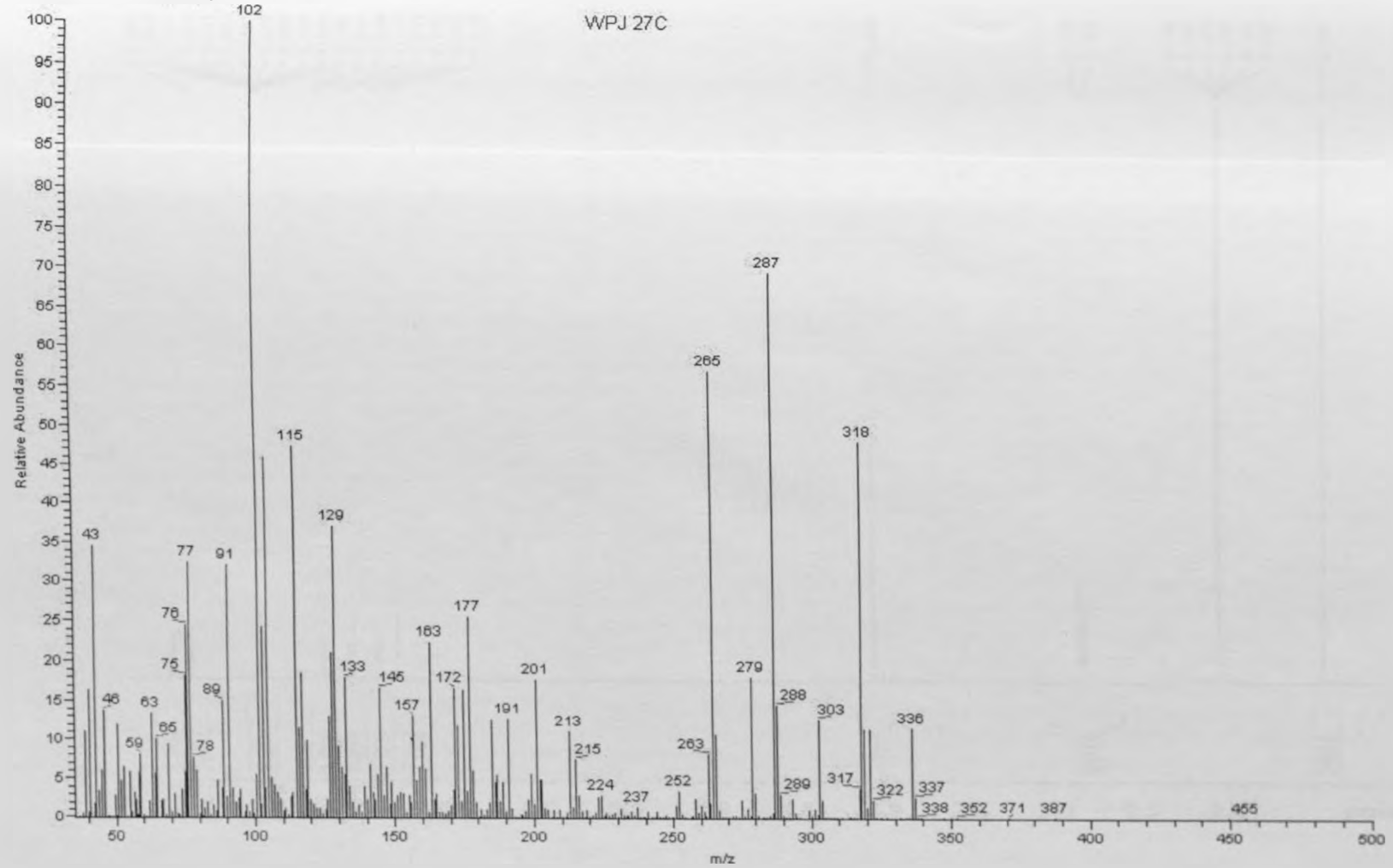


NOESY SPECTRUM EXPANSION FOR COMPOUND 8

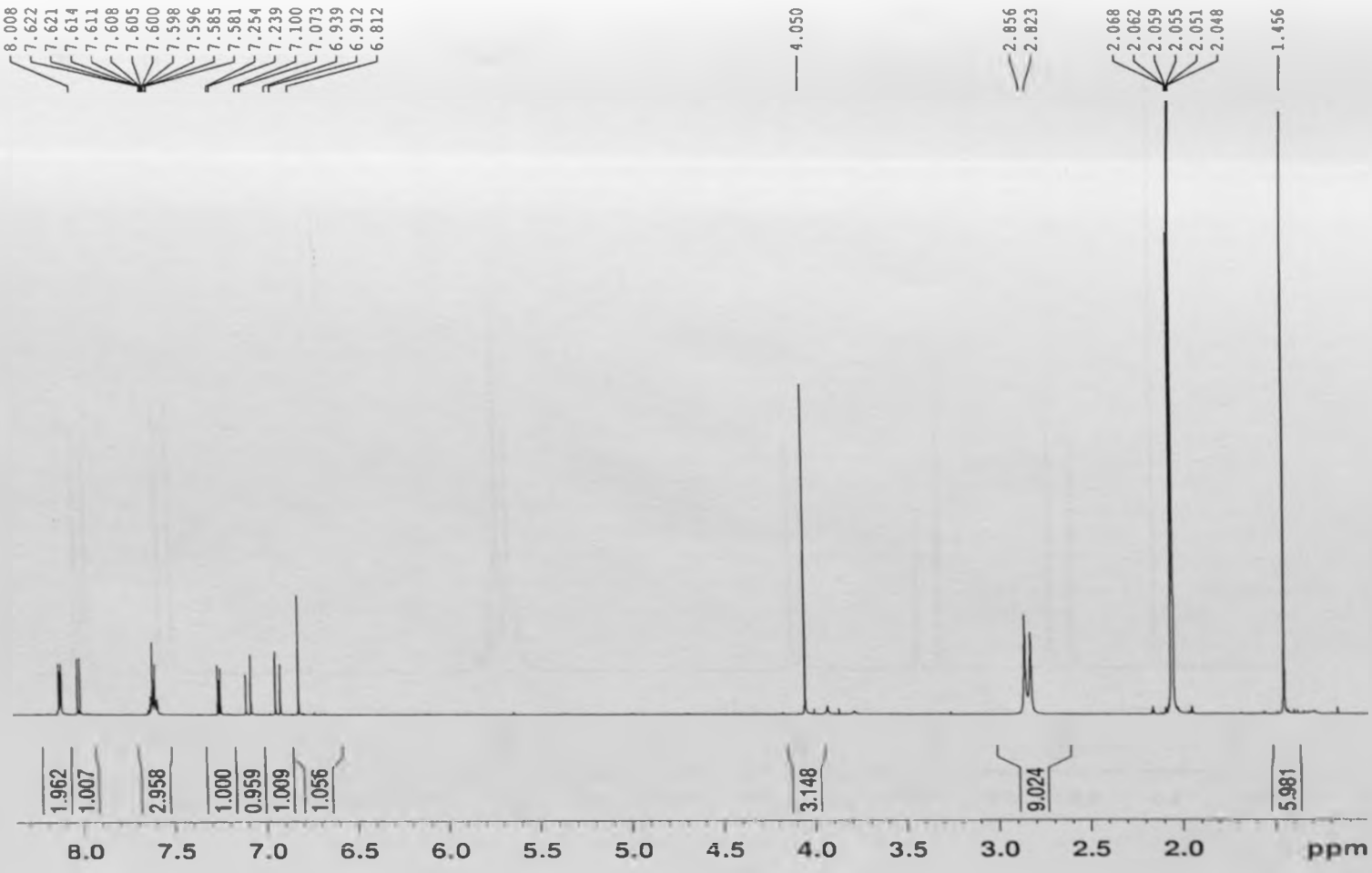


Appendix I: Spectra for compound 9

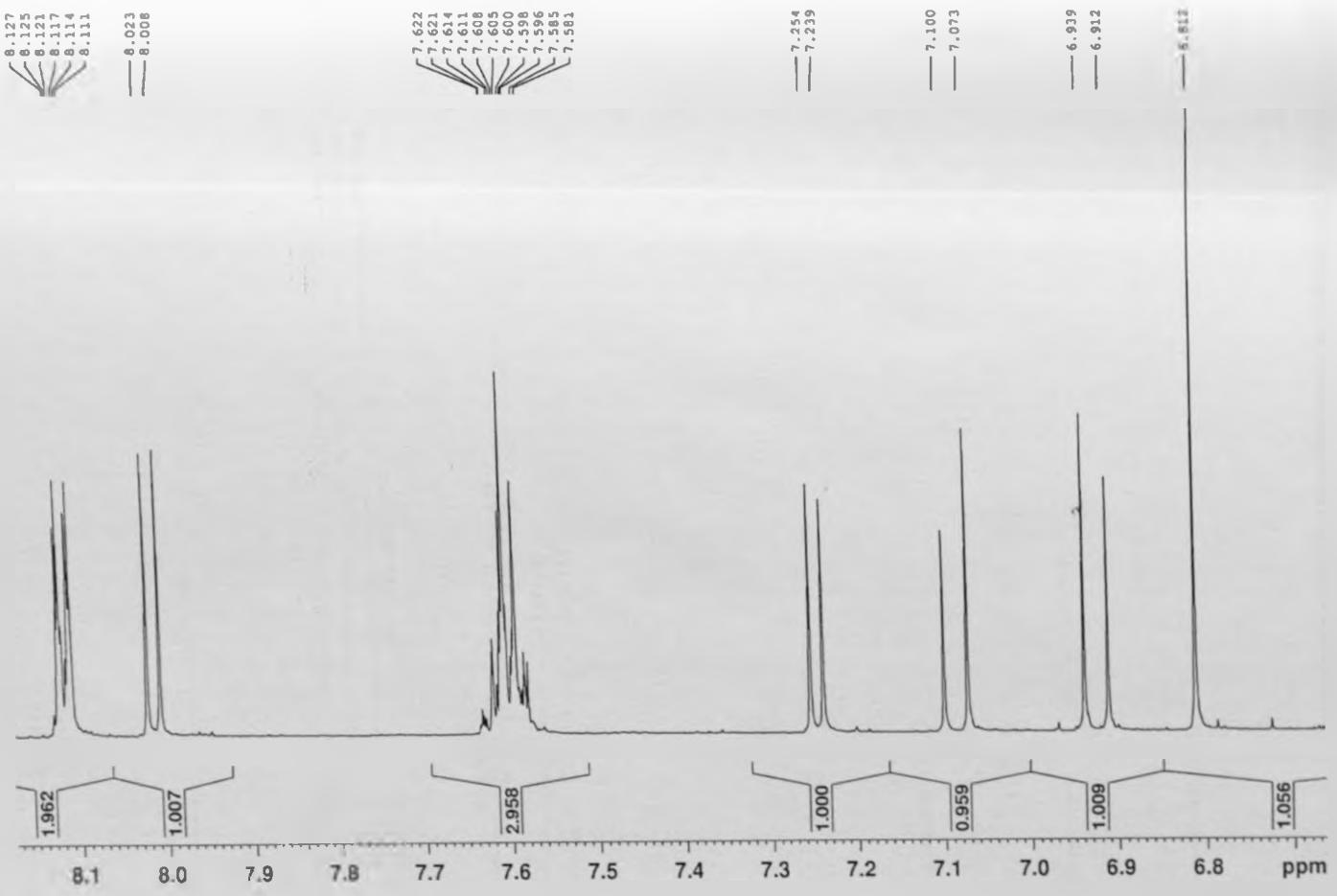
EI-MS SPECTRUM FOR COMPOUND 9

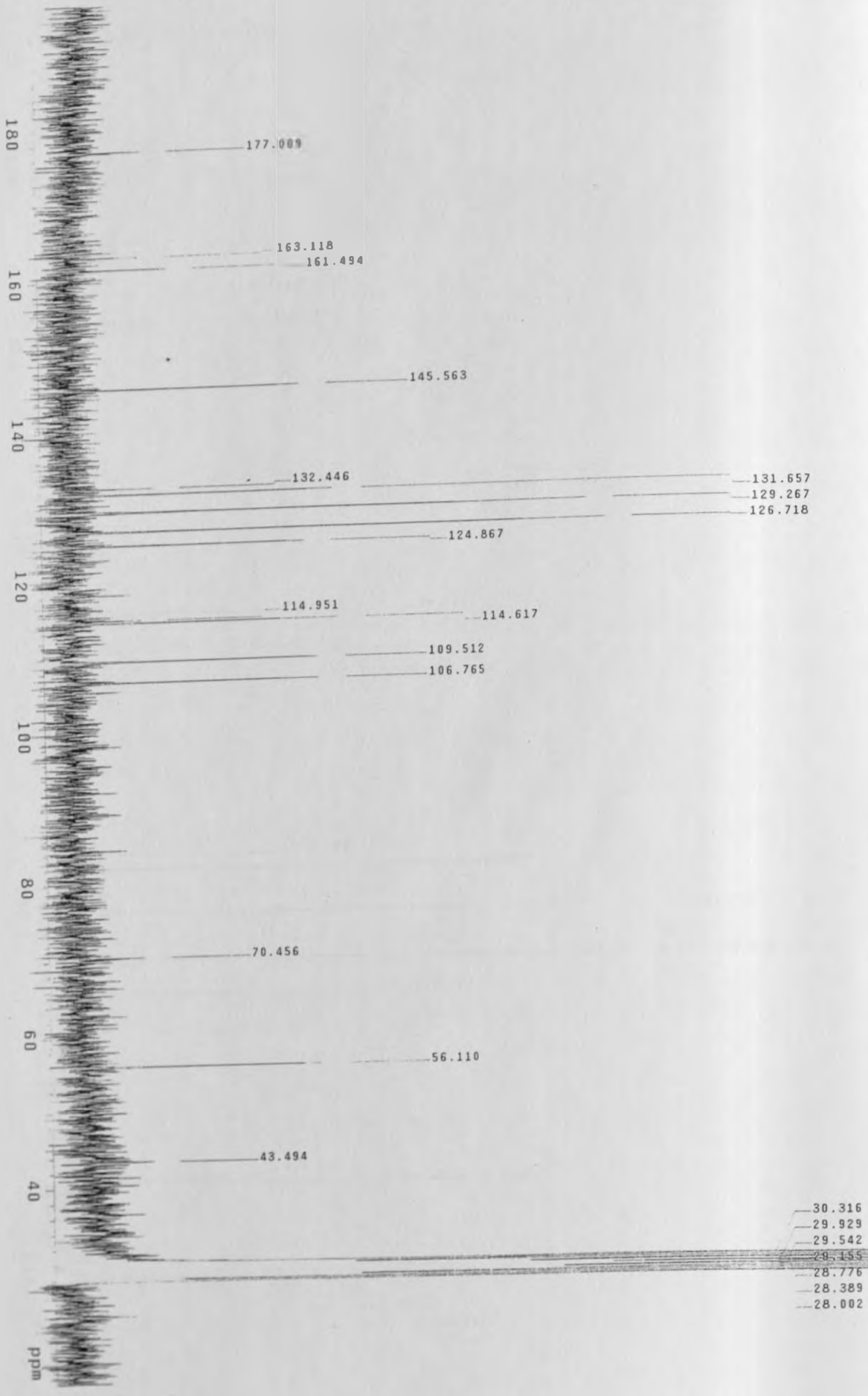


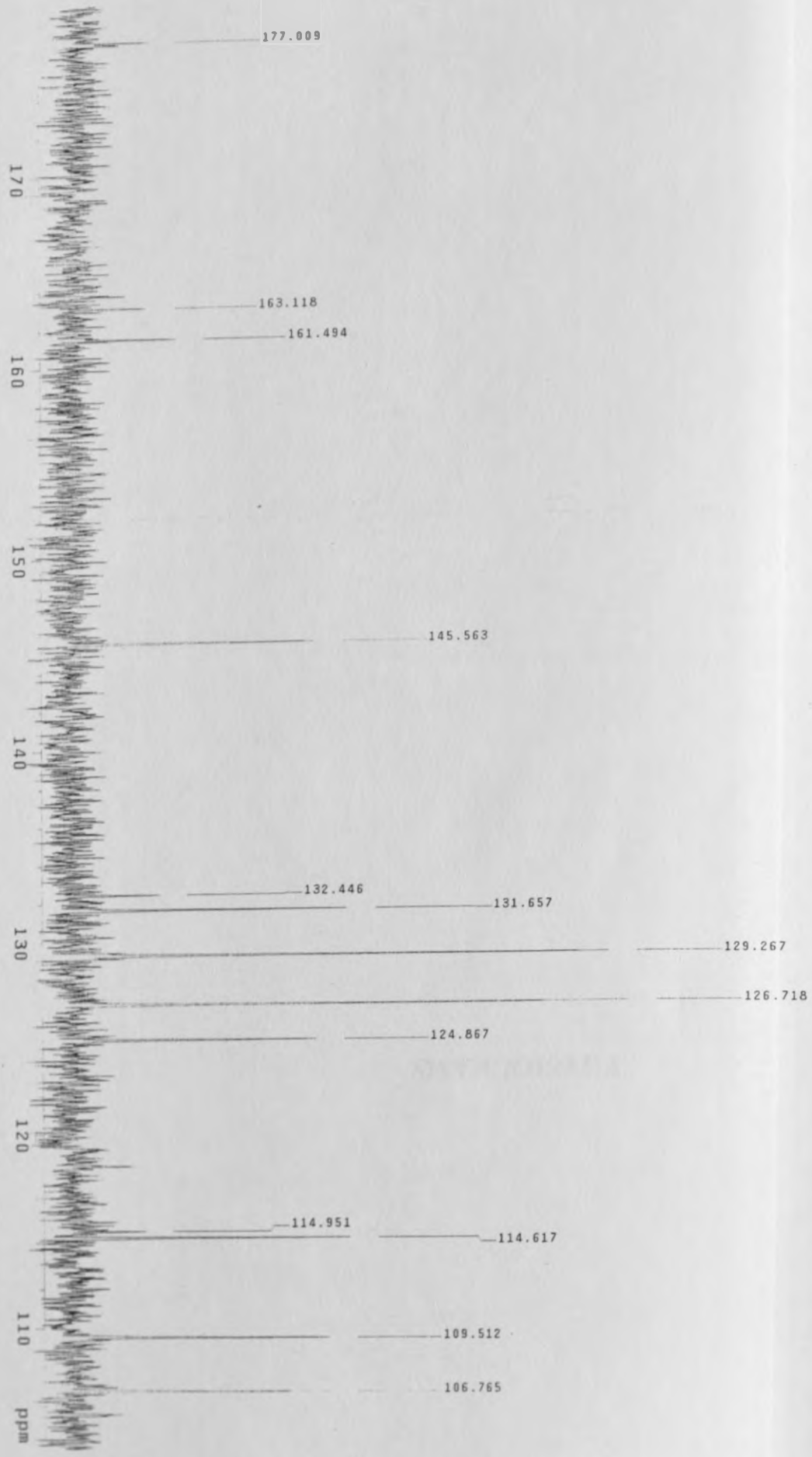
^{1}H NMR SPECTRUM FOR COMPOUND 9 (600 MHz, ACETONE-d_6)



^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 9




177.009
163.118
161.494
145.563
132.446
131.657
129.267
126.718
124.867
114.951
114.617
109.512
106.765
70.456
56.110
43.494
30.316
29.929
29.542
29.155
28.776
28.389
28.002
180
160
140
120
100
80
60
40
ppm

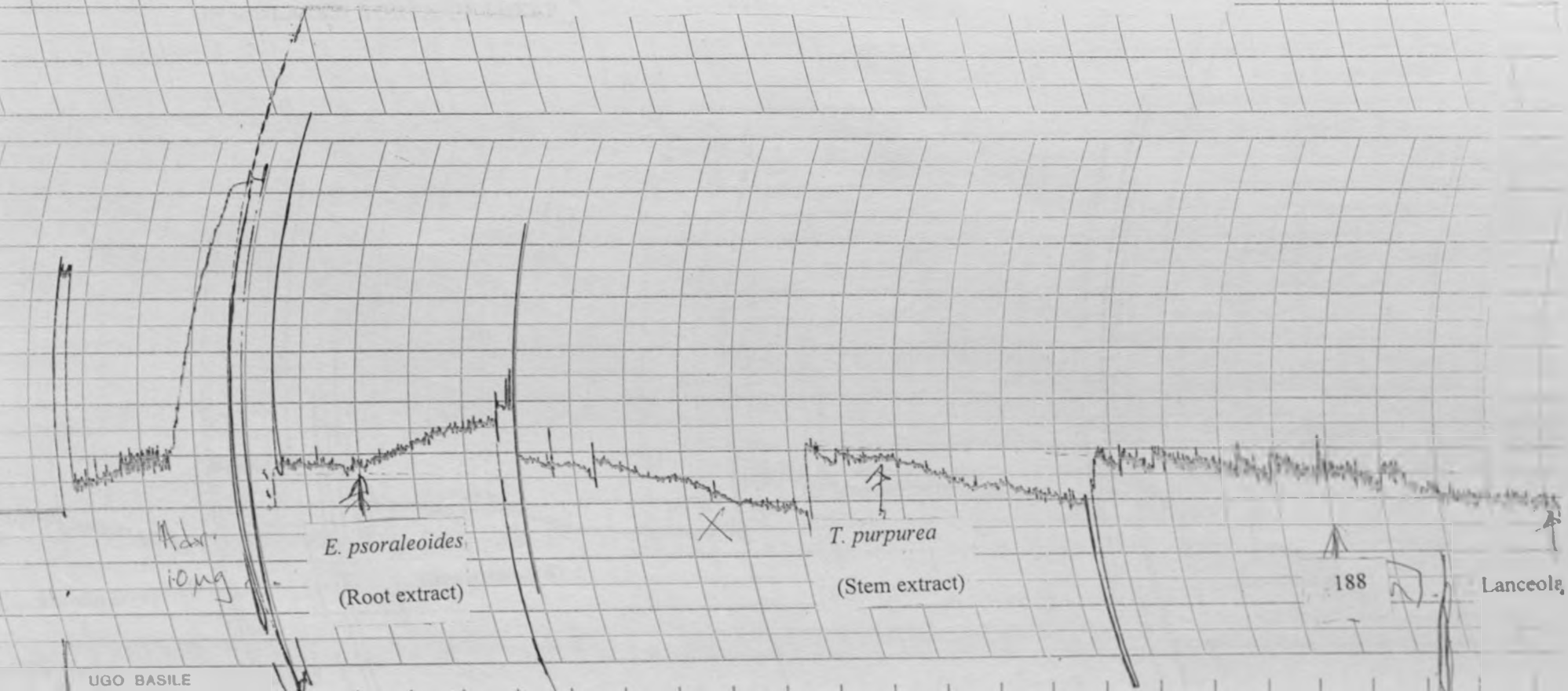

177.009
163.118
161.494
145.563
132.446
131.657
129.267
126.718
124.867
114.951
114.617
109.512
106.765
170
160
150
140
130
120
110
ppm

PHYSIOGRAMS

Appendix J: Physiograms of the effect of isolated compounds and extracts on isolated aorta

EFFECT OF ETHYL ACETATE FRACTION OF *E. PSORALEOIDES* (ROOT) AND *T. PURPUREA* (STEM) ON ISOLATED AORTA OF SHEEP

EFFECT OF ETHYL ACETATE FRACTION OF *E. PSORALEOIDES* (ROOT) AND *T. PURPUREA* (STEM) ON ISOLATED AORTA OF SHEEP

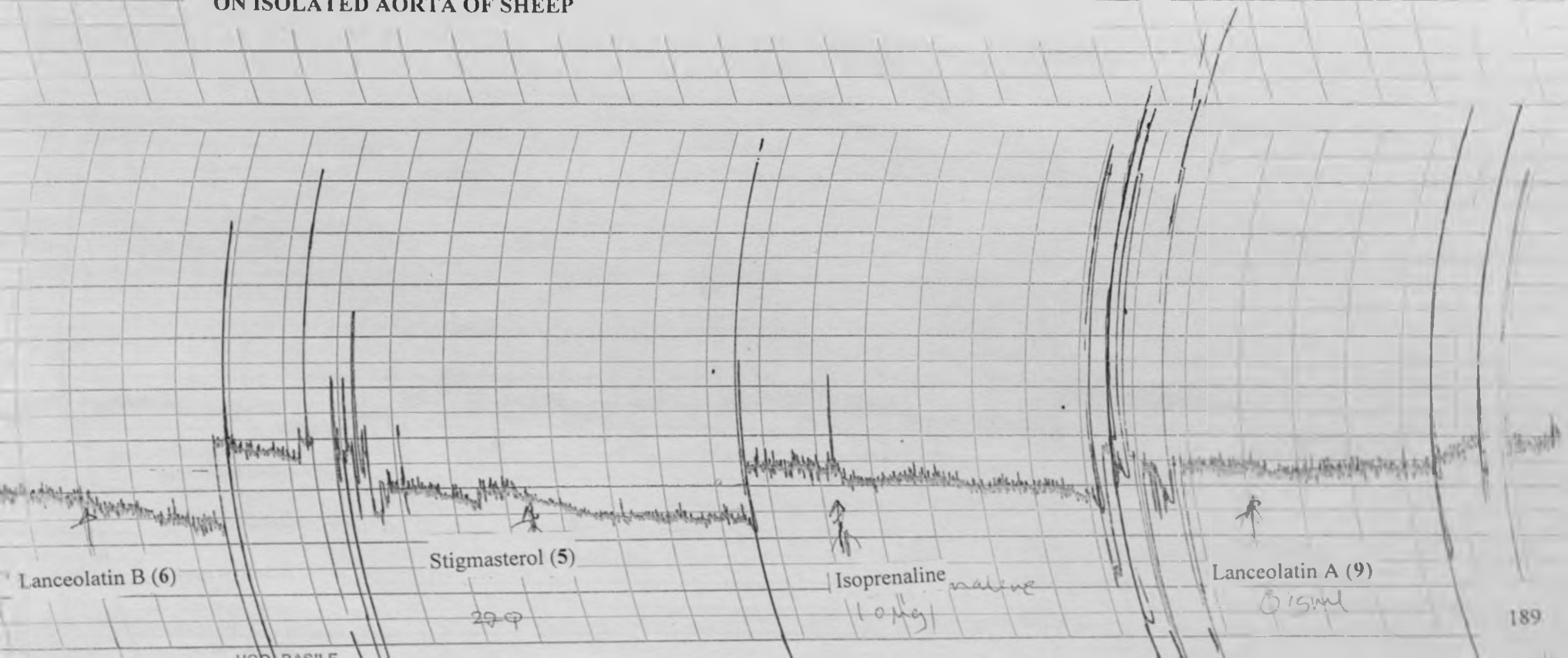


EFFECT OF LANCEOLATIN B (6), STIGMASTEROL (5) AND LANCEOLATIN A (9)

ON ISOLATED AORTA OF SHEEP

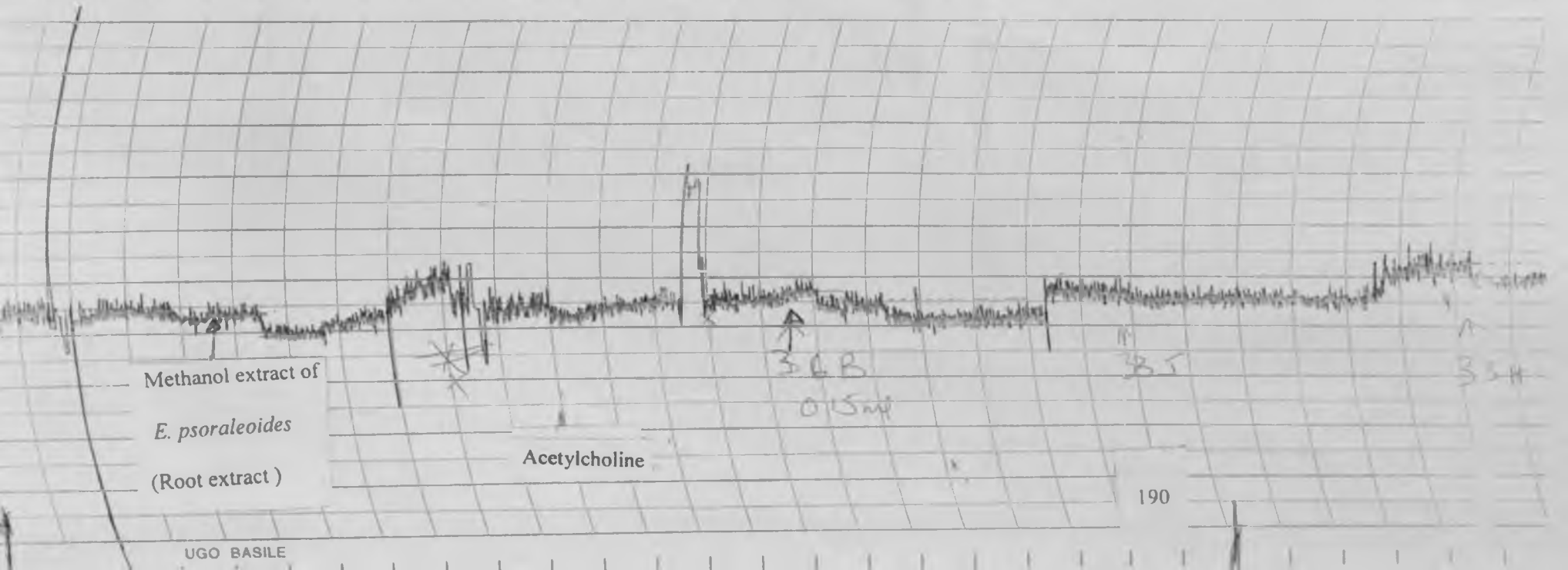
EFFECT OF LANCEOLATIN B (6), STIGMASTEROL (5) AND LANCEOLATIN A (9)

ON ISOLATED AORTA OF SHEEP



EFFECT OF METHANOLIC EXTRACT OF *E. PSORALEOIDES* (ROOT) ON ISOLATED AORTA OF SHEEP

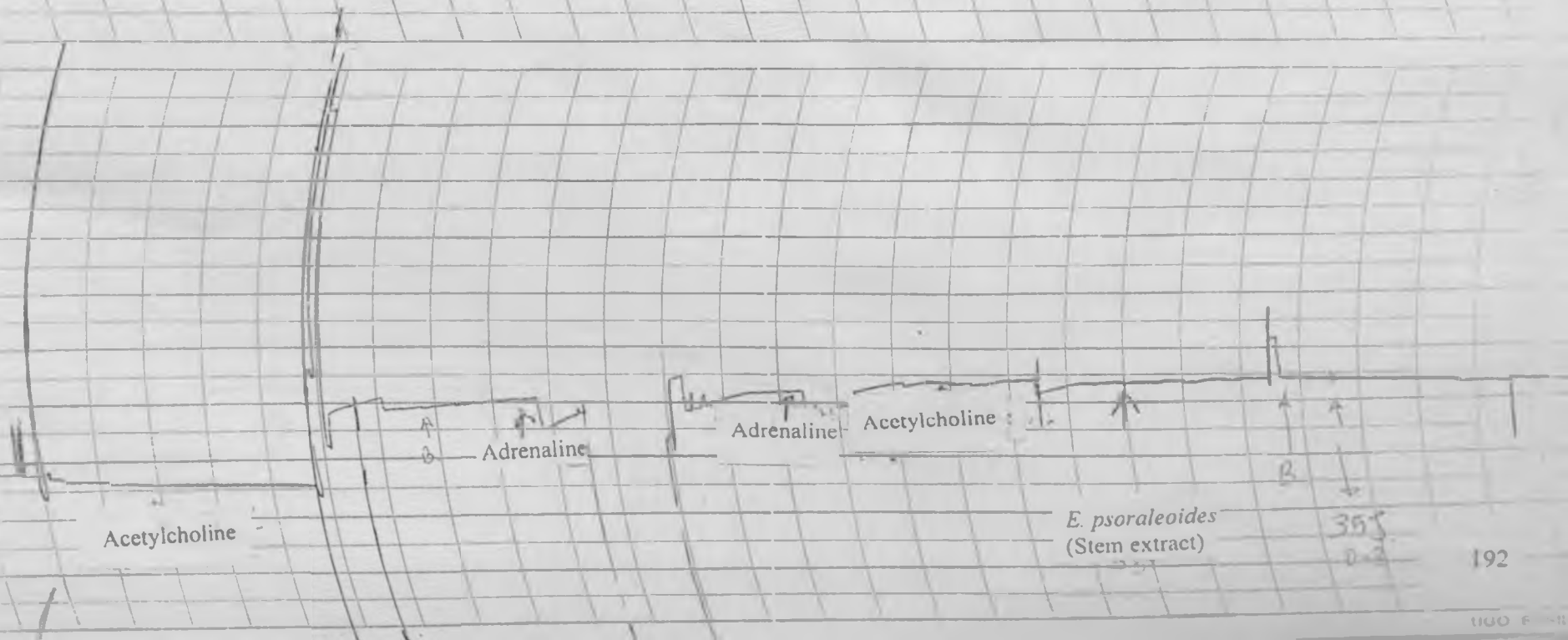
EFFECT OF METHANOLIC EXTRACT OF *E. PSORALEOIDES* (ROOT) ON ISOLATED AORTA OF SHEEP



Appendix K: Physiograms of the effect of isolated compounds and extracts on vasdeferens

EFFECT OF ADRENALINE, ACETYCHOLINE, AND ETHYL ACETATE FRACTION OF *E. PSORALEOIDES* STEM EXTRACT ON ISOLATED VAS DEFERENS OF A RAT

EFFECT OF ADRENALINE, ACETYCHOLINE, AND ETHYL ACETATE FRACTION OF *E. PSORALEOIDES* STEM EXTRACT ON ISOLATED VAS DEFERENS OF A RAT



EFFECT OF LANCEOLATIN A (9), SEMIGLABRIN (7), STIMGASTEROL (5) AND ETHYL ACETATE FRACTION OF *T. PURPUREA* STEM ON ISOLATED VAS DEFERENS OF A RAT

EFFECT OF LANCEOLATIN A (9), SEMIGLABRIN (7), STIMGASTEROL (5) AND ETHYL ACETATE FRACTION OF *T. PURPUREA* STEM ON ISOLATED VAS DEFERENS OF A RAT