SAPIENZA
UNIVERSITÀ DI ROMA

FACOLTA' DI FARMACIA E MEDICINA

Dottorato in Scienze Farmaceutiche

XXVI° Ciclo

Synthesis of bicyclo[3.2.1]octane tetracyclic diterpenes

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Introduction and objectives

Organic chemistry has always turned great attention to the synthesis of bioactive natural products both to determine their structure and to understand their chemical and biological behaviour or to supply greater amounts of those compounds whose natural occurrence is no sufficient. A particular interest concerns the synthesis of plant derived natural products, since many medicinal drugs belong to this class of compounds [1a]. Since most natural products are chiral, their obtaining in enantiopure form represents a further challenge for the organic chemist.

1.1. Terpenes

The term terpenes originates from turpentine (*lat.* balsanum terebinthinae). Turpentine, the so-called "resin of pine trees", is the viscous pleasantly smelling balsam which flows upon cutting or carving the bark and the new wood of several pine tree species (*Pinaceae*). Turpentine contains the "resin acids" and some hydrocarbons, which were originally referred to as terpenes. Traditionally, all natural compound built up from isoprene subunits and for the most part originating from plants are denoted as terpenes.

Terpenoids constitute a wide family of natural compounds structurally different from each other, deriving from isoprene units linked to each other from *head-to-tail*. Typical structures have carbon skeletons built up from $(C_5)_n$ units and are classified as Hemiterpens (C_5), Monoterpens (C_{10}), Sesquiterpens (C_{15}), Diterpens (C_{20}), Sesterterpens (C_{25}), Triterpens (C_{30}), Tetraterpens (C_{40}) and Polyterpens $(C_5)_n$ (Figure 1).

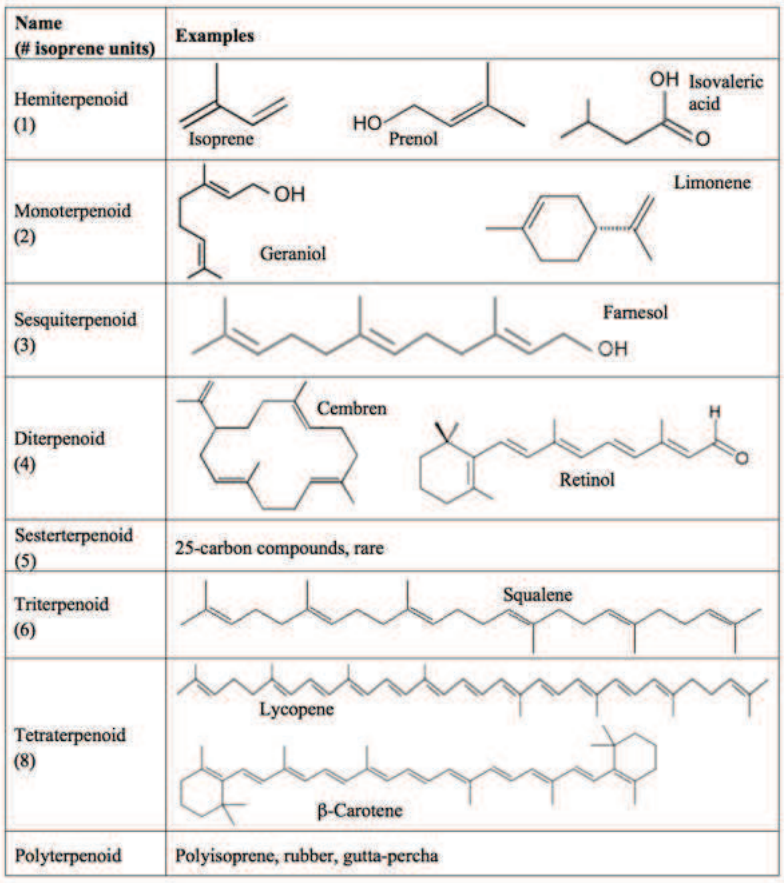


Figure 1: Examples of Terpens

These diterpenoids display a wide range of biological activities against cancer, malaria, inflammations and a variety of infections disease (viral and bacterial).

Isoprene is naturally produced, however, is not involved in the biogenesis of these compounds; have been identified, instead, as biologically active isoprene units, the diphosphate ester: dimethylallyldiphosphate (DMAPP) and isopentil diphosphate (IPP).

In Nature, terpenes occur predominantly as hydrocarbons, alcohols and their glycosides, ethers, aldehydes, ketones, carboxylic acids and esters. Terpenoids of different sizes and compositions are found throughout all classes of organisms; in fact Terpenoids represent the largest group of natural compounds, with over 35,000 are known in the literature.

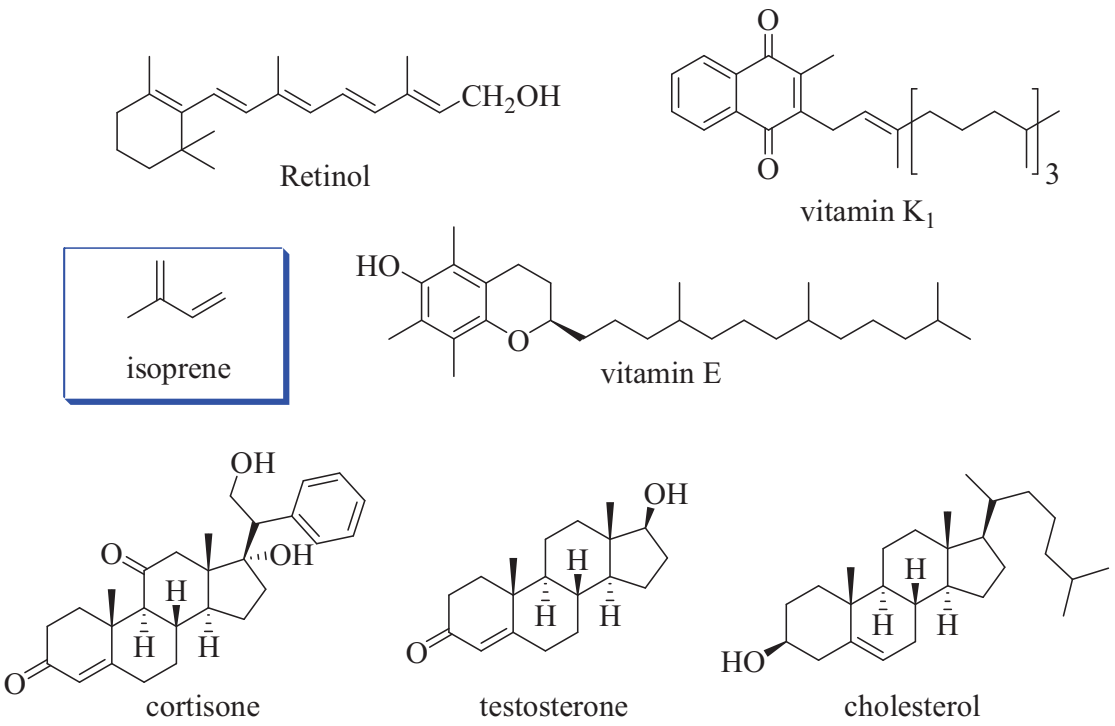


Figure 2: Common terpenoids.

Various complex terpenoids have biological activities and are used for the treatment of human disease. Many of them are essential components of human metabolism, such as cortisone and testosterone, the membrane component cholesterol, and the lipid soluble vitamins A, E, and K (Figure 2). Different terpenoids are involved in plants, fungi, bacteria, and insects secondary metabolism, leading to organisms prosperity in their specific environments. Terpenoids have always provided many lead compounds in disparate fields, such as the pharmaceutical and the agricultural ones. Among these pharmaceuticals, the anticancer drug Paclitaxel and the antimalarial drug Artemisinin are two of the most renowned terpene based-drug.

Paclitaxel or Taxol (Figure 3), is an important anticancer agent with a broad spectrum of activity against some forms of cancer that do not respond to other pharmaceutical remedies. It was isolated and identified from the bark of the Pacific yew (*Taxus brevifolia*) more than 35 years ago[1b]. Its total synthesis[1c] currently is not economically feasible because of the challenges of stereochemistry, low yield and high cost; α-Santonin (Figure 3) is one of the major anti-helminthic components of plants from *Artemisia* species, and it is effective for roundworm treatment. Artemisinin,

produced by the plant *Artemisia annua*, is a functionalized sesquiterpene active against malaria, including the quinine derivatives resistant strains; α-pyrethrins derived from *Chrysanthemum cinerariifolium* have potent insecticide activity. For this reason the extracts of *Chrysanthemum cinerariifolium* are used as biodegradable insecticides for agricultural applications. Pyrethrin are neurotoxins that attack the nervous systems of all insects. When present in amounts not fatal to insects, they still appear to have an insect repellent effect.

The sweet terpene glycoside stevioside, which is a natural sweetener extracted from the leaves of *Stevia rebaudiana* Bertoni, is responsible for the sweet taste of the leaves and it is used as a sweetener by the Center and South American indigenous peoples.

The smaller, more volatile terpenes are often used in flavours and perfumes.

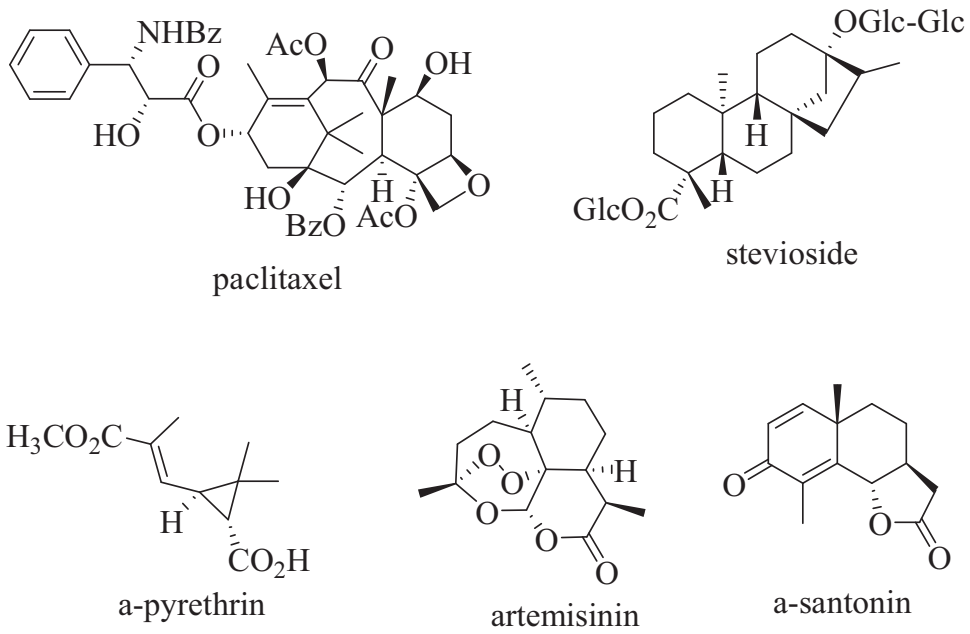
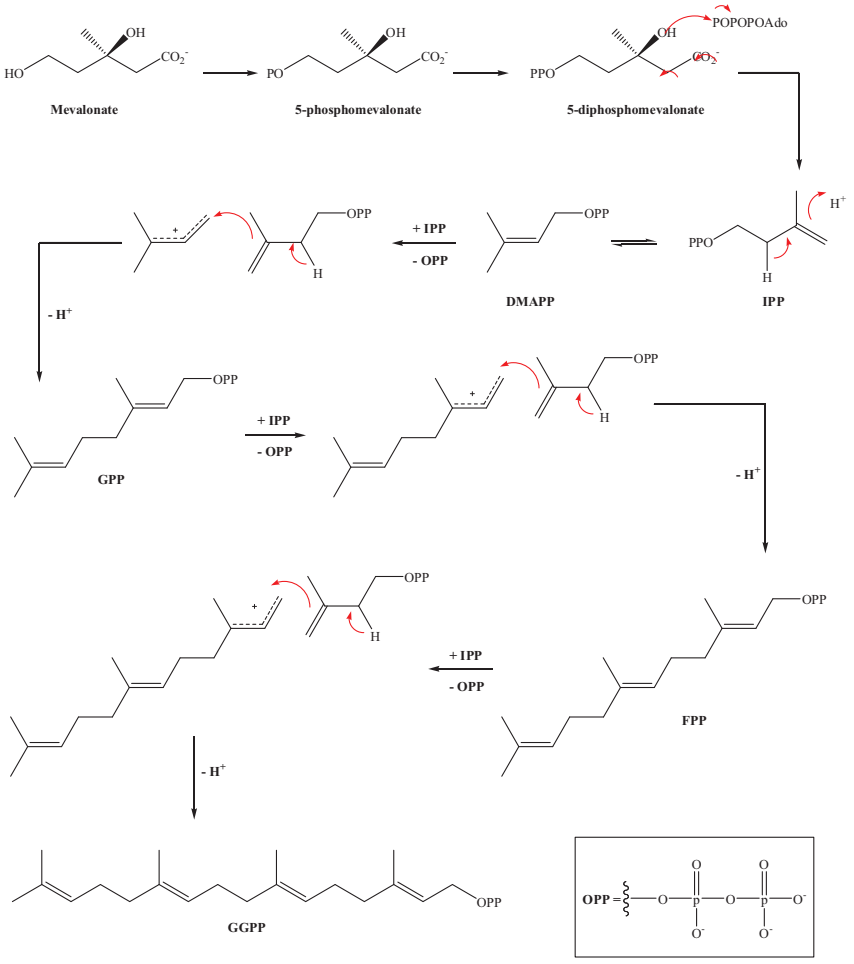


Figure 3: Terpenoids used in various practical applications.

Terpene biosynthesis is usually carried out by joining carbon atoms by electrophilic condensations and cyclizations. Specific enzymes known as prenyltransferases condense isoprene units, giving rise to polyprenyl diphosphates geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP), as reported in scheme 1. In the condensation reactions, dimethylallyl diphosphate

(DMAPP) loses diphosphate, and the resultant allylic carbocation is attacked by the nucleophilic double bond of isopentenyl diphosphate (IPP), with formation of GPP for the subsequent proton loss. In FPP and GGPP syntheses, additional IPP molecules are added in a similar fashion until the proper chain length is attained.

Cyclization of acyclic terpenoid substrates typically proceeds through several carbocationic intermediates, which readily undergo remarkable structural rearrangements. Specific enzymes named terpene cyclases, whose substrates are GPP, FPP, and GGPP, control these reactions providing a stereochemical template for cyclization and rearrangement. Larger terpenes can be generated by further condensations of these prenyl groups. The wide variety of arrangements of these linear and cyclic moieties results in the incredible structural diversity of terpenoid carbon skeletons observed in Nature.



Scheme 1: Biosynthesis of terpenes.

1.1.1. Diterpenes

Diterpenes, composed of four isoprene units (20 carbon atom), are well known for their biological activities in many complex species. They are universally present in small amounts in living organisms, where they play numerous vital roles in plant physiology as well as important functions in all cellular membranes. They are the most widely distributed terpenes in the plant kingdom and it is shown that the extraordinary variety they display can be due to ecological factors playing an evolutionary role[2a]. As shown in Figure 4, diterpene compounds possess a great structural variety.

Phytol, one of the most simple diterpenes, is an acyclic diterpene alcohol that can be used as a precursor for the synthetis of vitamin E[2a] and vitamin K.[2c]; geranylgeraniol, another diterpene alcohol which plays a role in several important biological processes. In fact it is an intermediate in the biosynthesis of other diterpenes, of vitamins E and K[2d] and it also used in the post-translational modification known as geranylgeranylation. Geranylgeraniol is a pheromone for bumblebees and a variety of other insects[2e].

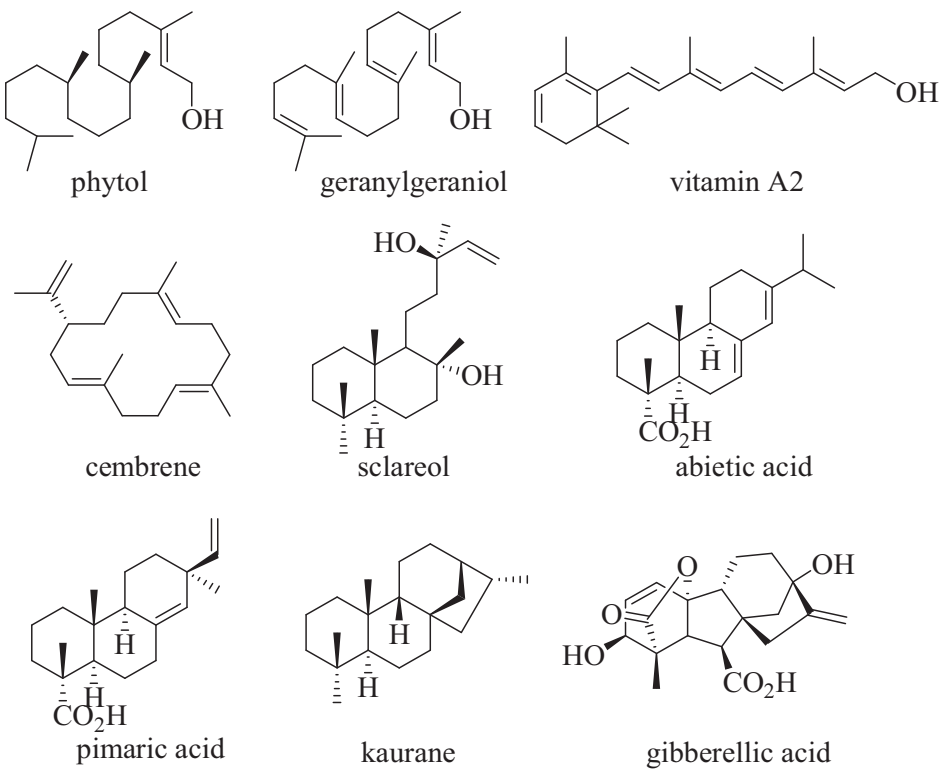
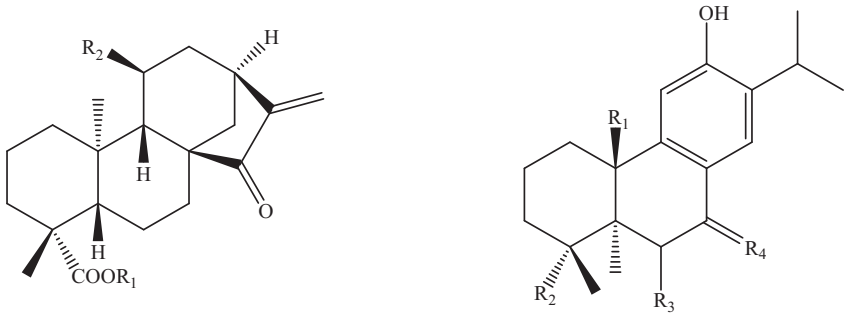


Figure 4: Common diterpenes.

Vitamin A1 (Figure 2), vitamin A2, and cembrene (Figure 4) are monocyclic diterpenes, the last of which belongs to a very wide variety of other natural products found both in plants and in animals[2f] and marine organisms. It was isolated from corals of the genus *Nephthea*[2g]. Sclareol (Figure 4), a bicyclic diterpenoid, is an important starting material for the preparation of perfumery chemicals. It is also able to kill human leukemic cells and colon cancer cells by apoptosis[2h,2i].

Resin acids (such as abietic and pimaric acids) are important constituents of tricyclic diterpenes: they are the major components of the oleoresin synthesized by grand fir (Abies grandis) as a defensive secretion against insect and pathogen agents attack. The tetracyclic diterpenes kaurane and gibberellic acid, constituents of a class that counts more than 100 different structures, play a significant role as plant growth hormones.

Several diterpenes show significant pharmacological activities, such as for example antitumor, anti HIV, antioxidant, analgesic, and neuroprotective ones, giving rise to a great pharmacological interest toward this class of natural products. The tetracyclic diterpenes ent-15-oxo-kaur-16-en-19-oic acids **32a-c**, isolated from Chrysobalanus icaco L. subsp. atacorensis (A. Chev.) F. White (Chrysobalanaceae family), showed in vitro activity in the NCI's anti-HIV screen[3], while the tricyclic diterpenes from the cones of Sequoia sempervirens genus (20-hydroxyferruginol derivatives **33a-e**) have been demonstrated to possess antitumor properties[4] (Figure 5).

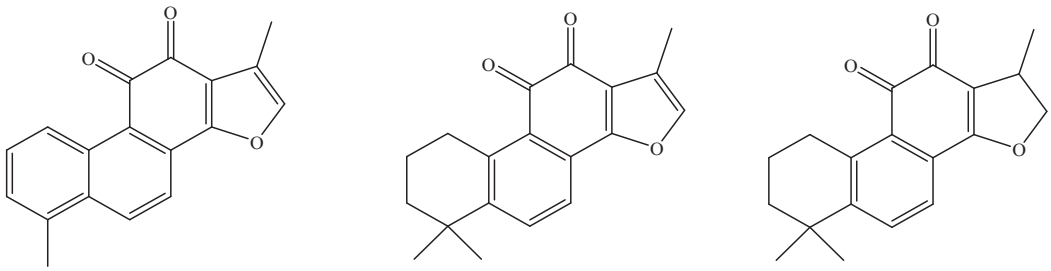


32a $R_1 = H, R_2 = H$
32b $R_1 = CH_3, R_2 = OH$
32c $R_1 = CH_3, R_2 = H$

34a $R_1 = CH_2OH, R_2 = CH_3, R_3 = H, R_4 = H_2$
34b $R_1 = CH_3, R_2 = CH_3, R_3 = H, R_4 = H_2$
34c $R_1 = CH_3, R_2 = CH_2OH, R_3 = H, R_4 = H_2$
34d $R_1 = CH_3, R_2 = CH_3, R_3 = H, R_4 = O$
34e $R_1 = CH_3, R_2 = CH_3, R_3 = OH, R_4 = O$

Figure 5: *Chrysobalanus icaco* L. subsp. *atacorensis* (A. Chev.) F. White and *Sequoia sempervirens* genus diterpenes.

Several diterpenes isolated from plants of the genus *Salvia*, especially from *Salvia miltiorrhiza*, showed other interesting pharmacological properties, such as antioxidant, antimicrobial, antiinflammatory, analgesic, antipyretic, hemostatic, hypoglycaemic, and antitumor[5]. The antibacterial, antitumor, antioxidant, antimutagenic, antiinflammatory and antiplatelet aggregation activities are attributed to the presence of the tanshinones diterpenes[6] (Figure 6). Recently, Mohsen Imanshahidi and Hossein Hosseinzadeh have examined important pharmacological effects on the central nervous system of several diterpenes isolated from different species belonging to the genus of *Salvia*, among which sedative, hypnotic, hallucinogenic, skeletal muscle relaxant, analgesic, memory enhancing, anticonvulsant, neuroprotective and antiparkinsonian activities, as well as the inhibition of ethanol and morphine withdrawal syndrome[7].



Tanshinone I **Tanshinone IIA** **Cryptotanshinone**

Figure 6: Some of the more than 30 kinds of tanshinones from the genus *Salvia miltiorrhiza*.

One of the most well known medicinally valuable terpenes is the diterpene, taxol. Taxol was first isolated from the bark of the Pacific yew (*Taxus brevifolia*) in the early 1960's, but it was not until the late 1980's that its value as an anticancer drug was determinate. It acts to stabilize the mitotic apparatus in cell, causing them to act as normal cells rather than undergo rapid proliferation as they do in cancer.

1.2. Stemarane diterpenes

Stemarane diterpenes are tetracyclic compounds characterized by a particular hydrocarbon skeleton named "stemarane skeleton" (Figure 7). The archetype of this class of compounds, Stemarin **33**[8] (Figure 8), was isolated in 1975 by Manchand and Blount from a rare littoral plant of the West Indies, belongs to the family of *Scrophulariaceae*[9], the *Stemodia Maritima* L (Figure 9). This plant is known in Jamaica as "poor man's strength", because in the Caribbean island of Curaçao extracts of its leaves are reputed to be a treatment for venereal disease[10].

The *Stemodia Maritima* L yielded several diterpenes, other than stemarin **33**, with mild cytotoxic activity: stemodin **34** and stemodinone **35**[11], 2-deoxystemodinone **36**[12], and maritimol **37**[13] (Figure 8). These diterpenes are structurally similar to the fungal metabolite aphidicolin **38**[14], another tetracyclic diterpene, isolated from the fungus *Cephalosporium aphidicola*, which shows various pharmacological activities including antiviral [15] and antitumor[16] ones.

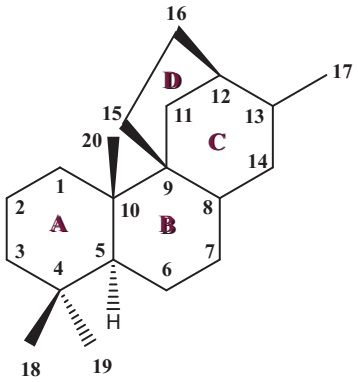
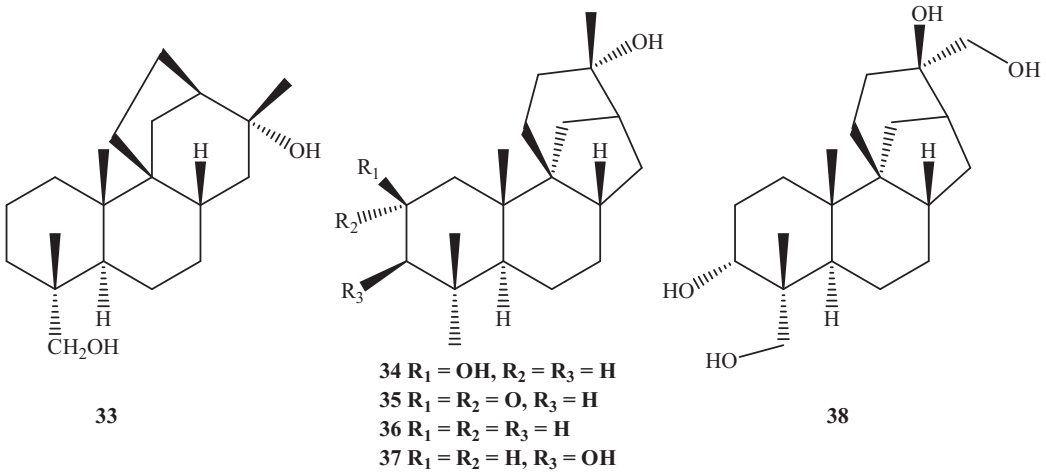


Figure 7: Carbon-skeleton of stemarane diterpenes.



33

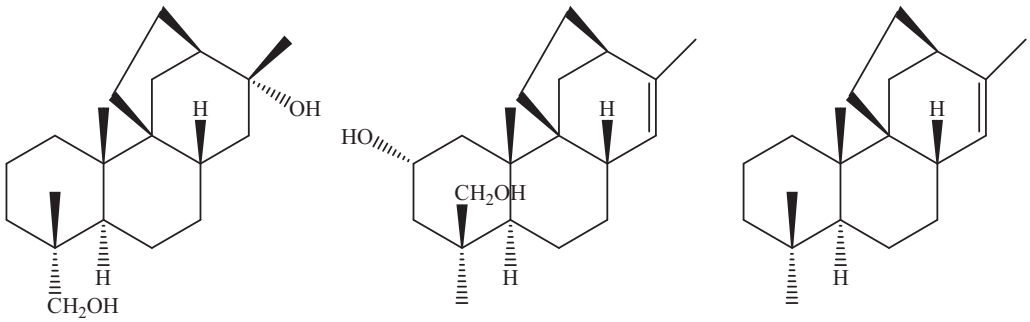
34 R_1 = OH, R_2 = R_3 = H
35 R_1 = R_2 = O, R_3 = H
36 R_1 = R_2 = R_3 = H
37 R_1 = R_2 = H, R_3 = OH

38

Figure 8: Stemarane, stemodane, and aphidicolane diterpenes.

Stemarane diterpenes are mainly found in plants, where they show several important roles such as defensive properties, and resistance to diseases caused by fungi and bacteria.

Figure 9: *Stemodia maritima* L. (left) and schematic representation of the *Calceolaria* genus (right).



33 **39** **40**

Figure 10: Structures of the stemarane diterpene: Stemarin 33, Oryzalexin S 39, and Stemar-13-ene 40.

The plant *Oryza Sativa* L., belonging to the *Poaceae* family[17], produces an important stemarane diterpenes, the phytoalexin oryzalexin S **39**, whose synthesis induction is stimulated by the pathogen attack of the rice blast fungus *Pyricularia oryzae* Cav. (the teleomorph is named *Magnaporthe grisea*), or by irradiation with UV-ray or treatment with heavy metals (Hg, Cu)(Figure 11). As mentioned before, Oryzalexin S **39** (Figure 10) was isolated in 1992 by Kodama and coworkers[17] by UV irradiation of the plant *Oryza sativa* L.

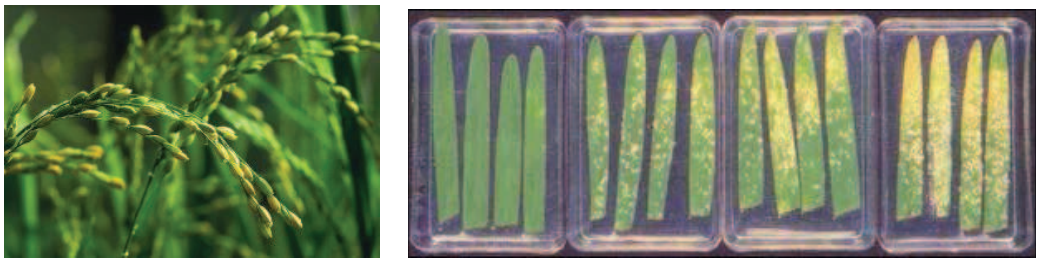


Figure 11: *Oryza sativa* L., rice plant and *Magnaporthe grisea* infected leaves.

In 2001 Oikawa and coworkers reported the isolation of stemar-13-ene **40**[18] (Figure 10) from the phytopathogenic fungus *Phoma betae* (Figure 12), and it is the only diterpene present in the fungi reign. The phytopathogenic fungus *Phoma betae*[19] also produces aphidicolin **38** together with less oxidized intermediates[20]. This co-occurrence of diterpenes in the *Phoma betae* fungus is especially noteworthy not only from a

biosynthetic point of view but also because stemarane diterpenes were found previously only in plants.

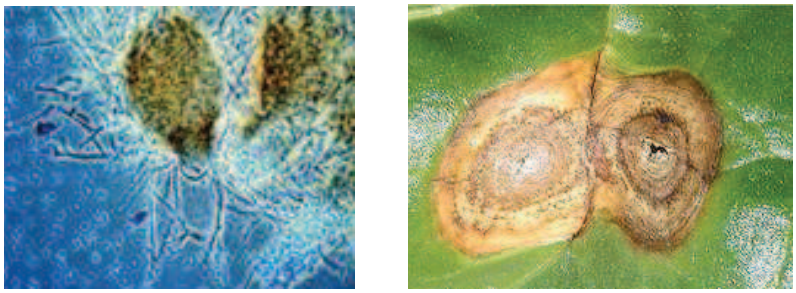


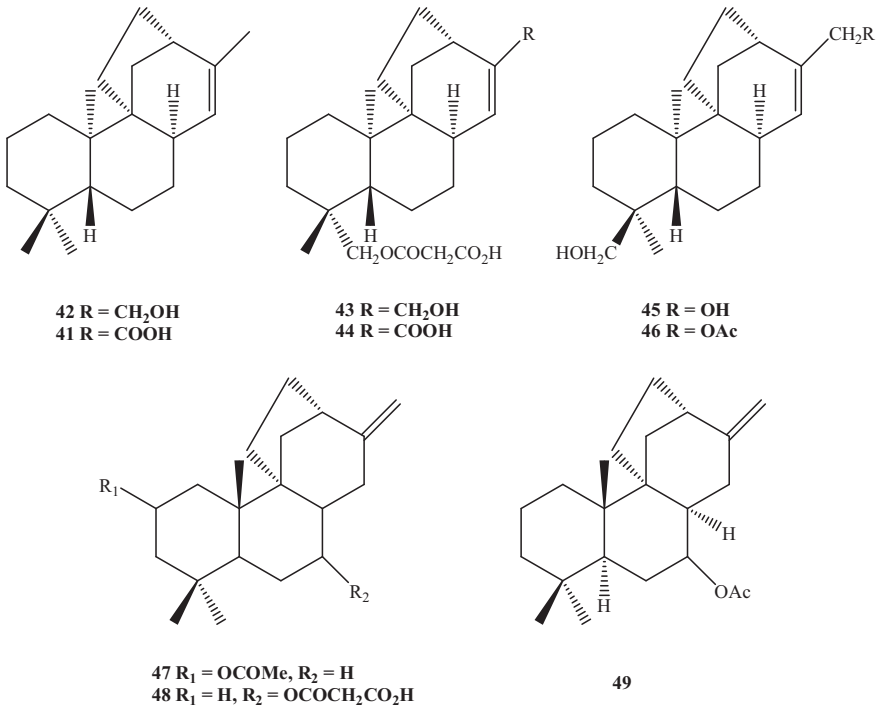
Figure 12: Fungus *Phoma betae*, (*left*) microscopic view and (*right*) naked-eye.

Stemarane diterpenes are also found in plants from the Calceolaria genus belonging to the *Scrophulariaceae* family (*Calceolaria Lepida*[21], *Calceolaria Latifolia*[22], *Calceolaria Kingii Phil.*[23], *Calceolaria Polifolia*[24], *Calceolaria Glabrata*[25], and *Calceolaria Dentata*[26])(Figure 13). The Calceolaria is one of the most abundant genus in the *Scrophulariaceae* family. There are more than 500 species distributed throughout New Zealand and especially Central and South America[27]; 86 species grow in Chile[28], which are used sometimes in popular medicine as stomach tonics, bactericidal agents and sweeteners.

Figure 13: *Calceolaria Polifolia* (left) and *Calceolaria Dentata* (right).

Several stemarane diterpenes was isolated by Garbarino and coworkers from Chilean plants of the *Calceolaria* genus (Universidad Tecnica Federico Santa Maria, Valparaiso,

Chile) (Figure 14). In particular, from a plant that grows in the coastal hills of central Chile, *Calceolaria Lepida*, Garbarino and coll.[21] isolated two new stemarane diterpenes: *ent*-stemar-13(14)-en-19-oic acid **41** and *ent*-stemar-13(14)-en-19-ol **42** (Figure 14). The last compound was also isolated from a plant that grows on hills of north and central Chile, *Calceolaria Latifolia* Benth.[22], and from a species which grows on the coastal hills of central Chile, *Calceolaria Polifolia*[24] (Figure 13). Furthermore, during these studies, has been reported the isolation and characterization of three new stemarane derivatives: 19-malonyloxy-*ent*-stemar-13(14)-ene **43** and 17-acetoxy-19-malonyloxy-*ent*-stemar-13(14)-ene **44** from *Calceolaria Polifolia*[24] and *ent*-stemar-13(14)-en-18-ol **45** from *Calceolaria Latifolia*[22]. From a plant which grows in north and central Chile, *Calceolaria Kingii* Phil., *ent*-stemara-13(14)-en-17-acetoxy-18-ol **46** has been isolated and then structurally elucidated[23]. From a perennial plant which grows in the middle-South of Chile, *Calceolaria Dentata* (Figure 14), 2-acetoxy-13-methylene-stemarane **47** and 7-malonyloxy-13-methylene-stemarane **48** were isolated and then structurally elucidated by spectroscopic analyses[26]. In the same way, from *Calceolaria Glabrata*, a shrub common in the southern part of Chile, the diterpene 13-methylene-7-acetoxy-stemarane **49** was isolated and then structurally elucidated[25].



42 R = CH_2OH
41 R = COOH

43 R = CH_2OH
44 R = COOH

45 R = OH
46 R = OAc

47 R_1 = OCOMe, R_2 = H
48 R_1 = H, R_2 = $OCOCH_2CO_2H$

49

Figure 14: Stemarane diterpenes isolated by Garbarino and coworkers.

1.2.1. Structure of stemarane diterpenes

The stemarane diterpenes carbon skeleton is reported in Figure 15, and its main structural characteristics are reported below:

- the bicyclic system C/D is constituted by a bicyclo[3.2.1]octane fused to the bicyclic A/B system in a different fashion with respect to other tetracyclic diterpenes possessing the bicyclo[3.2.1]octane system;
- oxygenated functions can be present at C(2), C(7), C(13), C(17), C(18), and C(19);
- two contiguous quaternary carbon atoms, the C(9) and C(10), are present, the former being a spirocyclic atom.

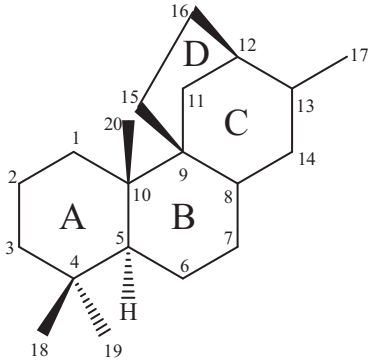
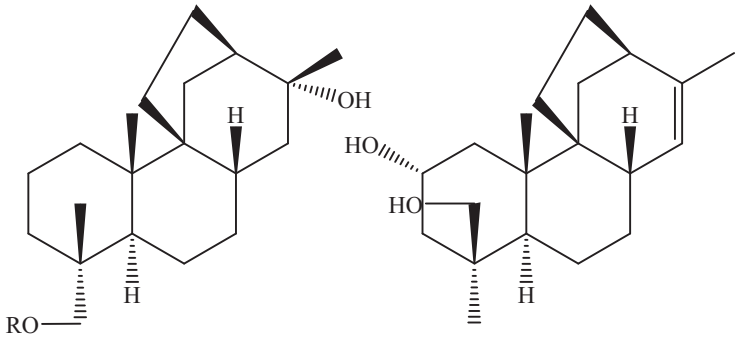


Figure 15: Stemarane diterpenes carbon skeleton

Only in the case of stemarin **33** the absolute configuration was determined by X-ray crystallographic analysis[8]. In particular, its absolute stereochemistry was determined through X-ray crystallographic analysis of its tosylate **33a** (Figure 16-17)**.**



33 R = H
33a R = $SO_2C_6H_4Me$-p

39

Figure 16: Structures of Stemarin 33, its tosylate 33a, and Oryzalexin S 39.

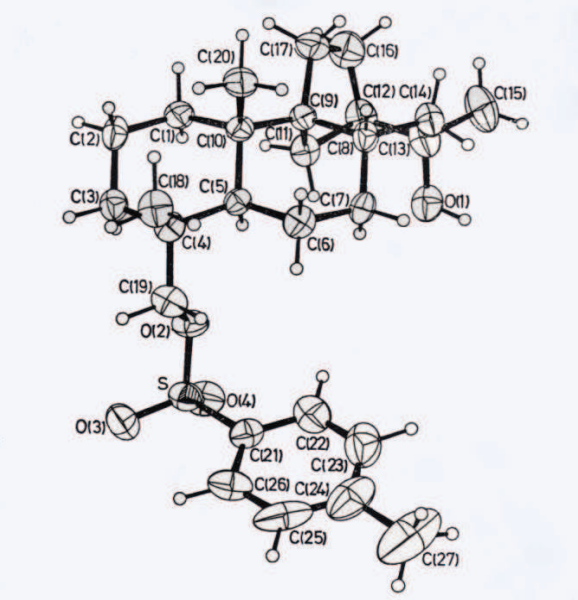


Figure 17: ORTEP drawing of stemarin tosylate 1a, image from P. S. Manchand, J. F. Blount, 1975, *J. Chem. Soc., Chem. Commun.*, 894).

On the contrary, oryzalexin S **39** absolute structure by means of crystallographic data is not available. The relative configuration of oryzalexin S **39** was established by a series of 2D-NMR experiments (^{13}C-^{1}H COSY, ^{1}H-^{1}H COSY, ^{13}C-^{1}H HOHAHA, ^{13}C-^{1}H COLOC and NOESY)[29], confirming the relative three-dimensional relationships between the functional groups (Figures 2.12-2.13).

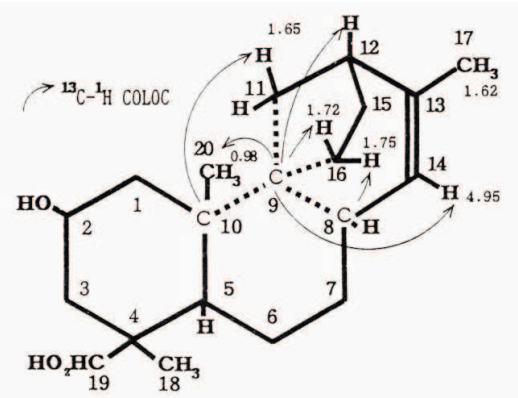


Figure 18: Structure of oryzalexin S, ^{13}C-^{1}H COLOC data (image from S. Tamogami, M. Mitani, O. Kodama, T. Akatsuka, 1993, *Tetrahedron*, *49*, 2025).

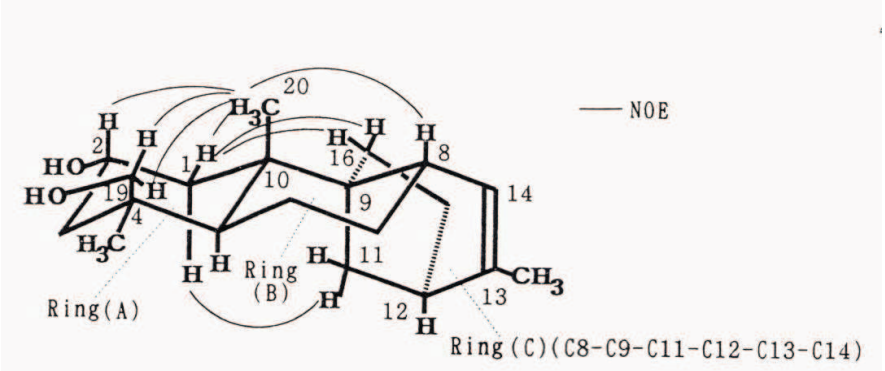
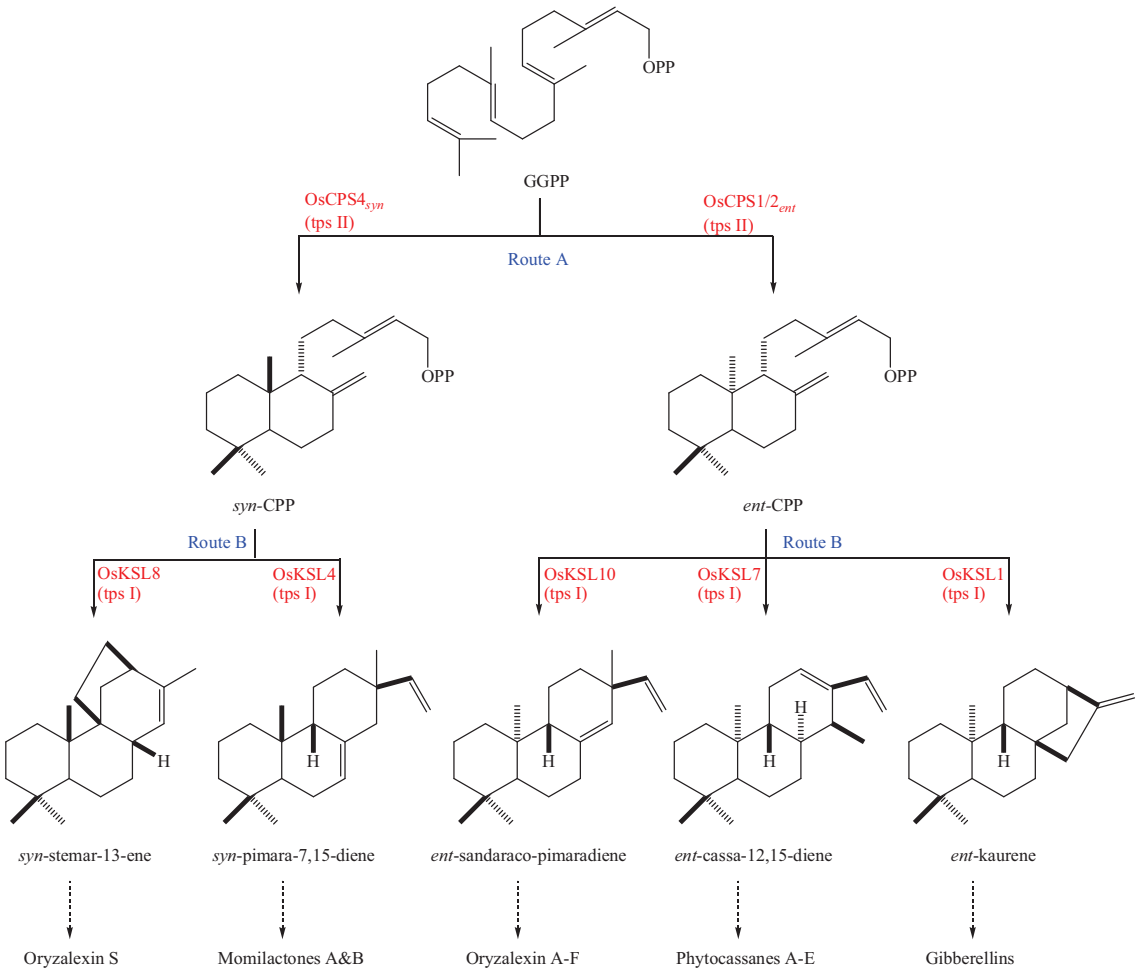


Figure 19: Structure of oryzalexin S, NOESY data (image from S. Tamogami, M. Mitani, O. Kodama, T. Akatsuka, 1993, *Tetrahedron*, *49*, 2025).

1.2.2. Biogenesis of stemarane diterpenes

Stemarane diterpenes biogenesis was deeply studied in the past years particularly in rice plants, since rice provides a model system to investigate labdane-related diterpenoid biosynthesis, as this genomically characterized plant[30] produces a number of such natural products, many of which are terpenoids (this class of compounds comprises, as discussed above, the largest class of natural products with nearly 30000 known members[31], among which labdane-related diterpenoids constitute over 10% of all of them).

As shown in scheme 2, rice became a model system for investigating the labdane-related diterpenoids metabolism. Biosynthesis of labdane-related diterpenoids is characterized by two subsequent cyclizations (route A and route B), catalyzed, respectively, by class II and class I terpene synthases.



Scheme 2: Known cyclization steps in rice. The corresponding enzymes are indicated, together with their products and the derived natural products.

In addition to the ubiquitous gibberellic acid phytohormones, rice produces more than 10 other labdane-related diterpenoids, which serve as phytoalexins[32,17], antibiotic compounds produced in response to microbial infection[33], and allelochemicals[34], secreted from the roots to suppress germination of neighbouring seeds[35]. The known rice diterpenoids fall into five structurally related families: momilactones A and B, phytocassanes A-E, gibberellins, oryzalexins A-F, and oryzalexin S **39**. They are polycyclic diterpenes characterized, respectively, by five different structures: *syn*-pimara-7,15-diene, *ent*-sandaracopimara-8(14),15-diene, *ent*-cassa-12,15-diene, *ent*-kaurene, and *syn*-stemar-13-ene, which is the stemarane diterpenes biogenetic

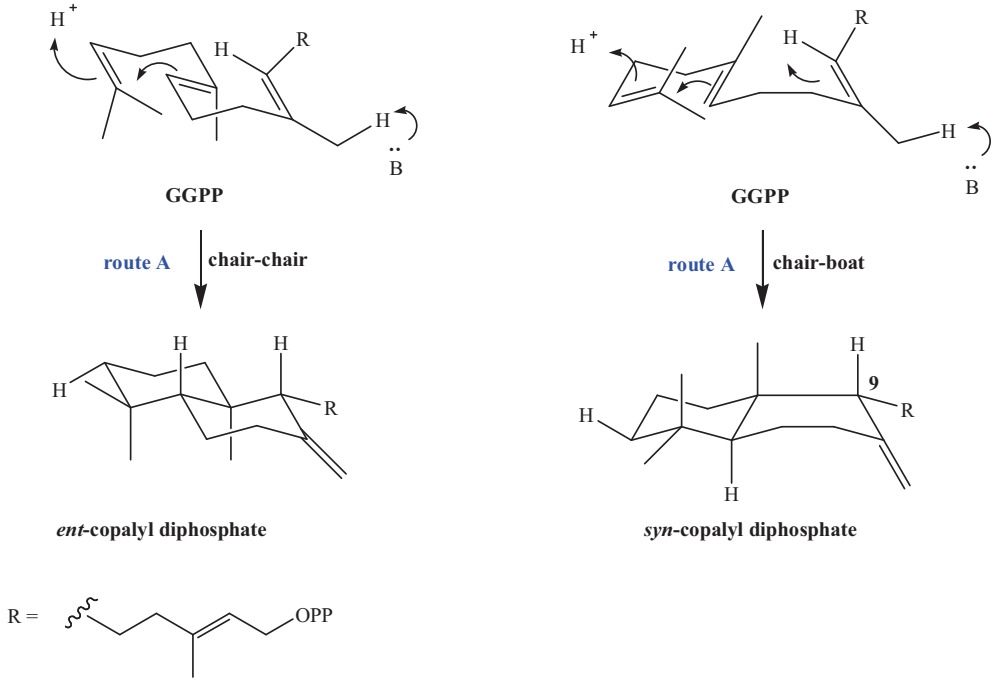
precursor[36]. Class II diterpene cyclases produce specific stereoisomers of labdanienyl/copalyl diphosphate (CPP)* from the universal diterpenoid precursor (E,E,E)-geranylgeranyl diphosphate (GGPP)[37]. This bicyclic core structure can then be further cyclised and/or rearranged by more typical (i.e., class I) CPP stereospecic terpene synthases to form various skeletal structures[38]. While prototypical plant class I terpene synthases contain two structurally defined domains[39], those involved in labdane-related diterpenoid metabolism invariably contain an additional N-terminal sequence (>>240 amino acids) termed the 'insertional' element[40].

However, whereas the class II cyclases contain a DXDD motif required for their protonation-initiated cyclization reactions[41], the class I enzymes contain a separately placed DDXXD motif, which is involved in the ligation of the divalent metal ion binding, required for the corresponding diphosphate ionization initiated reaction[42]. From the known sequence information available for rice, four class II and ten class I labdane-related diterpene synthases have been predicted. The class II genes were termed OsCPS1–4[43], and all the active class II enzymes produce CPP. Specifically, OsCPS1 and OsCPS2 produce *ent*-CPP for gibberellins, phytocassanes, and oryzalexins A-F biosynthesis, respectively, while OsCPS4 produces *syn*-CPP, and OsCPS3 is a pseudo-gene[44].

The class I genes were assigned as OsKS1-10[43,45], and only OsKS1 actually operates in gibberellin biosynthesis and, presumably, produces *ent*-kaurene. The synthases responsible for production of *ent*-cassa-12,15-diene, *syn*pimara-7,15-diene, and *syn*-stemar-13-ene have also been termed OsDTC1, OsDTS2, and OsDTC2, respectively[40,46]. To avoid confusion, it was suggested[30c] for these non-kaurene producing class I genes the use of OsKSL (rice kaurene synthase-like), with the corresponding number from Sakamoto *et al.* (2004)[43], where appropriate. Thus, OsKS1 presumably produces *ent*-kaurene[43,47], OsKSL4 (OsDTS2) produces *syn*-pimaradiene[40,45], OsKSL7 (OsDTC1) produces *ent*-cassadiene[46a], OsKSL8 (OsDTC2) produces *syn*-stemarene[46b], and OsKSL10 produces *ent*-sandaracopimaradiene[45]

* *Abbreviations used:* CPP, copalyl diphosphate; GC, gas chromatography; GGPP, (*E*,*E*,*E*)-geranylgeranyl diphosphate; KS, kaurene synthase; MS, mass spectrometry; NMR, nuclear magnetic resonance; OsCPS, rice CPP synthase; OsKSL, rice kaurene synthase-like; RT-PCR, reverse-transcription polymerase chain reaction; RACE, rapid amplification of cDNA ends.

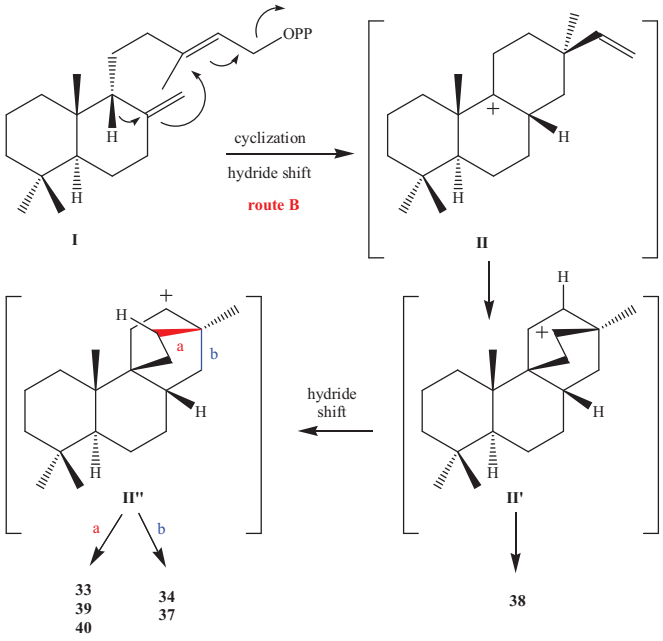
(Scheme 2). The different conformations that the universal diterpenoid precursor (E,E,E)-geranylgeranyl diphosphate (GGPP) can assume bring to different products in the first cyclization (Scheme 3, route A). Therefore, while the *chair-chair* conformation leads to the formation of *ent*-copalyl diphosphate, the *chair-boat* conformation leads to the formation of *syn*- copalyl diphosphate, also known as 9β-labdadienyl diphosphate for the beta orientation of H-C(9).



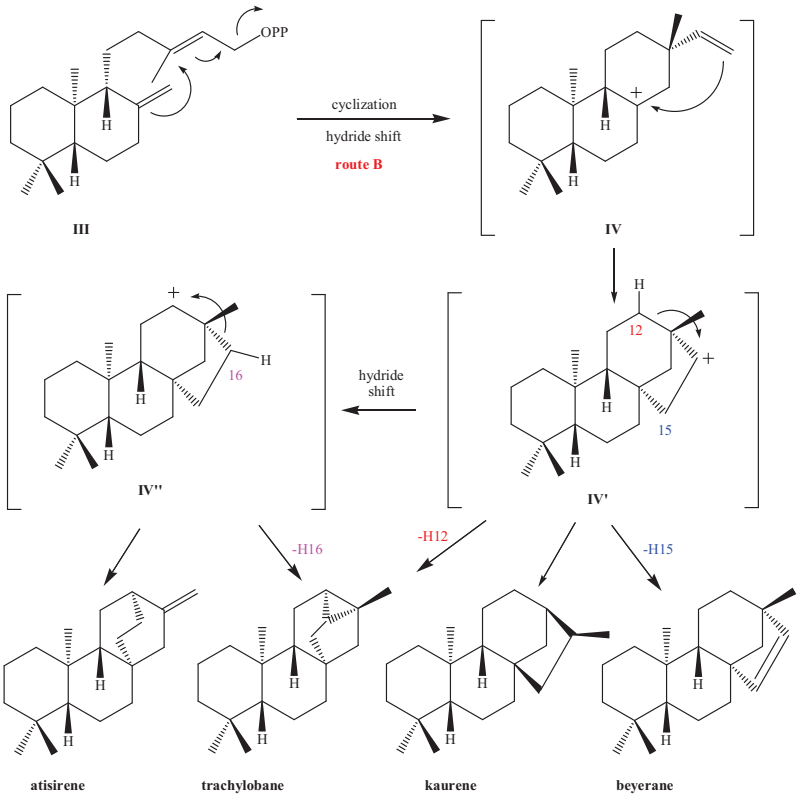
Scheme 3, route A: enzymatic cyclization of (E,E,E)-GGPP.

Diterpenes possessing a unique bicyclooctane skeleton in the C/D ring are divided into two classes: C9-ethano-bridged diterpenes (C9EBD) and C8-ethano-bridged diterpenes (C8EBD). C9EBD include compounds such as stemodin **34**, stemarin **33** and aphidicolin **38** (Scheme 4), whereas beyerane, kaurene, atisirene and trachylobane belong to C8EBD (Scheme 5). The cyclization route B would convert ent-copalyl diphosphate **III** and syn-copalyl diphosphate **I** into, respectively, transient pimarenyl intermediates **IV** and **II**, which after a series of rearrangements and proton shifts might form the diterpenes belonging to the two classes (Scheme 4[14b,48] and Scheme 5[49], respectively). As shown in scheme 4, aphidicolane, stemarane and stemodane skeletons

might derive from the same biogenetic pathway via compound **20**. The isolation of stemar-13-ene **40** in fungus *Phoma betae*[18] together with other biogenetic precursors of aphidicolin **38** confirms the above biosynthetic route. The proposed biogenetic pathway for oryzalexin S **39**[29] supports the idea that stemar-13-ene **40** is the probable hydrocarbon precursor of the stemarane phytoalexin; extracts of chitin-treated rice cells actually catalyze the biosynthesis of stemar-13-ene **40** from 9β–labdadienyl diphosphate **I**[30c].



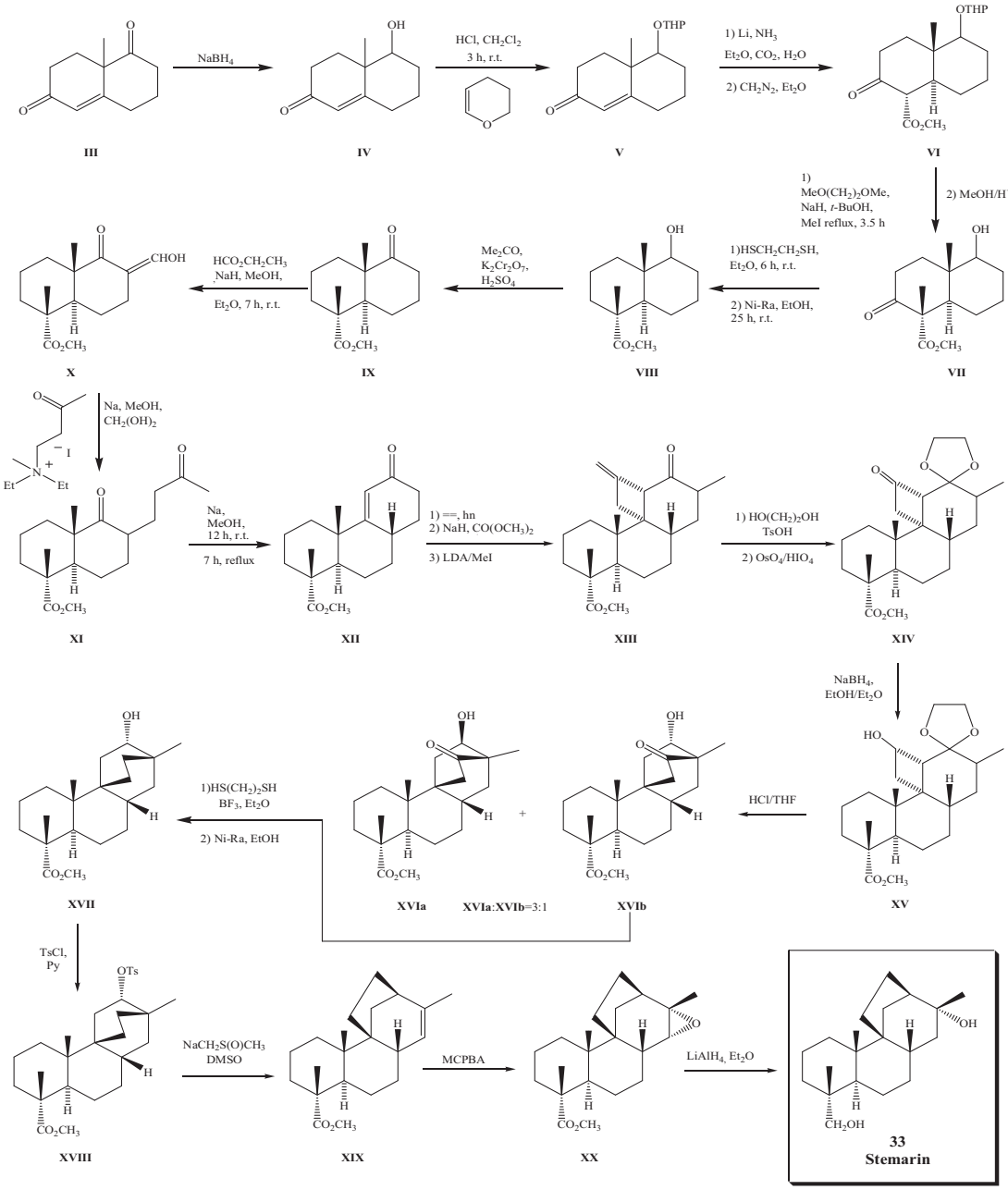
Scheme 4, route B: biosynthetic pathway to C9-ethano-bridged diterpenes.



Scheme 5, route B: biosynthetic pathway to C8-ethano-bridged diterpenes.

1.2.3. Previous synthesis of stemarane diterpenes

The first synthesis of a stemarane diterpene was reported in 1980 by Kelly and co-workers at the University of New Brunswick[67] who obtained stemarin **33** using as an intermediate tricyclic compound, previously synthesized by Spencer et al.[68] in racemic form in the course of the synthesis of the deisopropyldehydroabietic acid (scheme 6).



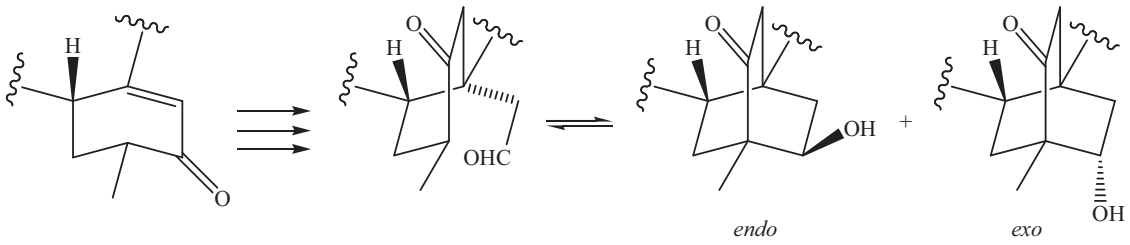
Scheme 6: Synthesis of (±)-stemarin 33.

The approach followed by Kelly is based on the method of photochemical annulation of Wiesner[69] and on the transposition of bicyclo [2.2.2] octane systems to bicyclo [3.2.1] octane systems[70] (Scheme 8).

According to the synthesis of Kelly, the diketone **III**, synthesized according to the known procedure of Robinson annulation[71], starting from 2-methyl-1,3-cycloesandione, it was selectively reduced to give the alcohol **IV**, according to the procedure of Boyce and Whitehurst[72] slightly modified from Spencer[73]. The compound **V** was obtained by reaction of the alcohol **IV**[74] with HCl and dihydropyran in dichloromethane. The next step was a "reductive carboxymethylation" of **V**, according to the methodology of Stork[75], that is a procedure that involves treatment with Li in liquid NH_3, followed by carbonation, acidification, and esterification with diazomethane. In particular, Spencer's procedure, which consists in the separation of the product of carbonation before acidification and subsequent esterification, has allowed to isolate directly the tetrahydropyranyl ether **VI**, which was then methylated to give **VII**. The compound **VII** is the majority product of the methylation β-ketoester **VI**, which is controlled stereoelettronically[76] in the reaction site by 1,3-diaxyal unfavorable interactions (by the β-methyl substituent, and two hydrogens 2β and 6β). In this conversion, in fact, in addition to β-methylation, which leads to an intermediate which has the stereochemistry of the abietic acid, the α-methylation was obtained, used by Spencer for the synthesis of podocarpic acid[77]. The conversion of **VII** to **IX** was achieved through the removal of the carbonyl group at C(3) (using *Raney*-Ni desulfurization with the corresponding thioacetal) and the subsequent oxidation of the alcohol group at C(9) with the Jones reagent[78].

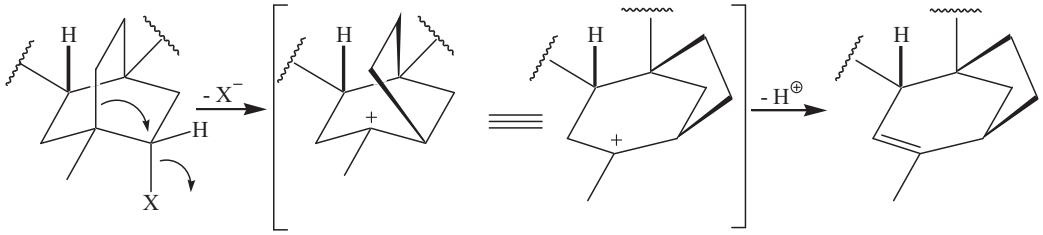
The formylation of the intermediate **X** and Robinson annulation led to the tricyclic enone **XII**, which was converted first by photochemical addition of allene and after by methylation to give methylencyclobutane **XIII**. After protection of the carbonyl group, the methylene group was subjected to oxidative cleaved, and the new carbonyl group **XIV** was quantitatively reduced to **XV**. By treatment of **XV** with diluted mineral acid, the deprotection of the carbonyl group was obtained, followed by a retroaldolic reaction, which was followed by a new aldol-condensation, leading to the formation of

the chetolo **XVIa** (in a ratio 3:1) and its epimer **XVIb** (differentiated by the different orientation of the hydroxyl group) (Scheme 7) .



Scheme 7 : Intramolecular aldol condensation.

Only the second epimer has the hydroxyl group oriented correctly for the subsequent transposition to stemaranic system (Scheme 8).



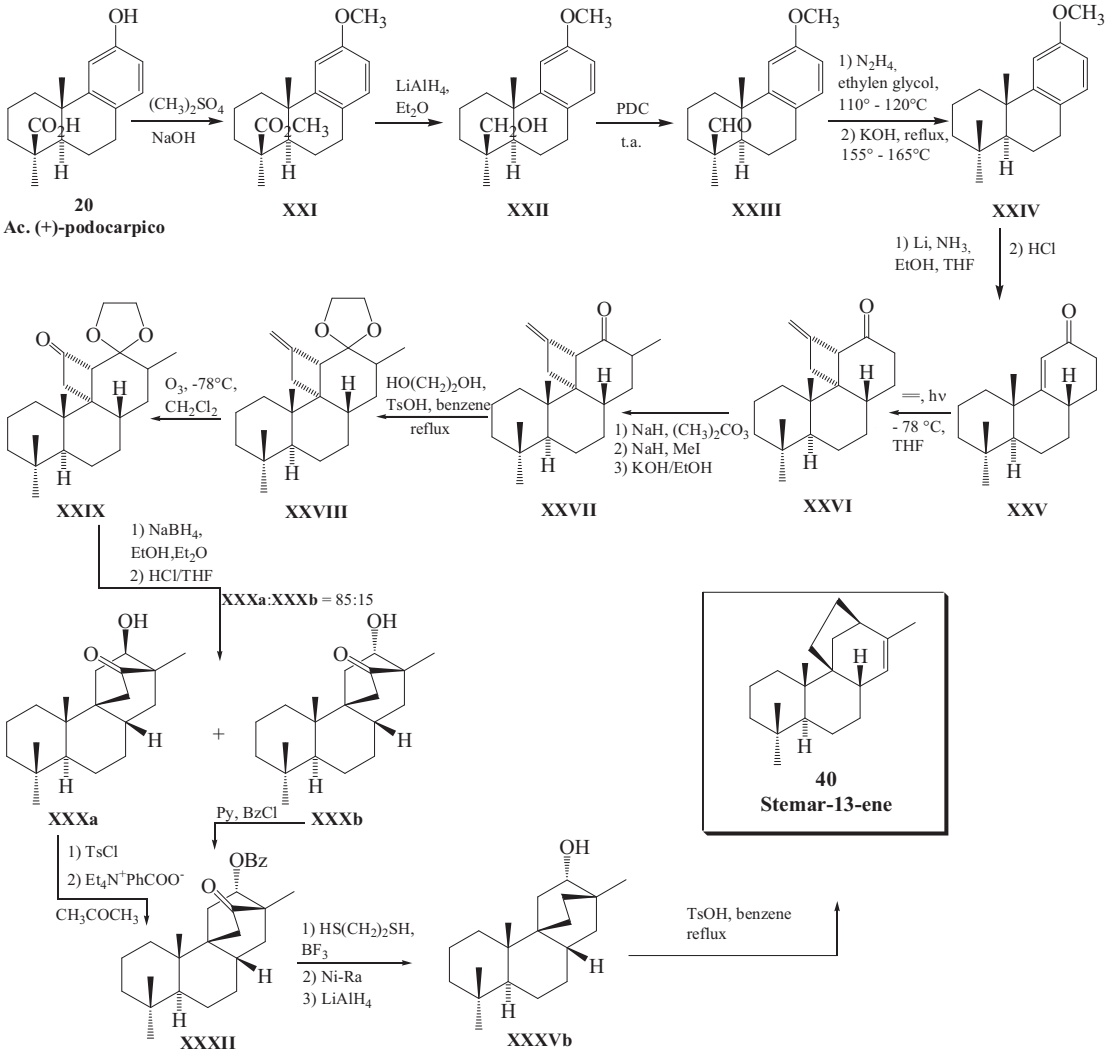
Schema 8 : Solvolitic rearrangement.

The deoxygenation of compound **XVIb** led to alcohol **XVII**, from which, by means of the solvolitic rearrangement reaction of its tosylate **XVIII**, the compound **XIX** was obtained. The conversion of **XIX** into stemarin **33** was obtained by stereoselective epoxydation and subsequent reduction of the ester group to a primary alcohol and by the reduction of the epoxide to tertiary alcohol.

This synthesis has a weaknesses: as can be seen in scheme 6, the aldol condensation gives two products: the epimer **XVIa**, in which the hydroxyl group is *endo*, and the other epimer **XVIb** in which the hydroxyl group is *exo* (in a ratio 3:1). Only the minority epimer has the HO-C(12) correctly oriented for subsequent rearrangement reaction to stemaranic system. The solvolitic rearrangement, in fact, is characterized by stereoelectronic requirements: the bond that migrates is the antiperiplanar bond regard

the leaving group (Scheme 7). the synthesis of Kelly proceeds only with the minor epimer of the aldol condensation (**XVIb**) and this entails a serious loss of efficiency.

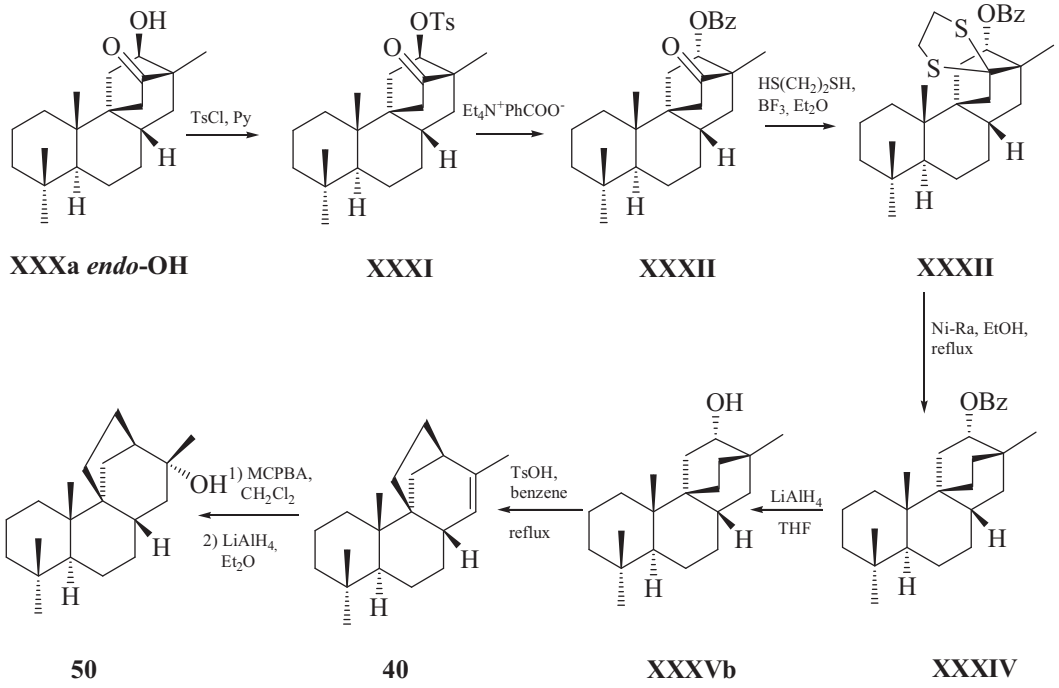
The second synthesis of a stemarane compound, stemar-13-ene **40**, was acheved in the laboratory where I've done this PhD thesis (Scheme 9). This summary is born from the need to overcome the difficulties encountered by Kelly in obtaining key intermediate bicyclo [2.2.2]-2-eightyolico.



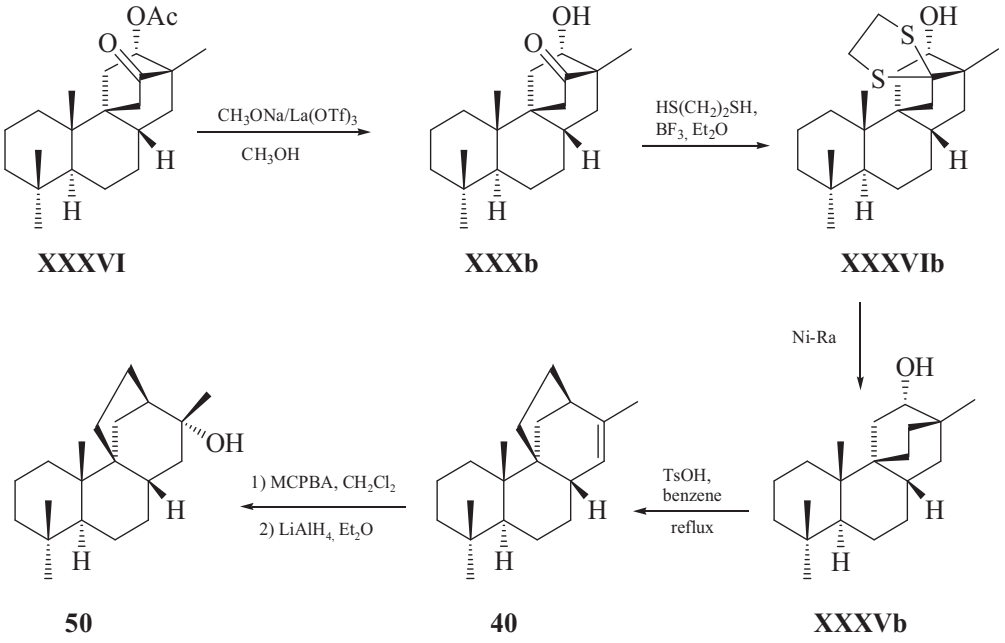
Schema 9 : Synthesis of (+)-stemar-13-ene 40.

The starting material was the natural product (+)-podocarpic acid, which was converted into desired enone **XXV** according to the methodology of Wenkert[79], which

provides the methylation of phenol group and the acid group to give homologous anisole esterified[80], the reduction of the carboxylic group with $LiAlH_4$[81], the oxidation of primary aliphatic hydroxyl group to give the homologous aldehyde, the removal of the aldehyde group by Wolff-Kishner reaction and finally the Birch reduction of the anisole to give the compound **XXV**. The latter was then transformed into photoadduct **XXVI**, which was then converted into the mixture of ketols **XXXa** and **XXXb** which was separated chromatographically. The ketol *endo* **XXXa** was then converted into the benzoate *exo* **XXXII** by treatment of its tosylate **XXXI** with $Et_4N^+PhCOO^-$, taking average of the effect electron withdrawing of the adjacent carbonyl group to the bridgehead position which does not allow the rearrangement[82]. The bicyclo[2.2.2]eighty-2-ol **XXXVb** was then obtained in two ways: from the ketoester **XXXII** for deoxygenation followed by removal of the benzoate with $LiAlH_4$ (Scheme 10), or for methanolysis[83] of the acetate **XXVI** (which was prepared from the ketol **XXXa** by inversion of configuration of the tosylate **XXXI**, followed by deoxygenation) (Scheme 11). The (+)-stemar-13-ene **40** was then obtained by treatment of the bicyclo[2.2.2]eighty-2-ol 30b with TsOH in benzene.



Scheme 10 : Trasformation of the ketol XXXa into (+)-stemar-13-ene 40 and into (+)-18-deoxystemarin 50.



XXXVI **XXXb** **XXXVIb**

XXXVb **40** **50**

Scheme 11 : Trasformation of the intermediate XXXVI into (+)-stemar-13-ene 40 and into (+)-18-deoxystemarin 50

1.2.4. Stemarane diterpenes biological activity

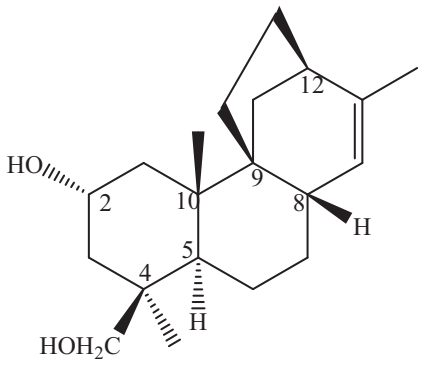
Stemarane diterpenes biological activity was not as deep evaluated as for other diterpenes, resulting in scarce literature references. An exception is found for the phytoalexin oryzalexin S **39**[17], whose antimicrobial activity was evaluated. As previously reported, the diterpenes were deeply studied in the past years especially in rice plants, with particular interest toward rice phytoalexins, not only from a biological activity point of view, but even for a better understanding of the phytoalexin mechanism of action, still unclear for most of the aspects.

1.2.5. Oryzalexin S

Orizalessina S **39** is a tetracyclic diterpene (Figure 20). It was isolated from Rice plant, *Oriza sativa* L., in 1992 by Kodama et al. at Ibaraki University (Japan) and its molecular structure was established by the same research group. We don't know the absolute configuration of the stereogenic centers but we know only the relative configuration, by means of a series of NMR experiments (^{13}C-^{1}H COSY, ^{1}H-^{1}H COSY, ^{13}C-^{1}H HOHAHA, ^{13}C-1H COLOC and NOESY)[50]. These experiments have allowed to establish the structural characteristics of this molecule:

- has a stemarane diterpene skeleton where the bicyclic system C/D is costituited by a bicycle[3.2.1]octanic system;
- there are seven stereogenic centers, five of which are contiguous [C (4), C (5), C (10), C (9) and C (8)], and two of which are adjacent quaternary carbons [C (9) and C (10)]

Orizalessina S **39** was obtained from rice by UV irradiation. As known, this radiation stimulates the production of phytoalexins. After irradiation the rice was leaves in a incubation box with high humidity condition for 3 days. Then by chromatography the different phytoalexins formed were separated: from 200 g of rice leafs, 10.9 mg of orizalessina S **39** were obtained[51]. The fact that this molecule have interesting biological activity, which is obtainable in small quantities from the natural source and which has a skeleton complex, making it a valid target synthetic.



39

Figure 20: Oryzalexin S

An efficient Oryzalexin S synthesis, in fact, not only would be a success from the academic point of view, but also allow to study the structure-activity relationships. Now, Oryzalexin S synthesis in literature are not reported, on the contrary have been developed several synthesis of other stemarane diterpenes.

1.2.6. Rice phytoalexin biological activity

Among the diterpene compounds, the most cited active ones are the kaurane derivatives for which, diverse biological activities have been described, including plant growth regulating, antimicrobial, antiparasitic, insect antifeedant, cytotoxic, antitumor, anti-HIV, steroidogenic, antifertility, hypotensive and antiinflammatory activities, described in the review published by Ghisalberti in 1997[52]. Some of these kaurane biological effects have also been cited in recent literature[53].

Considering the biological activity of stemarane diterpenes, it was not as deeply studied as for the other diterpenes, thus resulting in a few literature citations. The most common and the most studied biological activity is the antimicrobial one, and in particular, the phytoalexin activity of oryzalexin S **39**.

Phytoalexins from rice mainly involve two types of diterpenes and flavones, including momilactones A and B, oryzalexins A-F, phytocassanes A-E, sakuranetin, and the stemarane diterpene oryzalexin S **39**[54] (Figure 21). The first phytoalexin with diterpene structure isolated were the momilattoni A and B in 1981[32a], followed by oryzalexin A-F and S[29-32b,c,d-59a-64], and five phytocassanes[32h-65].The momilattoni A and B and the orizalessine A-F were classified as pimarane diterpenes, the sakuranetin is a flavanoid, while the oryzalexin S belongs to the class of stemarane diterpenes. Momilactones A and B, among the plants belonging to the *Gramineae* family, were initially isolated as plant inhibitors from rice husk[55], and were afterwards found to be produced in plant rice in response to either by the pathogen *P. oryzae* infection or by UV light irradiation[56,32a]. More recently, momilactones A and B have been found in rice root exudates, which participate in the defence against weeds[57].

Sakuranetin was identified from rice plants infected by *P. oryzae*, and it showed high antifungal activity and a large amount of accumulation in rice leaves[58]. Oryzalexins A-F and S were isolated from rice leaves infected by *P. oryzae*[59,17]. Oryzalexins A-C strongly inhibited the spore germination of *P. Oryzae*: their ED_{50} values were 130, 68 and 136 ppm, respectively. Complete inhibition of *P. oryzae* spore germination was observed at 200 ppm. Moreover, Oryzalexins A-C strongly suppressed the germ tube elongation of *P. oryzae*; indeed their ED_{50} values on *P. oryzae* germ tube elongation were 35, 18 and 35 ppm, respectively[59a].

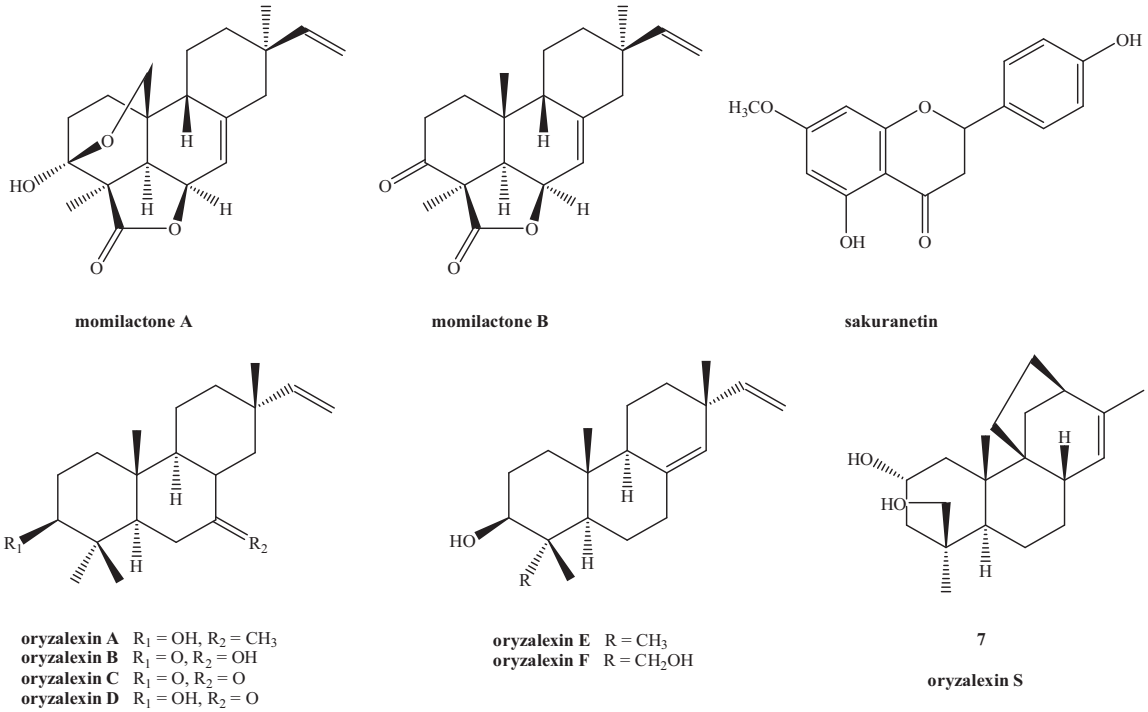


Figure 21: Common rice phytoalexins.

In the same way, Oryzalexins D-F and S significantly inhibited spore germination of the rice blast fungus[59b-c,17]. Noteworthy, Oryzalexins E had slightly lower antifungal activity than Oryzalexins D, but higher than Oryzalexins A-C[59c]. In 1987 some chemists from the University of Ibaraki investigated the different activities of the enantiomers of Oryzalexins A-D, showing the higher antifungal activity of the (+)-enantiomers and

thus elucidating the great importance of the absolute configuration of these natural compounds[60].

Phytocassanes A-E are produced by the rice leaves and stems infected with the fungi *P. oryzae* and *R. solani*, exhibiting high activity against these pathogenic fungi as shown by the low value of ED_{50}, reported in Figure 22[32h].

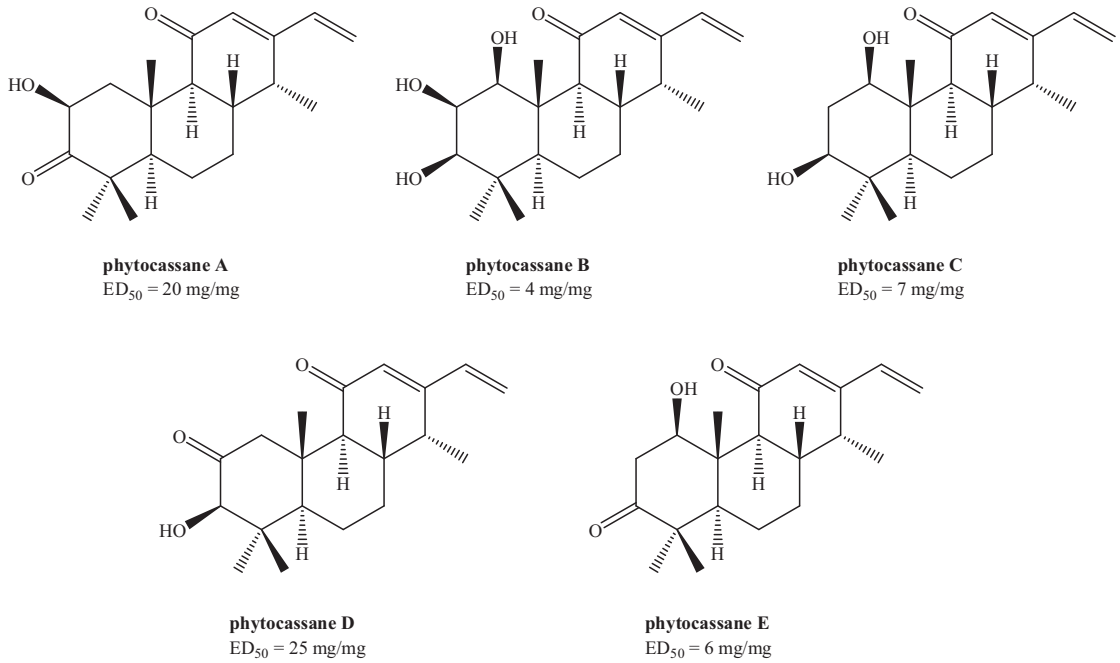


Figure 22: Structures of phytocassanes A-E and their antifungal activities (the ED_{50} values) on spore germination of *P. oryzae*.

The relative small amounts of these substances released by the plant, the small knowledge of the mechanism of action of phytoalexins, and the disease resistance are the main difficulties in determining the antifungal activities of rice phytoalexins, and hence of the stemarane diterpene oryzalexin S **39**. An increasing number of studies have shown that rice phytoalexins are induced by elicitors that are produced by pathogenic microorganisms and determine a field disease control by inducing the pathogen defence mechanism in rice[61]. Elicitors have been investigated extensively, and it has been demonstrated that jasmonic acid and its related compounds play an important role as signalling molecules eliciting the phytoalexins production in rice. Sakuranetin

production, for example, may be elicited by exogenously applied jasmonic acid in rice leaves, and its production by exogenously applied jasmonic acid was significantly counteracted by amino acids, cytokines, kinetin and zeatin[61b-c].

It has been shown in host-pathogen interactions that resistance reactions can be triggered by a large number of abiotic and biotic factors. Among the chemical factors, macromolecules of microbial origin are very important, stimulating plant defence responses at very low concentrations[62]. It was confirmed that some chemical substances may induce plant disease control by activating the natural resistance mechanisms of the host (for example, 2,2-dichloro-3,3-dimethyl cyclopropane carboxylic acid may exert its systemic fungicidal activity against the rice blast disease caused by *P. Oryzae*[63]). The application of methionine on wounded rice leaves induced the production of rice phytoalexins, sakuranetin and momilactone A. In the paddy field, methionine treatment has been demonstrated to reduce rice blast[58].

1.2.7. Photochemistry

At the beginning of the twentieth century G. Ciamician, in an article published in the journal Science[66], examined the problem of the development of photochemistry. After facing the question about the use of photochemical processes in plants (imagining a future in which the plants were grown in order to obtain their chemical compounds producted by photochemical processes), Ciamician wondered what would be the future development of photochemistry, which at that period was only at an early stage, and that could have been widen to different sectors in the years to come.

He also realized that often the photochemical behaved in a manner not easily predictable, unlike the ordinary reactions of organic chemistry, but remained extremely confident of the benefits that this new technique would lead to chemists of the future. At a time when the industry exploited the coal, was the first to talk about green chemistry and the use of solar energy.

On a very general photochemical processes involve two steps: 1. the production of an excited state and 2. events that determine the fate of the excited state. The absorption of

a quantum of radiation in the UV -VIS produces a high-energy species that dissipates its energy using mainly two fundamental processes of deactivation : 1. a chemical reaction that leads to a photoproduct and 2. a physical process that restores the ground state.

Absorbing light, molecules reach an electronically excited state. As a result, the distribution of electrons in the molecules is significantly different at these states when compared to the ground state.

In some cases, using photochemical steps significantly shortens a total synthesis, and frequently comple, polycyclic, or highly functionalized structures can be obtained from simple substrates. Photochemical substrate activation often occurs without additional reagents, which diminishes formation of byproducts. Due to this fact, photochemical reactions become particularly interesting in the context of green chemistry. Some of these reactions can be carried out with visible light or sunlight as a renewable energy source. Using light as a reagent also facilitates transformations inside supramolecular structures or crystals. Such fragile structures are not decomposed by aggressive reagents or heating. These transformations are often carried out in order to control the stereoselectivity of photochemical reactions; Irradiation with UV or visible light frequently improves the yields of metallocatalyzed reactions. For instance, ligand-exchange steps are often accelerated, and irradiation with light has become part of the standard reaction conditions. Irradiation is also used to initiate radical chain processes under mild conditions. Thus, particularly complex radical reactions such as certain radical tandem or multicomponent transformations have become possible.

1.2.7.1. [2+2]Photocycloaddition of α,β unsatured carbonyl compounds to alkenes: some examples on the total synthesis of certain natural compounds

Among photochemical reactions, the [2+2] photocycloaddition of α,β-unsaturated ketones or esters to alkenes, alkynes, or allenes leading to cyclobutanes[84] is certainly the most applied reaction in organic synthesis[85-88]. With α,β-unsaturated ketones, the reaction can be induced by simple light absorption. In many cases, the unsaturated

carbonyl compounds react at the $^3\pi\pi^*$ state with alkenes and 1,4-biradical intermediates are generated. Their behavior significantly affects the outcome of the reactions[86], particularly influencing the stereo- and regioselectivity. As shown in the examples reported below, through photocycloaddition reactions you can obtain complex structures in only one step without using and/or toxic reagent and this considerably simplifies the total synthesis of complex molecules such as natural products.

The [2+2] photocycloaddition of various α,β-unsaturated lactones such as **50** (Figure 23) has been studied in the context of an application to the total synthesis of Solanoeclepin A. As indicated by the arrows, only the crossed adduct was isolated. In one step, the tricyclic fragment containing a cyclobutane unit was obtained with the required relative configuration.

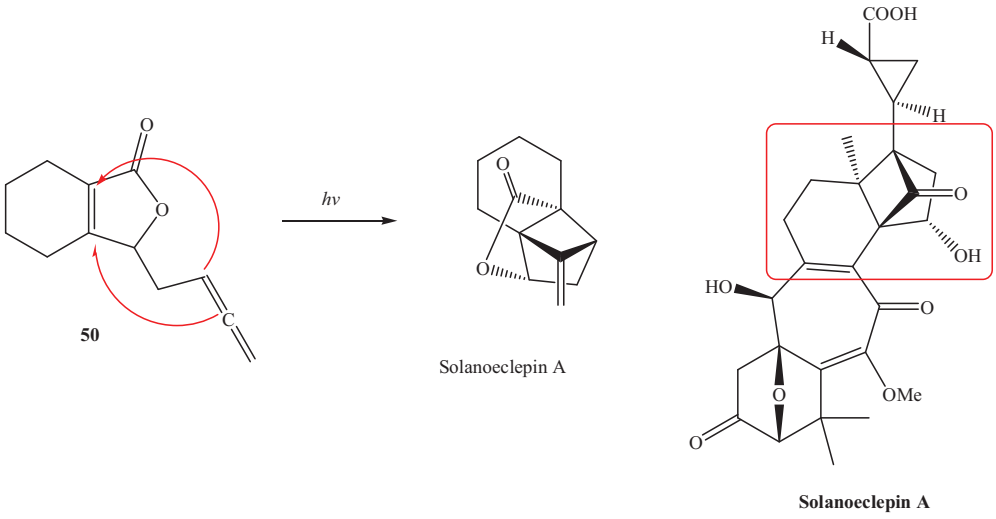


Figure 23

In an analogous approach, the dioxyenone **51** (Figure 24) was transformed into **52** via a [2+2] photocycloaddition. Compound **52** was then used as an intermediate in the first total synthesis of ingenol (Figure 24), a diterpenoid with unique architecture and has derivatives possessing important anticancer activity[89].

Another example of this reactions, reported in figure 24, is the first total synthesis of (+)-2β-hydroxysolanascone, the aglycone of the phytoalexin 2β-hydroxysolanascone-β-glucopyranoside isolated from flue-cured tobacco leaves (*N. tabacum* L., OCL), which possess antibacterial activity[90].

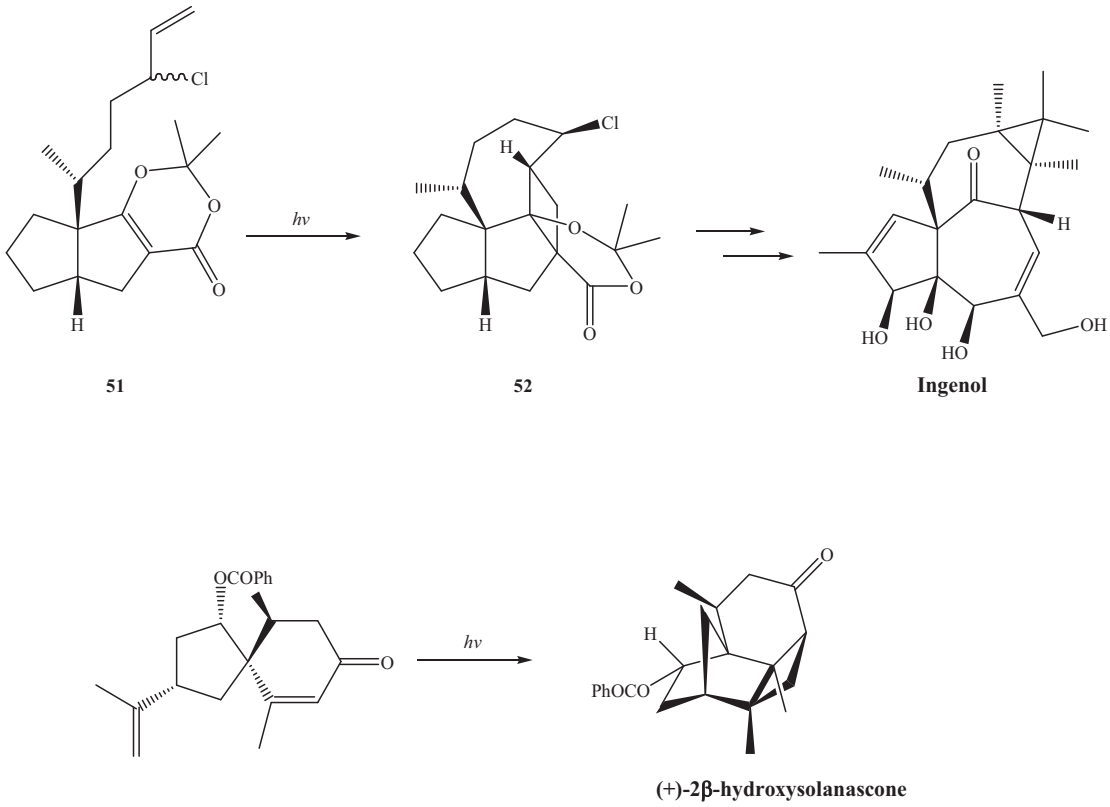


Figure 24

A [2+2] photocycloaddition was also used, by Crimmins et al., as key step in the synthesis of Ginkgolide B 1 (Figure 25), a natural product which possess a propellane core structure, and photochemical reactions have frequently been applied for the synthesis of such derivatives[91].

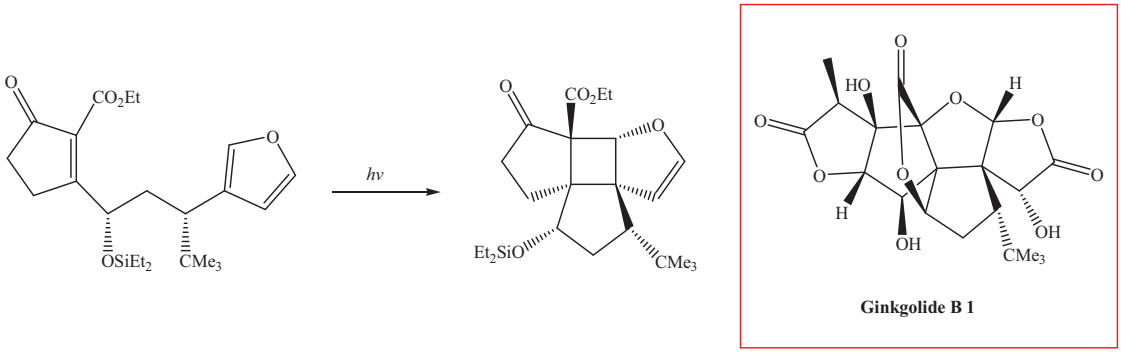
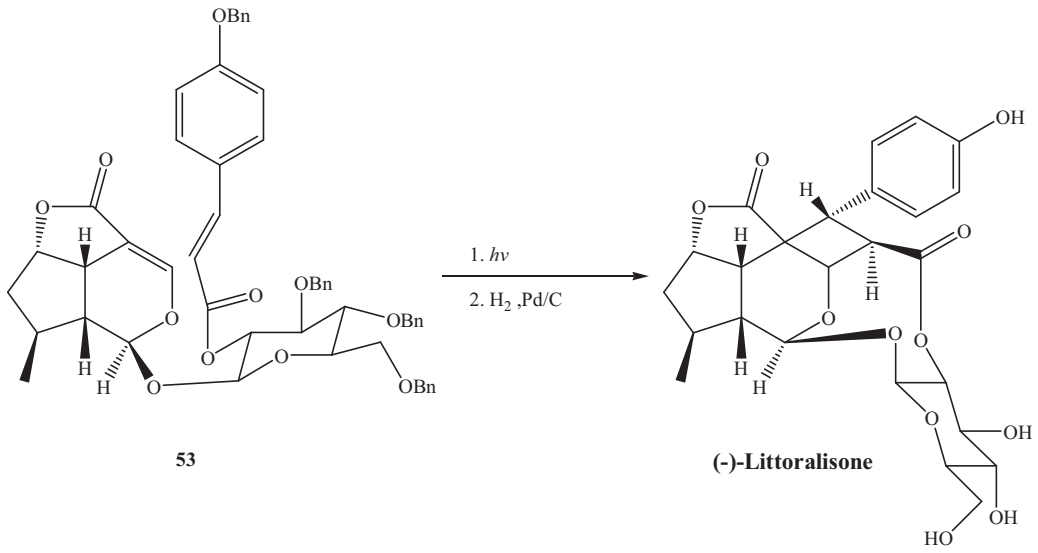


Figure 25: Crimmins' key step in the synthesis of Ginkgolide B 1.

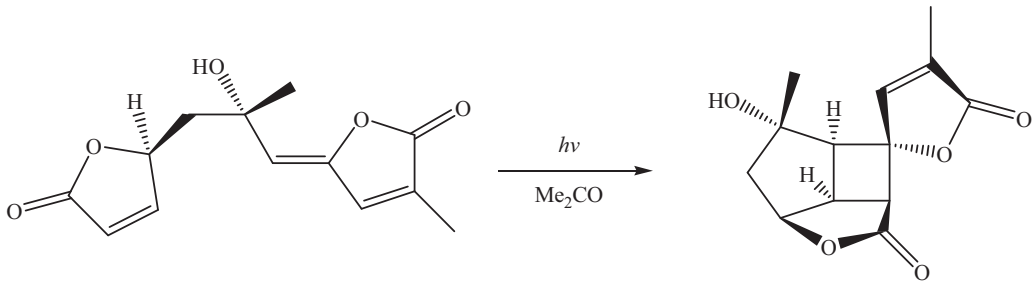
Under similar conditions, Mangion and MacMillan, reported the conversion in high yield of the cinnamic acid derivative **53** into (-)-Littoralisone via an interamolecular [2+2] photocycloaddition followed by hydrogenation (Figure 26).



53 **(-)-Littoralisone**

Figure 26: Final step of a synthesis of (-)-Littoralisone

Recently, Doroh and Sulikowski, studied the intramolecular [2+2] photocycloaddition of 5-alkylidene-2(5H)-furanone as a key step in the synthesis of Bielschowskysin (Figure 27), a diterpene possessing a [9.3.0.0] tetradecane ring system with 11 stereocenters.



5-alkylidene-2(5H)-furanone **Bielschowskysin**

Figure 27: Key step in the synthesis of Bielschowskysin

De Mayo reaction is a versatile variant, in synthetic application, of the [2+2] photocycloaddition. A recent example of this reaction is reported in figure 28:

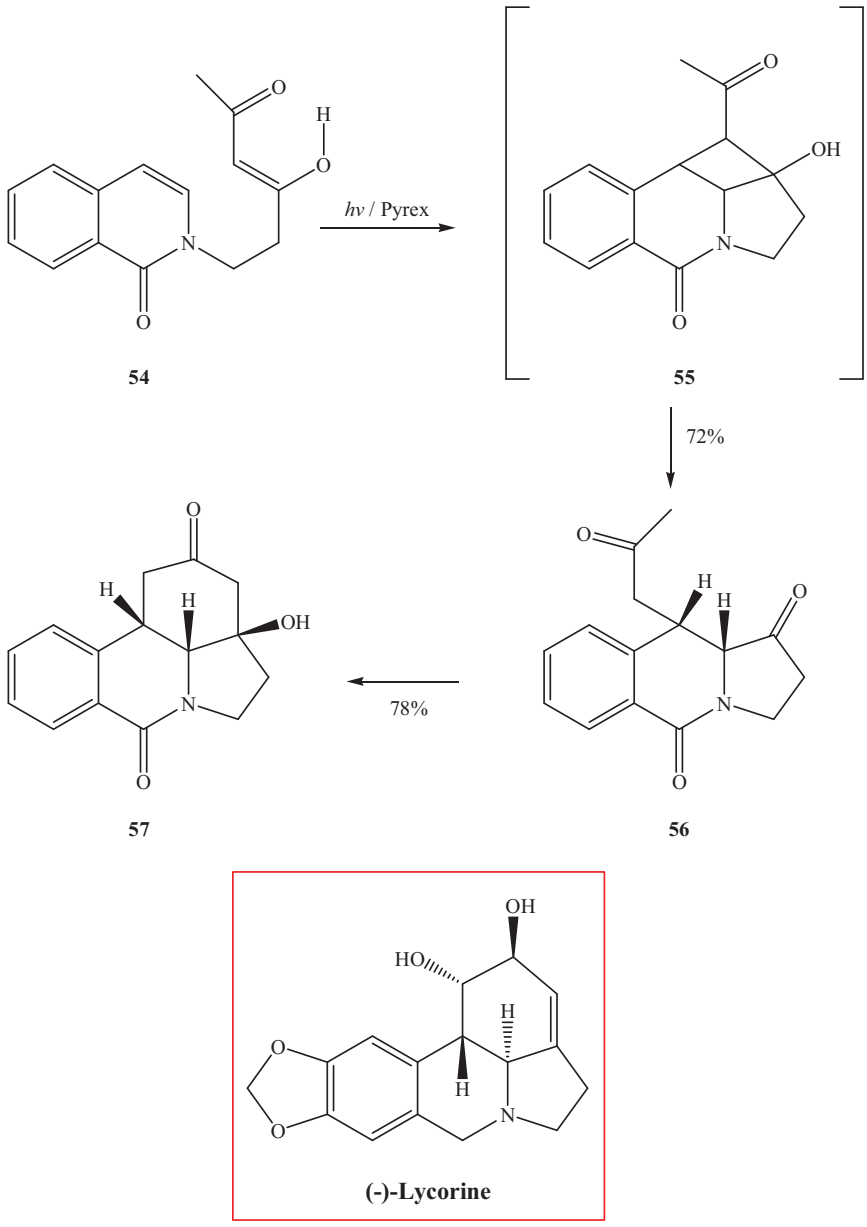
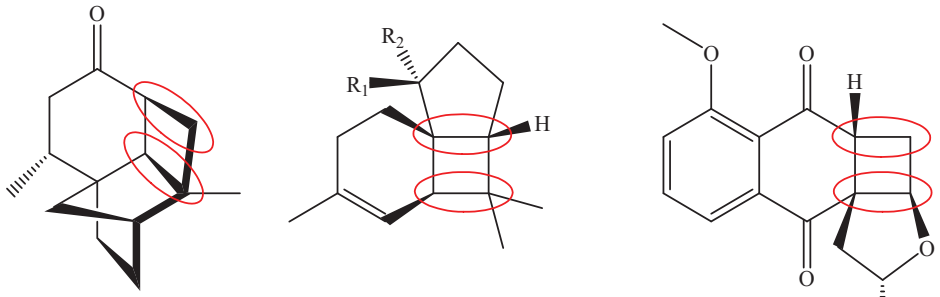


Figure 28: Synthetic application example of De Maio reaction

In its enol form In its enol form, a β-diketone reacts as an α,β-unsaturated ketone with an alkene. This step is followed by a retro-aldol reaction. In compound **54**, the mono-enolized β-diketone chromophore adds to the enamine part of the isoquinolone moiety and the tetracyclic aldol **55** is obtained as an intermediate[92]. Due to ring strain, the latter is immediately transformed into **57**. After an intramolecular aldol reaction, the

more stable tetracyclic hydroxyketone **56** is isolated. Compound **56** possess the galathane skeleton which is encountered in Lycorine alkaloids isolated from *Amaryllidaceae*[93].

As reported in figure 29, a range of naturally occurring cyclobutanes have been synthesized by using intramolecular [2+2] photocycloaddition. One such example is (+)-Solanascone **58**, the biosynthesis of which [from (-)-Solavetivone]is through to occur photochemically. Further examples include (±)-Italicene **59**, (±)-Isoitalicene **60**, (-)-Elecanacin **61**, (±)-Trihydroxydecipiadiene and (+)-Dehydrosolanascone.



58 **59 R_1 = Me; R_2 = H** **60 R_1 = H; R_2 = Me** **61**

Figure 29: The naturally occurring cyclobutanes (+)-Solanascone 58, (±)-Italicene 59, Isoitalicene 60, (-)-Elecanacin 61, which where synthesized by intramolecular [2+2] photo-cycloadditions.

As previously mentioned, photochemical reactions have frequently been performed in supramolecular structures since the reaction conditions are particularly mild. In the case of an intramolecular [2+2] photocycloaddition, stereoselectivity can be induced inside such a host/guest structure. For example, the quinolone derivative **62** was complexed with the template structure **64** (Figure 30)[94]. Photocycloaddition yielded the cyclobutane derivative **63** with high enantioselectivity. Numerous intra- and intermolecular photochemical reactions have been performed under analogous conditions using host structures such as **64**[95-96].

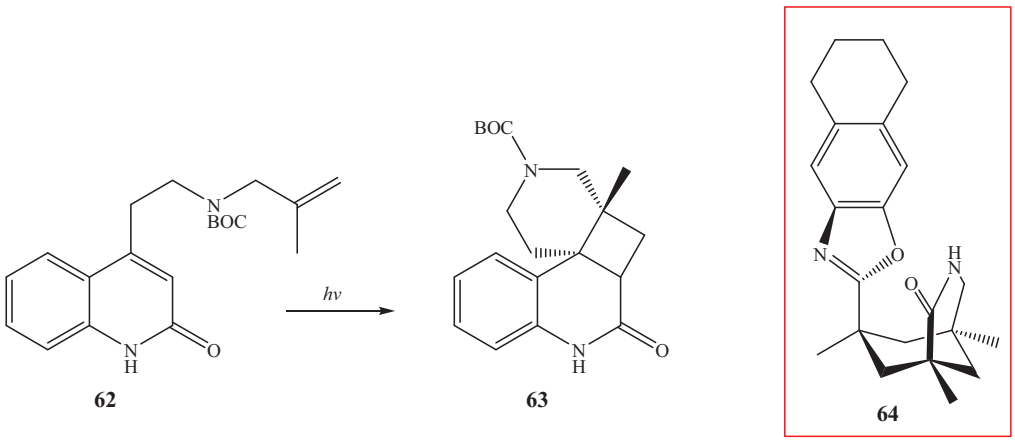


Figure 30

Intermolecular [2 + 2] photocycloadditions have also been studied and represent a facile and versatile access to cyclobutane derivatives. For example, the enantiomerically pure uracil derivative **65** reacted via a sensitized [2+2] photocycloaddition with ethylene to yield the corresponding cyclobutanes **66a** and **66b** (Figure 31)[97]. The diastereoselectivity of this reaction was low[98], but the stereoisomers were easily separated and then transformed into the β-amino acids **67** and **ent-67**.

Similar products resulting from intra- or intermolecular [2+2] photocycloaddition have frequently been applied to the synthesis of β-amino acids[99-100]. When incorporated in a peptide structure, such β -amino acids have a significant influence on the secondary and tertiary structure of these compounds. In particular, 2-aminocyclobutane-1-carboxylic acid moieties rigidify a peptide structure in such a way that a helix secondary structure is induced[101].

In this context, the acyclic β-amino acid of a Rhodopeptin derivative was replaced by a cyclobutane analog, prepared via [2+2] photocycloaddition of ethylene to a corresponding uracil derivative[102]. Rhodopeptins are cyclic lipopeptides isolated from *Rhodococcus sp.* Mer-N1033 possessing antifungal activities.

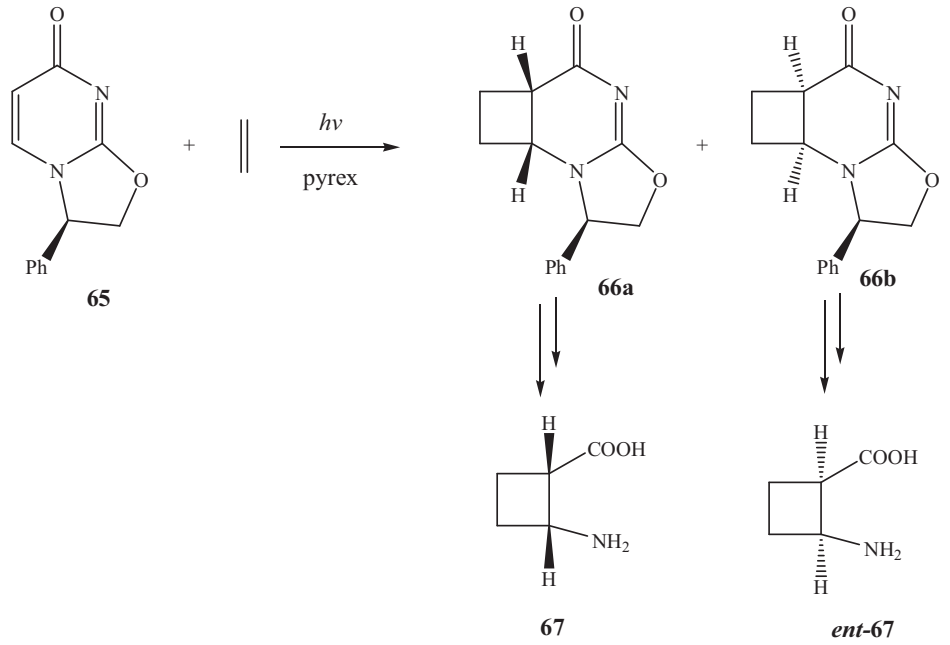


Figure 31

Another important class of photochemical reactions frequently used in organic synthesis use copper as catalyst: two examples are reported in figure 32.

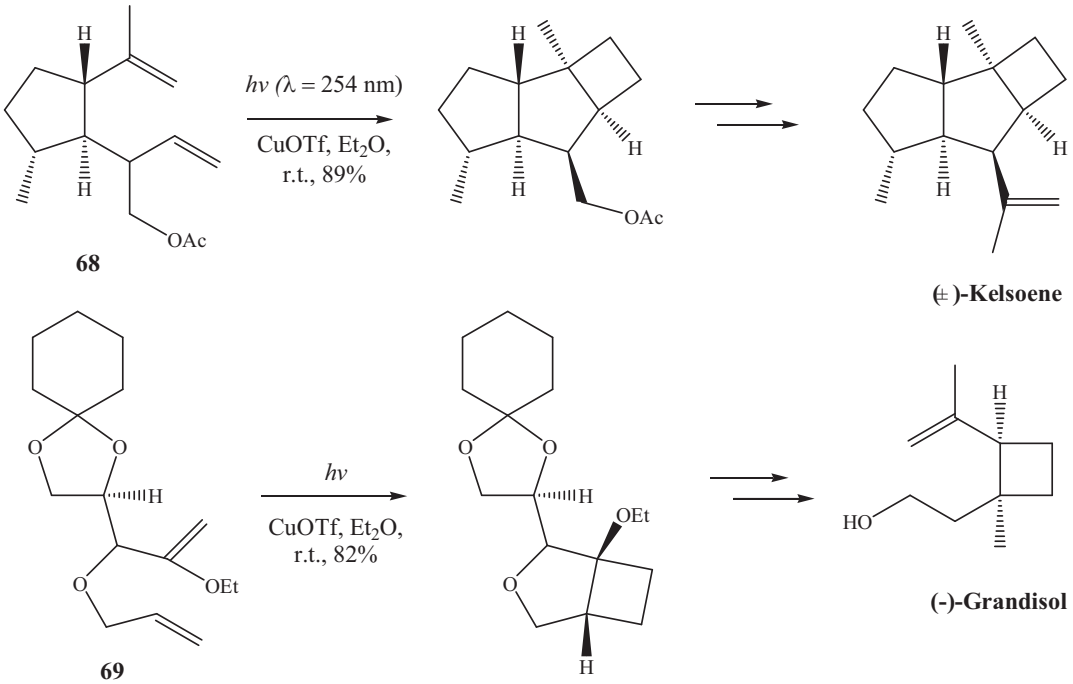


Figure 32

(±)-Kelsoene was synthesized from the diene **68** (Figure 32)[103]. This sesquiterpene was also obtained using the corresponding addition of ethylene to an α,β-unsaturated ketone[104]. The copper-catalyzed [2 + 2] photocycloaddition is very versatile and has often been applied to asymmetric synthesis. For instance, (-)-grandisol, a component of the aggregation pheromone of the male boll weevil (*Anthonomus grandis*) was obtained from compound **69** with a copper-catalyzed [2 + 2] photocycloaddition as the key step of the synthesis (Figure 32)[105].

1.3. Stemodane diterpenes

As previously reported, the *Stemodia Maritima* is a plant belonging to the family *Scrophulariaceae* and it is found in tropical and subtropical regions of the world[9].
In fact, this plant, grows widely in the Virgin Islands and the region to the North-East of Brazil, near the sea, where it is known as "*Melosa*"[106] . This plant is known for a long time in traditional medicine of the population in the north of South America and in most of the Caribbean islands, and is used for the treatment of venereal diseases [10a].

Although *Stemodia* includes about 40 species, the chemical investigation of this kind is limited to 5 species (*S. maritima*, *S. chilensis*, *S. kingii*, *S. viscose*, *S. foliosa*)[107] from which were isolated flavonoids, labdane diterpenes and some tetracyclic diterpenes with a rare skeleton called stemodane skeleton (Figure 33).

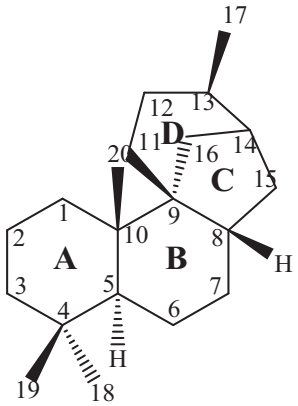
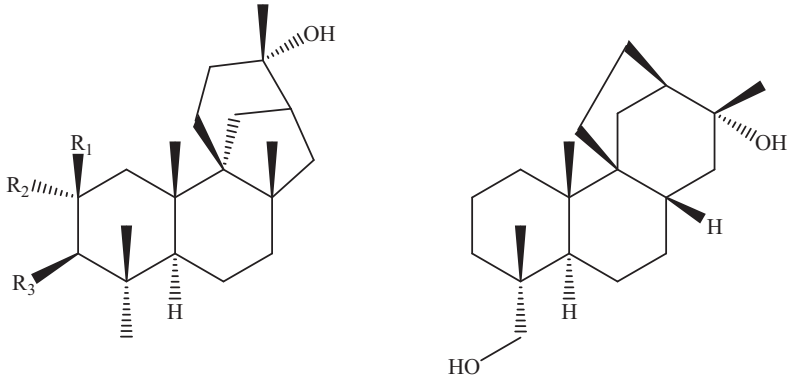


Figure 33: stemodane skeleton

Diterpenes with the stemodane skeleton, isolated from *Stemodia Maritima*, are stemodin **70**[11a], stemodinone **71**[11a], 2-deoxystemodinone **72**[11a] and maritimol **73**[10a] (Figure 34). From the same plant was also isolated stemarin **33**[8], the founder of the stemarane diterpenes. As point out before, the structure of stemarin **33** has been established by X-ray diffraction.



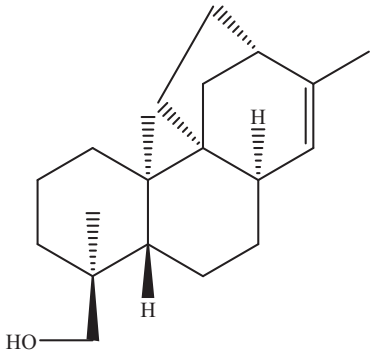
70 $R_1 = OH, R_2 = R_3 = H$
71 $R_1 = R_2 = O, R_3 = H$
72 $R_1 = R_2 = R_3 = H$
73 $R_1 = R_2 = H, R_3 = OH$

33

Figure 34: stemodin 70, stemodinone 71, 2-deoxystemodinone 72, maritimol 73 and stemarin 33.

In 1990 in Chile, Garbarino and co-workers described the isolation, from Chilean *Calceolaria* Lepida, of a new diterpenoid. Garbarino and co-workers had originally speculated that the diterpenes of *Calceolaria* could have a stemodane or stemarane skeleton, opting, then for the latter.

The structure of this new diterpenoid **15**, (-)-2-deoxyoryzalexin S, was attribuited, by Garbarino, on the basis of the ^{1}H and ^{13}C NMR spectra,. To this compound, as well as to its congeners, the absolute *ent*-stemarane configuration was assigned on biogenetic grounds.



15
(-)-2-deoxyoryzalexin S

1.3.1. Structure of stemodane diterpenes

The stemodane diterpenes carbon skeleton is reported in figure 35, and its main structural characteristics are reported below:

- the bicyclic system C/D is constituted by a bicyclo[3.2.1]octane fused to the bicyclic A/B system;
- there is a quaternary carbon spirocyclic, the C (9), adjacent to a second quaternary carbon, the C (10);
- oxygenated functions can be present at C(2), C(3), and C(13).

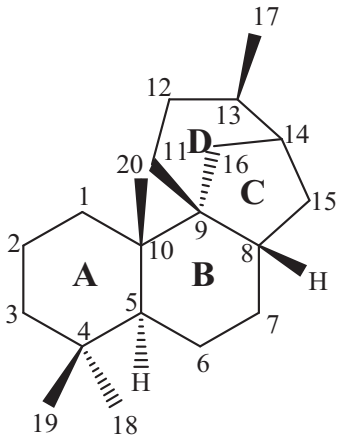
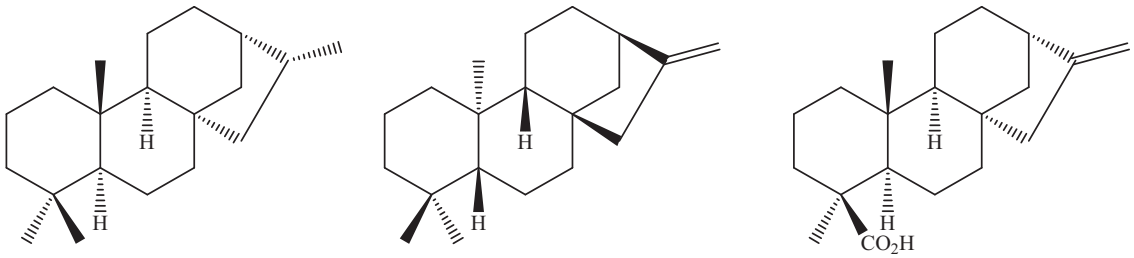


Figure 35: Stemodane diterpenes carbon skeleton

1.4. Kaurane diterpenes

Kaurane diterpenoids, whose parent compound is the kaurane (Figure 36), are an important class of natural product known from the beginning of this century and widespread in the plant kingdom.



73 **kaurane** **74** ***ent*-kaurene** **75** **kaurenoic acid**

Figure 36: kaurane (73), *ent*-kaurene(74) and kaurenoic acid (75)

Kaurene **73** (Figure 36) was first isolated by Hosting from the leaf oil of Kauri pine, a resinous conifer native of the island north of New Zealand and belonging to the family of *Agathis australis*. Later it has been obtained from *Podocarpus macrophyllus* as well.

The structures of kaurane diterpenes are well-known: they contain a rigid tetracyclic scheleton costituited by a perhydrophenantrene unit (A, B and C rings) fused with a cyclopentane unit (D ring) formed by a bridge of two carbons between C-8 and C-13 (Figure 37)[108].

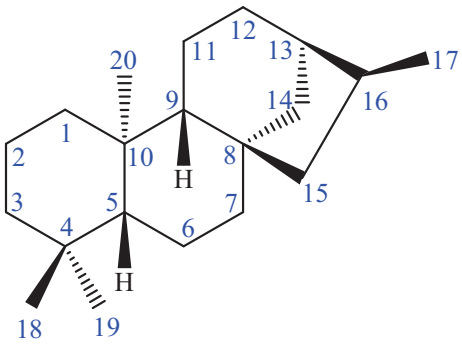


Figure 37: Carbon-skeleton of an *ent*-kaurane diterpene

Kaurane diterpenoids, and in particular kaurenoic acid **75** (Figure 36), are important intermediates in the biosynthesis of a number of plant and fungal metabolites, including Gibberellin, a well-known class of phytohormones involved in the regulation of plant growth.

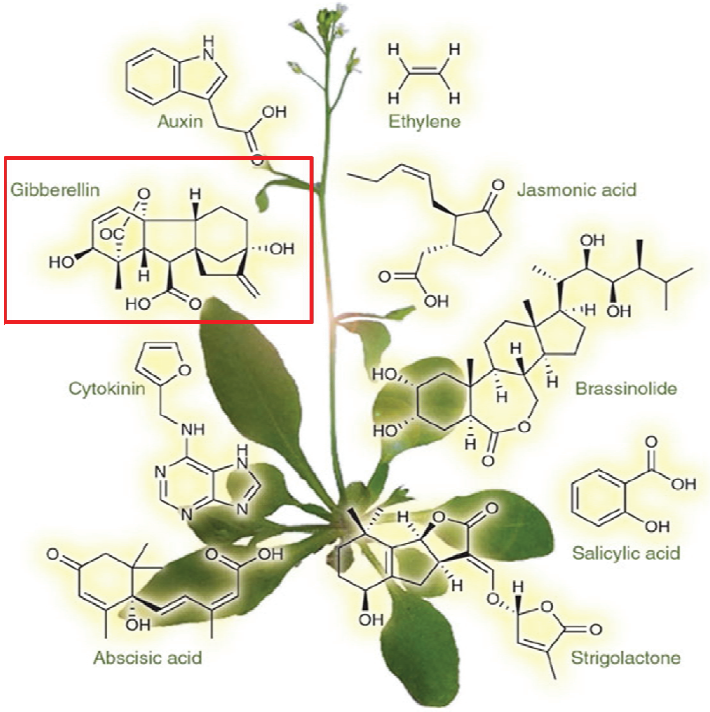


Figure 38

Several biological activities of kaurane diterpenes have been described in literature such as, antimicrobial[109], antiparasitic[109], antiinflamatory[109], hypotensive[109], insect antifeedant[109], antifertility[109], cytotoxic[109], antitumour[109], anti-HIV[109] and plant growth regulation[110].

Some of these kaurane biological effects have also been cited in the review published by Ghisalberti in 1997[52a].

1.4.1. Some examples of Kaurane diterpenes in Nature

In 1964, Henrick and Jefferies[111], from the ethereal extract of *Ricinocarpus stylosus* belonging to *Euphorbiaceae* family, obtained four new kaurenoid diterpenes: 19-hydroxy-16α-(-)-kauran-17-oic acid **79**, (-)-kaur-16-en-19-oic acid **78** and 16α-(-)-kauran-l7,19-dioic acid **76**, kauran-16α,-17,19-triol **77** (Figure 39).

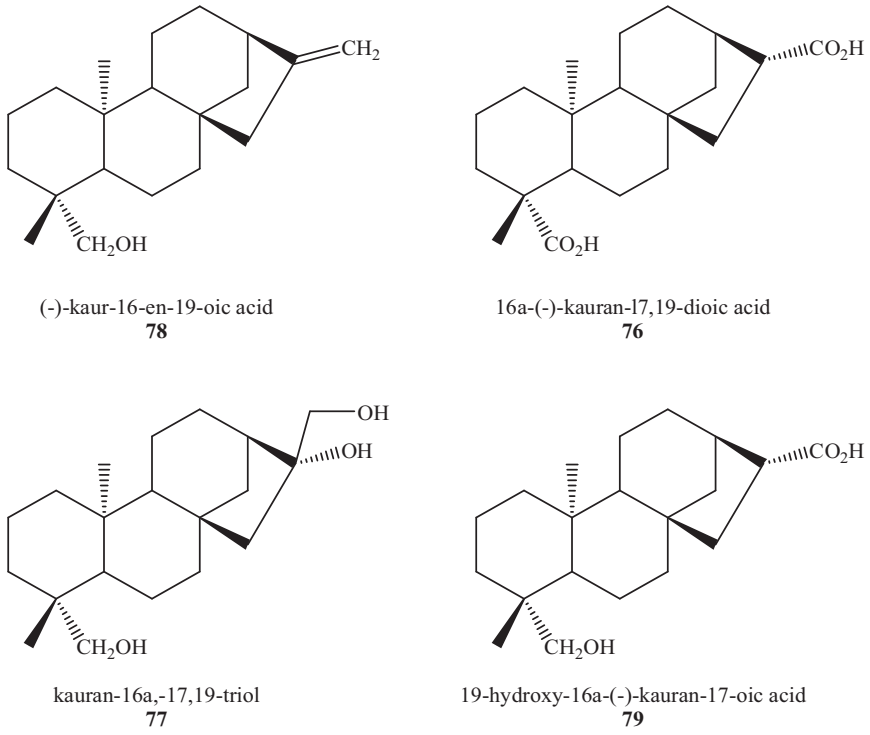


Figure 39: New kaurene derivatives by Henrick and Jefferies in 1964.

Moreover, starting from compound **76**, Henrick & Jefferies[111] obtained other derivatives, by the common chemical reactions.

In 1968, Mori *et al.*[112], carried out the total synthesis of kaurenoic acid **75 .** Four years later, in 1972, the same group accomplished the synthesis of the Gibberellins A_2

(**80**)[113], A_4 (**81**)[113], A_9 (**82**)[113] and A_{10} (**83**) [113] and Steviol (**84**)[114](Figure 40). In these papers they describes the synthesis of suitable kaurane derivatives which are functionalized on the ring A.

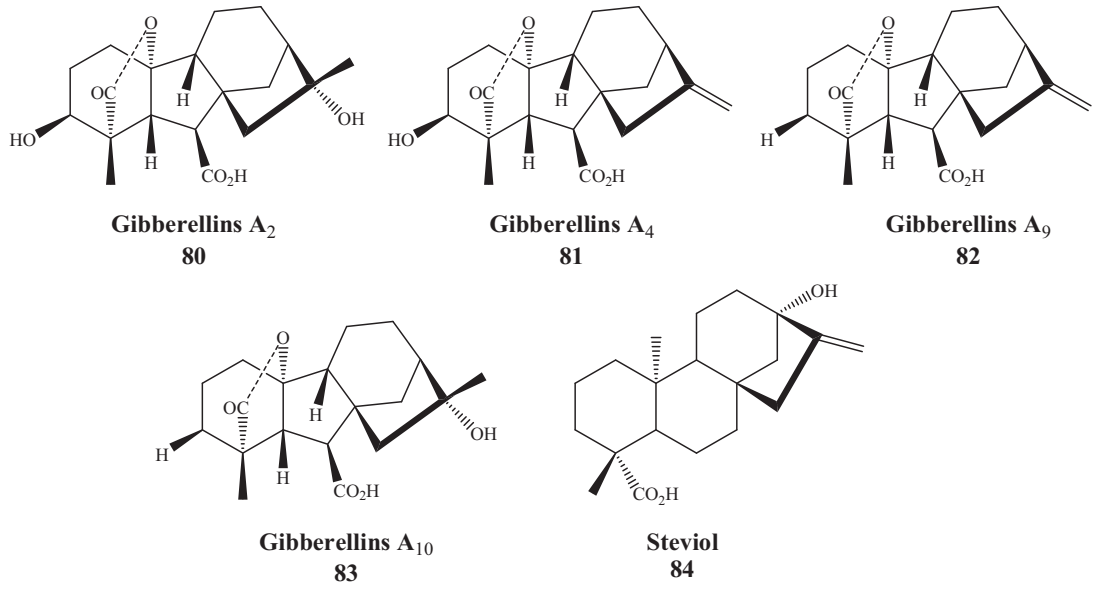
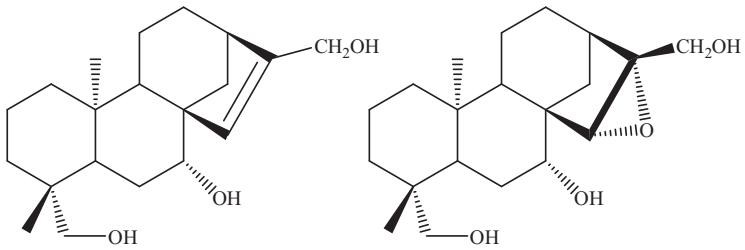


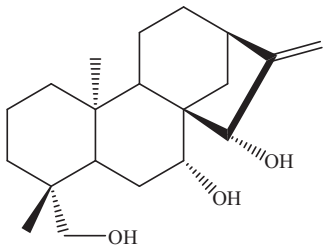
Figure 40.

In 1988, Bellino and Venturella[115], reported the synthesis of *ent*-7β,17,18-trihydroxykaur-15-ene **85** (sinfernol), *ent*-15β,16β-epoxy-7β- trihydroxykaurane **86** (epoxysinfernol) and *ent*-7β,15β-trihydroxykaur-16-ene **87** (canditriol) the constituents present in the aerial part of *Sideritis infernalis* a species endemic to the Canary Island, using as starting material an abundant diterpene of *Sideritis infernali,s ent*-7β,18β-dihydroxykaur-15-ene **88** (episideridiol) (Figure 41).

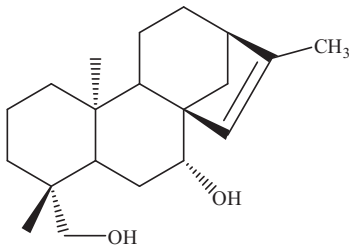


Sinfernol
85

Epoxysinfernol
86



Canditriol
87



Episideridiol
88

Figure 41.

In 1987, the Corey group[116a] achieved the total synthesis of (±)-atractyligenin **89**. Ten years later[116b], however, they reported a novel catalytic enantioselective synthetic route to the preparation of **89** with an enantiomeric excess over 87 % (Figure 43).

In 2004 Britton, Piers and Patrick reported the total synthesis of (±)-13-methoxy-15-oxozoapatlin **90** (Figure 42), an interesting diterpenoid which was found to be active as a cancer therapeutic agent and it has shown antimalarial activity. This synthesis consists of 21 steps and it is a new method for the construction of the functionalized bicyclo[3.2.1]octane unit.

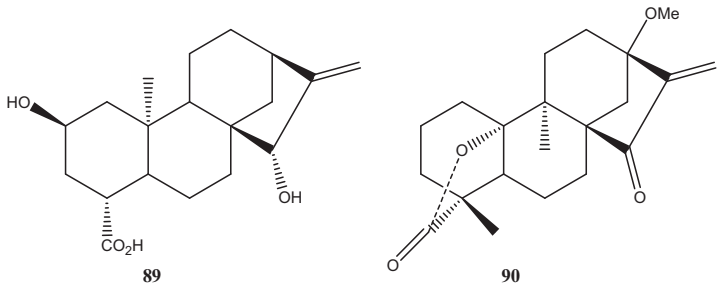


Figure 42.

In 2002, Vieira *et coll.*[117] reported the synthesis of 12 new derivatives of kaurenoic acid **75** with the aim either to improve the therapeutic activity against trypomastigote forms of *Trypanosoma cruzi*, either to reduce the lytic activity of the kaurenoic acid **75** on blood erythrocytes. Among the derivatives prepared, compound **92** (Figure 43) showed more enhanced trypanosomicidal activity *in vitro* than kaurenoic acid **75** but it kept discrete lytic activity on erythrocytes.

Moreover, they found that compound **91** (Figure 43) did not show lytic activity but has similar level of activity as kaurenoic acid **75**. All the other derivatives of kaurenoic acid **75** were completely inactive against Trypomastigote forms of *Trypanosoma cruzi* at the concentrations tested (2.27 – 0.57 mM).

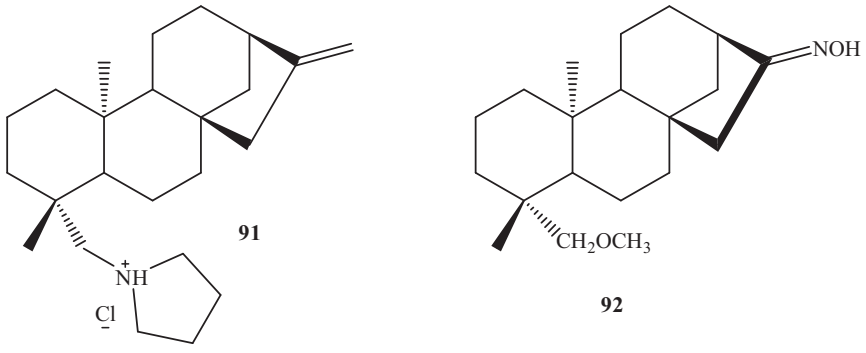
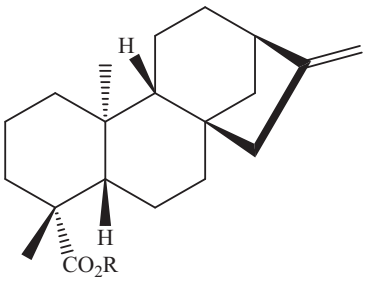
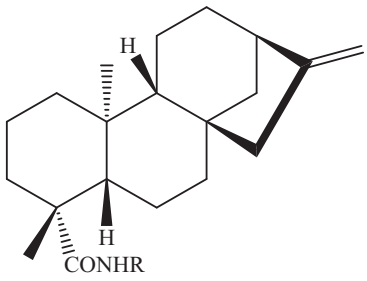


Figure 43: Two of the some derivatives synthesized by Vieira *et coll.*, in 2002

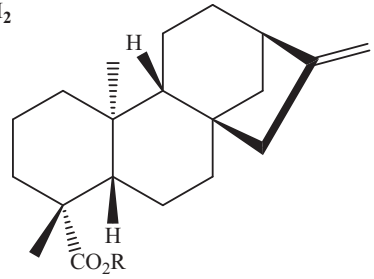
The well-known biological activities of kaurenoic acid **75** and the relatively high natural abundance of this diterpene, stimulated Boeck *et coll.*[118], to synthetize kauranoic acid **78** and derivatives in order to evaluate their potential pharmacological activities. Boeck *et coll.*, in 2005, tested all their synthetized compounds for antifungal activity against human opportunistic pathogenic fungi including yeast (such as *Candida albicans*, *C. tropicalis*, *Saccharomyces cerevisiae* and *Cryptococcus neoformans,* the filamentous fungi *Aspergillus niger*, *A. flavus* and *A. fumigates*, etc.), hialohyphomycetes and dermatophytes (*Microsporum canis, M. gypseum, Trichophyton rubrum*, *T. mentagrophytes* and *Epidermophyton flocco sum*). They observed that only kaurenoic acid **75** and derivatives containing the acidic -COOH group displayed a moderate activity against three dermatophytes (*Trichophyton rubrum*, *T. mentagrophytes* and *Epidermophyton flocco sum*), indicating that the presence of hydrophilic groups contributes to the antifungal activity.



75 R = H
93 R = CH_3
94 R = $CH_3CH_2CH_2CH_2$
95 R = $PhCH_2$
96 R = 4-$ClPhCH_2$
97 R = 4-$BrPhCH_2$



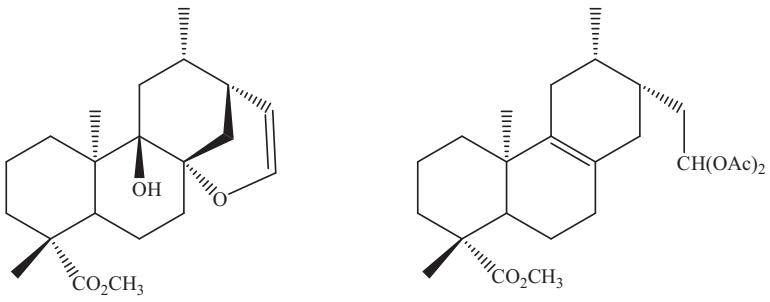
98 R = Ph
99 R = $PhCH_2CH_2$
100 R = $PhCH_2CH_2CH_2CH_2$



101 R = H
102 R = CH_3

Figure 44: Kaurenoic acid (75) and some derivatives (93-102) synthetized by Boeck *et coll.*

In 2005, Alonso *et coll.*[119], starting from (-)-kaur-9(11),16-dien-19-oate, which was isolated from Venezuelan species of *Espeletia* (Asteraceae), have synthetized the diterpenes **103** and **104** (Figure 45) evaluating their antimicrobial and antitumoral activity. The results obtained, showed that these compounds have high cytotixic and cytostatic activity against diverse neoplastic cancer cell lines (such as colon, lung, leukemia, melanoma, ovary, kidney and prostate).

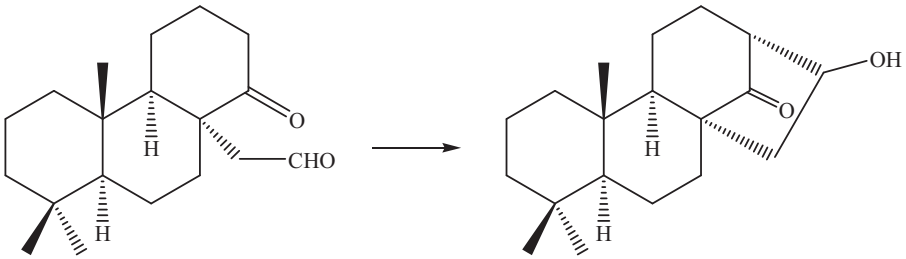


103 **104**

Figure 45: diterpenes prepared by Alonso *et coll.*

1.4.2. Previous synthesis of Kaurane diterpenes

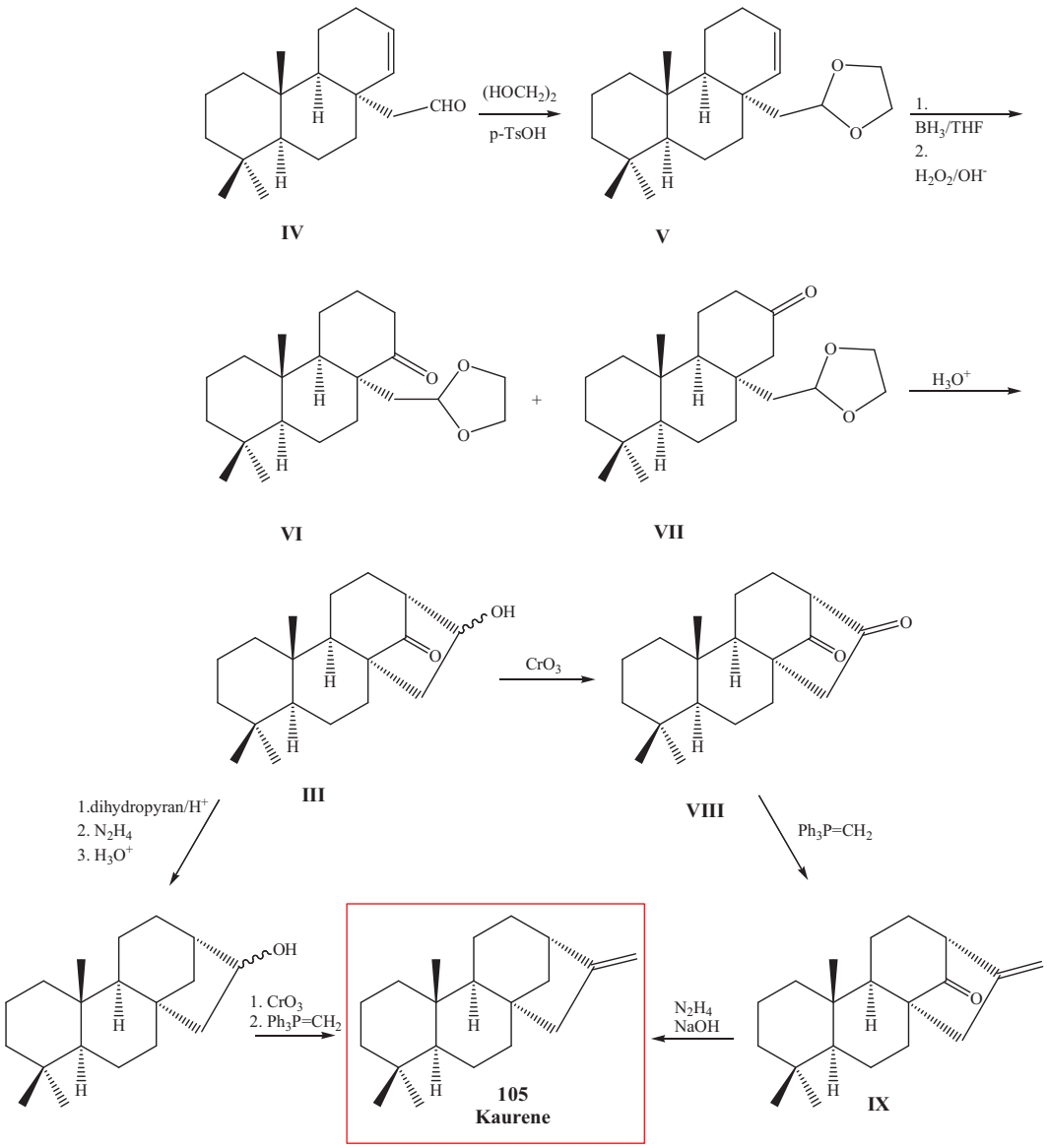
Many synthesis of kaurane diterpenes are reported in the literature and a review on this topic was published by Goldsmith. Common aspect of some synthesis reported by Ireland on Kaurene **105**, by Mori on Kaurenoic acid **75** and by Fujita on kaurendiol **106**, concerns the formation of the D ring of the kaurane system by cyclization of a phenanthrenic substituted system as shown in figure 46:



I **II**

Figure 46: D ring of the kaurane system by cyclization of a phenanthrenic substituted system.

In the synthesis of Ireland and co-workers (Scheme 12), there is a protection of the aldehyde function **IV** with the formation of the acetal group **V**. The latter was then subjected to hydroboration and oxidation and converted into ketones **VI** and **VII** in ratio 1.6:1.

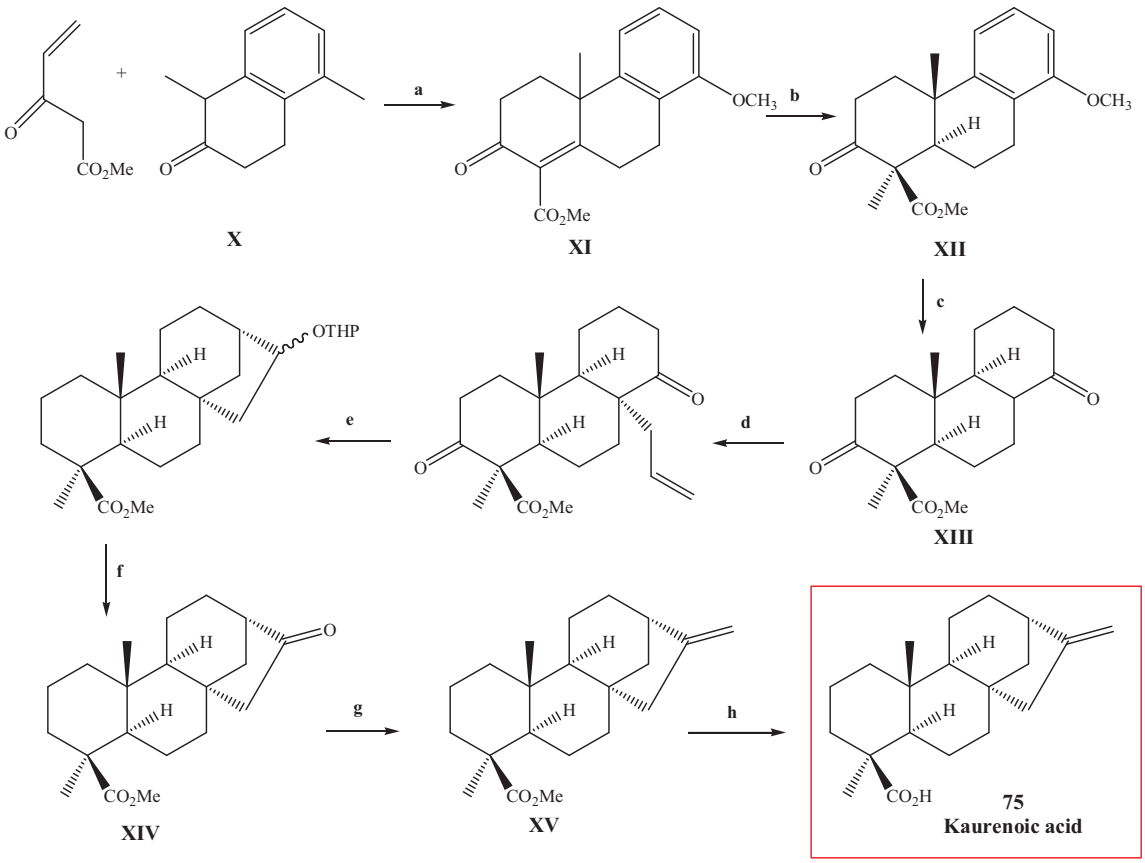


Scheme 12: Synthesis of Kaurene (105) by Ireland

The carbonyl function at C(14) allows, after acid treatment of the ketoacetalic system, the rearrangement to the kaurenoic skeleton through intramolecular aldol condensation. Then, the resulting compound **III** is converted into kaurene through two independent reaction pathways, as reported in the scheme 12 in one-way of these patways, the hydroxyl group is protected and the carbonyl group is removed by Wolff Kishner reduction. Then the hydrolysis of the protected group restores the carbonyl function and through a reaction of Wittig you will come to the formation of kaurene. the other way of these patways, concerns the selective methylation the dione **VIII**, which

was previously obtained by treating compound **III** with CrO_3, using a phosphorus ylide to give **IX**. The subsequent deoxygenation of **IX** leads to the formation of kaurene **105**. The defect of this synthesis is given by the fact that the ketone **VI** is obtained in a not regioselective way.

Mori and co-workers, for the synthesis of the kaurenoic acid **75**, follows the general approach used by Ireland for the kaurene synthesis (Scheme 13).

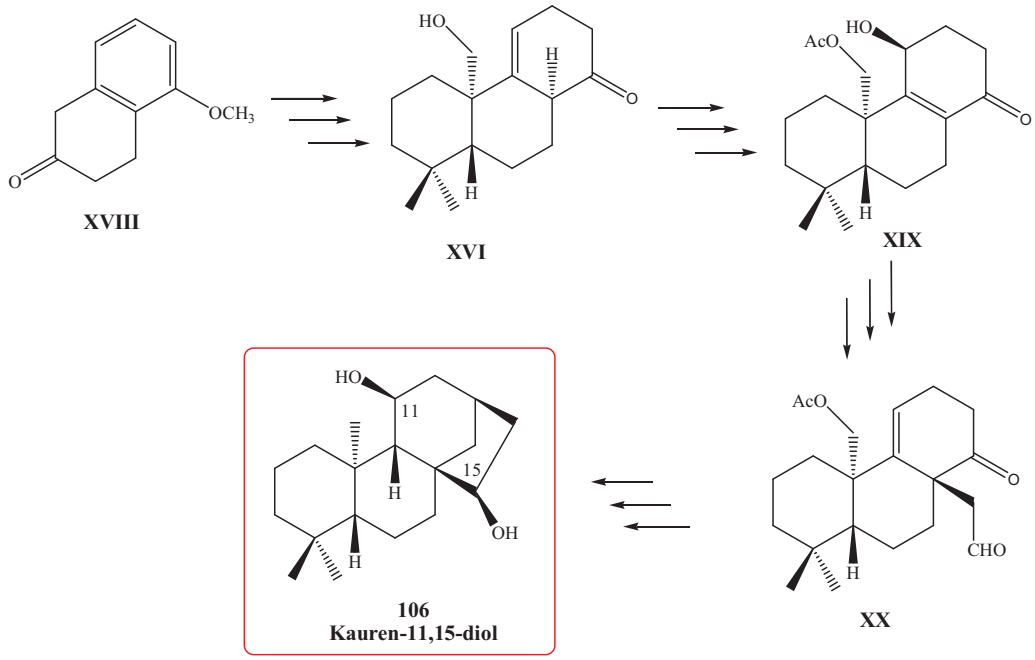


Scheme 13: Synthesis of the kaurenoic acid (Ic). a)$NaOCH_3$; b)1.KO-*t*-Bu/MeI, 2.$(HSCH_2)_2/BF_3$, 3.*Ra*-Ni; c)1. Rh-Pt/H_2, 2. CrO_3; d)1. $HCO_2Et/NaOCH_3$, 2.n-BuSH/TsOH, 3. KO-*t*-Bu/ CH_2=$CHCH_2Br$, 4.KOH; e)1.OsO_4,HIO_4, 2.$NaOCH_3$, 3.DHP/p-TsOH, 4.N_2H_4/KOH; f) 1.H_3O^+, 2.CrO_3; g)1.CH_2N_2, 2.$(HOCH_2)_2$/TsOH, 3.$LiAlH_4$, 4.HCl, 5.Ph_3=CH_2; h) CrO_3

The tricyclic compound **XI** was prepared by Robinson annulation of the β-tetralone **X**. Then, the alkylation followed by the reduction of the double bond and the ketone group led to the formation of the ester **XII**. The reduction of the aromatic ring, followed then by Jones oxidation, led to the tricyclic ketone **XIII**. The C/D rings system was then

builted using the approach by Ireland through the allylation of a protected ketone, the cleavage of the allylic unit, to give a keto-aldehyde, intramolecular aldol condensation and Wolff Kishner reduction. In this way the ketone **XIV** was obtained. The latter was then converted into kaurenol **XV** which was later transformed into kaurenoic acid **75**.

In the synthesis of the kaurene-11,15-diol **106**, by Fujita and Ochiai (Scheme 14), the key step is the regioselective formation of the unsaturated β,γ-ketone **XVI**.



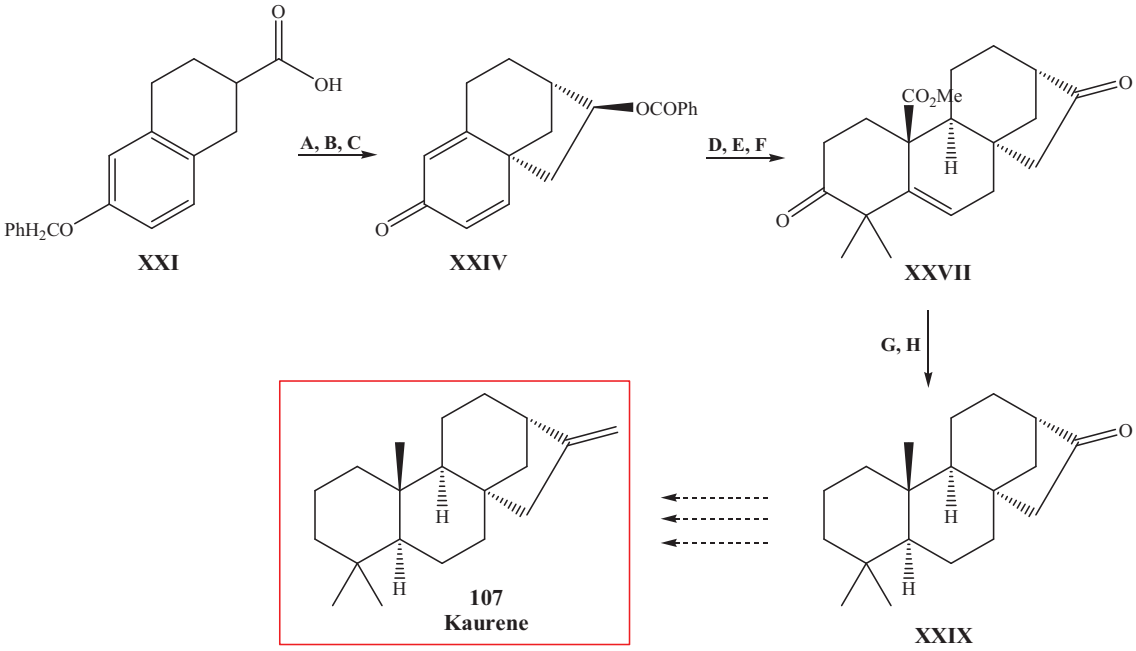
Scheme 14: Principal steps of the kaurendiol's synthesis (156) by Fujita and Ochiai

The compound **XVI** was prepared by Fujita and Ochiai by several synthetic steps (such as Robinson annulations, methylations, desulphurization and Birch reduction) using 5-metoxy-β-tetralone **XVIII** as starting material. Then by epoxidation and by elimination base-catalyzed, the hydroxyl-enone **XIX** was obtained. The two carbon atom unit, required for the five terms D ring, is formed selectively by using the hydroxyl group at C(11) of the compound **XIX** as orienting group. The latter was converted into compound **XX** by Claisen rearrangement.

The product of the Claisen rearrangement **XX**, through several steps (aldol condensations, Collin's oxidation, Wolff-Kishner reduction, hydroboration, Jones'

oxidation, isomerization and photosensitized oxygenation), is then transformed into the natural product kaurendiol **106**.

A synthesis of kaurene **107** was also conducted by Masamune and co-workers (Scheme 15). Unlike other researchers, they have built before the system of rings B/C and subsequently, by intramolecular cyclization, obtained the D ring. Then by Robinson annulation, A ring was built.



Scheme 15: Synthesis of the kaurene by Masamune. A)1. $(COCl)_2$, 2.CH_2N_2, 3. HBr; B)1.$NaBH_4$, 2.DHP/H^+, 3.Pd(C)/H_2; C)1.KO*t*-Bu, 2.H_3O^+, 3.PhCOCl; D)1.Pd($CaCO_3$)/H_2, 2.Ph_3CNa/CO_2, 3.CH_2N_2; E)1.Pent-1-en-3-one/OH^-, 2.DHP/H^+; F)1.KO*t*-Bu, 2.H_3O^+, 3.CrO_3; G)1.H_2/Pd, 2.$(HOCH_2)_2$/H^+, 3.N_2H_4/KOH; H)1.LAH, 2.CrO_3/pyr., 3. N_2H_4/KOH.

1.4.3. Some examples of Kaurane glycosides

In chemistry, **"Glycoside"** is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides are widely distributed innature and they play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis[120], which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body.

In the last 40 years, kaurane glycosides have assumed a great importance because of their toxicity, potential therapeutic and economic value have been demonstrated.

Some of the most important examples of these class are stevioside **110**, atractyloside **108**, carboxyatractyloside **109** and wedeloside **111**, the structures of which are shown in figure 47.

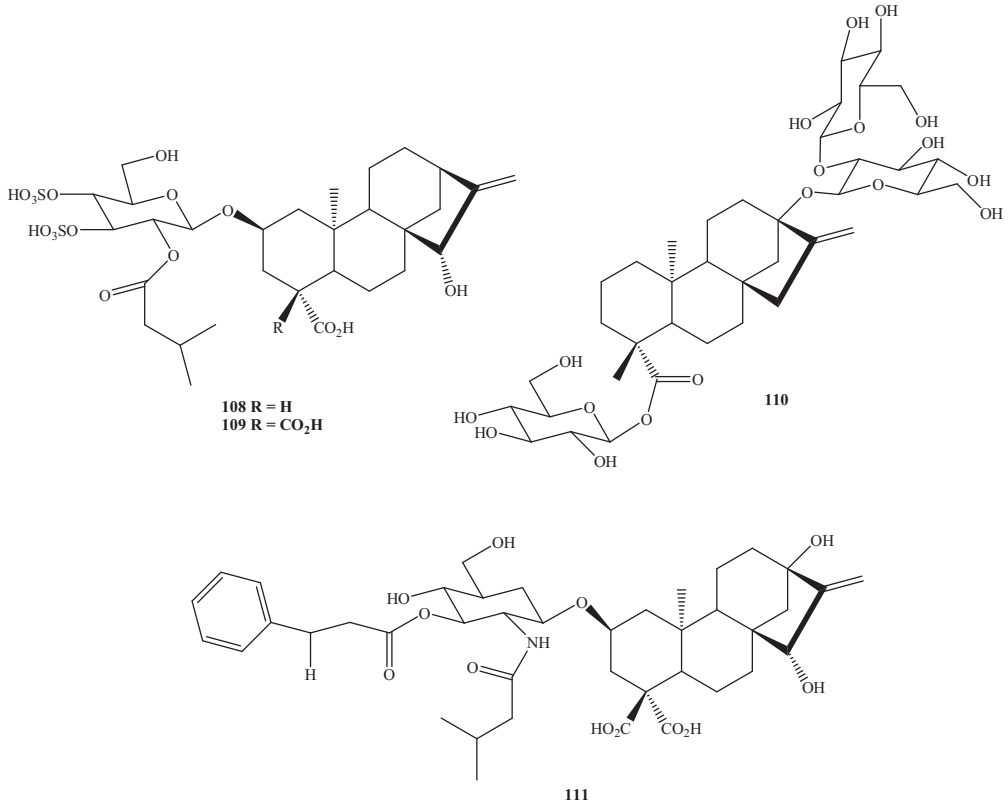


Figura 47

Stevia rebaudiana Bertoni (Asteraceae family)(Figure 48), is a native plant of Paraguay and Brazil; infact its leaves have been traditionally used for hundreds of years by natives of Paraguay and Brazil as a “sweet treat” and also for its medicinal benefits. Later it has been cultivated also in Israel, South and North Korea, China and Japan.

Figure 48: *Stevia rebaudiana* Bertoni leale

The first news of the existence of this plant came from its use by the indigenous *Guarani* which called **Caa-eh-è** (sweet grass) or sweet chrysanthemum and used it to cover the bitter taste of the “Ilex paraguayensis” by which they prepared a brew called "Mate "(Paraguayan tea) slightly stimulant for its low caffeine content.

Stevia rebaudiana was described for the first time by Moises Santiago Bertoni (1857-1929), a Paraguayan botanist, such as *Eupatorium rebaudianum*. Then it was classified in the genus *Stevia*, by William Botting Hemsley (1843-1924), a British researcher of the Kew’s Botanical Gardens. The name "*rebaudiana*" it has been given to this plant in honor of the chemist Rebaudi who first studied the chemical characteristics of the substances contained in the plant.

More than 150 species of *Stevia* have been described, but *rebaudiana* it is the only one possessing sweetening properties. Its leaves contain a complex set of diterpene glycosides. These substances are characterized by the presence in their structure of three molecules of glucose. About the four sweeteners (Stevioside, Rebaudioside A, Rebaudioside C and Dulcoside A), present in higher concentrations in the leaves, stevioside **110** (3-10% of the dry weight of the leaves) and rebaudioside A (1-3%) have

physical and sensorial properties well characterized with a sweetness of 110-270 and 180-400 times higher than sucrose, respectively. In fact for these properties and for the reason that do not show any carcinogenic activity, the industrial use of the stevioside **110** as a substitute for saccharose, in foods and dietary drinks is allowed in some countries around the world. In medicine it is used as an anti-hyperglycemic agent for the treatment of diseases of the skin, in the treatment of hypertension for its cardiotonic action and for many other diseases.

The study of some species of *Stevia* spp. has led to the isolation of several other kaurane glycosides, with or without sweetening properties[121].

Atractyloside **108** (Figure 47), initially isolated from rhizomes of *Atractylis gummifera* L. (Asteraceae)[122], and then from *Wedelia glauca* Ort. (Asteraceae)[123], it is also present in a number of plants used as ethnomedicines throughout Africa, the Mediterranean areas and the Far Eastern countries.

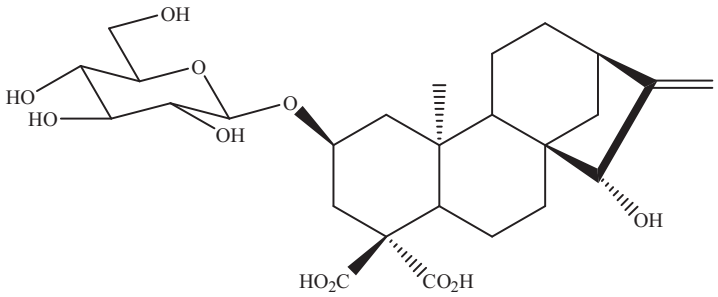
Figure 49: (left) *Wedelia glauca* Ort. and (right) *Atractylis gummifera* L.

Atractyloside **108** is a natural compound that functions as a specific inhibitor of the adenine nucleotide translocase (ANT), a mitochondrial ADP/ATP carrier. Additionally, Atractyloside is a proapoptotic ligand of ANT that induces pore formation by ANT, and results in the permeabilization of the mitochondria membrane. In fact it is the main compound responsible for the lethality of these plants in bovine cattle of some regions of Argentina, Brazil and Uruguay. Its toxic action is due to the inhibition of mitochondrial oxidative phosphorylation[122], causing hypoglycemia, respiratory depression, nephrotoxicity, hypoxemia and cell injury.

In 2001, Obatomi, Blackburn, and Bach[124] dimostrated that in humans these compounds can cause renal and hepatic failure, also followed by necrosis.

This toxic action is due to the inhibition of mitochondrial oxidative phosphorylation[122].

In 2005, Konopleva *et coll.*[125], from the aerial parts of *Gnaphalium sylvaticum* (Asteraceae family) isolated a new diterpene glucoside, named Sylviside **112** (Figure 50).



112
Sylviside

Figure 50: Sylviside

The structure of compound **112** was elucidated by spectroscophic analysis (^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, NOESY) and by X-ray crystallographic analysis. Sylviside **112** displayed cytotoxic activity against HeLa WT cells, human epitheloid cervical carcinoma.

Carboxyatractyloside **109** (Figure 47), have been isolated from *Atractylis gummifera* L., *Xanthium* spp. (Asteraceae), *Wedelia biflora* (Asteraceae), *Cestum paraqui* (Solanaceae) and *Iphiona aucheri* (Asteraceae)[126,127,128] and it is also contained in the seeds of *Xanthium strumarium* (Cocklebur), an herbaceous annual plant found in the U.S. along the shores of streams and ponds and in low-lying areas of farm fields.

Figure 51: ***Xanthium strumarium***

This plant is highly toxic to animals. It inhibits of the nucleotide translation through the mitochondrial membrane, causing 10 times more toxicity than atractyloside **108**. In fact Turgut *et coll*[134], reported that this glycoside is a highly selective and potent inhibitor of the Adenine nucleotide translocator (ANT), the nucleoside binding site of ANT on the cytoplasmic side of the inner membrane and blocks the exchange of matrix ATP and cytoplasmic ADP.

Carboxyatractyloside **109** showed, among other activities, antitumoral action against melanoma cells and Erlich tumour[129], beyond the insecticidal and herbicidal activities[130].

In 1981 Mac Leod, Lewis and Moeller[131] elucidated the structure of the diterpene aminoglycoside Wedeloside **111** (Figure 47), the major toxic constituent of the plant *Wedelia asperrima* Benth. (Asteraceae). Biochemical studies have shown that compound **111** is a powerful inhibitor of mitochondrial ADP/ATP transport, with a binding affinity to the carrier protein comparable to that of the related diterpene glycoside carboxyatractyloside **109**.

Figure 52: ***Wedelia asperrima*** **Benth.**

In 1990, Herigaya *et coll.*[132], reported the isolation of three *ent*-kauranoid diterpenoids **113**, **114** and **115** (Figure 53), from the acqueous extract of the aerial part of *Artemisia sacrorum* Ledeb. This plant distribuited in the Northeast District of China, is known as a Chinese folk medicine in the treatment of hepatitis.

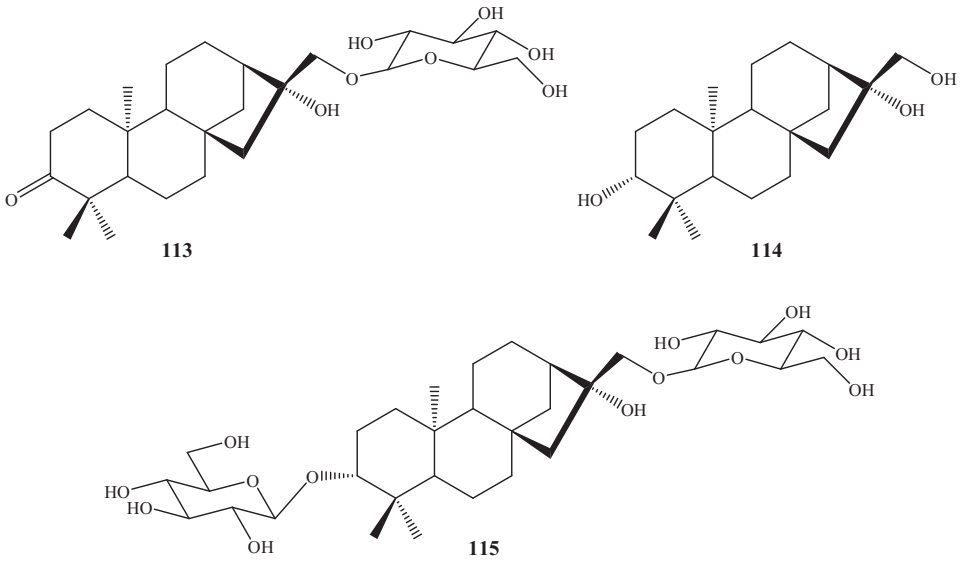


Figure 53

Herigaya *et coll.*[132], moreover,elucidated the structure of these three compounds by means of NMR analysis (DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and ^{1}H-^{1}H decoupling).

In 1996, Zhau *et coll.*[133], reported the isolation of two diterpene glycosides **116** and **117**(Figure 54) obtained from the flowers of *Inula britannica* L. (var. *chinensis*) (Asteraceae), used for the treatment of bronchitis and inflammation in the Chinese traditional medicine.

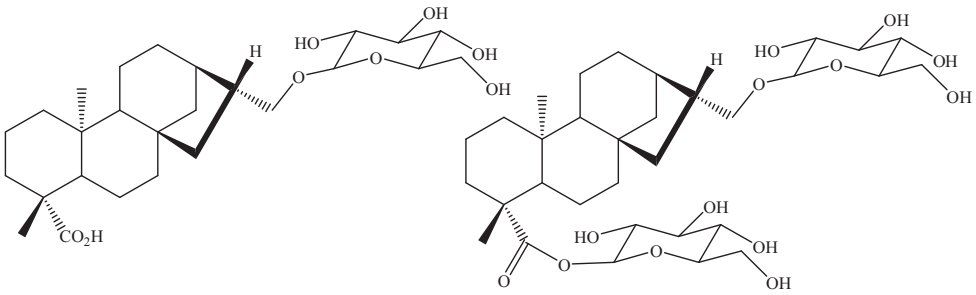


Figure 54: Two diterpene glycosides isolated by Zhau *et coll.* in 1996.

1.4. Objectives of this Ph.D. thesis

The first objective of this thesis was that of evaluating a methodology recently disclosed in our laboratory for the construction of stemarane diterpenoids. The first target was (+)-2-deoxyorizalexin S. Its obtaining would have also allowed to confirm the structure and absolute configuration of a Chilean *Calceolaria* diterpenoid to which the structure of (-)-2-deoxyorizalexin S had been attributed in the past. The original isolated material is, in fact, no longer available for direct comparison. The obtaining of (+)-2-deoxyorizalexin S would have also allowed to use this material to verify the role of HO-C(2) for phytoalexin activity.

The second objective derives from the first one. In fact obtaining of (+)-2-deoxyorizalexin S allowed also to demonstrate the incorrectness of the structure attributed to the diterpenoid isolated from the Chilean *Calceolaria*.

Searching for the structure of this unknown compound stemod-(13)-en-(19)-ol was therefore prepared from an intermediate of the previous synthesis. Also in this case no matching with the diterpenoid isolated from the Chilean *Calceolaria* was observed.

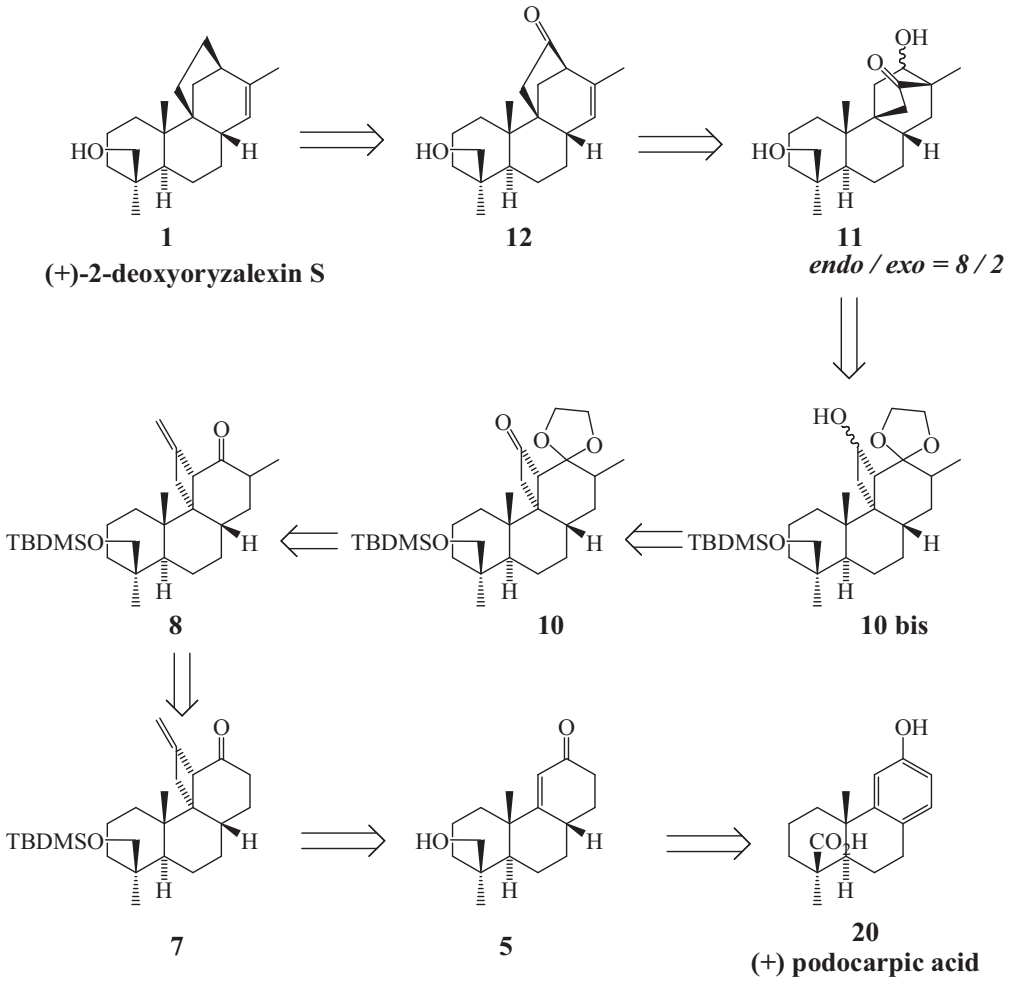
On conclusion of this section of the thesis the third objective was taken-up, that is a new approach to the synthesis of kaurane diterpenes whose importance was highlighted above.

This work has not been yet concluded.

Results and discussion

2.1 Retrosynthetic analysis for (+)-2-deoxyoryzalexin S

For the obtaining of the *target* (+)-2-deoxyoryzalexin S **1**, (+)-podocarpic acid, from which c*hiron* enone **5** was derived, was chosen as starting material and thus as *chiral template* (Scheme 16).



Scheme 16: Retrosynthetic approach to (+)-2-deoxyoryzalexin S 1.

A synthetic strategy that provides a serviceable approach to stereochemically complex structures can take advantage of the existence of the *chiral pool*, that is the set of natural compounds in which stereogenic centers are present in a defined configuration. Through a careful examination of the literature, Hanessian has collected several examples where a natural product is transformed into a chiral synthon, which he calls *chiron*. The *chiron* contain one or more stereogenic centers of the *template*.

At the beginning of his studies on the design of a synthesis, Corey had introduced the term *synthon*[1,2], an ideal fragment that you get through the process of disconnection of a bond. As previously mentioned, his innovative idea was then taken up and revised by Hanessian who brought the new concept of *chiron*. The term *chiron* means a "molecule or an enantiomerically pure intermediate that contains a high functional overlap and stereochemistry with a substructure of the *target* molecule"[3]. To use this strategy it is necessary, first, the examination of the molecular structure of the target, in order to recognize the configuration and position of the stereogenic centers present. They must then be placed in relation with those of the carbon backbone of *chiron*, which, in turn, is derived from the *chiral template*.

The combination of natural chirality, conformational constraints and topological constraints in carbohydrates leads to locate between their structures a skeleton carbonaceous able to ensure a high regio- and stereochemical control. The next step consists in the search for a good overlap between the stereogenic centers and the functions of the *template* with those of c*hiron* chosen for the preparation of a stereochemically complex compound. Sometimes the *chiron* may present a considerable degree of functional overlap and stereochemistry with target, and in part preserves its starting structure which constitutes the *template*. In other cases, however, it may be difficult to recognize inside the *chiron* the structure of the final compound, as happens when the stereocenters must undergo inversion or the rings must undergo cleavage. Thus, if a chiral product must be obtained from a carbohydrate, it is first necessary to compare the functional groups and the absolute configuration of the stereogenic centers of the desired product and the monosaccharide in question. This approach is valid if there is a similarity between the structure of the natural compound and that present in the *target* (or part of it).

If one wants to plan the synthesis of an organic compound it necessary to apply to retrosynthetic analysis[4-6] which consists in reconstruct backward stages of molecular construction that will constitute the synthesis process.

It is a technique to solve the problem of synthesis developed by E.J. Corey, described by him in 1990 in the Nobel Prize Lecture, based on the transformation of the molecule to be synthesized (synthetic target = synthetic TarGeT = TGT)[7] in easier structures until a commercially available precursor, is identified.

To the *target* structure is accompanied the application of a *transform*, the exact opposite of a synthetic step, and each structure identified by retro-analysis becomes, in turn, *target* for a subsequent retro-analytic operation. The repetition of the retro-analytic stages produces a tree of intermediate (EXTGT) with different hypothetical synthetical ways to obtain the *target* molecule. To each *transform* is related a *retron*, the structural unit key present in the *target*, which allows the application of the particular *transform*.

2.1.1 From (+)-podocarpic acid 20 to the intermediate 3, a precursor of *chiron* 5.

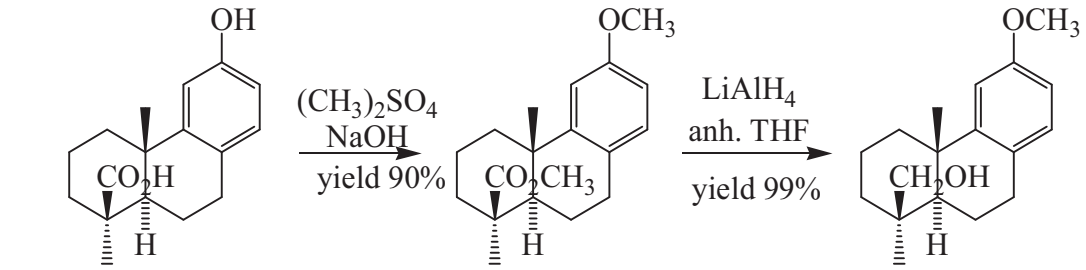
In the first step of the synthesis, both the carboxyl group at C(19) of (+)-podocarpic acid **20** and the phenolic function were methylated. The methylation reaction was conducted under basic conditions with dimethyl sulphate. The formation of the dimethylated product **2** was confirmed by the presence in the ^{1}H-NMR spectrum of a singlet at 3.77 ppm, relative to the aromatic methoxyl and of another singlet at 3.66 ppm, relative to the methyl ester group.

The ^{13}C-NMR spectrum showed a signal at 178 ppm relative to the carbonyl ester function and a signal at 55.4 ppm relative to the methoxyl carbon. The IR spectrum showed a band at 1719 cm^{-1} attributable to an ester carbonyl. Finally, as a further confirmation, GC-MS analysis gave a molecular peak at *m/z* = 302.

The ester group of compound **2** was then reduced with $LiAlH_4$, to give the alcohol **3** (Figure 55). The reduction reaction was carried out in anhydrous THF and the presence of the alcohol group was confirmed by the ^{1}H-NMR spectrum in which it appears an AB system, consisting of two doublets at 3.88 ppm and 3.52 ppm, attributable to the

protons of the group -CH_2-OH, and a multiplet at 2.31 ppm to 2.26 ppm relative to the proton of the hydroxy group. Moreover, we noted the disappearance of the singlet at 3.66 ppm relative to the three protons of the methyl ester function.

The ^{13}C-NMR spectrum showed a signal at 65.2 ppm, characteristic of a carbon directly bonded to an oxygen, and the disappearance of the signal at 178 ppm associated to the ester carbonyl. In the IR spectrum it was possible to note the disappearance of the band at 1719 cm^{-1} of the ester function. Finally, GC-MS analysis gave a molecular peak at *m/z* = 274.



20
(+) podocarpic acid

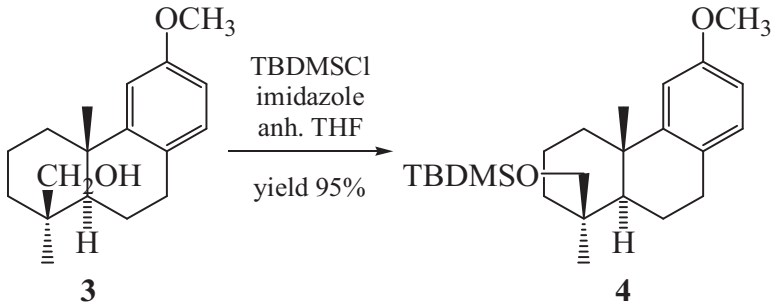
2

3

Figure 55: Synthesis of intermediate 3

2.1.2 Synthesis of *chiron* 5

Before reducing the aromatic ring by the Birch methodology, it was necessary to protect the alcohol group. TBDMSilyl ether was chosen as the protecting group for its stability under the conditions of the Birch reaction. Thus compound **3** was reacted with TBDMSiCl and imidazole in anhydrous THF (Figure 56).



3

4

Figure 56: Synthesis of intermediate 4

The formation of the compound **4** was confirmed by the presence in the ^{1}H-NMR spectrum of a singlet at 0.92 ppm relative to the nine protons of the *t*-butyl group and a doublet at 0.05 ppm attributable to the two methyls bound to silicon. The ^{13}C-NMR spectrum showed two signals around -5 ppm attributable to the two methyls bound to the silicon and a very intense signal at 25.9 ppm relative to the methyl of the *t*-butyl group. As a further confirmation GC-MS analysis, gave the peak of the molecular ion at *m/z* = 388.Compound **4** was then subjected to Birch reduction with lithium in liquid ammonia and *t*-BuOH to give a dienolether, which was then hydrolyzed in acid conditions to give the *chiron* **6** (Figure 57). During this step the removal of the protecting group of the HO-C (19) also occurred.

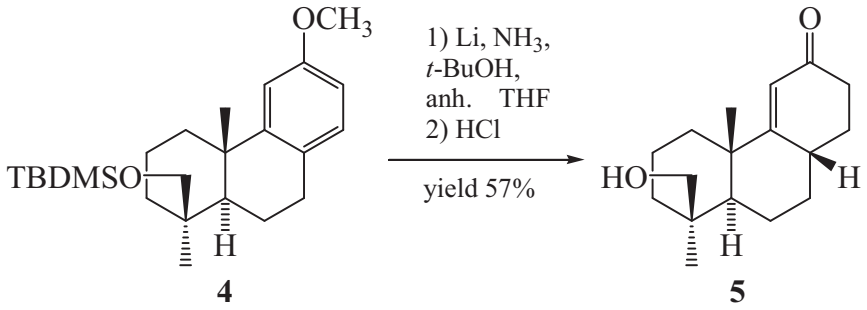
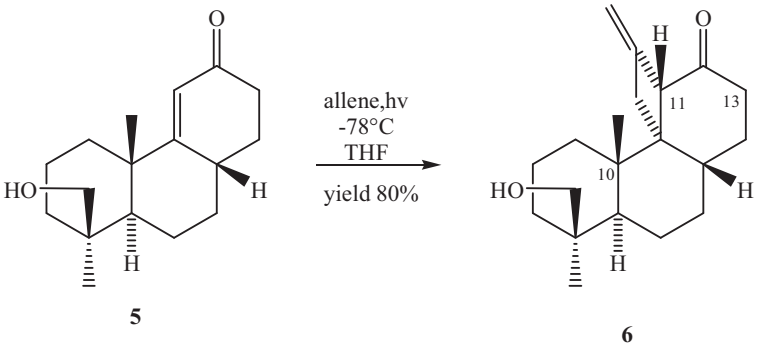


Figure 57: Synthesis of *chiron* 5

The ^{1}H-NMR spectrum confirmed the obtaining of the compound **5** through the disappearance of the signals at 6.98-6.66 ppm, a multiplet related to the three protons of the aromatic ring, and the singlet of the three protons of the methoxyl group at 3.79 ppm. The ^{1}H-NMR spectrum showed a singlet at 5.86 ppm attributable to the olefinic proton C(11). The IR spectrum showed the band relative to the stretching of conjugate C=O at 1661 cm^{-1}. Moreover the GC-MS analysis gave the peak of the molecular ion at m/z = 262. In this step (conducted under conditions which would permit equilibration at C(8)-vinylogous), compound **5** was obtained as a single product because the epimer that have the HC(8) β-oriented is the more stable.

Through photochemical addition of allene to compound **5** (Figure 58), a four-cycle term was built. The reaction was carried out in anhydrous THF, at -78 ° C after having condensed the gaseous allene inside the solution. The latter was then irradiated at -78 °

C through Pyrex with a Hg vapor lamp, which allowed the transition of type n-π* of the non-bonding electrons of the oxygen of the carbonyl. The formation of the photoadduct (+)-**6** was confirmed by NOESY experiments which showed a cross-peak between the HC(11) and CH_3-C(10), that allowing us to establish the stereochemistry of the newly formed cyclobutane ring.



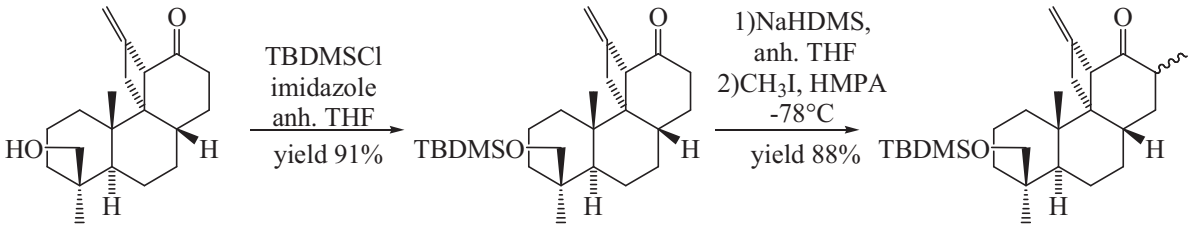
5 **6**

Figure 58: Synthesis of photoadduct 6

The regiochemistry of the photoaddition followed from the dowfield shift (about 1.5 ppm) of HC(11), adjacent to both a double bond and a carbonyl function, compared to that of $H_2C(13)$. The IR spectrum showed the band relative to the stretching of conjugate C=O at 1686 cm^{-1}.

2.1.3 Synthesis of compound 8

Prior to methylation at C(13), it was necessary to protect as the *tert*-butyldimethylsilyl ether the HO-C(19) of photoadduct **(+)-6**. The compound **6** was, therefore, reacted with TBDMSiCl and imidazole in anhydrous THF (Figure 59).



6 **7** **8**

Figure 59: Synthesis of intermediate 8

The formation of the intermediate **7** was verified by the ^{1}H-NMR spectrum in which it appears a singlet at 0.06 ppm relative to the six protons of the methyl groups on silicon and an intense singlet at 0.98 ppm attributable to the methyls of the *t*-butyl group. The ^{13}C-NMR spectrum showed two signals at -5.72 and -5.68 ppm relative to the two methyls on silicon and a signal at 25.8 ppm of *t*-butyl group. Further confirmation comes from the GC-MS where is present a peak at *m/z* = 359, due to the loss by fragmentation of the *t*-butyl group.

The protected photoadduct **7** was then methylated using a hindered base such as sodiobis(trimethylsilyl)amide (NaHMDS) at -78 °C and subsequent methylation with CH_3I in hexamethylphosphoroamide (HMPA). Compound **8** was obtained as epimeric mixture at C(13) and this mixture was reacted as such in the subsequent steps, because the stereochemistry of stereogenic center will be established in the desired manner during the subsequent reaction of aldolic condensation necessary for the formation of the bicyclo[2.2.2]octane system. The formation of methylated photoadduct **8** was confirmed by ^{1}H-NMR spectra of both epimers through the appearance of a doublet at 0.97 ppm and 1.22 ppm, corresponding to the three protons of the added methyl. The analysis of the GC-MS of both epimers showed retention times slightly different and a *m/z* value = 373 relative to the loss of the *t*-butyl group.

2.1.4 Synthesis of compounds 11a and 11b

Before the oxidative cleavage of the exocyclic double bond the methylated photoadduct **8** was converted into the acetal **9** (Figure 60). The reaction was carried out by treating the intermediate **8** with ethylene glycol, benzene and TsOH and removing the water formed with a Dean-Stark apparatus. A mixture epimeric at C(13) was obtained. The formation of compound **9** was confirmed by the IR spectra of both epimers by the disappearance of the band relative to the stretching of C=O. GC-MS confirmed the obtaining of two compounds with near retention times and having a value corresponding to the molecular ion of *m/z* = 474.

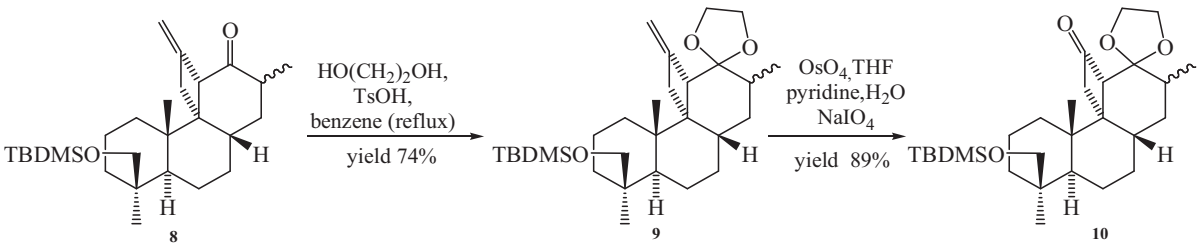


Figure 60: Synthesis of the intermediate 10

The epimeric mixture **9** was subjected to oxidative cleavage with OsO_4 and $NaIO_4$ to give the cyclobutanone **10** (Figure 60), a mixture of epimers at C(13) with different R_f. The ^{1}H-NMR spectra showed the disappearance of the signals at 4.98 ppm and 5.31 ppm of the two methylene protons of the C(16). The IR spectra showed the presence of the band relative to the stretching of C=O to 1771 cm^{-1}. Finally, GC-MS showed the presence of two peaks with a molecular ion *m/z* = 476 with retention times close to each other. The cyclobutanone **10** was then reduced with $NaBH_4$ in Et_2O/MeOH and afterwards treated with a mixture of THF/2N HCl at 80 °C to give compound **11** (Figure 61) in an approximate 80:20 *endo*/*exo* ratio. In this step, the carbonyl group was deprotected, the resulting hydroxy ketone underwent a retro-aldol reaction, which was followed by a new aldol reaction which finally led to compound **11**.

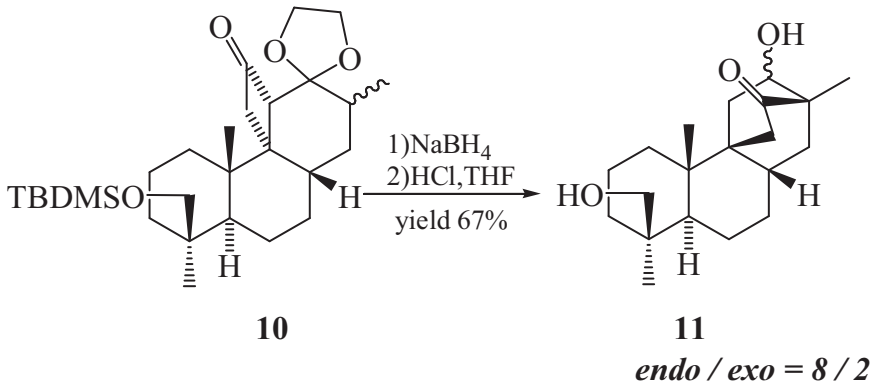


Figure 61: Synthesis of epimeric mixture 11a and 11b

The formation of ketols **11** has been confirmed by IR spectra in which a signal is present at 1715 cm^{-1} attributable to the stretching of C=O. In addition GC-MS showed two peaks with a molecular ion *m/z* = 320 and with very close retention times.

2.1.5 Synthesis of (+)-2-deoxyoryzalexin S 1

Epimers **11** were treated with *p*-toluenesulfonic acid, at 80 °C, in toluene at reflux, giving the transposition product **12** characterized by the bicyclo[3.2.1]octane system (Figure 62).

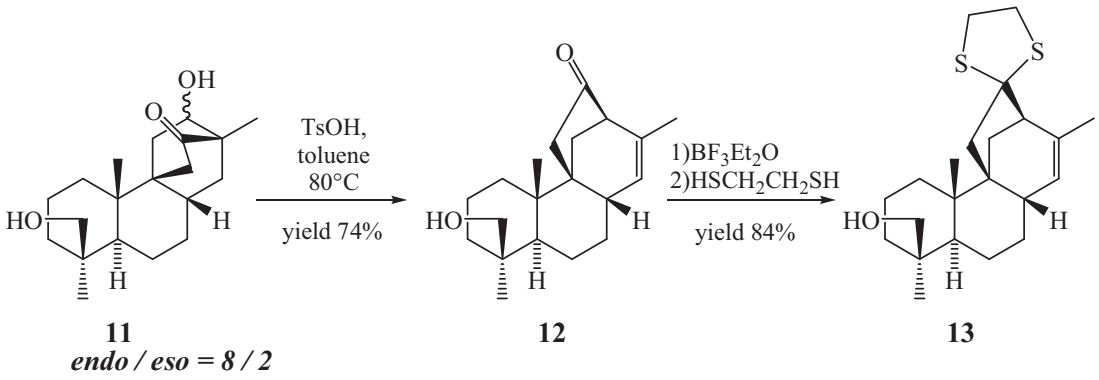
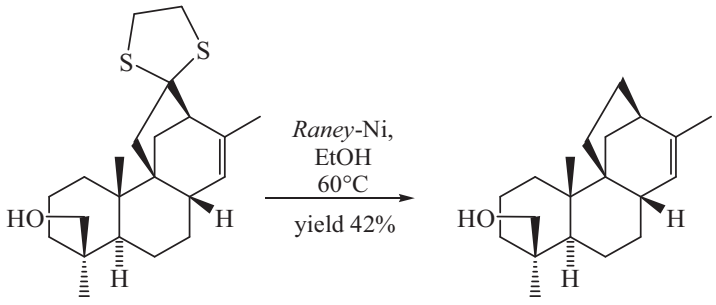


Figure 62: Synthesis of compound 13

The formation of **12** was confirmed by the presence in the ^{1}H-NMR spectrum of the signal of the olefinic proton at 5.31 ppm. The IR spectrum showed the signal at 1728 cm^{-1} relative to the stretching of C=O and from GC-MS a molecular ion peak at *m/z* = 302 was obtained. Thioacetalization of **12** with ethanedithiol and $BF_3 \cdot Et_2O$ at 0°C afforded then compound **13**, which was transformed into **1** by the action of *Raney*-Ni in EtOH at 60°C (Figure 63).



13 **1**

(+)-2-deoxyoryzalexin S

Figure 63: Synthesis of (+)-2-deoxyoryzalexin S 1

The formation of the compound **13** was confirmed by the disappearance in the IR spectrum of the signal at 1728 cm^{-1} relative to the C=O. The ^{1}H-NMR spectrum showed the signal of the olefinic proton at 5.0 ppm, and finally GC-MS analysis revealed the peak of the molecular ion at *m/z* = 378. The formation of the final product **1** was confirmed by GC-MS which showed a peak with the molecular ion at *m/z* = 288. In the ^{1}H-NMR spectrum is present a signal of an olefinic proton at 4.93 ppm.

2.1.6 The key step of the (+)-2-deoxyoryzalexin S synthesis.

The key step of this synthesis is the acid-catalyzed transposition of a 6-hydroxy-bicyclo[2.2.2]octane-2-one intermediate to the bicyclo[3.2.1]octane system typical of stemarane compounds. On the basis of the stereoelectronic requirements of the bicyclo[2.2.2]octane to bicyclo[3.2.1]octane rearrangement and on the *endo*/*exo* 6-hydroxy-bicyclo[2.2.2]octan-2-one equilibrium, under the reaction conditions adopted, for the rearrangement we propose the rationale reported in figure 64.

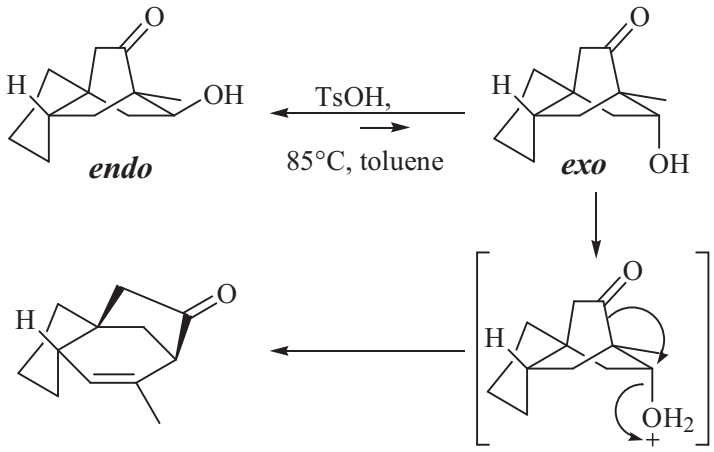
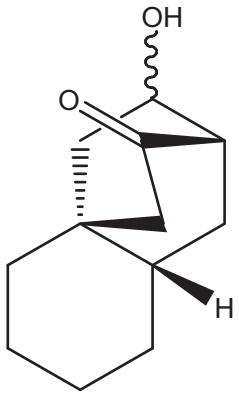


Figure 64: Acid-catalyzed rearrangement mechanism proposed

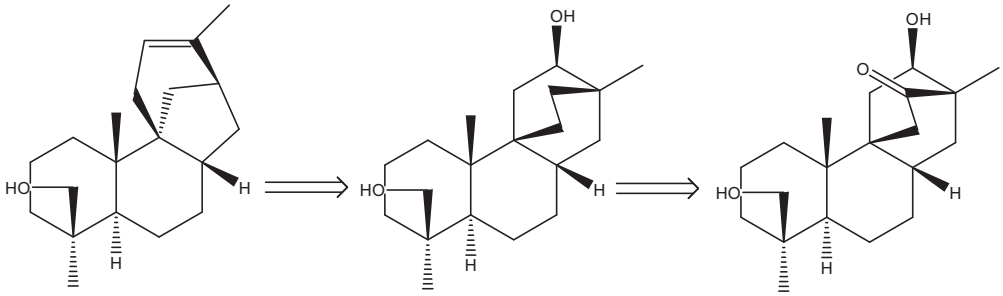
This equilibrium is due to an unfavorable 1,3 boat-axial interaction experimented in the *exo* epimer by the *pseudo*-axially oriented hydroxyl group. The protonated *exo* hydroxyl compound act as the leaving group and the acyl group migration occurs from the *anti* side. The *endo* epimer does not undergo rearrangement because the carbonyl function at C(2) prevents the development of a positive charge on the adjacent bridged carbon. Moreover the role of the bridgehead methyl in stabilizing the carbocation resulting from the rearrangement was confirmed by the fact that the same experimental conditions were applied to known **212** no reaction occurred.



212

2.2 Retrosynthetic analysis for stemod-(13)-en-(19)-ol.

For the obtaining of *target* stemod-(13)-en-(19)-ol **19** ketol **11a**, an intermediate of the (+)-2-deoxyoryzalexin S synthesis (Scheme 17), was chosen as starting material.

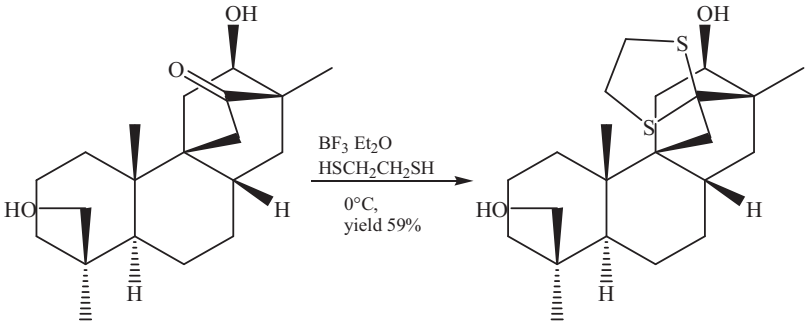


19 **18** **11a**

Scheme 17: Retrosynthetic approach to stemod-(13)-en-(19)-ol

2.2.1 Synthesis of stemod-(13)-en-(19)-ol

The thioketalization was carried out on **11a** whose HO-C(12) is correctly oriented for the final reaction of transposition. Thus *endo* **11a** was reacted with ethanedithiol and $BF_3 \cdot Et_2O$ at 0 °C to give the thioacetal **17** (Figure 65).

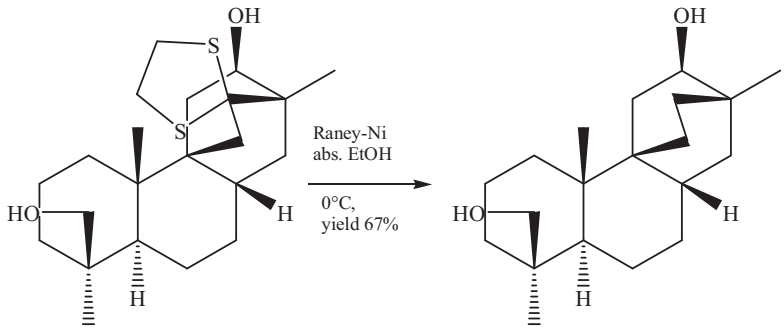


11a **17**

Figure 65: Synthesis of compound 17

The formation of thioacetal **17** was confirmed by the disappearance, in the IR spectrum, the signal at 1715 cm^{-1} relative to the stretching of C=O.

In the ^{1}H-NMR spectrum two doublets at 3.82 ppm and 3.45 ppm relative to the AB system of $–CH_2OH$ group, and two triplets at 3.38 ppm and 3.15 ppm relative to two CH_2 directly linked to the two sulfur atoms of the thioacetal group are observed. The thioketal **17** was afterwards subjected to desulphurization by treatment with Raney-Ni, at 60°C, in absolute EtOH under reflux to give the compound **18** (Figure 66).

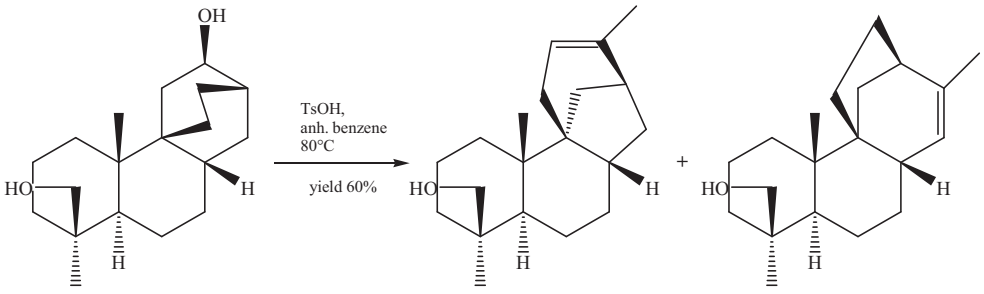


17 **18**

Figure 66: Synthesis of compound 18

The formation of compound **18** was confirmed by the disappearance, in the ^{1}H-NMR spectrum, the two triplets at 3.38 ppm and 3.15 ppm relative to the thioacetal group. In addition GC-MS analysis showed a molecular ion peak with *m/z* = 306.

Finally, the reaction of trasposition was carried out by treating compound **18** with *para*-toluensulphonic acid at 80 °C, in anhydrous benzene under reflux. Surprisingly along with the expected stemodane compound **19** (+)-2-deoxyoryzalexin S **1** was also formed, in ratio 1/1.6 (Figure 67).



18 **19** **1**
(+)-2-deoxyoryzalexin S

1 : 1.6

Figure 67: Rearrangement intermediate 18

Stemod-(13)-en-(19)-ol **19** was isolated and characterized. The formation of the stemod-(13)-en-(19)-ol **19**, was confirmed by the appearance in the ^{1}H-NMR spectrum of a signal at 5.5 ppm relative to the olefinic proton at C-(12). In addition GC-MS analysis shows a molecular ion peak with *m/ z* = 288.

2.2.2 A hypothesis for the formation of the stemarane system.

From the results obtained, we proposed the following hypothesis concerning the formation of the stemarane system (figure 68).

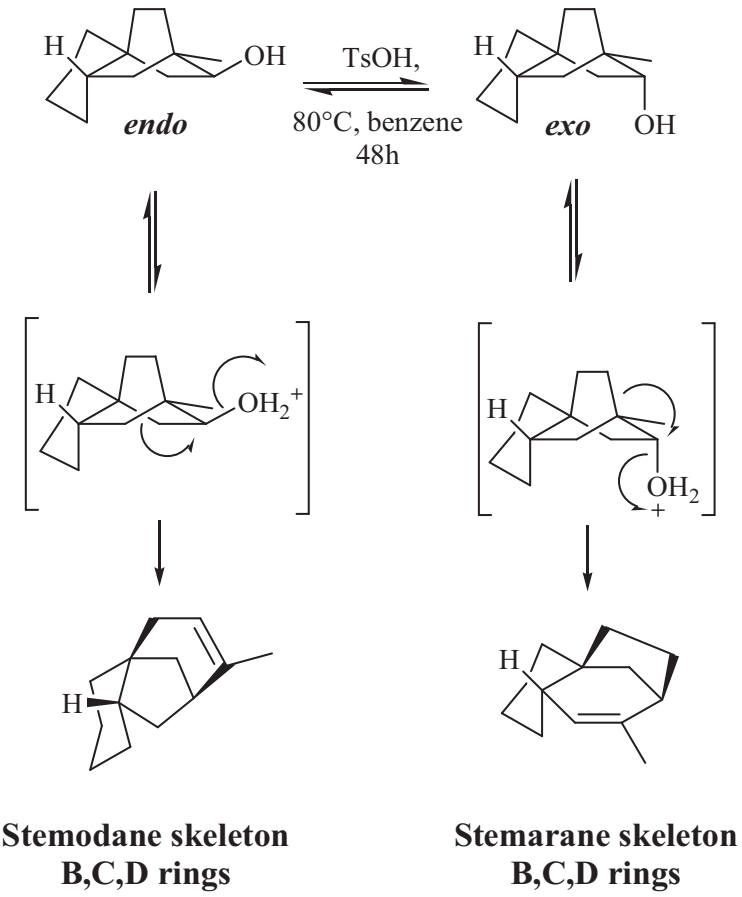


Figure 68: Mechanism hypothesis

To confirm this hypothesis we submitted a secondary chiral alcohol, (*R*)-(-)-*sec*-butanol, to the same reaction conditions (figure 69). After 48 hours the analysis of the reaction mixture revealed complete recemization of the starting (*R*)-(-)-*sec*-butanol.

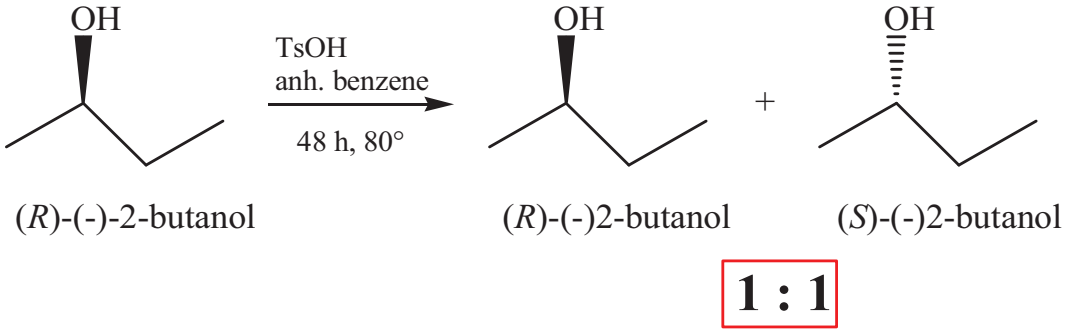
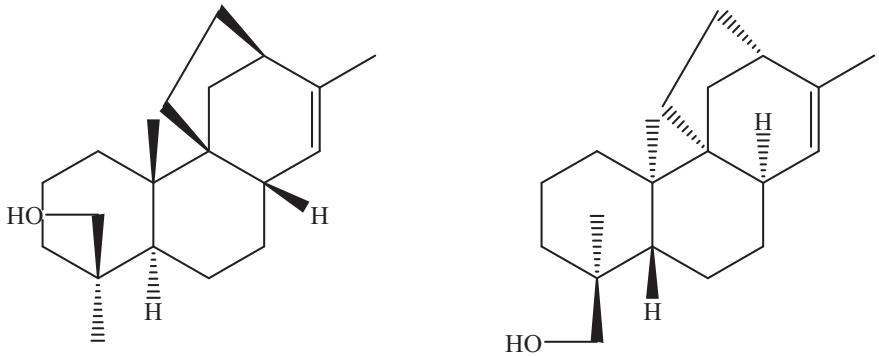


Figure 69

Having obtained *(+)-2-deoxyoryzalexin S* **1**, it was thought to confirm the structure and the absolute configuration of a compound isolated in Chile by Garbarino from plants of the genus *Calceolaria*, to which to the structure of *2-deoxyoryzalexin S* **15** had been attributed (Figure 70) .



1
(+)-2-deoxyoryzalexin S

15
(-)-2-deoxyoryzalexin S

Figure 70

The NMR data recorded by us for the *(+)-2-deoxyoryzalexin S* **1** and those reported in literature for Garbarino for compound **15** were therefore compared. We can observe,

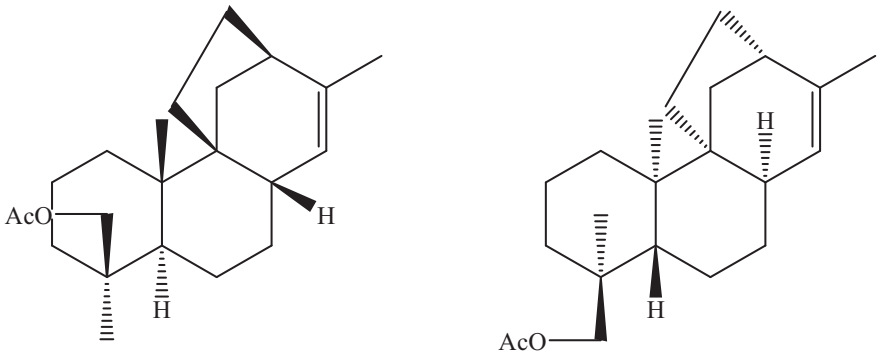
on table 1, the differences in the ^{13}C NMR data regarding C(5), C(7), C(8), C(11), C(12), C(15), and C(16).

Position	*(+)-2-deoxyoryzalexin S* [a]		*2-deoxyoryzalexin S* [b]	
	δC type	δH (J in Hz)	δC type	δH (J in Hz)
1	32.0, CH_2 [d]	1.75-1.62, m 1.22-1.12, m	31.8	
2	18.4, CH_2	1.57-1.52, m 1.48-1.41, m	18.3	
3	35.6, CH_2	1.86-1.79, m 0.95-0.85, m	36.3	
4	38.7, C [e]		38.5 [d]	
5	50.2, CH	1.27-1.22, m	49.4	
6	22.09, CH_2	1.65-1.57, m 1.36-1.31, m	21.5	
7	31.8, CH_2 [d]	1.75-1.62, m 1.22-1.12, m	35.3	
8	44.4, CH	2.01-1.93, m	42.2	
9	51.1, C		51.4	
10	38.3, C [e]		38.2 [d]	
11	30.0, CH_2	1.66-1.60, m 1.49-1.43, m	24.5	
12	43.3, CH	2.19 *t*(4.4)	41.7	
13	138.9, C		138.6	
14	123.9, CH	4.94 *pd* (4.3)	123.4	4.77 *bs*
15	33.4, CH_2	1.65-1.57, m 1.54-1.44, m	34.2	
16	32.4, CH_2	1.51-1.44, m 1.44-1.37, m	28.1	
17	22.12, CH_3	1.62 *pt*(1.3)	21.8	1.62 *d* (1.0)

18	27.2, CH_3	0.95 *s*	26.7	0.97 *s*
19	65.5, CH_2	3.80 *d* (10.9) 3.42 *dd* (10.5, 1.0)	65.0	3.78 *d* (11.0) 3.38 *d* (11.0)
20	17.9, CH_3	0.93 *s*	18.1	0.93 *s*

Table 1: NMR Spectroscopic Data for (+)-2-deoxyoryzalexin S and 2-deoxyoryzalexin S. [a] ^1H and ^{13}C NMR at 400.13 and 100.61 MHz, respectively; δ in ppm relative to the residual solvent peak of $CDCl_3$ at 7.26 and 77.0 ppm for ^1H and ^{13}C respectively. [b] ^1H and ^{13}C NMR at 250 and 63 MHz, respectively; δ in ppm relative to TMS as internal standard.

(+)-2-deoxyoryzalexin S acetyl derivative **14** has also been synthesized and characterized. The data of compound **14** were compared with the data of the acetyl derivative **16** prepared by Garbarino from **15** (Figure 71).



14
(+)-2-deoxyoryzalexin S
acetyl derivative

16
(-)-2-deoxyoryzalexin S
acetyl derivative

Figure 71

The results (table 2 and 3) showed, also in this case, that the two products are not identical. Not identical, are in fact the spectra of ^1H and ^{13}C-NMR, the melting points and the absolute $[\alpha]^{20}_D$ value. Differences was found particularly in the ^{13}C NMR of C(5), C(7), C(8), C(11), C(12), C(15), and C(16).

	$[\alpha]^{20}_{D}$	Melting point
14	+ 58.2	118.2-119.7°C
16	-17.02	96-97°C

Table 2: Melting points and specific rotations of acetyl derivatives 14 and 16

Position	Compound **14** [a]		Compound **16** [b]	
	δC type	δH (J in Hz)	δC type	δH (J in Hz)
1	31.9, CH_2 [d]	1.76-1.63, m 1.23-1.12, m	31.7	
2	18.4, CH_2	1.58-1.50, m 1.47-1.40, m	18.4	
3	36.3, CH_2	1.76-1.69, m 0.99-0.89, m	36.1	
4	37.2, C		36.8 [d]	
5	50.2, CH	1.27-1.22, m	49.4	
6	22.1, CH_2	1.66-1.60, m 1.41-1.35, m	21.7	
7	31.8, CH_2 [d]	1.75-1.63, m 1.23-1.12, m	36.3	
8	44.3, CH	2.01-1.96, m	42.2	
9	51.1, C		51.4	
10	38.8, C		38.5 [d]	
11	29.9, CH_2	1.65-1.60, m 1.48-1.43, m	24.6	
12	43.3, CH	2.19 *t*(4.4)	41.8	
13	138.9, C		138.4	
14	123.9, CH	4.94 *pd* (3.2)	123.2	4.77 *bs*
15	33.4, CH_2	1.65-1.58, m 1.52-1.44, m	34.2	
16	32.2, CH_2	1.52-1.45, m	28.1	

		1.44-1.37, m		
17	22.1, CH_3	1.62 *pt*(1.3)	22.0	1.63 *d* (1.0)
18	27.7, CH_3	0.93 *s*	27.3	0.93 *s*
19	67.2, CH_2	4.27 *d* (10.5) 3.87 *d* (10.5)	66.8	4.28 *d* (11.0) 3.84 *d* (11.0)
20	17.9, CH_3	0.96 *s*	18.0	1.02 *s*
21	171.6, C		171.1	
22	21.2, CH_3	2.04 *s*	21.0	2.03 *s*

Table 3: NMR Spectroscopic data for acetyl derivatives of (+)-2-deoxyoryzalexin S 14 and 2-deoxyoryzalexin S 16. [a] ^{1}H and ^{13}C NMR at 400.13 and 100.61 MHz, respectively; δ in ppm relative to the residual solvent peak of $CDCl_3$ at 7.26 and 77.0 ppm for ^{1}H and ^{13}C respectively. [b] ^{1}H and ^{13}C NMR at 250 and 63 MHz, respectively; δ in ppm relative to TMS as internal standard.

Moreover, the structure of the (+)-2-deoxyoryzalexin S acetyl derivative **14** was confirmed by X-Ray crystallographic analysis using synchrotron radiation data collected at ELETTRA (XRD-1 beamline) by Dr. Doriano Lamba, C.N.R., Area Science Park – Basovizza, Trieste (Figure 72).

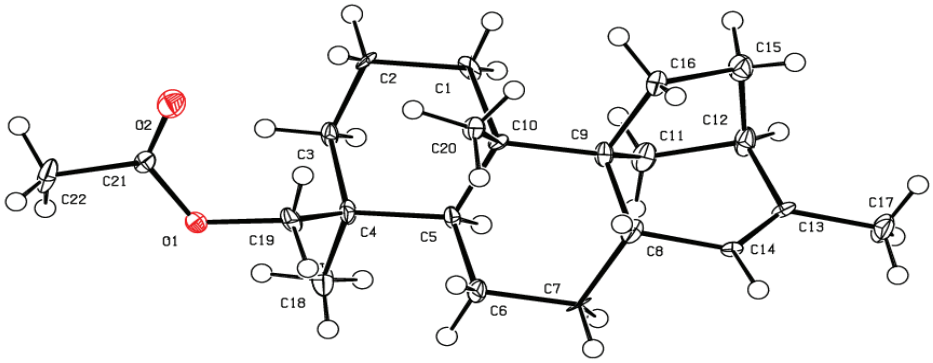
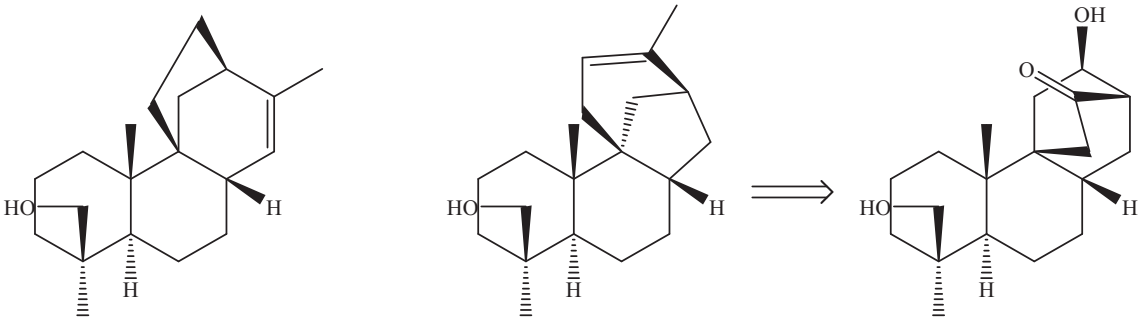


Figure 72: X-Ray structure of A. Thermal ellipsoids are shown at the 50% probably level

Because Garbarino had originally speculated that *Calceolaria* diterpenes could have a stemodane or stemarane skeleton, opting, then, for the latter, as a result of non-identity of the (+)-2-deoxyoryzalexin S acetyl derivative **14** with the product isolated from *Calceolaria*, we decided to prepare from **11a** an intermediate of *(+)-2-deoxyoryzalexin*

S **1** synthesis, stemod-(13)-en-(19)-ol **19** which differs from **1** in the C/D ring system (Figure 73). In this way it would be possible to check if the structure of the product isolated by Garbarino from *Calceolaria*.



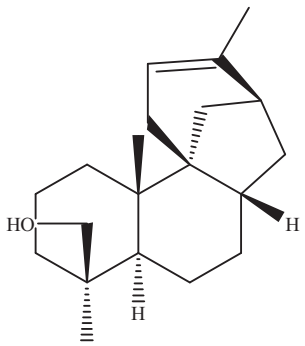
1
(+)-2-deoxyoryzalexin S

19
(+)-stemod-(13)-en-(19)-ol

11a
ketol *endo*

Figure 73.

Comparing the ^{13}C-NMR spectrum of the compound **19** with that of the compound isolated from Garbarino from *Calceolaria* it could be established, however, the non-identity of the two compounds (Table 4).



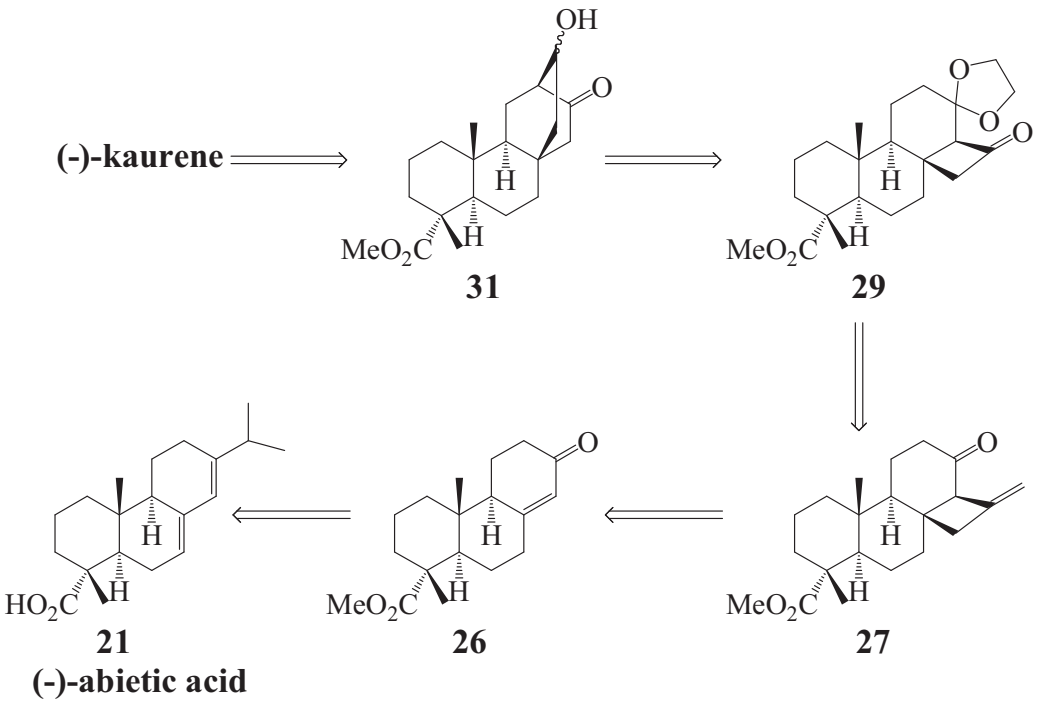
19
(+)-stemod-(13)-en-(19)-ol

19	**15**
142.11	138.8
117.05	123.3
65.82	68.1
50.82	51.5
48.55	49.6
46.83	42.3
42.97	41.7
38.45	38.5
38.09	37.1
37.33	36.4
35.66	36.1
35.22	34.1
32.59	31.8
31.75	28.1
29.51	27.3
27.66	24.7
22.19	22.1
21.37	21.7
20.76	18.4
18.45	18.2

Table 4: ***left:*****19) ^{13}C-NMR (ppm) of stemod-(13)-en-(19)-ol and 15) ^{13}C-NMR (ppm) of the compound isolated from *Calceolaria* (-)-2deoxyoryzalexin S. *right*: Structure of stemod-(13)-en-(19)-ol and of (-)-2deoxyoryzalexin S.**

2.3 Retrosynthetic approach to (-)-kaurene

For the obtaining of *target* (-)-kaurene, (-)-abietic acid **21** from which the *chiron* enone **6** was derived (Scheme 18), was chosen as starting material, and thus as *chiral template*.

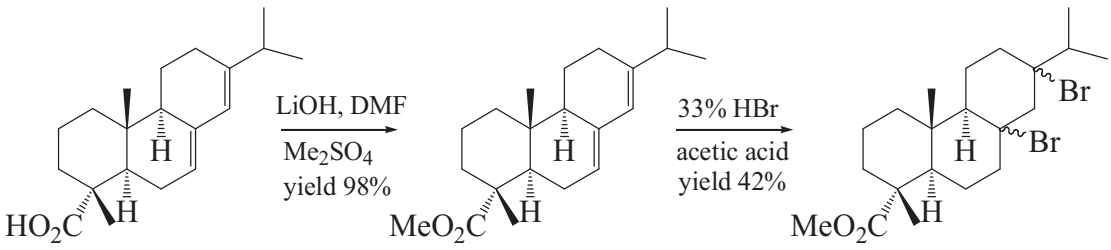


Scheme 18: Retrosynthetic approach to (-)-Kaurene

2.3.1 From (-)-abietic acid 21 to the c*hiron* 6

In the first step of the synthesis, the carboxyl group of (-)-abietic acid **21** was methylated (figure 74). The methylation reaction was carried out under basic conditions using dimethyl sulphate, to give 98% of methyl abietate **22**. The formation of methyl abietate **22** was confirmed by the presence in the ^{1}H-NMR spectrum of a singlet at 3.6 ppm, relative to the ester group. The ^{13}C-NMR spectrum showed a signal at 179.1 ppm

relative to same group. The IR spectrum showed a band at 1722 cm^{-1} attributable to an ester carbonyl. Finally, as further confirmation, GC-MS analysis gave the molecular peak at *m/z* = 316.



21
(-)-abietic acid

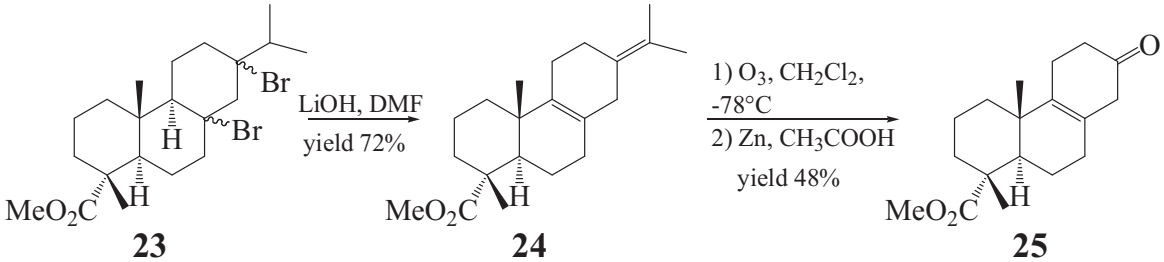
22

23

Figure 74.

Methyl abietate **22** was then treated with a solution of 33% hydrobromic acid in acetic acid to give 42% of dibromo derivative **23** (figure 75). The formation of compound **23** was confirmed by the disappearance of the signals related to the olefinic protons in the ^{1}H-NMR spectrum and by the absence, in the ^{13}C-NMR spectrum, of signals in the region 140-115 ppm relative to the two carbon-carbon double bonds. The IR spectrum showed a band at 1722 cm^{-1} attributable to an ester carbonyl.

The dehydrobromination of **23** was carried out with lithium hydroxide in dimethylformamide at 80°C, to give the diene **24** (figure 75). The formation of diene **24** was confirmed by the presence in the ^{13}C-NMR spectrum of signals at 138.8, 128.4, 126.2, 120.9 ppm relative to the two tetrasubstituted double bonds. The IR spectrum showed a band at 1720 cm^{-1} attributable to an ester carbonyl. Finally, as further confirmation, GC-MS analysis gave the molecular peak at *m/z* =316.



23

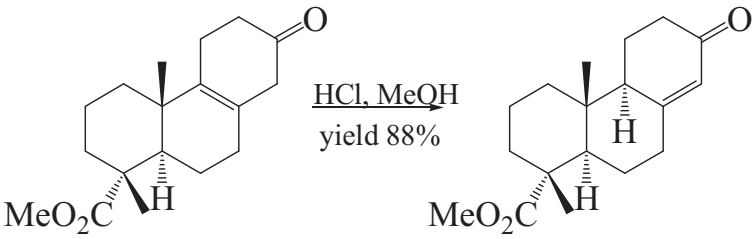
24

25

Figure 75

The diene **24** was subjected to ozonolysis, using a stream of ozone in dichloromethane at -78°C; the ozonide was then reacted with zinc in acetic acid, to give 48% of β,γ-enone **25** (figure 75). The formation of compound **25** was confirmed by the presence in the ^{13}C-NMR spectrum of a signal at 211.5 ppm relative to the unconjugated carbonyl function. The latter was also observed in the IR spectrum which showed a band at 1715 cm^{-1}. Finally, as further confirmation, GC-MS analysis gave the molecular peak at *m/z* = 290.

Isomerization of β,γ-enone **25** with hydrochloric acid in methanol gave 88% of α,β-enone **26** (figure 76). The formation of compound **26** was confirmed by the presence in the ^{1}H-NMR spectrum of a singlet at 5.86 ppm relative to the olefinic proton at C(9). The ^{13}C-NMR spectrum showed a signal at 199.6 ppm relative to the carbonyl function of a α,β-unsatured ketone. The IR spectrum showed a band at 1660 cm^{-1} attributable to the carbonyl function of a α,β-unsatured ketone. Finally, as further confirmation, GC-MS analysis gave the molecular peak at *m/z* = 290.



25 **26**

Figure 76

Photoaddition of allene to **26** at -78°C proceeded from the β-face and gave the expected cyclophotoadduct **27** with a yield of 67% (figure 77)[1,2]. The formation of compound **27** was confirmed by the presence in the ^{1}H-NMR spectrum of a singlet at 4.84 ppm relative to the proton of the exocyclic double bond. The ^{13}C-NMR spectrum showed the signals at 142.1 ppm and at 210.5 ppm relative to the olefinic carbon C(15) and C(13). The IR spectrum showed a band at 1715 cm^{-1} attributable to the carbonyl function. Finally, as further confirmation, GC-MS analysis gave the molecular peak at *m/z* =330.

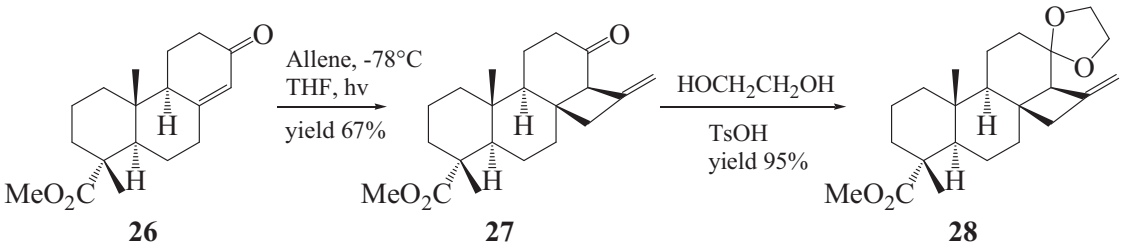


Figure 77

Before the oxidative cleavage of the exocyclic double bond, compound **27** was converted into the acetal **28** with a yield of 95%, by standard methods (Figure 77). The reaction was carried out by treating the intermediate **7** with ethylene glycol, benzene and TsOH, removing the water formed with a Dean-Stark apparatus. The formation of compound **28** was confirmed by the IR spectra by the disappearance of the band relative to the stretching of C=O. In the ^{1}H-NMR spectrum of a multiplet at 3.80-4.00 ppm relative to the four acetal protons was present. The ^{13}C-NMR spectrum showed the signals at 64.1 ppm and at 64.3 ppm relative to the OCH_2CH_2O. Moreover the ^{13}C-NMR spectrum showed the disappearance of a signal at 210.5 relative to the C=O in the compound **7**, and the presence of the signal at 110.4 relative to the protected carbonyl C(13). GC-MS gave the molecular peaks at *m/z* = 376.

Acetal **28** was converted into the cyclobutanone **29** by the action of OsO_4 and $NaIO_4$ (figure 78). The formation of compound **29** was confirmed by the IR spectra by the presence of the band relative to the stretching of C=O at 1782 cm^{-1}.

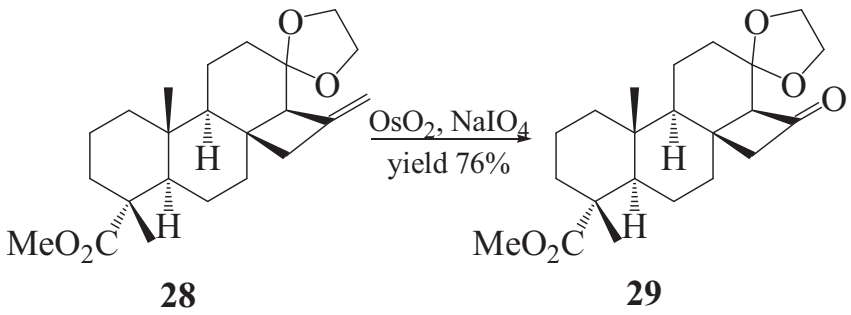
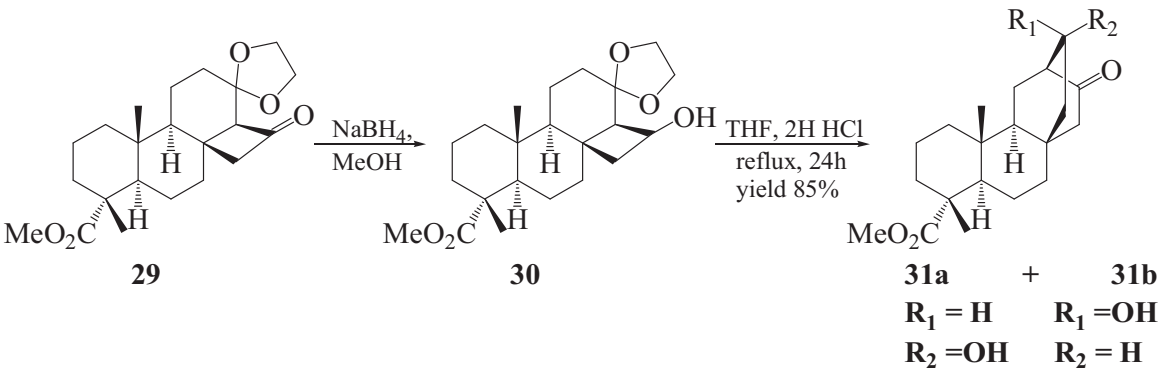


Figure 78

The carbonyl function of the compound **29** was then converted, by reduction, into the hydroxyl compound **30** (figure 79), which was used in the next step without purification. Treatment of **30** with a 2:1 mixture THF/2N HCl under reflux for 24 h gave hydroxyl ketone **31a** and 3**1b** in a *ca.* 9:1 ratio.



29 **30** **31a** + **31b**

R_1 = H **R_1 =OH**

R_2 =OH **R_2 = H**

Figure 79

The ^{1}H-NMR spectrum of **31a** and **31b** showed the signals related to the protons of the hydroxyl group at 4.12 ppm and 4.20 ppm, respectively. The ^{13}C-NMR spectrum showed the signals at 55.1 ppm and at 54.7 ppm relative to C(15). Moreover the IR spectra showed the presence of the band relative to the stretching of C=O at 1720 cm^{-1}.

In accordance with precedent cases[140], the major epimer was attributed the structure **31a**, which the HO-C(13) is *endo*-configurated. The assignment was confirmed by comparing the ^{13}C chemical shifts of the HO-C(13) of **31a** (69.1ppm) and **31b** (65.5 ppm): these values are diagnostic in that the signal of the HO-C(13) in the *exo*-epimer appears at higher field. The same $\Delta\delta$ is displayed by the C(11)-atom in **31a** and **31b** the C(11)- and C(13)-atoms in **30b** seem, therefore, to be in sterically more crowded environment than the corresponding C-atoms in **31a**.

Unfortunately when compound **31**, dissolved in benzene, was refluxed in the presence of TsOH no reaction occurred. It appears, therefore, that this rearrangement must be encouraged by some means like for instance the presence of an *exo* olefinic bond. We hope to be able to prove in the near future this hypothesis.

Conclusions

In the frame of this Ph.D. work the first synthesis of *(+)-2-deoxyorizalexin S* from (+)-podocarpic acid was achieved. *(+)-2-deoxyoryzalexin S* was characterized also as its acetyl derivative. The structure of the latter was confirmed by X-ray crystallographic analysis. Comparison of the data of the *(+)-2-deoxyoryzalexin S* and its acetyl derivative with those reported in letterature by Garbarino did not confirm the structure of *(-)-2-deoxyoryzalexin S* originally assigned to the diterpenoid isolated from Chilean *Calceolaria* species.

(+)-stemod-(13)-en-(19)-ol was then synthesized from an intermediate of the synthesis of *(+)-2-deoxyoryzalexin S*. *(+)-stemod-(13)-en-(19)-ol* was characterized as the acetyl derivative. Comparison of the data of *(+)-stemod-(13)-en-(19)-ol* and its acetyl derivative with those reported in letterature by Garbarino did not confirm the structure of *(-)-2-deoxyoryzalexin S*.

Intermediates of this work will be tested to evaluate their phytoalexin activity particularly those possessing a stemarane skeleton.

After having concluded this part of the work, we used the same methodology in order to get the kaurane system. Starting from pre-formed system of (+)-abietic acid, an advanced intermediate was obtained, which hopefully will allow us to get the kaurane system in the near future.

Experimental Part

4.1 Materials and methods

All solvents were evaporated to dryness under vacuum. All solvents were anal. grade. All reagents and anhydrous solvents were ALDRICH, they were used without further purification if not indicated.

TLC: Merck silica gel 60 F_{254}. Column chromatography (CC): silica gel 60, 70-230 mesh ASTM. Melting point: Mettler-FP-61 apparatus (uncorrected).

IR Spectra: FT-IR spectrometer, Shimadzu-8400S IR scattering, NaCl cell; in cm^{-1}.

^{1}H- and ^{13}C NMR spectra (compound **8**): Bruker AC 300 at 300.13 and 75.48 MHz, respectively; δ in ppm relative to the residual solvent peak of C_6D_6 at 7.15 and 128.02 ppm and $CDCl_3$at 7.26 and 77.0 ppm for ^{1}H- and ^{13}C, respectly.

^{1}H- and ^{13}C NMR spectra: Brucker AVANCE 400 at 400.13 and 100.61 MHz, respectly; δ in ppm relative to the residual solvent peak of C_6D_6 at 7.15 and 128.02 ppm and $CDCl_3$at 7.26 and 77.0 ppm for ^{1}H- and ^{13}C, respectly; *J* in Hz.

HPLC analysis: Shimadzu SCL-10 A VP; RID detector (RID 10-A) and UV-VIS detector (SPD 10-A). HPLC experiments were performer at room temperature. Analytical columns, EC 250/4 Nucleosil 100-5, EC 250/4 Nucleodur 100-5; flow rate of 0.8 mL/min; semipreparative column, VP 250/10 Nucleodur 100-5; flow rate of 6 mL/min.

GC-MS analysis: Shimadzu GCMS-QP5000, (Equity^{TM}-5 capillary column, n° 32809-02B, 30 m x 0.25 mm, film thickness 0.25 μm).

Optical rotations: DIP 370 Jasco digital polarimeter.

HRESIMS spectra: Micromass Q-TOF Micromass spectrometer (Waters) in the electrospray-ionization mode.

4.2 Abbreviations and notes

TLC : thin layer chromatography

CC : column chromatography

r.t.: room temperature

s.s.: saturated solution

THF : tetrahydrofuran

TsOH = *p*-toluenesulfonic acid

TBDMSCl = *t*-butyl dimethyl silyl chloride

m.p. = melting point

brine = NaCl saturated solution

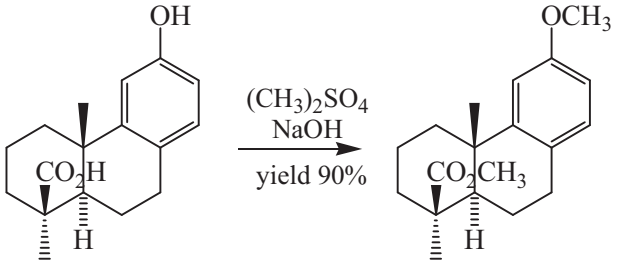
DEG = diethylenglicol

HMPA= hexamethylphosphoramide

NMR: J = coupling constant in Hz; s = singlet; *d* = doublet; *t* = triplet; *m* = multiplet; *b* = broad, *dd* = doublet of doublets, *ddd* = doublet of doublets of doublets, *dtd* = doublet of triplet of doublets, *o* = overlapping signals. GC-MS: peak intensity expressed in % in round bracket (relative to base peak).

4.3 Preparations

Synthesis of **2** from (+) podocarpic acid **20**:



20
(+) podocarpic acid

2

To a solution of (+)-podocarpic acid **20** (20 g, 73 mmol) in aqueous $EtOH_{50\%}$ (300 mL), NaOH (9 g, 225 mmol) was added, until achieving a basic pH. After that dimethyl sulphate (20.5 mL, 216 mmol) was added dropwise through dropping funnel. The mixture was stirred at reflux (for 48 h) until TLC (eluent: AcOEt/hexane = 3/7) showed the disappearance of the starting material.

The mixture was cooled to r.t. and washed with Et_2O, brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced pressure.

The residue obtained, was crystallized from hexane, giving **2** (19.8 g, 65.6 mmol, yield 90%).

m.p.: 124.3 - 126.9°C.

$[\alpha]_D$: +129 (c = 3.0 g/100 mL, $CHCl_3$).

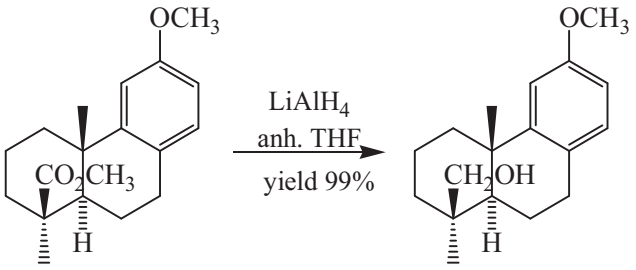
IR ($CHCl_3$): 2951, 1719 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 1.02-1.14 (m, 1H), 1.03 (s, 3H), 1.27 (s, 3H), 1.34-1.65 (m, 3H), 1.87-2.30 (m, 5H), 2.67-2.89 (m, 2H), 3.66 (s, 3H), 3.77 (s, 3H), 6.65-6.98 (m, 3H).

^{13}C-NMR ($CDCl_3$): 20.2, 21.3, 23.1, 28.7, 31.4, 37.8, 38.8, 39.6, 44.2, 51.4, 53, 55.4, 111.3, 111.4, 127.8, 130, 149.5, 157.9, 178.

GC-MS: (t_R 10.6) *m/z* = 302 (M^+ ,47), 287 (4), 243 (2), 228 (20), 227 (100), 213 (1), 199(3), 185 (7), 173 (13), 171 (20), 161 (15), 159 (10), 147 (15), 134 (11), 128 (9), 121 (12), 115 (11), 91 (10), 77 (6), 69 (6), 59 (6), 55 (10), 53 (4).

Synthesis of **3** from **2**:



2 **3**

To a solution of **2** (19.8 g, 65.6 mmoli) in anhydrous THF (396 mL), $LiAlH_4$ (2.50 g, 65.8 mmol) was added. The mixture was stirred under an Ar atmosphere, until TLC (eluent: AcOEt/hexane = 3/7) showed the disappearance of the starting material (12 h).

The excess hydride was destroyed carefully with drops of H_2O at 0 °C and then a 2N HCl solution was added to obtained a neutral solution.

The mixture was extracted with Et_2O, and the organic layer washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced pressure.

The residue obtained, was crystallized from hexane/Et_2O, giving **3** (18 g, 65.7 mmol, yield 99%).

m.p.: 89.2 – 90.8 °C.

[α]$_D$: +67 (c= 2.0 g/100 mL, $CHCl_3$)

IR ($CHCl_3$): 2930, 1261 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 1.06 (s, 3H), 1.19 (s, 3H), 1.26-2.01 (m, 9H), 2.27-2.31 (m, 1H), 2.71-2.92 (m, 2H), 3.52-3.88 (m, 2H), 3.78 (s, 3H), 6.65-6.97 (m, 3H).

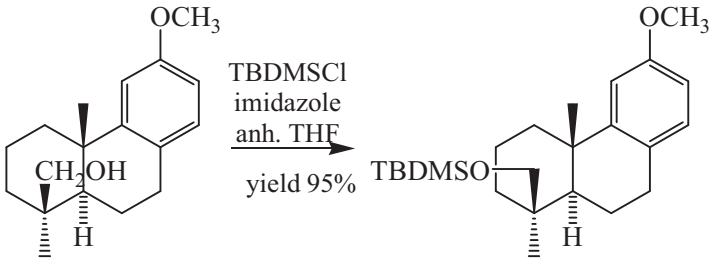
^{13}C-NMR ($CDCl_3$): 19.2, 19.4, 25.8, 27, 30.3, 35.4, 38.1, 39, 51.4, 55.3, 65.2, 110.6, 111.1, 127.2, 130.0, 151.1, 157.8.

GC-MS: (t_R 11.1) *m/z* = 274 (M^+, 80), 272 (6), 259 (10), 241 (42), 229 (9), 201 (19), 199 (12), 187 (12), 185 (16), 174 (11), 173 (43), 171 (29), 161 (93), 159 (33), 158 (20), 148 (14), 147 (100), 141 (11),

135 (19), 134 (18), 129 (17), 128 (23), 121 (43), 115 (29), 91 (28), 81 (19), 77 (16), 55 (32), 53 (10).

HPLC: eluent: AcOEt/hexane =15/85, flow: 0.8 mL/min, t_R 16.7 min.

Synthesis of **4** from **3**:



3 **4**

To a solution of **3** (18.0 g, 65.7 mmol) in anhydrous THF (69.2 mL), *tert*-butyldimethylsilyl chloride (TBDMSCl, 11.7g, 78.0 mmol) and imidazole (5.75 g, 84.5 mmol) were added.

The mixture was stirred at r.t. until TLC (eluent: AcOEt/hexane = 3/7) showed the disappearance of the starting material (14 h). *n*-Hexane was added, the suspension filtered through Celite, the organic phase evaporated, and the residue purified by CC (AcOEt/*n*-hexane, 2.5:97.5) to give **4** (24.3 g, 62.5 mmol, yield 95%).

m.p.: 52.9-53.9 °C.

[α]$_D$: +50 (c = 1.47 g/100 mL, $CHCl_3$)

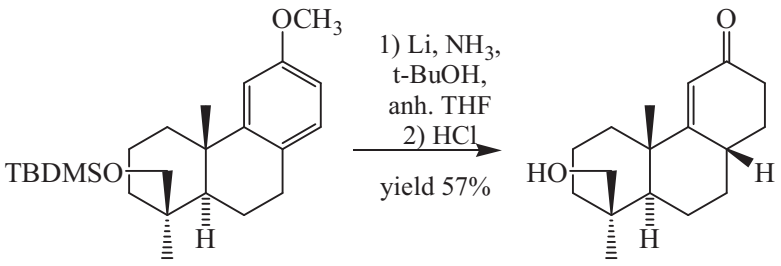
IR ($CHCl_3$): 2930, 1250, 1092 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 0.05-0.06 (d, 6H), 0.92 (s, 9H), 1.01 (s, 3H), 1.21 (s, 3H), 1.44-1.49 (m, 2H), 1.60-1.76 (m, 3H), 1.85-1.89 (m, 1H), 1.96-2.03 (m, 1H), 2.26-2.30 (m, 1H), 2.70-2.91 (m, 2H), 3.50-3.53 (d, 1H), 3.70-3.73 (d, 1H), 3.79 (s, 3H), 6.66-6.98 (m, 3H).

^{13}C-NMR ($CDCl_3$): 157.6, 151.2, 129.7, 127.3, 110.7, 110.3, 65.2, 55.2, 50.9, 39.0, 38.8, 37.9, 35.5, 30.2, 27.4, 25.9, 25.6, 19.4, 19.0, 18.2, -5.5, -5.6.

GC-MS: (t_R 13.3) *m/z* = 388 (M^+ , 21), 331 (25), 255 (28), 241 (18), 199 (12), 185 (37), 173 (20), 171 (13), 161 (51), 159 (17), 147 (40), 121 (14), 103 (16), 95 (14), 89 (17), 83 (10), 81 (12), 75 (100), 73 (47), 59 (11), 55 (16).

Synthesis of **5** from **4**:



4 **5**

In a three neck flask equipped with a mechanical stirring, ammonia (600 mL) at -78°C under Ar was condensed. Then anhydrous THF (600 mL), *t*-BuOH (14.5 mL) and Li (10 g, 1.43 mol) were added under stirring. A solution of **4** (23 g, 59.3 mmol) was then added to the mixture, kept under stirring and at -50°C, until TLC (eluent: Et_2O/hexane = 2.5/7.5) showed the disappearance of the starting material (7 h). Then, absolute EtOH was added up to solution decolouration.

After evaporation of the ammonia at r.t., water (142 mL), MeOH (56 mL) and 6N HCl solution were added, allowing the mixture to stir overnight. The mixture wae then, neutralized with a $NaHCO_3$, and the organic layer extracted with Et_2O, washed with brine, dried on Na_2SO_4 and concentrated under reduced pressure.

The residue obtained, was first purified by CC (eluent: AcOEt/hexane = 30/70) and then crystallized (distilled Et_2O /AcOEt = 1/1) giving α,β-unsatured ketone **5** (8.9 g, 34.0 mmol, yield 57%).

m.p.: 112.4-113.3°C.

$[\alpha]_D$: -32 (c = 2.989 g/100 mL, $CHCl_3$)

IR ($CHCl_3$): 2934, 1661 cm^{-1}.

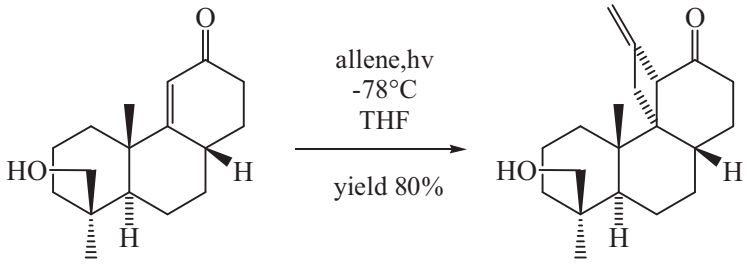
^{1}H-NMR ($CDCl_3$): 0.62-0.92 (m, 3H), 0.75 (s, 3H), 0.92 (s, 3H), 1.01-1.60 (m, 9H), 1.86-2.00 (m, 4H), 2.16-2.27 (m, 1H), 3.27-3.31 (d, 1H), 3.56-3.60 (d, 1H), 5.86 (s, 1H).

^{13}C-NMR ($CDCl_3$): 201.4, 176.1, 119.6, 65.0, 53.6, 40.8, 39.2, 36.7, 35.7, 35.5, 35.1, 34.3, 29.3, 26.8, 22.0, 21.4, 18.3.

GC-MS: (t_R 25.9) *m/z* = 262 (M^+, 1), 232 (11), 231 (29), 149 (9), 124 (10), 123 (100), 121 (18), 110 (35), 109 (14), 107 (15), 105 (14), 95 (10), 93 (13), 91 (21), 81 (14), 77 (12), 67 (12), 55 (26).

HPLC: Eluent: AcOEt/hexane = 30/70, flow: 1 mL/min, t_R 17.7 min.

Synthesis of **6** from **5**:



5 **6**

A Pyrex vessel containing a solution of **5** (6.9 g, 26.2 mmol) in THF (78 mL) was cooled to -78°C, and an excess of allene was condensed into it. The vessel was irradiated under Ar at -78°C with a 500 W mercury vapor lamp until TLC (eluent: Et_2O 100%) showed the disappearance of **5** (4 h). The solution was slowly warmed to r.t., the solvent evaporated, and the residue purified by CC (eluent: AcOEt/hexane = 3.5/6.5) and then crystallized (distilled Et_2O /AcOEt) to give the photoadduct **8** (6.3 g, 20.8 mmol, yield 80%) as a white powder.

m.p.: 174.2-175.8 °C.

$[\alpha]_D$: +27 (c = 3.253 g/100 mL, $CHCl_3$)

IR ($CHCl_3$): 2941, 1686.

^{1}H-NMR ($CDCl_3$, 400.13 MHz): 4.91 (1H, pq, J = 2.6 Hz), 4.89 (1H, pq, J = 2.8 Hz), 3.72 (1H, d, J = 10.8 Hz), 3.47- 3.42 (1H,m), 3.40 (1H, d, J = 10.8 Hz), 2.79 (1H, dt, J_t = 2.5 Hz, J_d = 17.4 Hz), 2.63 – 2.51 (2H,m), 2.14 (1H, dddd, J = 0.8 Hz, J = 7.7 Hz, J = 11.5 Hz, J = 19.1 Hz), 1.95 – 1.25 (12H,m), 1.11(1H, pq, J_d = 4.5 Hz, J_q= 12.7 Hz), 1.03 (1H, dd, J = 2.9 Hz, J= 12.9 Hz), 0.94 (3H,s), 0.89 (1H, td, J_d = 4.4 Hz, J_t= 13.7 Hz), 0.81 (3H,s).

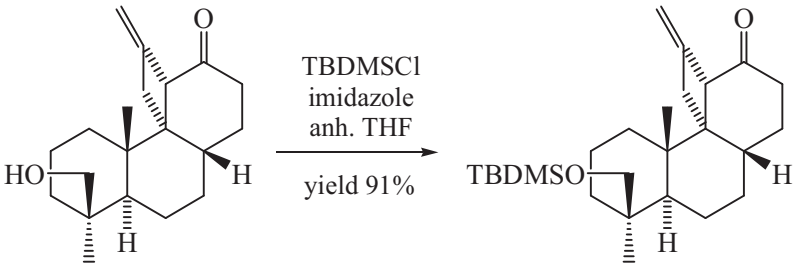
^{13}C-NMR ($CDCl_3$, 100.61 MHz): 211.4, 140.8, 109.7 (CH_2), 64.8 (CH_2), 55.6 (CH), 49.7, 47.2 (CH), 39.1, 38.5 (CH_2), 38.2, 36.5 (CH), 35.4 (CH_2), 31.7 (CH_2), 31.6 (CH_2), 28.9 (CH_2), 26.8 (CH_3), 25.3 (CH_2), 21.9 (CH_2), 18.1 (CH_2), 16.7 (CH_3).

HRESIMS: *m/z* = 325.2159 (calcd for $C_{20}H_{30}O_2$ $[M + Na]^+$, 325.2144).

GC-MS: (t_R 25.9) *m/z* = 262 (M^+, 1), 232 (11), 231 (29), 149 (9), 124 (10), 123 (100), 121 (18), 110 (35), 109 (14), 107 (15), 105 (14), 95 (10), 93 (13), 91 (21), 81 (14), 77 (12), 67 (12), 55 (26).

HPLC: Eluent: AcOEt/hexane = 30/70, flow: 1 mL/min, t_R 10.9 min.

Synthesis of **7** from **6**:



6 **7**

To a solution of **3** (5.9 g, 19.5 mmol) in anhydrous THF (20.25 mL), *tert*-butyldimethylsilyl chloride (TBDMSCl, 7.7 g, 50.9 mmol) and imidazole (3.0 g, 45.0 mmol) were added.

The mixture was stirred at r.t. until TLC (eluent: AcOEt/hexan = 6/4) showed the disappearance of the starting material (5 h). *n*-Hexane was added, the suspension filtered through Celite, the organic phase evaporated, and the residue purified by CC (AcOEt/*n*-hexane, 2.5:97.5) to give **7** (7.2 g, 17.3 mmol, yield 91%) as a white powder.

m.p.: 114.6-116.3 °C.

$[\alpha]_D$: +9 (c = 3.11 g/100 mL, CCl_4)

IR (CCl_4): 2932, 1697, 1092 cm^{-1}.

^{1}H-NMR (C_6D_6, 400.13 MHz): 5.02 (1H, q, J = 2.7 Hz), 4.82 (1H, q, J = 2.6 Hz), 3.63 (1H, d, J = 9.6 Hz), 3.55- 3.49 (1H,m), 3.37 (1H, d, J = 9.6 Hz), 2.52 (1H, A of ABX_2, J_{AB} = 17.5 Hz, J_{AX2} = 2.7 Hz), 2.47 (1H, dd, J = 5.6 Hz, J = 18.8 Hz), 2.30 (1H, B of AYX_2, J_{AB} = 17.5 Hz, J_{BY} = 3.1 Hz, J_{BX2} = 2.7 Hz), 2.05 – 1.86 (2H,m), 1.66 – 1.04 (10H, m), 0.99 (9H, s), 0.97 (3H, s), 0.92 – 0.71 (3H, m), 0.64 (3H, s), -0.07 (3H, s).

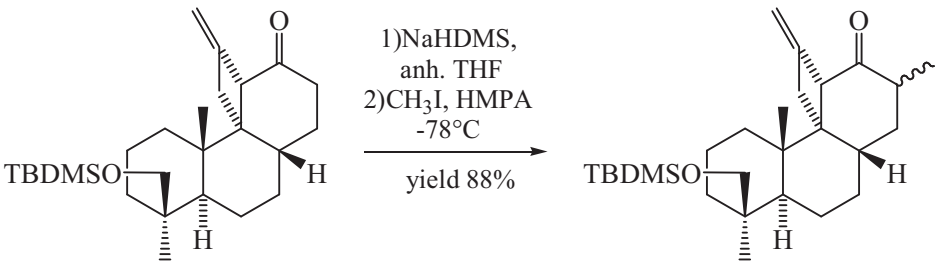
^{13}C-NMR (C_6D_6, 100.61 MHz): 208.1, 141.7, 109.2 (CH_2), 65.1 (CH_2), 55.6 (CH), 49.7, 47.1 (CH), 39.1, 38.6 (CH_2), 38.5, 36.5 (CH), 36.0 (CH_2), 31.7 (CH_2), 31.5 (CH_2), 29.2 (CH_2), 27.6 (CH_3), 26.2 (3xCH_3),

25.5 (CH_2), 22.2 (CH_2), 18.6, 18.4(CH_2), 16.5 (CH_3), -5.29 (CH_3), -5.33 (CH_3).

HRESIMS: *m/z* = 439.3021 (calcd for $C_{26}H_{44}O_2Si$ $[M + Na]^+$, 439.3008).

GC-MS: (t_R 15.5) *m/z* = 359 (M^+ - t-butyl) (9), 269 (10), 187 (11), 173 (19), 159 (12), 145 (12), 131 (13), 119 (12), 105 (16), 89 (22), 75 (100), 67 (12), 55 (27).

Synthesis of **8** from **7**:



7 **8a +8b**

A solution of sodium bis(trimethylsilyl) amide (NaHDMS, 17 mL, 1M in THF, 17 mmol) was added at -78°C to a solution of **7** (7.0 g, 17 mmol) in THF (43 mL). After 1 h the resulting sodium enolate was added to a solution of CH_3I (2.03 mL, 33 mmol) in hexamethylphosphoramide (HMPA, 8.7 mL, 50 mmol). The solution was stirred at -78°C until TLC showed the disappearance of **7** (4h).

The mixture was warmed to r.t., neutralized with 2N HCl, diluted with Et_2O, washed, dried and evaporated. The residue was purified by CC (eluent: AcOEt/hexane = 2.5/97.5) to give a mixture of **8a** and **8b** (6.2 g, 14 mmol, 82%, **8a**:**8b**, 3:1; R_f **8a** > R_f **8b**). For analytical purposes the mixture was separated by HPLC (AcOEt/hexane = 1/99; flow 1 mL /min):

(+) - **8a** ($t_{R\ HPLC}$ =11.7 min): white powder.

m.p.: 106.7 – 108.0 °C.

[α]$_D$: +21 (c = 3.13 g/100 mL, CCl_4)

IR (CCl_4): 2932, 1699 cm^{-1}.

^{1}H-NMR (C_6D_6, 300.13 MHz): 4.90 (1H, pq, J = 2.6 Hz), 4.81 (1H, q, J = 2.5 Hz), 3.58 (1H, A of AB, J_{AB} = 9.6 Hz), 3.56 (1H, pq, J = 2.8 Hz), 3.36 (1H, B of AB, J_{AB} = 9.6 Hz), 2.66 – 2.45 (2H, m), 2.33(1H, dq, J_d = 17.4 Hz, J_q = 2.7 Hz), 2.07 – 1.87 (2H, m), 1.66 – 0.68 (27H, m), 0.64 (3H, s), 0.07 (6H, s).

^{13}C-NMR (C_6D_6, 75.48 MHz): 212.5, 142.2, 109.3 (CH_2), 64.9 (CH_2), 55.9 (CH), 51.2, 47.0 (CH), 39.4(CH), 39.2, 38.5 , 35.8(CH_2), 34.3 (CH_2), 32.7 (CH), 31.8 (CH_2), 31.0 (CH_2), 29.2 (CH_2), 27.5 (CH_3), 26.2 (3xCH_3), 22.0 (CH_2), 18.6, 18.4 (CH_2), 17.6 (CH_3), 16.3 (CH_3), -5.29 (CH_3), -5.33 (CH_3).

GC-MS: (t_R 15.5) *m/z* = 373 (M^+ - t-butyl), 283 (10), 187 (15), 173 (14), 159 (12), 145 (15), 131 (10), 119 (15), 105 (17), 89 (25), 81 (18), 75 (100), 73, 67 (14), 55 (24).

HRESIMS: *m/z* = 453.3143 (calcd for $C_{27}H_{46}O_2Si$ $[M + Na]^+$, 453.3165).

(-) – **8b** ($t_{R\ HPLC}$ =15.3 min): white solid.

m.p.: 150.3 - 151.7 °C

$[\alpha]_D$: -8.2860, (c = 4.757 g/100 mL, CCl_4)

IR (CCl_4): 2932, 1697 cm^{-1}.

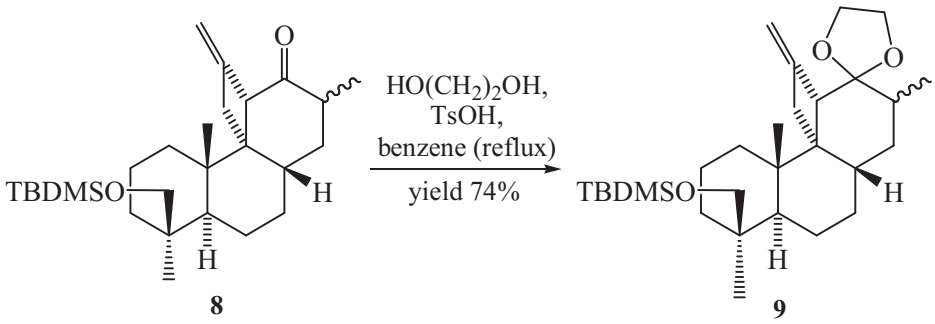
^{1}H-NMR (C_6D_6, 400.13 MHz): 5.07 (1H, q, J = 2.8 Hz), 4.82 (1H, pq, J = 2.6 Hz), 3.64 (1H, d, J = 9.7 Hz), 3.55 3.50 (1H, m), 3.40 (1H, d, J = 9.6 Hz), 2.51(1H, A of ABX_2, J_{AB} = 17.4 Hz, J_{AX2} = 2.5 Hz), 2.31 (1H, B of $ABYX_2$, J_{AB} = 17.4 Hz, J_{BY} = 3.1 Hz, J_{BX2} = 2.7 Hz), 1.97 – 1.84 (2H, m), 1.61 – 1.10 (13H, m), 1.00 (9H, s), 0.99 (3H, s), 0.94 – 0.71 (3H, m), 0.65 (3H, s), 0.08 (6H, s).

^{13}C-NMR (C_6D_6, 100.61 MHz): 209.9, 141.6, 109.4 (CH_2), 65.1 (CH_2), 55.2 (CH), 49.6, 47.1 (CH), 45.5 (CH), 39.0, 38.6 , 36.8(CH), 36.0 (CH_2), 34.2 (CH_2), 31.84 (CH_2), 31.78 (CH_2), 29.1 (CH_2), 27.6 (CH_3), 26.2 (3xCH_3), 22.3 (CH_2), 19.1 (CH_3), 18.6, 18.4 (CH_2), 16.6 (CH_3), -5.29 (CH_3), -5.32 (CH_3).

GC-MS: (t_R 15.5) *m/z* = 373 (M^+ - t-butyl), 285 (11), 187 (16), 173 (13), 159 (10), 145 (16), 119 (14), 105 (16), 89 (19), 81 (16), 75 (100), 73, 67 (13), 55 (23).

HRESIMS: *m/z* = 453.3186 (calcd for $C_{27}H_{46}O_2Si$ $[M + Na]^+$, 453.3165).

Synthesis of **9** from **8**:



8 **9**

A solution of **8** (**8a** + **8b**) (6.1 g, 14 mmol) ethane-1,2-diol (38 mL, 0.55 mmol) and TsOH (0.13 g, 0.71 mmol) in benzene (0.60 L) was placed into a two-neck flask fitted with a Dean-Stark apparatus, a condenser, and a $CaCl_2$ tube. The mixture was refluxed until TLC showed the disappearance of **8** (24 h).

After cooling to r.t., the mixture was diluted with Et_2O, washed with a satured $NaHCO_3$ solution, dried, and evaporated. The residue was purified by CC (eluent: Et_2O/hexane = 2/98) yielded a mixture of **9a** and **9b** (5.2 g, 11 mmol, 78%, **9a**:**9b**, 1:9; R_f **9a** > R_f **9b**). For analytical purposes the mixture was separated by HPLC (AcOEt/hexane = 0.5/99.5; flow 1 mL /min).

(-) – 9a ($t_{R\ HPLC}$ =7.2 min): white solid.

m.p.: 117.3-118.3 °C

[α]$_D$: -42.783 (c = 1.291 g/100 mL, $CHCl_3$)

IR ($CDCl_3$): 2930, 1213, 1092 cm^{-1}.

^{1}H-NMR (C_6D_6, 400.13 MHz): 5.51 – 5.47 (1H, m), 5.01 – 4.97 (1H, m), 3.82 (1H, d, J=9.6 Hz), 3.58 – 3.43 (5H, m), 2.96 (1H,bs), 2.52 (1H, A of ABX_2, J_{AB} = 17.3 Hz, J_{AX2} = 2.4 Hz), 2.46 (1H, B of $ABYX_2$, J_{AB} = 17.3 Hz, J_{BY} = 2.8 Hz, J_{BX2} = 2.7 Hz), 2.16 – 1.98 (3H, m), 1.71 – 1.25 (12H, m), 1.11 (3H, m), 1.05 (3H, s), 1.03 – 0.82 (12H, m), 0.094 (3H, s), 0.091 (3H, s),.

^{13}C-NMR (C_6D_6, 100.61 MHz): 144.4 (CH_2), 111.4, 109.5 , 65.2 (CH_2), 64.4 (CH_2), 63.5 (CH_2), 49.2, 47.5 (CH), 47.2 (CH), 39.4(CH), 39.0, 38.7 , 36.0(CH_2), 35.7 (CH_2), 32.5 (CH), 32.2 (CH_2), 31.1 (CH_2), 29.6 (CH_2), 27.7 (CH_3), 26.2 (3xCH_3), 22.3 (CH_2), 20.3 (CH_3), 18.7(CH_2), 18.6, 16.5 (CH_3), -5.25 (CH_3), -5.31 (CH_3).

GC-MS: (t_R 17.9) *m/z* = 474 [M^+] (7), 329 (13), 205 (9), 113 (100), 100 (7), 75 (29), 69 (13), 55 (9).

HRESIMS: *m/z* = 497.3450 (calcd for $C_{29}H_{50}O_3Si$ [M + Na]$^+$, 497.3427).

(+) – 9b ($t_{R\ HPLC}$ = 8.4 min): white solid.

m.p.: 80.6-81.8 °C.

$[\alpha]_D$: +27.2, (c = 4.514 g/100 mL, $CHCl_3$)

IR ($CDCl_3$): 2932, 1086 cm^{-1}.

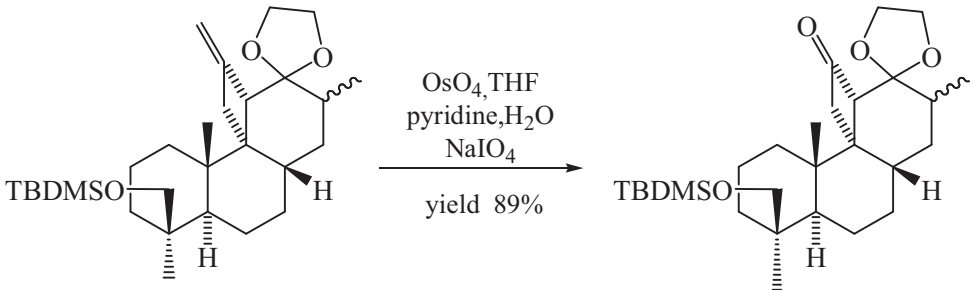
^{1}H-NMR (C_6D_6, 400.13 MHz): 5.37 – 5.31 (1H, m), 5.01 – 4.97 (1H, m), 3.79 (1H, d, J=9.6 Hz), 3.64 – 3.50 (4H, m), 3.44 (1H, d, J = 9.6 Hz), 2.83 - 2.79 (1H, m), 2.51 – 2.35 (3H, m), 2.16 – 1.98 (2H, m), 1.67 – 1.35 (7H,, m),0.97 (3H, s), 0.95 – 0.68 (2H, m), 0.093 (3H, s), 0.087 (3H, s).

^{13}C-NMR (C_6D_6, 100.61 MHz): 146.1, 113.5, 108.9 (CH_2), 65.3 (CH_2), 65.2 (CH_2), 64.7 (CH_2), 49.7, 48.2 (CH), 47.6 (CH), 39.2, 38.6 , 36.0(CH_2), 35.8 (CH_2), 31.9 (CH_2), 31.3 (CH), 30.9 (CH_2), 30.64 (CH), 30.59 (CH_2), 27.7 (CH_3), 26.2 (3xCH_3), 22.3 (CH_2), 18.6 (CH_2), 16.1 (CH_3), 15.9 (CH_3), -5.25 (CH_3), -5.29 (CH_3).

GC-MS: (t_R 17.9) *m/z* = 474 [M^+] (7), 329 (14), 205 (9), 113 (100), 100 (9), 89 (7), 73 (41), 69 (14), 55 (9).

HRESIMS: *m/z* = 497.3423 (calcd for $C_{29}H_{50}O_3Si$ [M + Na]$^+$, 497.3427).

Synthesis of **10** from **9**:



9 **10**

A solution of OsO_4 (43 mg, 0.17 mmol), pyridine (1.0 mL), and H_2O (9 mL) was treated with a solution of **9** (4.1 g, 8.6 mmol) in THF (121 mL). After stirring in the dark for 10 min, the solution was treated with a suspension of $NaIO_4$ (21 g, 98 mmol) in H_2O (65 mL), and the resulting mixture stirred until TLC showed the disappearance of **9** (92 h). The suspencion was filtered through Celite, the filtrate diluted with Et_2O, and organic phase washed, dried, and evaporated. The residue was purified by CC (eluent: AcOEt/hexane = 1/9) to give **10** (3.7 g , 7.8 mmol) as a mixture of **10a** and **10b** (**10a** : **10b**, 1:9; R_f **10a** > R_f **10b**).

(-) – 10a : white solid.

m.p.: 141-142.3°C

[α]$_D$: -2 (c = 1.89 g/100 mL, $CHCl_3$)

IR ($CDCl_3$): 2932, 1773, 1094 cm^{-1}.

^{1}H-NMR (C_6D_6, 400.13 MHz): 3.98 (1H, pq, J = 7.4 Hz), 3.76 (1H, td, J_d = 4.2 Hz, J_t = 6.8 Hz), 3.66 – 3.57 (2H, m), 3.46 – 3.34 (2H, m), 3.03 (1H, d, J = 16.3 Hz), 3.00 (1H, d, J = 6.7 Hz), 2.54 (1H, dd, J = 6.7 Hz, J = 16.3 Hz), 1.83 (1H, pd, J = 13.5 Hz), 1.71 – 1.51 (3H, m), 1.46 – 1.04 (9H, m), 0.98 (9H, s), 0.97 (3H, s), 0.94 (3H, s), 0.81 (3H, s), 0.78 – 0.64 (2H, m), 0.07 (3H, s), 0.06 (3H, s).

^{13}C-NMR (C_6D_6, 100.61 MHz): 202.9, 110.1, 67.1(CH), 65.9(CH_2), 65.1(CH_2), 65.0(CH_2), 48.0(CH), 47.1 (CH_2), 46.6, 40.6(CH), 40.2, 38.7, 36.5(CH), 35.9 (CH_2), 34.4 (CH_2), 32.5(CH_2), 30.5 (CH_2), 27.7(CH_3), 26.2 (3xCH_3), 23.0(CH_2), 18.6, 18 .5(CH_2), 17.1 (CH_3), 14.6 (CH_3), -5.30 (CH_3), -5.32 (CH_3).

GC-MS: (t_R 21.1) *m/z* = 476 [M^{+}] (5), 207 (5), 147 (5), 134 (33), 119 (9), 113 (37), 105 (8), 100 (6), 87 (100), 81 (9), 75 (51), 69 (12), 55 (13).

HRESIMS: *m/z* = 499.3241 (calcd for $C_{28}H_{48}O_4Si$ [M + Na]$^{+}$, 499.3220).

(+) – 10b : white solid.

m.p.: 101.6-103.0 °C.

[α]$_D$: +34 (c = 2.908 g/100 mL, $CHCl_3$)

IR ($CDCl_3$): 2936, 1771, 1088 cm^{-1}.

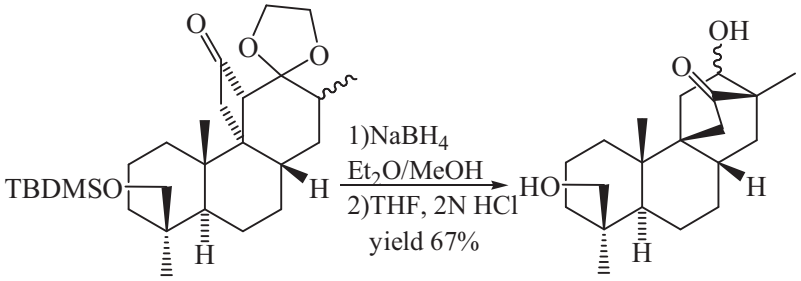
^{1}H-NMR (C_6D_6, 400.13 MHz): 3.69 (1H, d, J = 9.6 Hz), 3.65 – 3.57 (1H, m), 3.54 – 3.41 (3H, m), 3.39 (1H, d, J = 9.6 Hz), 3.04 – 2.99 (1H, m), 2.67 (1H, A of ABX, J_{AB} = 18.4 Hz, J_{AX} = 4.6 Hz), 2.59 (1H, B of ABX, J_{AB} = 18.4 Hz, J_{BX} = 2.1 Hz), 2.22 – 2.09 (1H, m), 1.97 – 1.78 (2H, m), 1.57 – 1.35 (3H, m), 1.33 – 1.06 (6H, m) 0.99 (9H, s), 0.98 (3H, s), 0.96 – 0.95 (1H, m), 0,94 (3H, d, J = 6.6 Hz), 0.89 (3H, s) 0.74 (1H, td, J_d = 3.8 Hz, J_t = 13.6 Hz), 0.61(1H, qd, J_d = 4.2 Hz, J_q = 12.8 Hz), 0.07 (6H, s).

^{13}C-NMR (C_6D_6, 100.61 MHz): 204.8, 111.2, 65.2 (CH_2), 65.00 (CH_2), 64.0 (CH), 48.4 (CH), 47.0 (CH_2), 46.5, 39.2, 38.5, 35.8 (CH_2), 35.1 (CH_2), 33.9 (CH), 31.6 (CH_2), 31.3 (CH_2), 30.1 (CH), 27.6 (CH_3), 26.2 (3xCH_3), 22.3 (CH_2), 18.6, 18.3 (CH_2), 16.6 (CH_3), 15.5 (CH_3), -5.30 (CH_3), -5.34 (CH_3).

GC-MS: (t_R 22.4) *m/z* = 476 [M^{+}] (1), 377 (4), 147 (6), 134 (29), 121 (5), 113 (66), 105 (8), 100 (6), 93 (7), 87 (100), 81 (8), 75 (49), 69 (11), 55 (11).

HRESIMS: *m/z* = 499.3221 (calcd for $C_{28}H_{48}O_4Si$ $[M + Na]^+$, 499.3220).

Synthesis of **11** from **10**:



10 **11**

endo / exo = 8 / 2

A solution of **10** (1.2 g, 2.5 mmol) in Et_2O/MeOH (1:1, 51 mL) was treated with $NaBH_4$ (0.49 g, 13 mmol) at 0°C, and the mixture stirred until TLC showed the disappearance of **10** (20 min). H_2O was added slowly at 0°C to quench excess $NaBH_4$. After neutralization with 2N HCl the solution was diluted with Et_2O, washed, dried, and evaporate, to give 1.2 g of crude.

A solution of the crude (1.2 g) in THF/2N HCl (4:1; 210 mL) was refluxed for 7 h (until TLC showed the disappearance of it). After cooling to r.t., the whole was diluted with AcOEt, washed with saturated $NaHCO_3$ and brine, dried, and evaporated. The residue was subjected to CC (AcOEt/hexane 3/7) to give **11** (0.72 g, 2.2 mmol, yield 88%) as a mixture of **11a** (*endo*) and **11b** (*exo*) (**11a:11b**, 82:18; R_f **11a** > R_f **11b**), which for analytical purpose was separated by CC.

(+) – 11a: white powder.

m.p.: 191-192.3 °C

[α]$_D$: +13.7(c = 2.842 g / 100 mL, $CHCl_3$)

IR ($CDCl_3$): 2928, 1715 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 400.13 MHz): 3.84 – 3.77 (2H, m), 3.44 (1H, d, J = 10.7 Hz), 2.61 (1H, ddd, J = 3.0 Hz, J = 9.2 Hz, J = 14.1 Hz), 2.15 (1H, A of AB, J_{AB} = 18.5 Hz,), 2.11 (1H, B of ABX, J_{AB} = 18.5 Hz, J_{BX} = 3.1

Hz), 1.91 – 1.61 (7H, m), 1.54 – 1.11 (8H, m), 0.99 (3H, s), 0.98 (3H, s), 0.97 – 0.85 (5H, m).

^{13}C-NMR ($CDCl_3$, 100.61 MHz): 216.0,74.1(CH), 65.5(CH_2), 48.9, 47.5(CH), 44.3(CH_2), 43.4, 38.63, 38.58, 37.8 (CH_2), 35.5 (CH_2), 33.6(CH_2), 33.1 (CH_2), 32.96(CH), 32.95 (CH_2), 27.6(CH_3), 22.1 (CH_2), 18.5 (CH_2), 17.4 (CH_3), 16.1 (CH_3).

GC-MS: (t_R 16.4) *m/z* = 320 [M^+] (5), 289 (25), 276 (25), 260 (32), 253 (11), 245 (13), 229 (17), 207 (11), 173 (11), 161 (21), 147 (26), 145 (20), 137 (15), 136 (13), 135 (38), 123 (93), 105 (73), 91 (65), 81 (96), 67 (70), 55 (100).

HRESIMS: *m/z* = 343.2232 (calcd for $C_{20}H_{32}O_3Si$ [M + Na]$^+$, 343.2249).

(-) – 11a: white powder.

m.p.: 184.5-186.5 °C

[α]$_D$: +37.1 (c = 2.455 g / 100 mL, $CHCl_3$)

IR ($CDCl_3$): 2934, 1715 cm^{-1}.

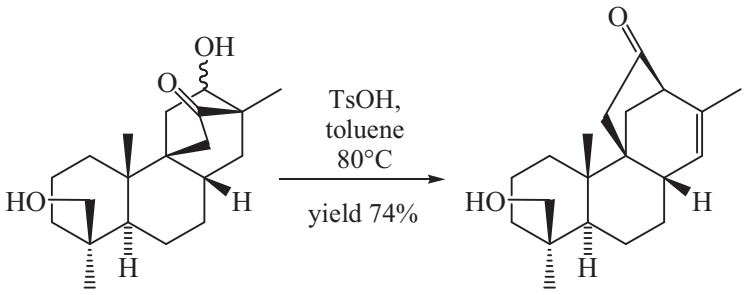
^{1}H-NMR ($CDCl_3$, 400.13 MHz): 3.81 (1H, d, J = 10.8 Hz), 3.74 – 3.67 (1H, m), 3.45 (1H, d, J = 10.8 Hz), 2.14 – 1.79 (6H, m), 1.77 – 1.22 (13H, m), 0.98 (3H, s), 0.96 (3H, s), 0.93 – 0.84 (4H, m).

^{13}C-NMR ($CDCl_3$, 100.61 MHz): 216.9, 70.5(CH), 65.5(CH_2), 49.1, 47.2(CH), 44.3(CH_2), 42.4, 38.7, 38.6, 35.5 (CH_2), 33.2 (CH_2), 33.1 (CH), 32.6 (CH_2), 31.8 (CH_2), 31.6 (CH_2), 27.5 (CH_3), 22.2 (CH_2), 18.5 (CH_2), 17.1 (CH_3), 15.9 (CH_3).

GC-MS: (t_R 16.1) *m/z* = 320 [M^+] (6), 289 (24), 276 (13), 271 (30), 229 (19), 173 (10), 159 (20), 147 (27), 135 (19), 123 (100), 121 (33), 105 (52), 91 (48), 81 (67), 69 (40), 55 (71).

HRESIMS: *m/z* = 343.2256 (calcd for $C_{20}H_{32}O_3Si$ [M + Na]$^+$, 343.2249).

Synthesis of **12** from **11**:



11
endo / eso = 8 / 2

12

A solution of **11** (**11a**+**11b**, 0.35 g, 1.1 mmol) and TsOH (0.19 g, 1.0 mmol) in toluene (54 mL) was heated at 85°C until TLC showed the disappearance of **11** (48 h). After cooling to r.t., the whole was diluted with AcOEt, washed with satured $NaHCO_3$, brine, dried, and evaporated.

The residue was purified by CC (eluent: AcOEt/hexane 1:9) to give **12** (0.22 g, 0.73 mmol, 66%) as a white powder.

m.p.: 165.1 – 166.8°C

[α]$_D$: +539 (c = 2.7 g / 100 mL, $CHCl_3$)

IR ($CDCl_3$): 2936, 1728, 1213 cm^{-1}.

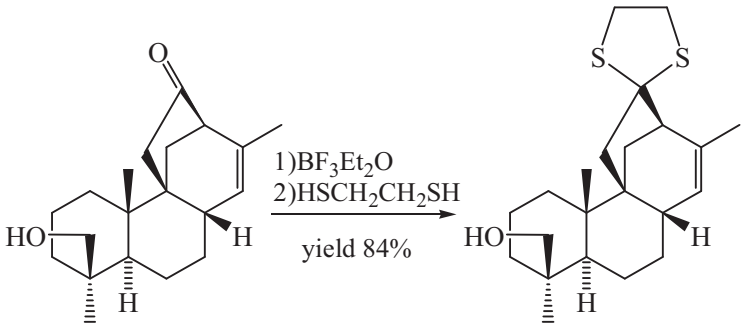
^{1}H-NMR (C_6D_6, 400.13 MHz): 5.10 (1H, d, J = 2.8 Hz), 3.53 (1H, d, J = 10.4 Hz), 3.15 (1H, d, J = 10.4 Hz), 2.51 (1H, bs), 2.06 (1H, d, J = 18.3 Hz), 1.95 – 1.76 (3H, m), 1.65 (3H, s), 1.61 – 1.53 (1H, m), 1.49 – 0.94 (10H, m), 0.91 (3H, s), 0.81 (1H, bs), 0.74 (1H, td, J_d = 3.9 Hz, J_t =13.4 Hz), 0.64 (3H, s).

^{13}C-NMR (C_6D_6, 100.61 MHz): 207.9, 131.5, 128.5 (CH), 64.8(CH_2), 55.5 (CH),, 48.5(CH), 48.0, 44.0 (CH_2), 42.2(CH), 38.74, 38.66, 35.5 (CH_2), 32.6 (CH_2), 32.2 (CH_2), 27.5 (CH_3), 26.3 (CH_2), 22.3(CH_2), 21.7 (CH_3), 18.5 (CH_2), 18.1 (CH_3).

GC-MS: (t_R 13.4) *m/z* = 302 [M^+] (24), 284 (14), 271 (16), 229 (14), 161 (14), 157 (11), 147 (26), 131 (28), 119 (52), 105 (100), 91 (79), 81 (56), 67 (43), 55 (59).

HRESIMS: *m/z* = 325.2152 (calcd for $C_{20}H_{30}O_2Si$ $[M + Na]^+$, 325.2144).

Synthesis of **13** from **12**:



12 **13**

To a solution of **12** (0.18 g, 0.60 mmol) in 1,2-ethanedithiol (1.1 mL, 13 mmol), cooled to 0°C, was added $BF_3 \cdot Et_2O$ (0.47 mL, 3.8 mmol) while stirring until TLC showed the disappearance of **12**(10 min). The mixture was diluted with CH_2Cl_2 (30 mL), washed with 2N NaOH (3 x 6 mL), H_2O until neutral, brine, dried, and evaporated. The mixture was purified by CC (eluent: AcOEt/hexane 93/7) to give **13** (191 mg, 0.5 mmol): white powder.

m.p.: 171.7 – 173.4°C

[α]$_D$: +105 (c = 3.2 g /100 mL, $CHCl_3$)

IR ($CDCl_3$): 2965 cm^{-1}.

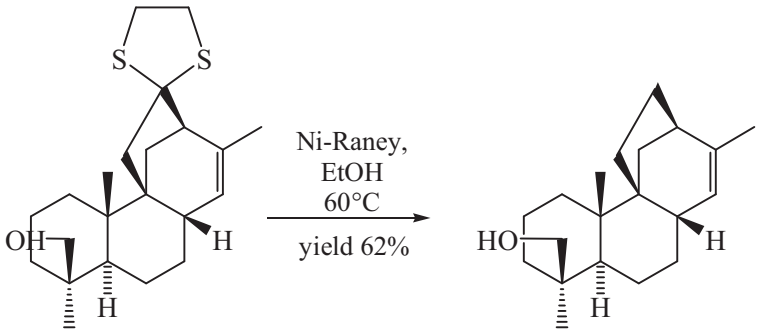
^{1}H-NMR ($CDCl_3$, 400.13 MHz): 5.11 (1H, pd, J = 2.0 Hz), 3.77 (1H, d, J = 10.8 Hz), 3.39 (1H, d, J = 10.8 Hz), 3.37 – 3.15 (4H, m), 2.47 (1H, d, J = 14.6 Hz), 2.33 (1H, d, J = 3.8 Hz), 2.13 – 2.05 (1H, m), 2.01 (1H, dd, J = 3.8 Hz, J = 11.6 Hz), 1.71 – 1.28 (8H, m), 1.25 – 1.11 (2H, m), 0.97 – 0.85 (7H, m).

^{13}C-NMR ($CDCl_3$, 100.61 MHz): 137.3, 128.6 (CH), 76.5, 65.4 (CH_2), 56.2 (CH), 50.57, 50.47 (CH_2), 49.5(CH), 44.5 (CH), 40.1 (CH_2), 39.3 (CH_2), 38.65, 38.63, 35.4 (CH_2), 32.6 (CH_2), 31.3 (CH_2), 30.2 (CH_2), 27.1 (CH_3), 24.5 (CH_3), 22.0(CH_2), 18.2 (CH_2), 17.8 (CH_3).

GC-MS: (t_R 23.2) *m/z* = 378 [M^+] (14), 260 (16), 210 (8), 182 (11), 157 (11), 144 (22), 131 (21), 119 (32), 105 (100), 91 (41), 81 (24), 67 (20), 55 (32).

HRESIMS: *m/z* = 401.1961 (calcd for $C_{22}H_{34}O_2Si$ [M + Na]$^+$, 401.1949).

Synthesis of **14** from **13**:



13 **1**

(+)-2-deoxyoryzalexin S

A solution of **13** (0.17 g, o.44 mmol) in EtOH (58 mL) was stirred at 60°C with Raney-Ni until TLC showed the disappearance of **13** (30 min). The catalyst was removed by filtration through Celite, the solvent evaporated, and the mixture purified by CC (eluent: AcOEt/hexane 5:95) to give **1** (79 mg, 0.27 mmol): white solid.

m.p.: 116.1 – 117.6°C

$[\alpha]_D$: +58 (c = 1.8 g /100 mL, $CHCl_3$)

IR ($CDCl_3$): 2965 cm^{-1}.

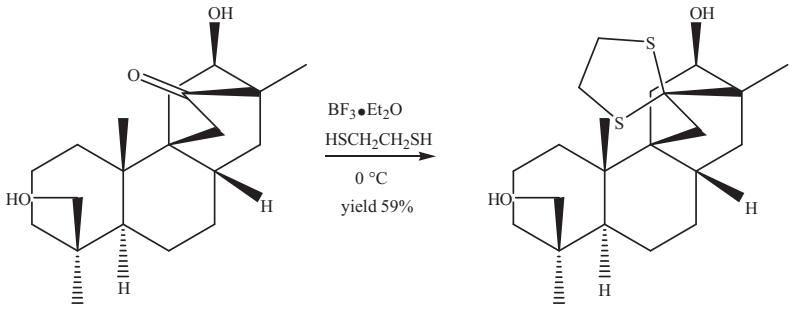
^{1}H-NMR ($CDCl_3$, 400.13 MHz): 4.94 (1H, pd, J = 4.3 Hz), 3.80 (1H, d, J = 10.9 Hz), 3.41 (1H, dd, J = 1.0 Hz, J = 10.9 Hz), 2.19 (1H, t, J = 4.4 Hz), 2.01 – 1.93 (1H, m), 1.87- 1.79 (1H, m), 1.75 – 1.10 (19H, m), 0.98 – 0.85 (7H, m).

^{13}C-NMR ($CDCl_3$, 100.61 MHz): 138.9, 123.9 (CH), 65.5 (CH_2), 51.1, 50.2 (CH), 44.4 (CH), 43.3 (CH), 38.8, 38.7, 35.6 (CH_2), 33.4 (CH_2), 32.4 (CH_2), 32.0 (CH_2), 31.8 (CH_2), 30.0 (CH_2), 27.7 (CH_3), 22.12 (CH_3), 22.09 (CH_2), 18.4 (CH_2), 17.9 (CH_3).

GC-MS: (t_R 11) *m/z* = 288 [M^+] (20), 257 (54), 229 (8), 201 (8), 187 (20), 175(15), 161 (33), 145 (33), 131 (59), 119 (43), 105 (100), 91 (78), 81 (72), 67 (47), 55 (77).

HRESIMS: *m/z* = 311.2353 (calcd for $C_{20}H_{32}O$ [M + Na]$^+$, 311.2351).

Synthesis of **17** from **11a**:



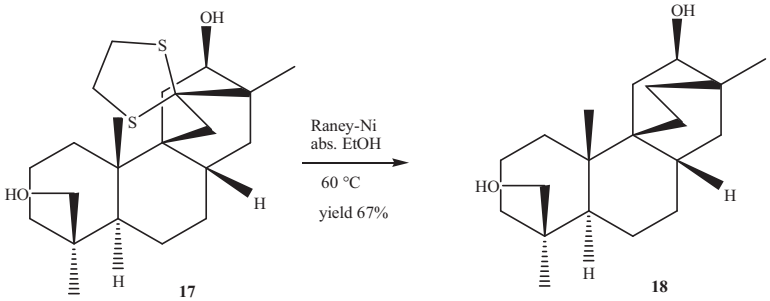
11a **17**

To a solution of **11a** (0.164 g, 0.51 mmoli) in 1,2-ethanedithiol (0.97 ml, 11.6 mmoli), cooled to 0°C, was added $BF_3 \cdot Et_2O$ (0.39 mL, 3.17 mmol) while stirring until TLC (AcOEt/hesane = 5/5) showed the disappearance of the starting material.

The mixture was diluted with CH_2Cl_2 , washed with 2N NaOH, H_2O until neutral, brine, dried, and evaporated. The mixture was purified by CC (AcOEt/hexane 15/85) to give **17** (0.120 g, 0.3 mmol, yield 59%) as a white powder.

m.p.:	161.6-163.2 °C
$[\alpha]_D$:	2.7 (c = 4.03 g / 100 mL, $CHCl_3$)
IR ($CHCl_3$):	3684, 3628, 3018, 2935, 2399 cm^{-1}.
^{1}H-NMR ($CDCl_3$):	0.72-0.76 (m, 1H), 0.90 (s, 3H), 0.97 (s, 3H), 1.34 (s, 3H),1.17-1.93 (m, 13H), 1.98-2.0 (d, 3H), 2.32-2.33 (d, 2H), 2.40-2.46 (m, 1H), 3.14-3.24 (m, 3H), 3.34-3.37 (m, 1H), 3.42-3.44 (d, 1H), 3.69-3.72 (m, 1H), 3.79-3.82 (d, 1H).
^{13}C-NMR ($CDCl_3$):	75.3, 72.6, 65.5, 51.1, 47.7, 41.8, 41.7, 41.5, 41.2, 38.5, 37.7, 35.5, 34.1, 33.1, 33.0, 32.8, 27.5, 22.2, 19.9, 18.4, 17.0.
HPLC:	(eluent: AcOEt/hexane = 35/65), flow (1ml/min), t_r 6.1 min.

Synthesis of **18** from **17**:



17 **18**

A solution of **17** (0.123 g, 0.31 mmoli) in abs. EtOH (42.1 mL) was stirred at 60°C with Raney-Ni until TLC (AcOEt/hesane 4/6) showed the disappearance of **13** (3h). After cooling, the catalyst was removed by filtration through Celite, the solvent evaporated, and the mixture purified by CC (eluent: AcOEt/hexane 20/80) to give **18** (64 mg, 0.21 mmol, yield 67%): white solid.

m.p.: 167.8-168.5 °C

[α]$_D$: -18 (c = 2.997 g / 100 mL, CH_2Cl_2)

IR ($CHCl_3$): 3684, 3620, 3018, 2937, 2399

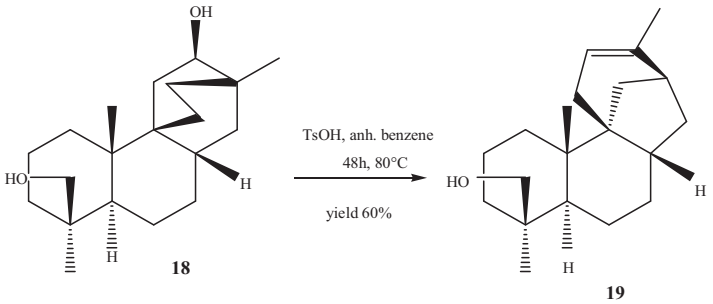
^{1}H-NMR ($CDCl_3$): 0.67-0.71 (m, 1H), 0.78 (s, 3H), 0.92 (s, 3H), 0.97 (s,3H), 1.05-1.89 (m, 20H), 2.37-2.43 (m, 1H), 3.33-3.35 (d, 1H), 3.47-3.51 (m, 1H), 3.81-3.83 (d, 1H).

^{13}C-NMR ($CDCl_3$): 73.2, 63.8, 41.19, 39.5, 38.6, 38.2, 35.3, 33.8, 33.77, 3.73, 32.7, 32.2, 26.8, 26.3, 25.5, 22.6, 22.0, 18.1, 15.7.

GC-MS: (t_R 13.3) *m/z* = 306 (M^+, 0.31), 288 (6), 275 (32), 257 (36), 245 (4), 229 (3), 215 (4), 201 (6), 187 (9), 175 (10), 161 (15), 147 (15), 133 (21), 123 (61), 105 (52), 91 (59), 81 (100), 67 (67), 55 (92).

HPLC: (eluent AcOEt/hexane = 35/65), flow (1ml/min), t_R 2.47 min.

Synthesis of **19** from **18**:



A solution of **18** (0.0458 g, 0.149 mmol) and TsOH (0.00141 g, $7.45 \cdot 10^{-3}$ mmol) in anh. benzene (5.4 mL) was heated at 80°C (4h). After cooling to r.t., the reaction mixture was diluted with AcOEt, washed with satured $NaHCO_3$, brine, dried, and evaporated.

The residue was first purified by CC (SiO_2; AcOEt/hexane 5:95) and then by semipreparative HPLC (Nucleodur; AcOEt/hesane 5/95) because after chromatography the GC-MS showed the presence of two compounds.

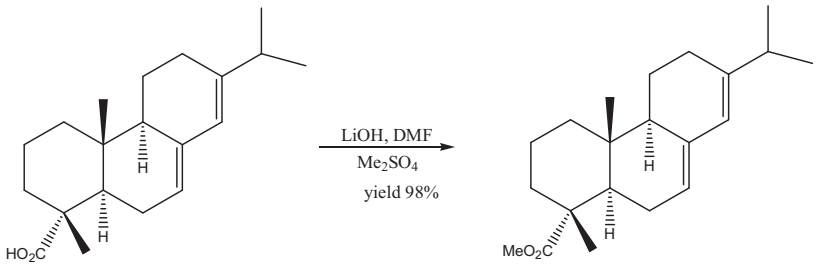
After semipreparative HPLC, **19** (0.0076 g, $2.64 \cdot 10^{-2}$ mmol, 17.7%) was obtained.

^{1}H-NMR ($CDCl_3$): 1.01-1.02 (d, 6H), 1.10-2.18 (m, 22H), 2.38-2.42 (d, 1H), 3.52-3.55 (d, 1H), 3.90-3.93 (d, 1H), 5.05 (s, 1H).

^{13}C-NMR ($CDCl_3$): 142.1, 117.0, 65.8, 50.8, 48.5, 46.8, 42.9, 38.4, 38.0, 37.3, 35.6, 35.2, 32.5, 31.7, 29.5, 27.6, 22.1, 21.3, 20.7, 18.4.

GC-MS: (t_R 11.06) *m/z* = 288 (M^+, 18), 273 (11), 257 (47), 241 (1), 231 (10), 213 (5), 206 (13), 187 (11), 175 (15), 163 (28), 147 (25), 133 (31), 119 (37), 105 (75), 91 (94), 81 (99), 67 (68), 55 (100).

Synthesis of Methyl abietate **22** from abietic acid **21**:



21
abietic acid

22

A solution of abietic acid **21** (20 g, 0.066 mol), and lithium hydroxide monohydrate (6 g, 0.132 mol) in dimethylformamide (80 mL) was stirred for 4h at r.t. and then cooled by means of a ice-water bath. After addition of dimethyl sulphate (20 mL) stirring was continued for 15 min. The mixture was poured into water and extracted with hexane.

The combined extracts were washed with 5% HCl solution, 10% $NaHCO_3$ solution and brine. After drying and concentration in vacuo 20,47 g (0,065 mol, 98%) of methyl abietate **22**, as an oil, was obtained.

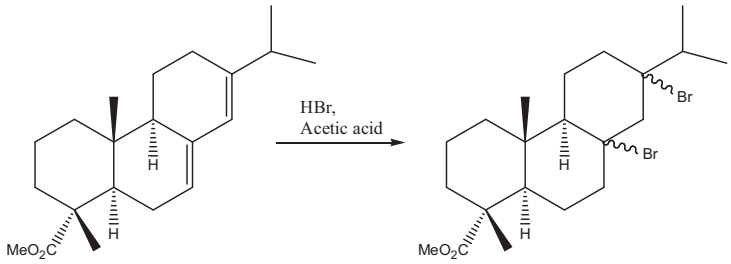
IR (KBr): 1722 cm^{-1}

^{1}H-NMR ($CDCl_3$, 400.13 MHz): 5.76 (s, 1H); 5.35 (d,1H); 3.61 (s, 3H); 2.29-1.48 (m, 10H); 1.24 (s,3H); 1.02-0.96 (m, 6H); 0.81 (s,3H).

^{13}C-NMR ($CDCl_3$, 400.13 MHz): 179.1, 145.4, 135.7, 122.5, 120.7, 51.9, 51.1, 46.7, 45.2, 38.5, 37.2, 35.0, 34.7, 27.6, 25.8, 22.6, 21.5, 20.9, 18.2, 17.1, 14.1.

GC-MS: (t_R 11.14) *m/z* = 316 (M^+, 50), 281 (3), 273 (7), 256 (100), 241 (68), 213 (64), 213 (6), 185 (54), 157 (14), 143 (29), 131 (56), 121 (91), 105 (87), 91 (82), 79 (52), 67 (43), 55 (66).

Synthesis of **23** from **22**:



22 **23**

A solution of methyl abietate **22** (5 g, 0.016 mmol) in acetic acid (15 mL) was added to a solution of 33% HBr in acetic acid (15 mL) and stirred for 6 h at r.t.

The precipitated solid was collected by vacuum filtration, the filtrate separated, and the solid washed with acetic acid and water. After drying in vacuo over P_2O_5, 3.21 g ($6.72x10^{-3}$ mmol, yield 42%) of the compound **23** was obtained.

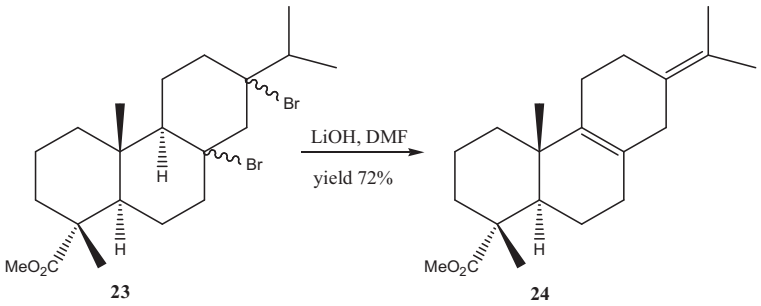
m.p.: 146- 148 °C (lit. 148 °C)

IR (KBr): 1722 cm^{-1}

^{1}H-NMR ($CDCl_3$, 400.13 MHz): 3.66 (s, 3H); 2.40-2.27 (m, 2H); 2.15-1.78 (m, 7H); 1.76 (s, 6H); 1.62-1.44 (m, 5H); 1.35-1.20 (m, 2H); 1.18 (s,3H); 1.15-1.10 (m, 1H); 1.05 (s, 3H); 0.95-0.79 (m, 2H).

^{13}C-NMR ($CDCl_3$, 400.13 MHz): 179.3, 77.9, 73.0, 59.6, 52.1, 50.7,48.3, 46.9, 46.2, 38.9, 37.0, 33.1, 32.9, 28.9, 22.5, 22.1, 17.6, 16.7, 16.5.

Synthesis of **24** from **23**:



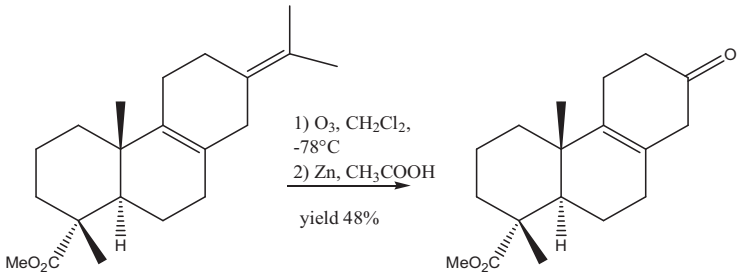
An intimate mixture of **23** (5 g, 10.4 mmol) and powdered lithium hydroxide monohydrate (0.9 g) in dimethylformamide (40 mL) was stirred while the temperature was raised over 30 min. to 80°C. The resulting solution was stirred for 3 h and 30 min at the same temperature and the reaction mixture was then poured into water and extracted with hexane. The hexane extract was washed with 5% HCl solution, 10% $NaHCO_3$ solution and brine.

The white residue obtained after drying, was then crystallized from methanol to give **24** (2.4 g, 7.5 mmol, yield 72%).

m.p.:	104 – 105°C (lit. 104.5-106 °C)
IR ($CHCl_3$):	1720 cm^{-1}.
^{1}H-NMR ($CDCl_3$):	3.65 (s, 3H); 2.77-2.32 (m, 2H); 2.15-1.47 (m, 18H); 1.18 (s, 3H); 0.96 (m, 3H).
^{13}C-NMR ($CDCl_3$):	16.5, 18.3, 19.5, 19.7, 20.2, 21.7, 24.9, 27.6, 31.8, 34.8, 35.4, 36.8, 37.2, 46.5, 47.8, 51.9, 120.8, 126.2, 128.4, 138.3, 179.4.
GC-MS:	(t_R =11.09) *m/z* = 316 (M^+, 25), 301 (10), 269 (2), 257 (6), 241 (29), 237 (4), 213 (6), 199 (3), 181 (10), 159 (12), 148 (35), 135 (100), 121 (39), 105 (29), 91 (37), 79 (22), 67 (17), 55 (31).

.

Synthesis of **25** from **24**:



24 **25**

In a three neck flask, under magnetic stirring, a stream of ozone was passed for approximately 20 min at -78°C through a solution of **24** (1 g, 3.16 mmol) in dicloromethane (50 mL).

After 30 min, the excess ozone was removed with a stream of Argon. The mixture was then treated with Zinc powder (2.1 g) and acetic acid (3 mL) and removed from the cooling bath. The resulting mixture was stirred at r.t. for 30 min. The reaction mixture was filtered with dicloromethane on Celite to remove the excess of zinc powder

The combined extracts were washed with satured solution $NaHCO_3$, water until neutrality, brine, dryied on Na_2SO_4, concentrated and chromatographed on silica gel (hexane/ether = 9/1).

After chromatographic separation **25** (0.44 g, 1.52 mmol, yield 48%) was obtained.

m.p.: 108-110°C

$[\alpha]_D$: +157 (c = 1.81 g/100 ml, $CHCl_3$)

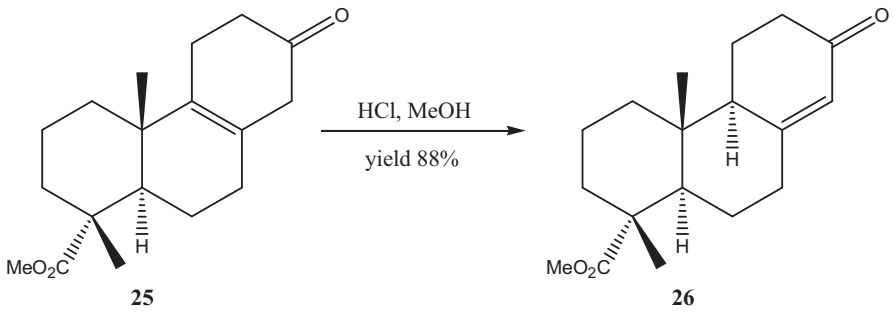
IR ($CHCl_3$): 1715 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 3.66 (s, 3H); 2.69 (m, 2H); 2.52-2.24 (m, 9H); 1.33-1.12 (m, 4H); 1.04 (m, 3H).

^{13}C-NMR ($CDCl_3$): 16.5, 18.1, 19.7, 21.3, 23.8, 31.3, 35.4, 36.7, 37.4, 39.4, 44.6, 46.3, 47.6, 49.8, 52, 124.9, 139.1, 179.1, 179.6, 211.5.

GC-MS: (t_R =10.63) *m/z* = 290 (M^+, 29), 275 (19), 231 (31), 215 (100), 197 (20), 173 (44), 159 (48), 149 (24), 131 (20), 121 (24), 105 (41), 91 (58), 79 (42), 67 (22), 55 (58).

Synthesis of **26** from **25**:



A solution of **25** (1 g, 3.44 mmol) and conc. HCl (26 mL) in methanol (10 mL) was refluxed, under Argon, for 40 min. The reaction mixture was diluited with water and extracted with Et_2O.

The organic phase was washed with satured solution $NaHCO_3$, brine, dried on Na_2SO_4, concentrated in vacuum and chromatographed on silica gel (hexane/ether = 8/2).

After chromatographic separation **26** (0.88 g, 3.03 mmol, yield 88%) was obtained.

m.p.: 125-127°C.

[α]$_D$: +30 (c = 1.15 g/100 ml, $CHCl_3$)

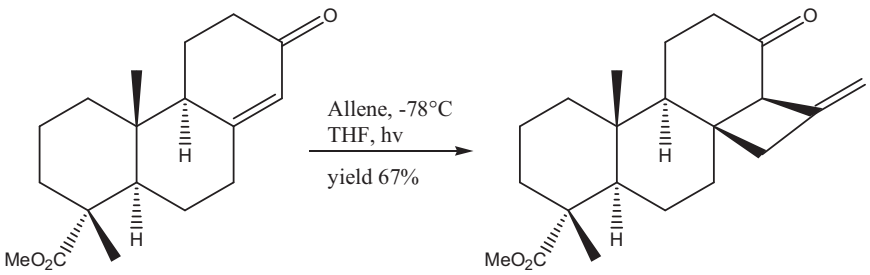
IR ($CHCl_3$): 1720, 1660, 1615 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 400.13 MHz): 5.86 (s,1H); 3.67 (s,3H); 2.56-1.92 (m, 6H); 1.86-1.45 (m, 6H); 1.39-1.11 (m, 4H); 0.83 (s, 3H).

^{13}C-NMR ($CDCl_3$, 400.13 MHz): 15.7, 17.1, 18, 20.4, 24.2, 35.3, 36.8, 37, 38.4, 47.4, 48.3, 48.2, 51.7, 52.2 126.3, 164.5, 179, 199.6.

GC-MS: (t_R =11.71) *m/z* = 290 (M^+, 5), 275 (1), 258 (0.4), 231 (9), 215 (5), 197 (1), 181 (13), 159 (6), 149 (2), 135 (4), 121 (50), 110 (100), 91 (16), 79 (16), 67 (12), 55 (21).

Synthesis of **27** from **26**:



26 **27**

A Pyrex vessel containing a solution of **26** (6.9 g, 23.7 mmol) in THF (78 mL) was cooled at -78°C, and an excess of allene was condensed into it. The vessel was irradiated under Ar at -78°C with a 500 W mercury vapor lamp until TLC (Et_2O/hexane = 4/6) showed the disappearance of **26**.

The reaction vessel was then removed from the cooling bath and kept overnight under the fumehood to allow the unreacted allene to evolve. Evaporation of the organic solvent gave a residue which was purified by CC (SiO_2; petroleum ether(40°-70°)/Et_2O = 7/3).

After chromatographic separation **27** (5.26 g, 15.9 mmol, yield 67%) was obtained.

m.p.: 97.7 – 98.8 °C

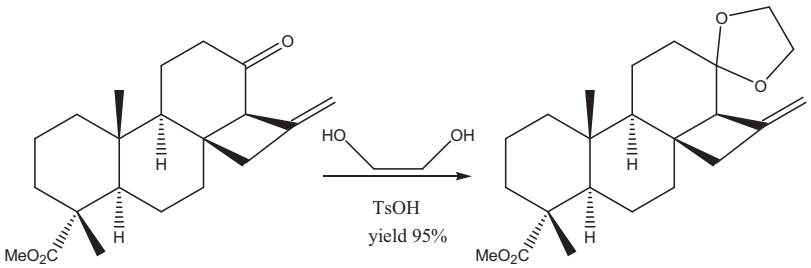
IR (CCl_4): 1715 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 0.77 (s, 3H); 1.09 (s, 3H); 3.59 (s, 3H); 4.84 (m, 1H); 4.92 (m, 1H).

^{13}C-NMR ($CDCl_3$): 14.9, 16.2 (C(19), C(20)); 17.2, 18.2, 21.6 (C(2), C(6), C(11)); 36.6, 37.5, 37.6, 37.9, 39.5, 39.9, 40.4 (C(1), C(3), C(7), C(8), C(10), C(12), C(16)); 47.4 (C(4); 50.2, 51.8, 53.7 (C(5), C(9), (MeO)); 62.9 (C(14)); 110.0 (=CH_2); 142.1 (C(15)); 179.2 (C(18)); 210.5 (C(13)).

EI-MS: 330 (23, M^+). Anal. calc. for $C_{21}H_{30}O_3$ (330.47): C 76.33, H 9.15; found C 76.22, H 9.17.

Synthesis of **28** from **27**:



27 **28**

To a solution of **27** (0.501 g, 1.51 mmol) in anh. benzene (80 mL) an excess of ethylenglycol and catal. amount of TsOH were added; the mixture was refluxed under N_2 with azeotropic removal of water (*Dean-Stark* trap), until TLC (petroleum ether (40°-70°)/EtO_2 7/3) indicated the complete disappearance of the starting material. The mixture was then cooled to r.t., diluited with ether, washed with $NaHCO_3$ solution, water (till neutral), brine, dried on Na_2SO_4, and evaporated.

The residue was purified by CC (SiO_2; petroleum ether (40°-70°)/EtO_2 9/1) and after chromatographic separation, **28** (0.539 g, 1.44 mmol, yield 95%) was obtained.

m.p.: 81.2 – 83.1°C

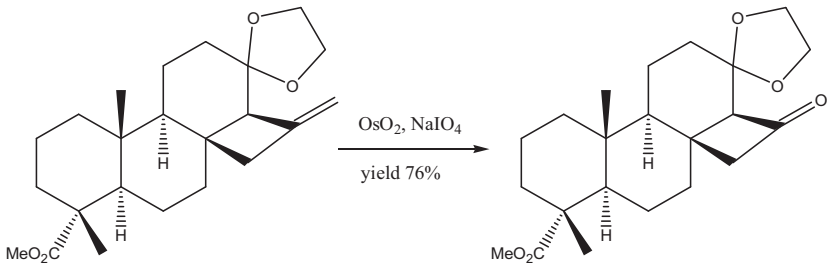
IR (CCl_4): 1718 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 0.75 (s, 3H); 1.10 (s, 3H); 3.60 (s, 3H); 3.80-4.00 (m, 4H); 4.83 (m, 1H); 4.94 (m, 1H).

^{13}C-NMR ($CDCl_3$): 14.1, 16.4 (C(19), C(20)); 17.3, 17.5, 21.9 (C(2), C(6), C(11)); 28.6 (C(12)); 36.7, 37.3, 37.5, 38.0, 38.9, 41.0 (C(1), C(3), C(7), C(8), C(10), C(16)); 47.4 (C(4)); 48.0, 50.2, 51.7 (C(5), C(9), (MeO)); 51.7 (C(14)); 64.1, 64.3 (OCH_2CH_2O); 109.8, 110.4 (C(13), ($=CH_2$)); 145.9 (C(15)); 179.5 (C(18)).

EI-MS: 374 (100, M^+). Anal. calc. for $C_{23}H_{34}O_4$ (374.52): C 73.76, H 9.15; found C 73.59, H 9.20.

Synthesis of **29** from **28**:



28 **29**

A solution of OsO_4 (20 mg,) in THF (2 mL) was added to a solution of **28** (103 mg, 0.27 mmol) in dioxane (5 mL). After stirring in the dark for 15 min, water (1 mL) and pyridine (1 mL) were added. A solution af $NaIO_4$ (235 mg, 1.10 mmol) in water (10 mL) was then added dropwise. The mixture was stirred in the dark for 45 h. The mixture was then filtered under reduced pressure through a Celite pad, and the filter and the flask were washed with methanol. The organic solvent was then evaporated and the residue taken up with water and extracted with Et_2O.

The combined organic extracts were then washed with $NaHCO_3$ solution, water (till neutral), brine, dried on Na_2SO_4, and evaporated.

The residue was purified by CC (SiO_2; petroleum ether (40°-70°)/EtO_2 9/1) and after chromatographic separation **28** (77 mg, 0.20 mmol, yield 76%) was obtained.

m.p.: 155.6 – 156.1°C

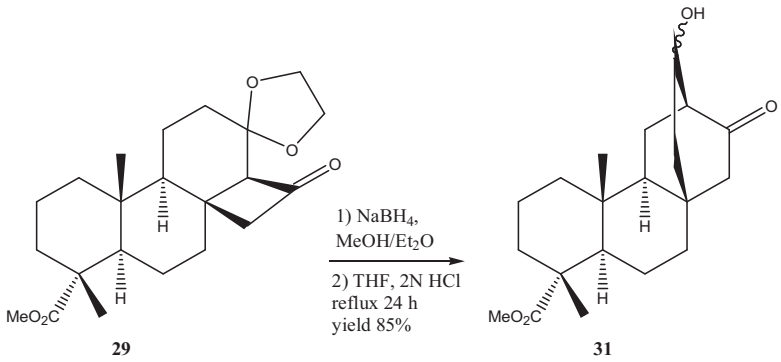
IR (CCl_4): 1725, 1782 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 0.75 (s, 3H); 1.11 (s, 3H); 2.33 (*dd*, J = 6.5, J =16.3, 1H); 2.69 (*dd*, J = 2, J = 6.5, 1H); 3.34 (*d*, J = 16.3, 2H); 3.61 (s, 3H); 3.70-4.00 (m,4H).

^{13}C-NMR ($CDCl_3$): 14.6, 16.1 (C(19), C(20)); 17.4, 17.4, 22.0 (C(2), C(6), C(11)); 34.5, 34.6, 36.7, 37.5, 37.5, 40.6 (C(1), C(3), C(7), C(8), C(10), C(12)); 47.3 (C(4)); 50.3, 51.2, 51.8 (C(5), C(9), (MeO)); 52.2 (C(16)); 64.3, 64.9 (OCH_2CH_2O);73.1 (C(14)); 107.3 (C(13)); 179.3 (C(18)); 205.8 (C(15)).

EI-MS: 376 (19, M^+). Anal. calc. for $C_{22}H_{32}O_5$ (376.49): C70.19, H 8.57; found C 70.23, H 8.59.

Synthesis of **31** from **29**:



29 **31**

To a stirred solution of **29** (67 mg, 0.18 mmol) in a 1:1 MeOH/Et_2O mixture (5 mL), $NaBH_4$ was added. When TLC (petroleum ether (40°-70°)/Et_2O 1/1, R_f (**30**) < R_f (**29**)) indicated the complete disappearance of **29**, the solution was evaporated and the residue taken up with water and extracted with $CHCl_3$.

The combined organic extracts were then washed with water (till neutral), brine, dried on Na_2SO_4, and evaporated. The crude product **30** could be used in the subsequent reaction without purification.

A solution of **30** dissolved in 2:1 THF/2N HCl (7.5 mL) was refluxed under N_2 for 24h. After neutralization (4N NaOH) and evaporation of the organic solvent, the residue was taken up with water and extracted with Et_2O. The combined organic extracts were then washed with water, brine, dried on Na_2SO_4, and evaporated.

The residue was purified by CC (SiO_2; petroleum ether (40°-70°)/EtO_2 1/1) gave **31a** (*endo* -OH) and **31b** (*exo* -OH) in a 76.6% and 8.3% yield, respectively. TLC (petroleum ether (40°-70°)/EtO_2 2/8): R_f(**31a**) < R_f(**31b**).

Data of **31a** (*endo* -OH)

m.p.: 221.2 – 223.2°C

IR (CCl_4): 1705, 1720, 3400 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 0.94 (s, 3H); 1.15 (s,3H); 3.63 (s,3H); 4.12 (m,1H).

^{13}C-NMR ($CDCl_3$): 14.5, 16.3 (C(19), C(20)); 16.9, 21.0, 21.9 (C(2), C(6), C(11)); 36.4, 36.6, 37.0, 37.5, 38.3, 39.1 (C(1), C(3), C(7), C(8), C(10), C(14)); 47.1 (C(4)); 50.2, 50.3, 51.5, 51.9 (C(5), C(9), C(12), (MeO)); 55.1 (C(15)); 69.1 (C(13)); 179.4 (C(18)); 215.3 (C(16)).

EI-MS: 334 (25, M^+). Anal. calc. for $C_{20}H_{30}O_4$ (334.45): C70.82, H 9.04; found C 71.92, H 9.03.

Data of **31b** (*exo* -OH)

m.p.: 186.7- 187.8°C

IR (CCl_4): 1710,1720, 3420 cm^{-1}.

^{1}H-NMR ($CDCl_3$): 1.10 (s, 3H); 1.13 (s,3H); 3.61 (s,3H); 4.20 (m,1H).

^{13}C-NMR ($CDCl_3$): 15.4, 16.3 (C(19), C(20)); 17.0, 17.5, 21.1 (C(2), C(6), C(11)); 36.3, 36.7, 37.4, 38.1, 38.3, 38.5 (C(1), C(3), C(7), C(8), C(10), C(14)); 47.2 (C(4)); 50.1, 51.8, 51.8, 52.2 (C(5), C(9), C(12), (MeO)); 54.7 (C(15)); 65.8 (C(13)); 179.5 (C(18)); 215.5 (C(16)).

EI-MS: 334 (4, M^+). Anal. calc. for $C_{20}H_{30}O_4$ (334.45): C71.82, H 9.04; found C 71.89, H 9.03.

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