CHAPTER I

Introduction

1. *EUPHORBIA* L. GENUS

1.1. GENERAL CONSIDERATIONS

The Euphorbiaceae family consists of about 300 genera and 7500 species of cosmopolitan distribution, but better developed in tropical and subtropical regions. By far, the largest genus is *Euphorbia* L. with over 2000 species found in the tropical and subtropical regions of Africa and America, and also in temperate zones worldwide. *Euphorbia* species (commonly named spurge)[1] range from annual or perennial herbs, woody shrubs, trees and succulent plants, characterized by a caustic and toxic milky latex that in contact with the skin may cause inflammation and rash. Some other large genera of this family (in number of species) include *Croton* (700), *Phyllanthus* (400), *Acalypha* (400), *Macaranga* (250), and *Antidesma*, *Drypetes*, *Tragia*, *Jatropha* and *Manihot*, each one with 150 species (Cronquist, 1981; Judd *et al*, 2002).

Euphorbia species have been widely used in traditional medicine all over the world, to treat several diseases, like tumors and warts (Hartwell, 1969). For example, *E. peplus* and *E. platyphylla* were used externally to treat dermatosis (Rivera and Óbon, 1995); *E. pekinensis* [2] is used in traditional Chinese medicine, where it is regarded as one of the 50 fundamental herbs, for the treatment of oedemas and retention of urine (Xue *et al*, 2007); the roots of *E. fisheriana* and *E. ebracteolata* are, along with *Stellera chamaejasme* (Thymelaeceae), the constituents of a traditional chinese medicine named "Lang-Du"[3] that has been used as expectorant and for the treatment of oedema and indigestion (Qin and Xu, 1998).

Many members of the *Euphorbia* genus are of great economic importance as a source of potential petroleum substitutes, due to their rich oil content. This is the case of *Euphorbia lagascae*, one of the species that was studied in this work. *Euphorbia lagascae* has been cultivated for the production of 12-epoxyoctadeca-*cis*-9-enoic acid (vernolic acid), which is found at high levels in the seeds of this species. Vernolic acid is an unusual C_{18} epoxidated fatty acid with potential industrial value because of the unique chemical properties associated with the Δ^{12} epoxy group. Vernolic acid-enriched seed oils, for example, can be

[1] The common name "spurge" derives from to purge, due to the use of the plant latex as purgative. The botanical name *Euphorbia* derives from the greek Euphorbius, in honour to the physician of Juba II of Mauritania, who is supposed to have used in his treatment a certain plant (*E. resinifera*) with a milky latex (Appendino and Szallasi, 1997).

[2] Common name: Jing Da Ji (Peking spurge).

[3] Lang-Du in chinese, means extremely toxic.

used as plasticizers of polyvinylchloride, a market that is currently served by petroleum-derived compounds such as phthalates and to a lesser extent by chemically epoxidized soybean and linseed oil. In addition, the ability of the epoxy group to crosslink makes vernolic acid containing oils useful in adhesives and coating materials such as paint (Cahoon *et al*, 2002; Cuperus *et al*, 1996).

Many species are also cultivated for their brilliant, showy bracts, as well as for their frequently colourful foliage. This is the case of *E. pulcherrima* (poinsettia) that is cultivated for ornamental purposes as a popular Christmas decoration (Judd *et al*, 2002).

1.2. *EUPHORBIA LAGASCAE* AND *EUPHORBIA TUCKEYANA*

Euphorbia lagascae Sprengel and *Euphorbia tuckeyana* Steud are the two species studied in this work. *Euphorbia lagascae* is a species characteristic from the Iberic Peninsula and Corsica (Figure 1.1). It is an annual plant with smooth light green stems, about 20 – 60 cm tall that flowers in early spring and fruits in Abril and May depending upon climate (Flora Iberica, 1989; Krewson and Scott, 1966). It appears in cultivated ground around towns, generally in fallow land rich in nitrogen. As referred previously, *Euphorbia lagascae* seeds are an abundant source of vernolic acid (Krewson and Scott, 1966).

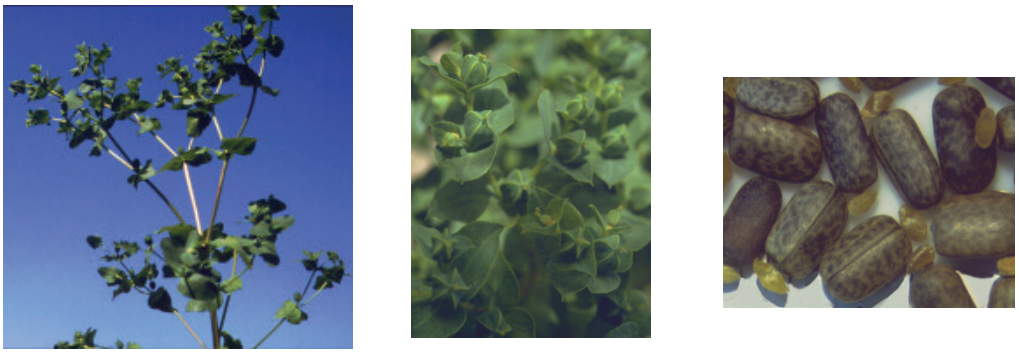


Figure 1.1. *Euphorbia lagascae* (aerial parts, flower detail and seeds)[4].

[4] http://www.n.f-2000-org/publications (January 2002)

Euphorbia tuckeyana (common name Tortolho) is an endemic species from Cape Verde archipelago (Figure 1.2). It is an evergreen shrub that grows up to 2.0 m tall and appears in rocky grounds at 400 – 900 m altitude. It flowers at late spring and the stems are thick and woody without inferior leaves but with superior compact foliage (Figueiredo, 1996). It is traditionally used in leather tanning procedures.[5]

Figure 1.2. *Euphorbia tuckeyana* (whole plant and flower detail).[6]

1.2.1. *Terpenoids: biogenetic considerations*

All living organisms possess similar metabolic pathways, essential for their survival, by which they synthesize and utilize certain essential chemical species: sugars, amino-acids, fatty-acids, nucleotides, etc. This is called primary metabolism and the produced compounds primary metabolites. On the other hand, most organisms also utilize other metabolic pathways (secondary metabolism) which produce compounds with no apparent utility, that are called secondary metabolites (Mann, 1987).

The three main starting materials for secondary metabolism are shikimic acid, aminoacids and acetate. The first two are, respectively, the precursors of many aromatic compounds and alkaloids. Acetate is either the precursor of polyacetylenes, prostaglandins and macrocyclic antibiotics *via* the stepwise addition of C_2 units, and isoprenoids

[5] http://www.caboverde.com/nature/plan-32.jpg (November, 2007)
[6] Pictures taken by the author (Garcia de Orta Garden, Lisbon, January 2005)

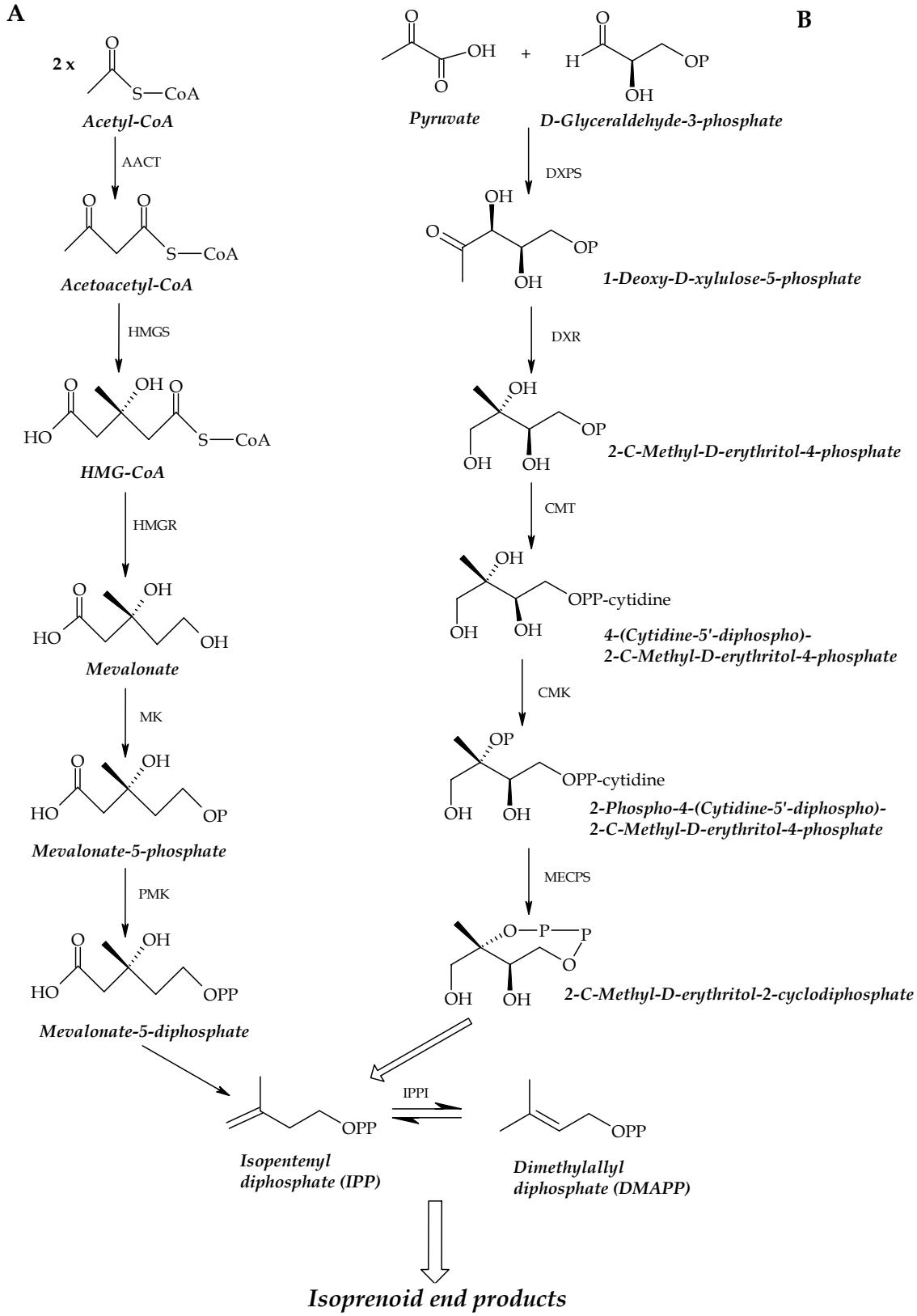
(terpenoids) *via* the mevalonate pathway and the mevalonate-independent pathway (Mann, 1987; Lange *et al*, 2000).

Terpenoids are present in all living organisms. Plant terpenoids could be classified as primary metabolites necessary for cellular function and maintenance (*e.g.* carotenoids and sterols which serve basic functions as photoprotection and membrane permeability), and secondary metabolites that are not involved in growth and development but are often commercially attractive because of their uses as flavour and colour enhancers, agriculture chemicals and medicines (Roberts, 2007). Formally, they are derived from the branched C_5 carbon skeleton of isoprene. This is known as the "isoprene rule" and hence the term "isoprenoids". However, there were apparent exceptions to this rule that lead to the formulation of the "biogenetic isoprene rule". That is, terpenoids were assembled from C_5 units (isoprene-like) and the number of repetitions of this motif, cyclization reactions, rearrangements and further oxidation of the carbon skeleton are responsible for the enormous diversity of structures (Mann, 1987; Rohmer, 1999).

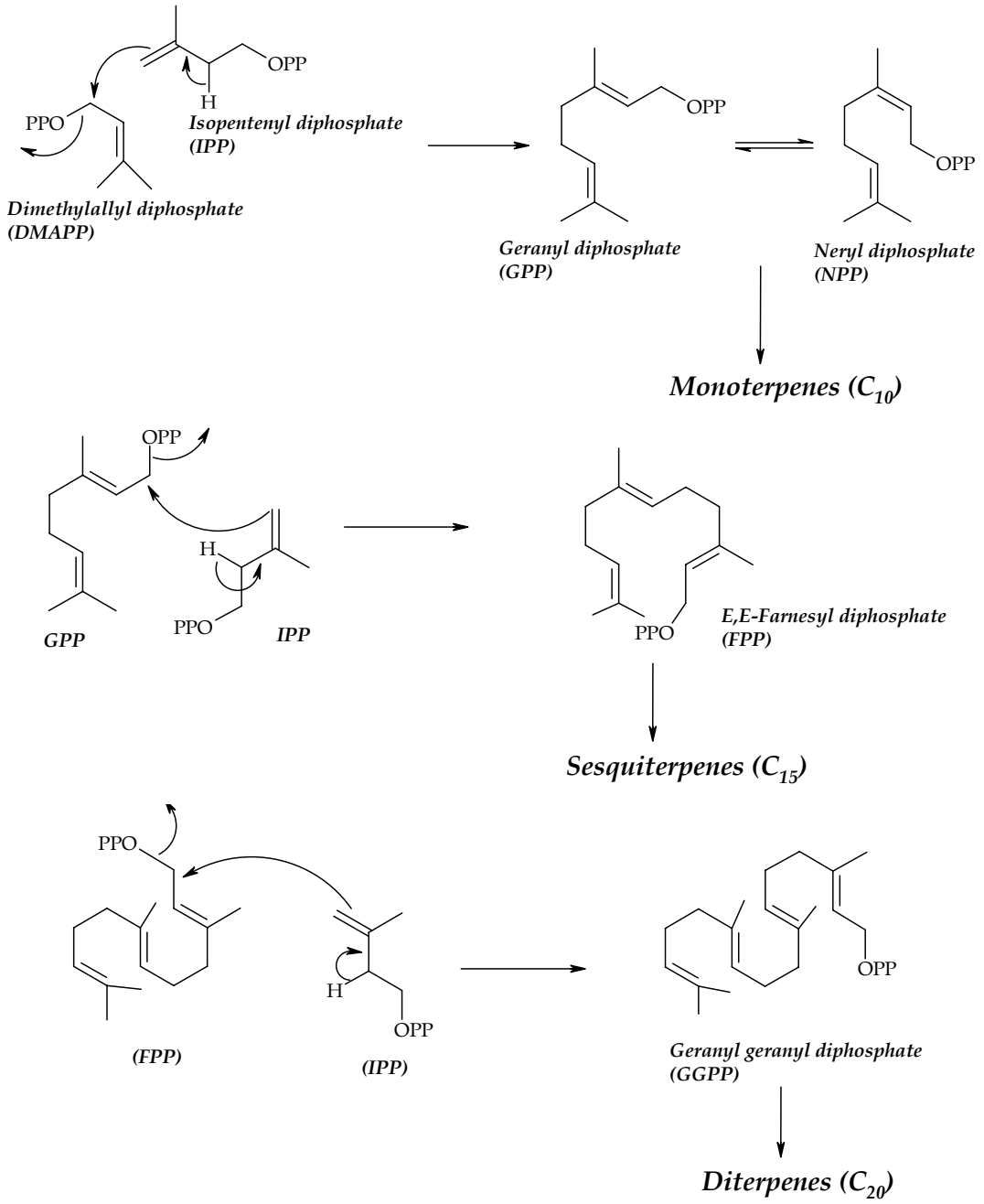
The terpenoid biosynthesis could be divided into two main stages:

a) The first stage includes the synthesis of isopentenyl diphosphate (IPP), isomerisation to dimethylallyl diphosphate (DMAPP), (Scheme 1.1), followed by prenyltransferase-catalyzed condensation of these two C_5 units to geranyl diphosphate (GDP) and the subsequent 1',4-additions of isopentenyl diphosphate to generate farnesyl (FDP) and geranyl geranyl diphosphate (GGDP), (Scheme 1.2).

b) In the second stage, the prenyl diphosphates undergo a range of cyclizations based on variations of the same mechanistic theme (head-to-tail) to produce the parent skeletons of each class. Thus, GDP (C_{10}) gives rise to monoterpenes, FDP (C_{15}) to sesquiterpenes and GGDP (C_{20}) to diterpenes (Bohlmann *et al*, 1998), (Scheme 1.2). Alternatively, the isoprenoid units may be linked in an irregular fashion, as in the triterpene squalene, which is a product of two tail-to-tail coupled molecules of farnesyl diphosphate (FDP), (Thomas, 2004). These cyclizations are catalyzed by the terpenoid synthases (cyclases) and may be followed by a variety of redox modifications on the present skeletal type to produce the many thousands of different terpenoid metabolites (Bohlmann *et al*, 1998).



Scheme 1.1. Biosynthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) by the mevalonate pathway (A) and the DXP pathway (B), (Lange *et al*, 2000).



Scheme 1.2. Suggested pathways for the biosynthesis of monoterpenes, sesquiterpenes and diterpenes (Mann, 1987).

As can be observed in Scheme 1.2, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are the central intermediates in the biosynthesis of terpenoids. Two distint pathways generate these universal C_5 percursors (Scheme 1.1): the

mevalonate pathway (MVA) and the deoxyxylulose-5-phosphate pathway (DXP), (Lange *et al*, 2000; Roberts, 2007).

The mevalonate pathway (MVA)

The MVA pathway was discovered in the 1950's and was assumed to be the only source of the terpenoid precursors IPP and DMAPP. Mevalonic acid has a branched chain C_6-skeleton that undergoes phosphorylation to mevalonic acid phosphate, followed by further phosphorylation and decarboxylation to form the essential C_5-intermediates isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), (Thomas, 2004).

The MVA pathway used to be universally accepted for the biosynthesis of all isoprenoids in all living organisms despite some contradictory results essentially obtained in the field of the isoprenoid biosynthesis in plants (Rohmer, 1999). In particular, isotopically labelled MVA and acetate were usually not or were only very poorly, incorporated into carotenoids, monoterpenes and diterpenes in plant systems. In contrast, these precursors were always efficiently incorporated into sterols, triterpenoids and quite often into the sesquiterpenoids. An independent IPP biosynthesis via the MVA pathway was consequently postulated in the plastids, although the possible presence of another route was not excluded (Rohmer, 1999).

The mevalonate-independent or deoxyxylulose phosphate pathway (DXP)

There are nowadays several terminologies in use for this pathway, including mevalonate-independent pathway, non-mevalonate pathway, glyceraldehyde-3-phosphate/pyruvate pathway, deoxyxylulose phosphate pathway (DXP or DOXP) and methylerythritol phosphate pathway (MEP), (Dewick, 2002).

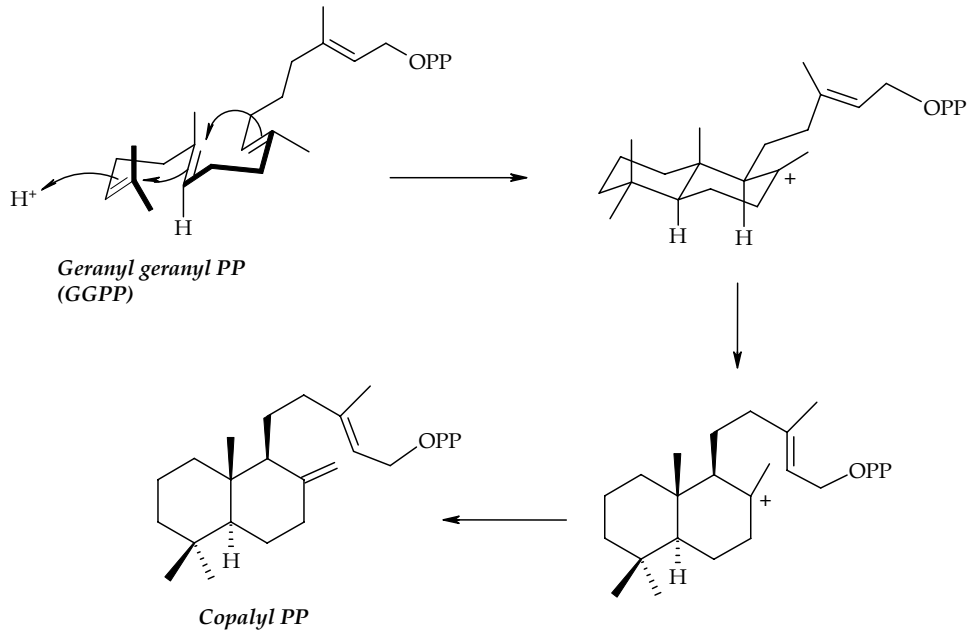
The first initial step in the DXP pathway is the formation of 1-deoxy-2-xylulose-5-phosphate (DXP) by the condensation of D-glyceraldehyde-3-phospate and pyruvate, catalyzed by DXP synthase (Kuzuyama and Seto, 2003), (Scheme 1.1). The target precursors IPP and DMAPP are obtained *via* 2-methyl-D-erythritol-4-phosphate, 2- methyl-D-erythritol-2,4-cyclodiphosphate and 1-hydroxy-2-methyl-2(*E*)-butenyl-4-diphosphate. Although last

steps in the formation of IPP and DMAPP are not yet clarified, the overall sequence requires the initial reductive isomerisation of 1-deoxy-2-xylulose-5-phosphate (DXP) to 2-methyl-D-erythritol-4-phosphate and two subsequent reductive steps (Thomas, 2004).

Whereas the mevalonate pathway enzymes are localized in the cytoplasm, the DXP pathway enzymes appear to be plastid-related. In this way, the mevalonate pathway provides cytosolic metabolites, particularly triterpenoids and steroids, plus some sesquiterpenoids. The DXP pathway leads to plastid-related metabolites, monoterpenes and diterpenes, some sesquiterpenes, tetraterpenes (carotenoids), and the prenyl-side chains of chlorophyll and plastoquinones. There are examples of cooperation between the cytosolic and plastidial pathways, especially in the biosynthesis of stress metabolites. The DXP pathway is not known to operate in mammals (Dewick, 2002; Rohmer, 1999).

Diterpenoids

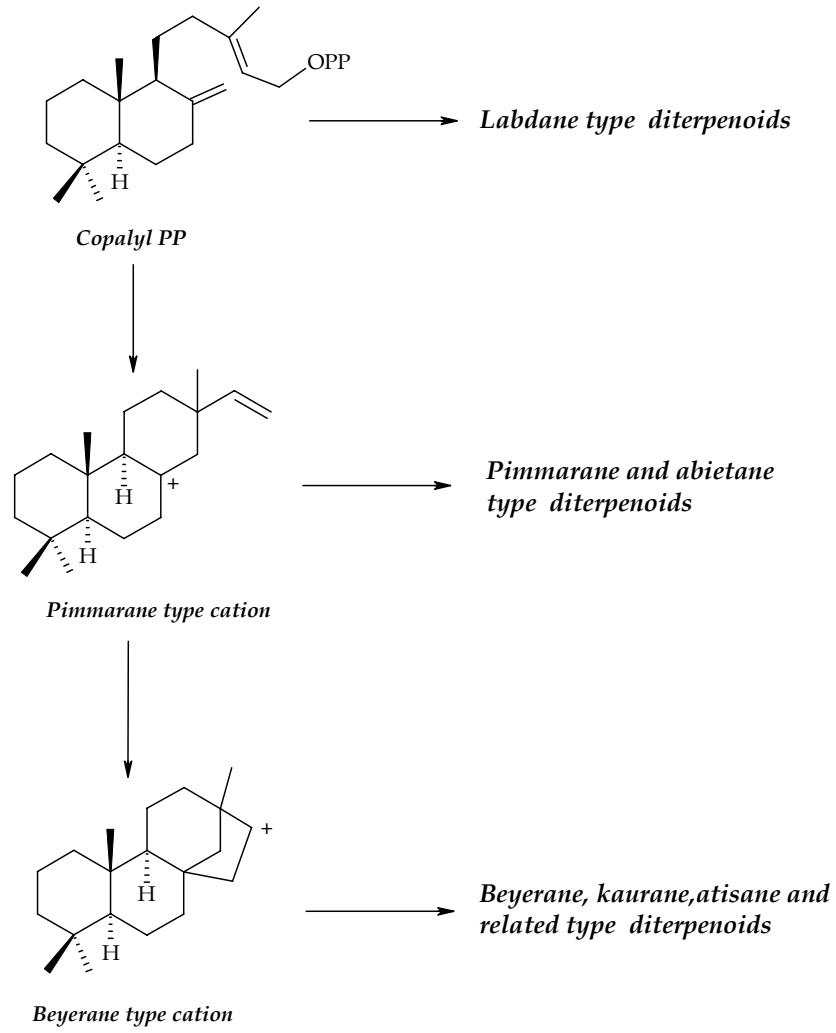
Diterpenoids constitute the second largest class of terpenoids with over 130 distinct skeletal types (Rowe, 1989). The precursor of diterpenoids is geranyl geranyl PP (GGPP), (Scheme 1.3).



Scheme 1.3. Cyclization of GPP to copalyl PP (Rowe, 1989).

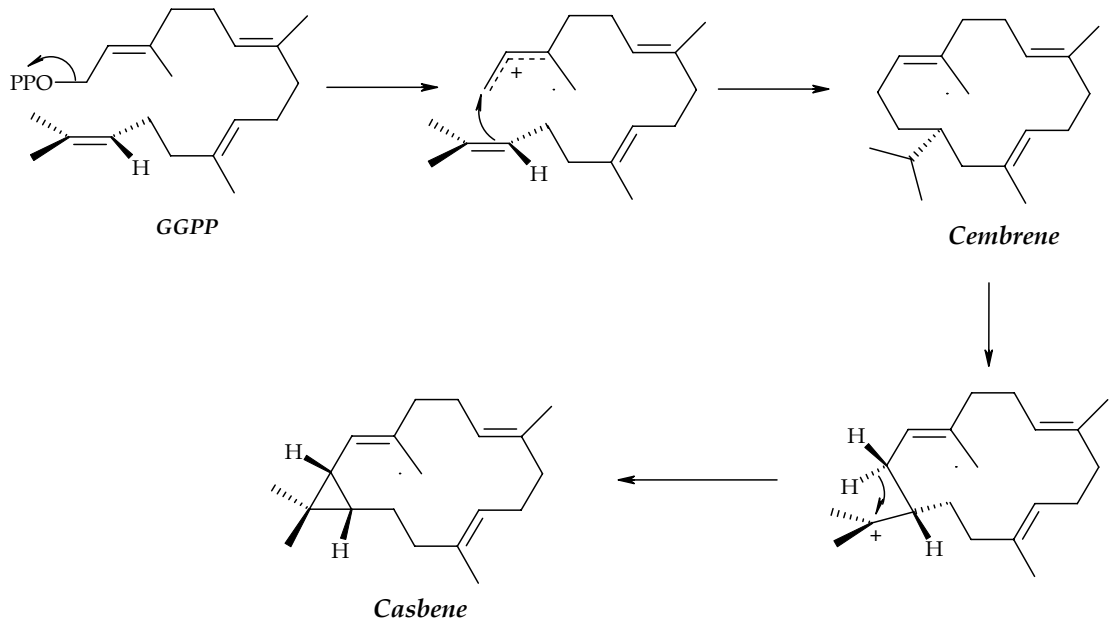
The initial enzymatic cyclization of GGPP can occur from either end of the molecule although cyclization from the head (mostly initiated by H^+) is by far the most dominant mode in diterpenes. The most important cyclization reaction of GGPP is the generation of copalyl diphosphate (copalyl-PP) that play a central role in the biosynthesis of most bi-, tri- and tetracyclic diterpenoids, as can be seen in Scheme 1.4 (Mann, 1987; Rowe, 1989).

Copallyl PP is the precursor of labdane diterpenoids. These diterpenoids could be formed with both normal (5α, 10β) and *ent*- (5β, 10α) configurations, which may arise through different modes of coiling of the open chain precursor on the cyclase enzyme surface (Hanson, 1991). Pimmaranes and abietanes derived from copallyl PP through the diphosphate acting as a leaving group (Scheme 1.4).

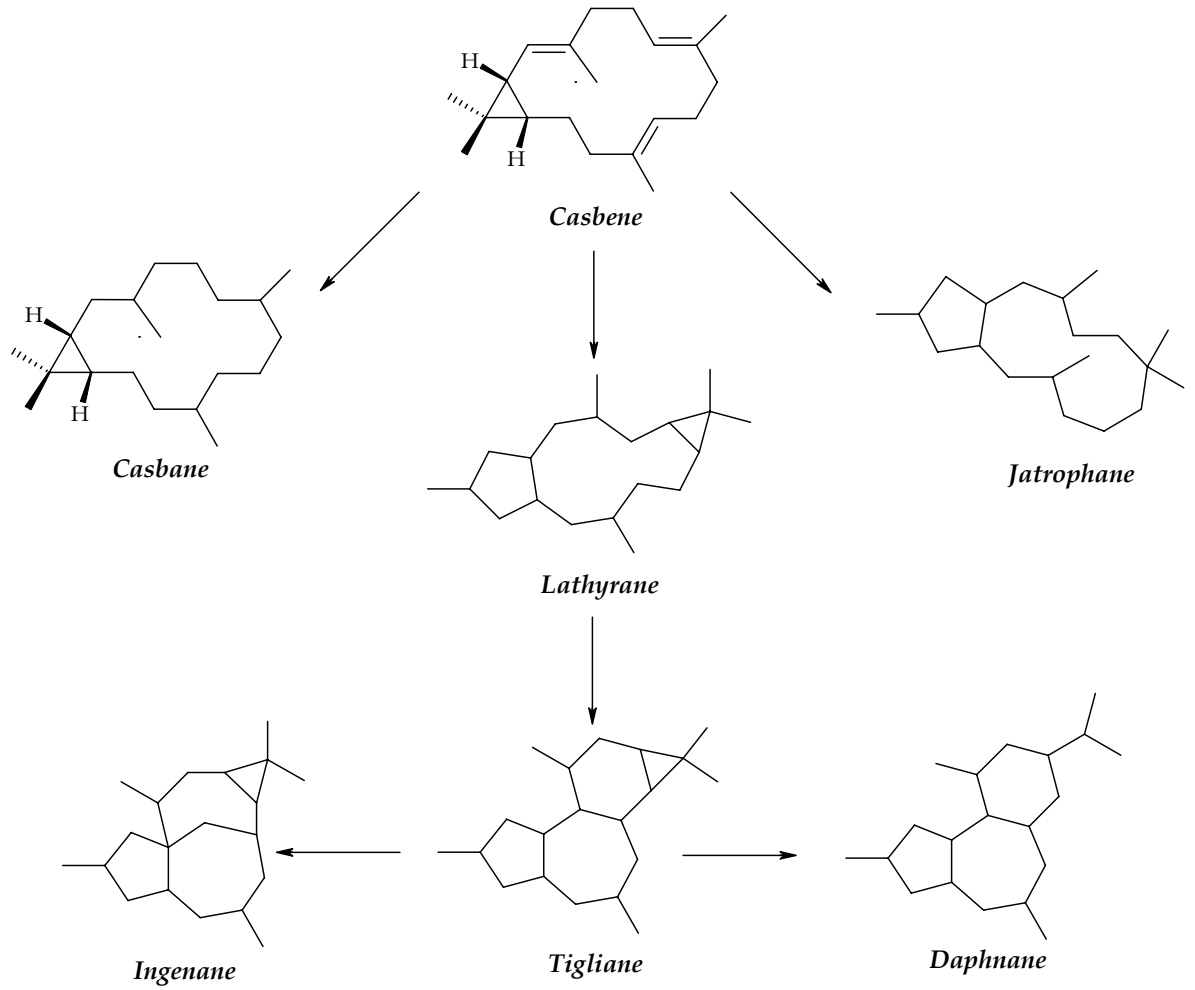


Scheme 1.4. Biogenesis of polycyclic diterpenoids (Rowe, 1989).

Another mode of geranyl geranyl PP cyclization leads to macrocyclic diterpenoids and their related compounds (Scheme 1.5). This is initiated by the terminal diphosphate group of GGPP acting as a leaving group and generating a formal carbocation that alkylates a double bond of the distal isoprene unit to form the diterpenes cembrene and casbene (Hanson, 1991). Casbene and its saturated analogue have been considered to be the biogenetic precursors of macrocyclic diterpenes, lathyranes and jatrophanes, as well as the precursors of the polyfunctional diterpenes of the tigliane, daphnane and ingenane types (Scheme 1.6), (Evans and Taylor, 1983).



Scheme 1.5. Cylization of GGPP leading to the macrocyclic diterpenoids cembrene and casbene (Dewick, 2002).



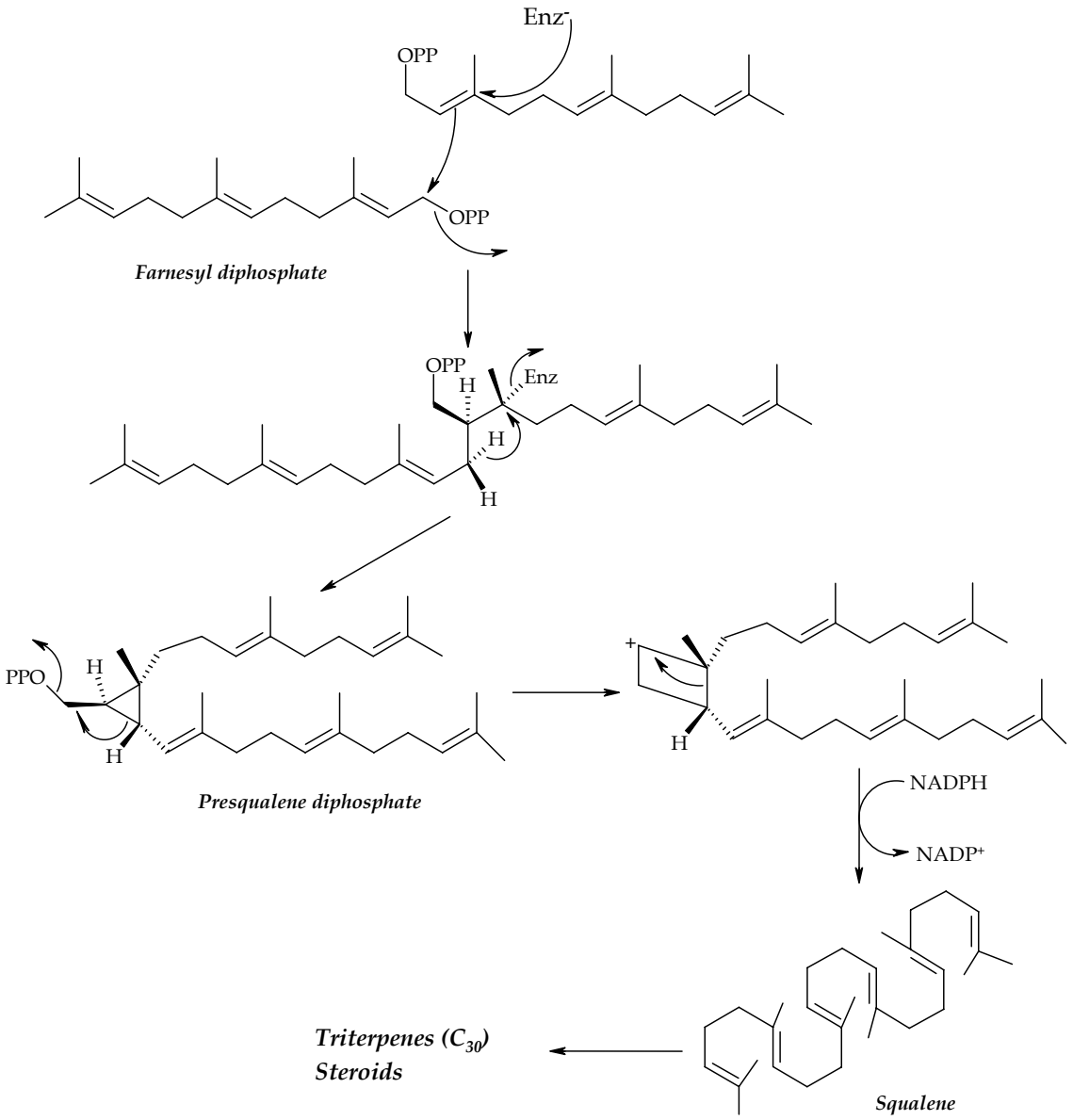
Scheme 1.6. Biogenesis of macrocyclic and polycyclic diterpenoids derived from casbene (Mann, 1987; Evans and Taylor, 1983).

Triterpenoids and steroids

Triterpenoids constitute a large family of terpenoids embracing over 200 skeletal types currently known (Connolly and Hill, 2007). According to the "biogenetic isoprene rule", all-*trans*-squalene is the immediate precursor of all cyclic triterpenoids and steroids. Squalene is derived from two farnesyl diphosphate units (FPP) by tail-to-tail coupling that is catalyzed by a membrane-bound enzyme (Scheme 1.7), (Rowe, 1989).

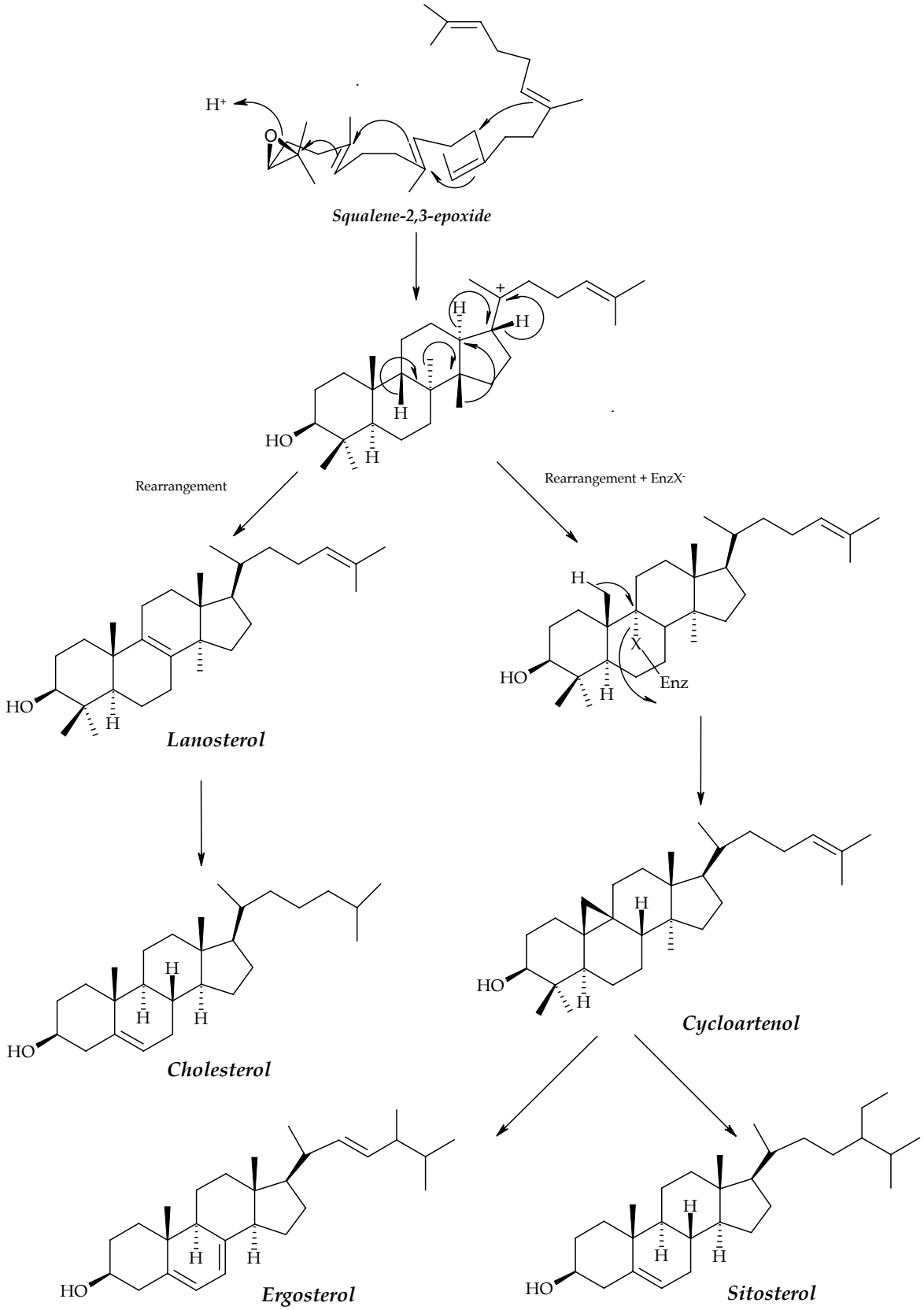
Cyclization of squalene proceeds in the vast majority of cases, by its oxidation first to squalene 2,3-epoxide, in which the quirality at C-3 is usually *S*. The polycyclic structures formed from squalene can be rationalized in terms of the conformations in which squalene chain may be folded on the enzyme surface, into a chair (C) or boat (B) conformations, or a

part remaining unfolded (U). The cyclization is usually initiated by acid-catalized ring opening of the squalene epoxide, and probably occurs through a series of carbocationic intermediates (Mann, 1987; Rowe, 1989).



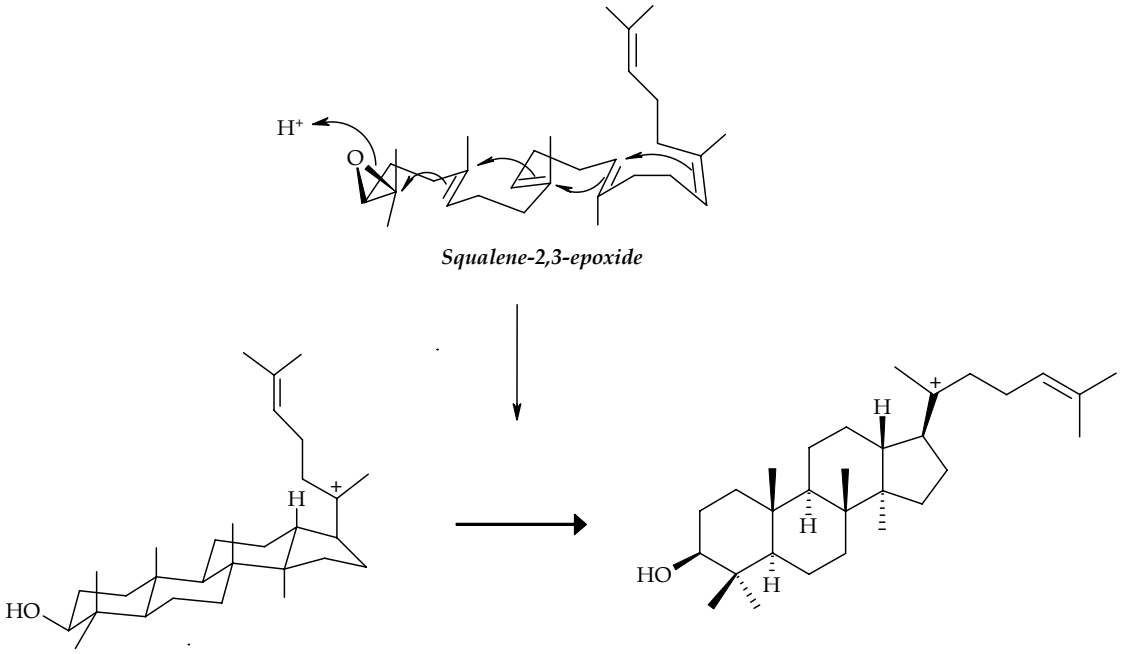
Scheme 1.7. Biosynthesis of squalene, the precursor of steroids and triterpenes (Rowe, 1989).

a) *Chair-boat-chair-boat-unfolded*: this mode of cyclization leads to the lanostane, protostane and cycloartane group, and steroids (Scheme 1.8), (Rowe, 1989). In general, animals employ the lanosterol to cholesterol pathway, while plants utilize the cycloartenol to phytosterol (ergosterol and sitosterol) pathway (Mann, 1987).



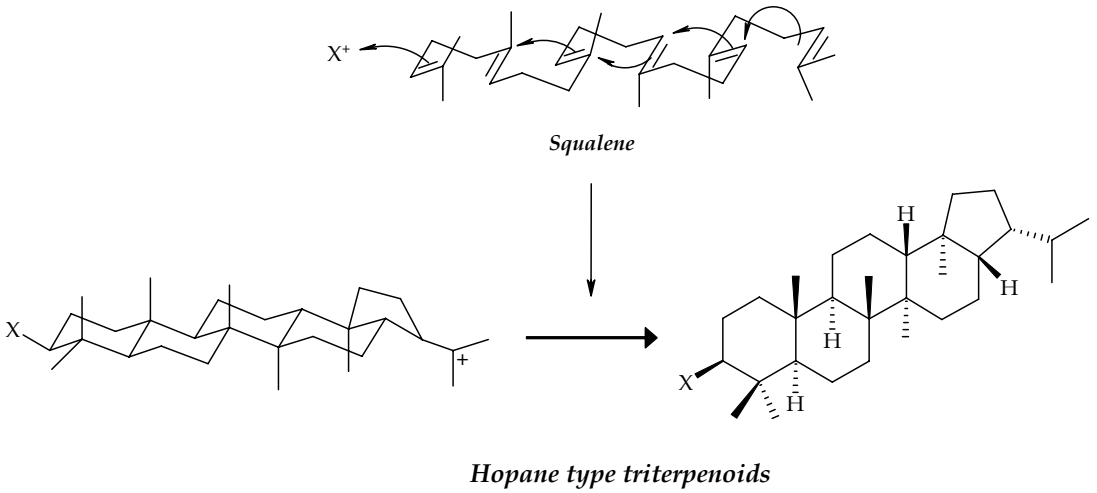
Scheme 1.8. Cyclization of squalene epoxide to lanostane and cycloartane-type triterpenoids and steroids (chair-boat-chair-boat-unfolded cyclization), (Mann, 1987).

b) *Chair-chair-chair-boat-unfolded*: cyclization of squalene epoxide in this type of conformation leads to a cation that is the precursor of the dammarane, euphane and tirucallane triterpenes (Scheme 1.9).



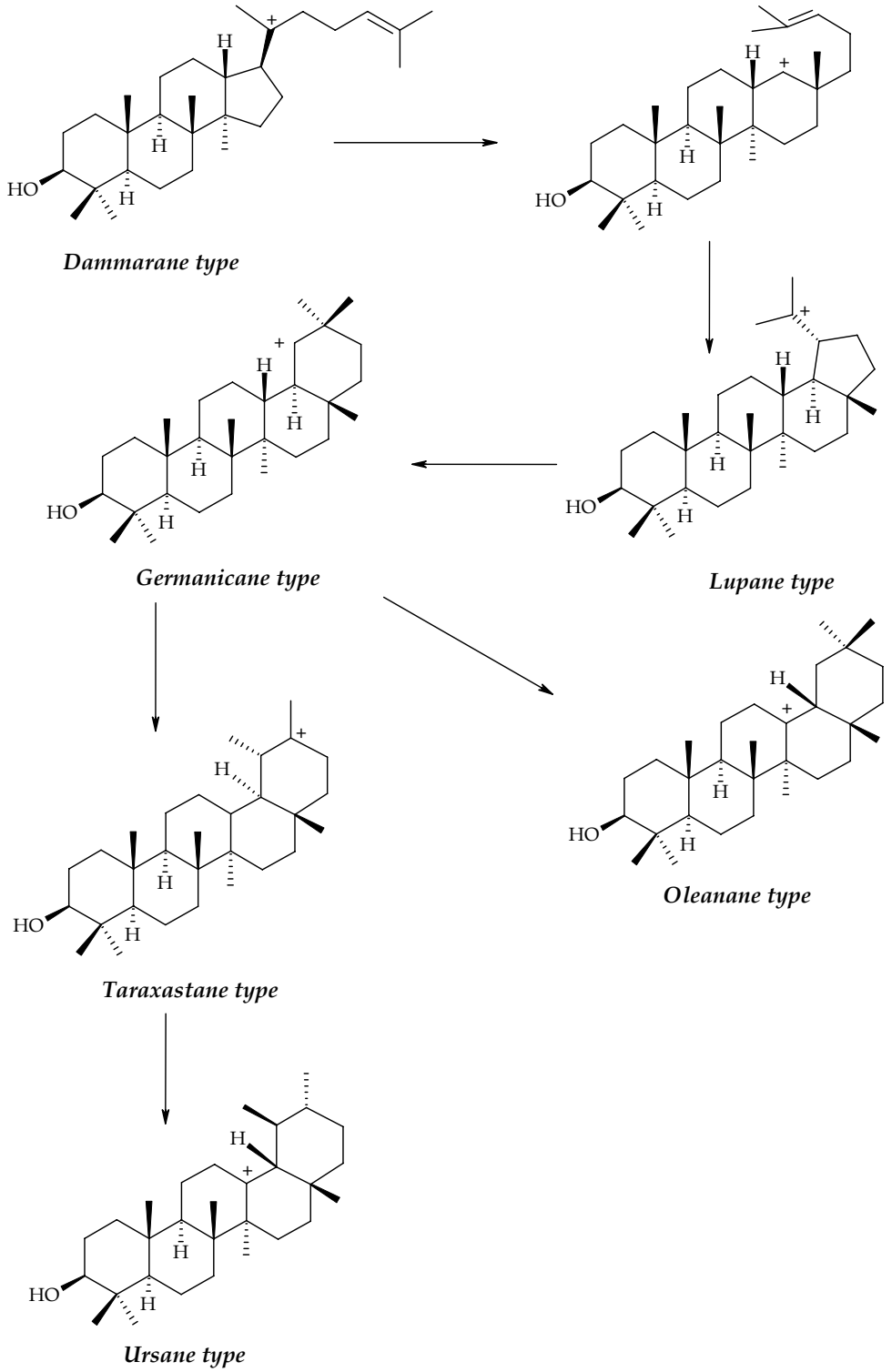
Scheme 1.9. Cyclization of squalene epoxide to dammarane-type triterpenoids (chair-chair-chair-boat-unfolded cyclization), (Rowe, 1989).

c) *Chair-chair-chair-chair-chair*: cyclization of squalene in all-chair conformation leads to the precursor ion of the hopane class of triterpenoids (Scheme 1.10), (Rowe, 1989).



Scheme 1.10. Biogenesis of hopane type triterpenoids (all-chair cyclization), (Rowe, 1989).

d) *Chair-chair-chair-boat*: from the tetracyclic dammarane type cation, expansion of the D ring is envisaged to furnish another carbocation which can cyclise further to generate the precursor of lupanes, germanicanes, taraxastanes, oleananes, ursanes and related classes of compounds (Scheme 1.11).



Scheme 1.11. Biogenesis of lupane, germanicane, taraxastane, oleanane and ursane type triterpenoids (Rowe, 1989).

2. LITERATURE REVIEW

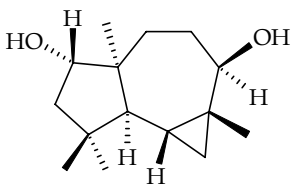
The central point of this work has been the isolation and structural characterization of terpenic compounds from two species of *Euphorbia* genus: *E. lagascae* and *E. tuckeyana*. Some phenolic compounds were also isolated and characterized. In this section, a literature review of the new terpenic (sesquiterpenes, diterpenes, triterpenes and steroids), and phenolic compounds isolated from *Euphorbia* species between 2002 and 2007 is presented.

2.1. SESQUITERPENES

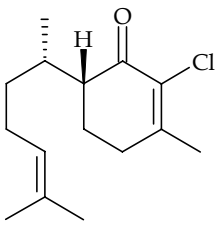
The new sesquiterpenes isolated from *Euphorbia* genus between 2002 and 2007 are summarized in Table 1.1 (**1.1 - 1.6**). These include a guaiane derivative (**1.1**) and a bisabolane-type sesquiterpene (**1.2**), as well as a drimane-type sesquiterpene coumarin ether (**1.3**), which has been reported for the first time from *Euphorbia* species.

Table 1.1. New sesquiterpenes isolated from *Euphorbia* sp. (2002 –2007).

Euphorbia sp.	Analysed part	Extract (fraction)	Compounds	References
E. chrysocoma	Dried roots	EtOH 95% (petr. ether fr.)	**1.2**	Shi *et al*, 2005
E. ebracteolata	Dried roots	EtOH 95% (EtOAc fr.)	**1.1**	Yin *et al*, 2005
E. portlandica	Dried whole plant	Acetone (Et_2O fr.)	**1.3**	Madureira *et al*, 2004 [a]
E.resinifera	Fresh latex	EtOAc (MeCN fr.)	**1.4 – 1.6**	Fattorusso *et al*, 2002



1.1



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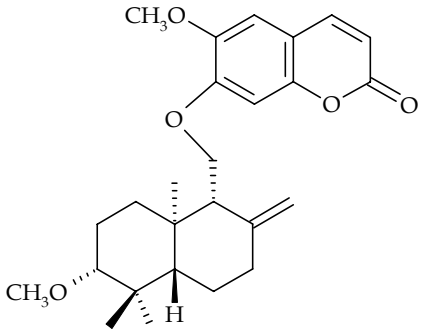

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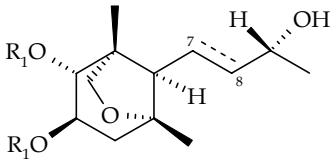


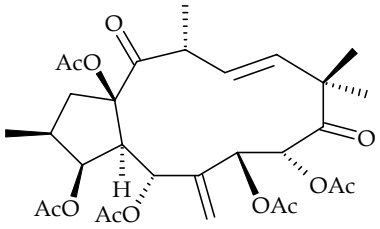
	R_1	
1.4	β-D-Glu	$\Delta^{7,8}$
1.5	β-D-Glu	7,8-dihydro
1.6	H	$\Delta^{7,8}$

2.2. MACROCYCLIC DITERPENES AND THEIR CYCLIZATION DERIVATIVES

The new macrocyclic diterpenes isolated within this period are described in Tables 1.2 and 1.3. They have the jatrophane (**1.7 – 1.91**), lathyrane (**1.101 – 1.106**) and ingol (**1.112 – 1.125**) skeletons. New compounds with the paraliane (**1.92** and **1.93**), pepluane (**1.94** and **1.95**) and segetane (**1.96 – 1.100**) skeletons have been isolated and are considered to be rearranged derivatives of the jatrophane scaffold. In the same way, some rearranged derivatives of lathyrane diterpenes have also been described (**1.107 – 1.109**). Lathyranoic acid (**1.110**), a secolathyrane diterpenoid, and lathyranone A (**1.111**), a rearranged lathyrane-type diterpene, both having unprecedent skeletons have been isolated from *Euphorbia* genus for the first time. In the period described above, a new casbane diterpene (**1.167**) has been isolated from *Euphorbia pekinensis*. This compound has the peculiarity of having a *trans*-ring junction between the macrocycle and the cyclopropane ring. The new diterpenes with tigliane (**1.126 – 1.138**) and ingenane skeletons (**1.139 – 1.152**) are reported in Table 1.4. The new myrsinane-type diterpenes are represented in Table 1.5 (**1.153 – 1.166**).

Table 1.2. New diterpenes with jatrophane, paraliane, pepluane and segetane skeletons isolated from *Euphorbia* sp. (2002 – 2007).

Euphorbia sp.	Analysed part	Extract (fraction)	Compounds	References
E. altotibetic	Fresh whole plant	EtOH 90% (petr. ether fr.)	**1.38 – 1.41**	Li *et al*, 2003
E. amygdaloides	Fresh whole plant	EtOAc	**1.12 – 1.23**	Corea *et al*, 2005 [a]
E. characias	Fresh whole plant	EtOAc	**1.24 – 1.35**	Corea *et al*, 2004 [a]
E. dendroides	Latex	EtOAc	**1.75 – 1.80**	Corea *et al*, 2003 [a]
			1.42 – 1.50	Corea *et al*, 2003 [b]
E. esula	Air-dried whole plant	EtOH ($CHCl_3$ fr.)	**1.59, 1.74**	Liu *et al*, 2002
E. helioscopia	Air-dried whole plant	EtOH 95% (EtOAc fr.)	**1.63**	Zhang and Guo, 2006
			1.60 –1.62	Zhang and Guo, 2005
E. hyberna	Air-dried roots	$CHCl_3$ (acetone fr.)	**1.11**	Ferreira *et al*, 2002
E. kansui	Dried roots	EtOH (petr. ether fr.)	**1.56, 1.57, 1.71**	Pan *et al*, 2004
	Dried roots	EtOH 60% ($CHCl_3$ fr.)	**1.58, 1.72**	Wang *et al*, 2003 [a]
			1.73	Wang *et al*, 2002
E. mongolica	Air-dried plant	MeOH (CH_2Cl_2 fr.)	**1.10, 1.51, 1.52**	Hohmann *et al*, 2003 [a]
E. paralias	Fresh whole plant	EtOAc	**1.92, 1.94**	Barile *et al*, 2007 [a]
			1.96 – 1.98	Barile *et al*, 2007 [b]
E. peplus	Fresh whole plant	EtOAc	**1.36, 1.37, 1.53 – 1.55**	Corea *et al*, 2004 [b]
			1.95	Corea *et al*, 2005 [b]
E. platyphyllos	Air-dried whole plant	$CHCl_3$	**1.65, 1.81, 1.83**	Hohmann *et al*, 2003 [b]
E. portlandica	Air-dried whole plant	Acetone (Et_2O fr.)	**1.99, 1.100**	Madureira *et al*, 2006
			1.93	Madureira *et al*, 2004 [b]
E. pubescens	Air-dried whole plant	MeOH (Et_2O fr.)	**1.70**	Valente *et al*, 2004 [a]
			1.8, 1.64	Valente *et al*, 2004 [b]
			1.9	Valente *et al*, 2004 [c]
			1.67 to 1.69	Valente *et al*, 2003
E. serrulata	Fresh whole plant	MeOH (*n*-hexane fr.)	**1.81, 1.86, 1.87, 1.89, 1.90**	Rédei *et al*, 2003
		MeOH (*n*-hexane fr.)	**1.66, 1.84, 1.85, 1.88, 1.91**	Hohmann *et al*, 2002
E. turczaninowii	Whole plant	EtOH	**1.7**	Liu *et al*, 2006



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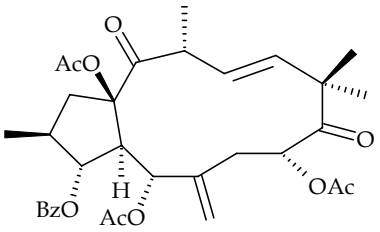

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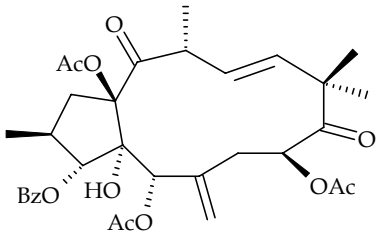

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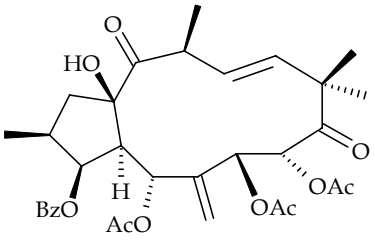

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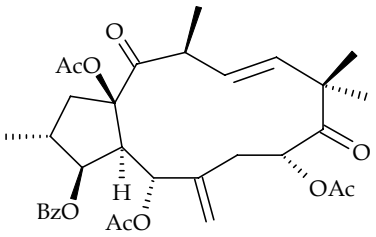

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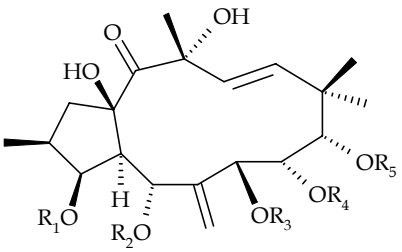
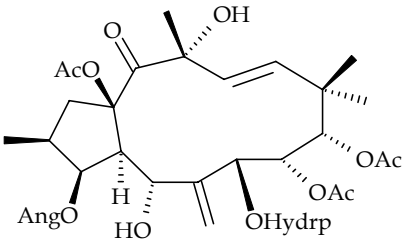
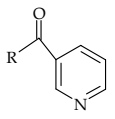




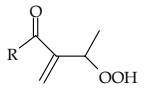
1.23

	R_1	R_2	R_3	R_4	R_5
1.12	Ac	Ac	Ang	Ang	Nic
1.13	Ang	H	Ang	Ac	Nic
1.14	Hydrp	H	Ang	Ac	Nic
1.15	Ang	H	Ang	Ac	Ac
1.16	Ang	H	Hydrp	Ac	Ac
1.17	Ang	Ac	H	Hydrp	Ac
1.18	Hydrp	Ac	H	Ang	Ac
1.19	Ac	Hydrp	H	Ang	Ac
1.20	Hydrp	Ac	Ang	H	Ac
1.21	Ac	Hydrp	Ang	H	Ac
1.22	Ang	Ac	Hydrp	H	Ac



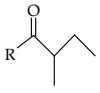
Nic

Ang

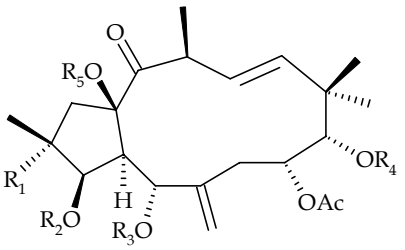


Hydrp

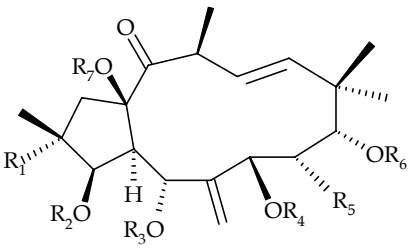
***i*Bu**



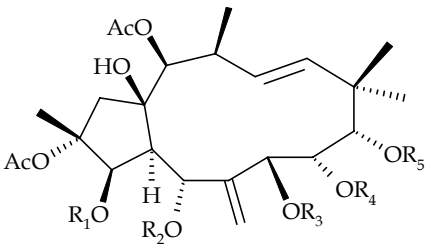
MeBu



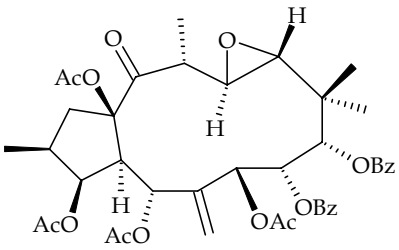
	R_1	R_2	R_3	R_4	R_5
1.24	OH	Bz	Ac	Nic	Ac
1.25	OH	Bz	Ac	Nic	H
1.26	OH	Bz	Ac	Bz	H
1.27	OH	MeBu	Ac	Ac	Ac
1.28	H	Bz	Ac	Ac	H
1.29	H	Bz	Ac	Nic	Ac
1.30	H	iBu	Ac	Nic	H
1.31	H	iBu	Ac	Nic	Ac
1.32	H	Pr	Ac	Nic	Ac
1.33	H	Ac	Ac	Nic	Ac
1.34	H	iBu	H	Nic	Ac
1.35	OH	Bz	H	Nic	Ac



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1.36	H	Ac	Ac	Ac	H	Ac	Ac
1.37	OAc	Bz	Ac	iBu	OH	Nic	H
1.38	H	Ac	Bz	Ac	OAc	Ac	Ac
1.39	H	Ac	Bz	Pr	OAc	Ac	Ac
1.40	OH	Ac	Bz	Ac	OAc	Ac	Ac
1.41	OH	Ac	Bz	Pr	OAc	Ac	Ac
1.42	OAc	H	iBu	Bz	OAc	Ac	H
1.43	OAc	H	MeBu	Bz	OAc	Ac	H
1.44	OAc	H	Nic	Bz	OAc	Ac	H
1.45	H	H	iBu	Bz	OAc	Ac	H
1.46	H	Ac	iBu	Bz	OAc	Ac	H
1.47	OH	Ac	iBu	Bz	OAc	Ac	H
1.48	OAc	Nic	Ac	iBu	OAc	Ac	H
1.49	H	Bz	Ac	iBu	OAc	Ac	H
1.50	ONic	Ac	iBu	Ac	OAc	ONic	OAc
1.51	H	Bz	Ac	Ac	OAc	Ac	Ac
1.52	H	Ac	Bz	Ac	OAc	Ac	Ac



	R_1	R_2	R_3	R_4	R_5
1.53	Bz	Ac	Ac	Ac	Nic
1.53	Bz	Ac	MeBu	H	Nic
1.55	Ac	iBu	Bz	Ac	Ac



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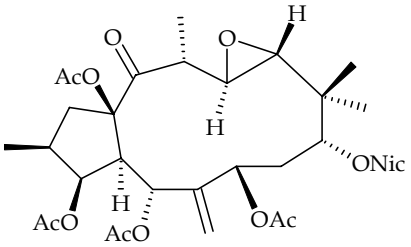

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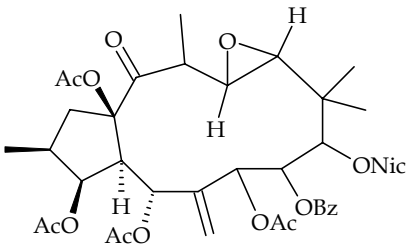
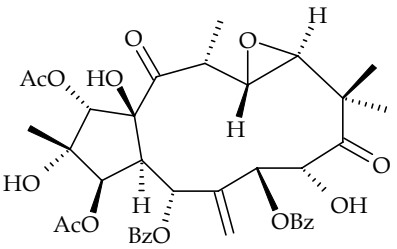


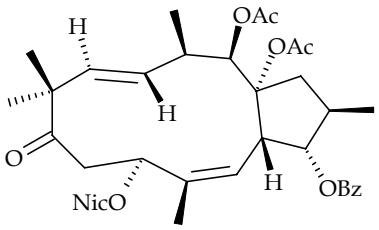
1.58



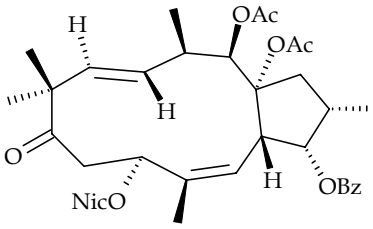
1.59

[7]

[7] The sterochemistry of C-7, C-8, C-9, C-11, C-12 and C-13 (compound **1.58**) is not determined in the original paper.



1.60



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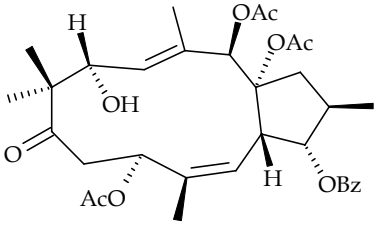

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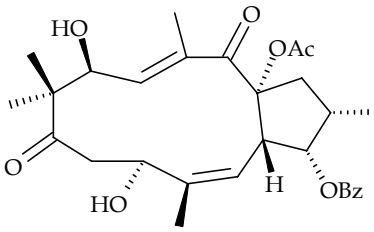
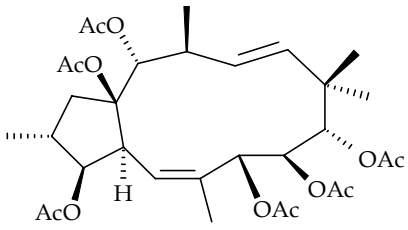
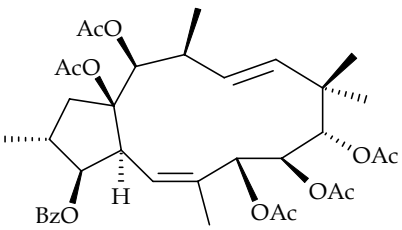


1.63

[8]



1.64



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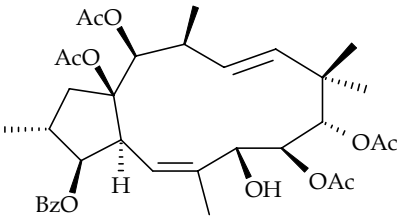
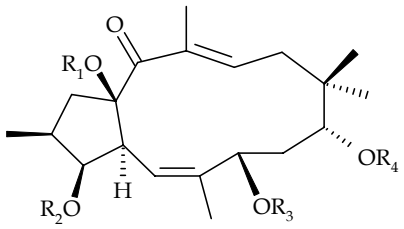
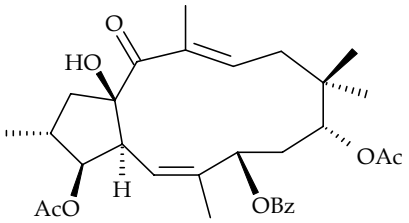


1.66

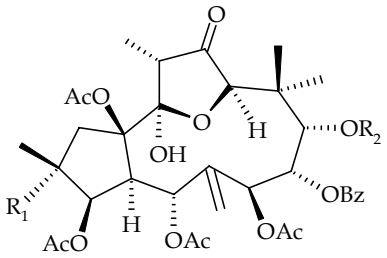
[8] Structures **1.60** to **1.63** are depicted exactly as in the original papers.



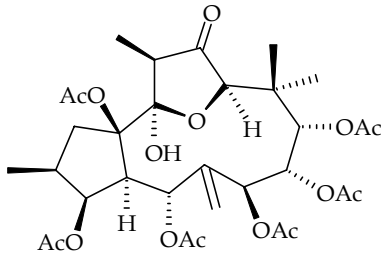
	R_1	R_2	R_3	R_4
1.67	Ac	Ac	Bz	Ac
1.68	Ac	Ac	Bu	Ac
1.69	H	Ac	Bz	Ac



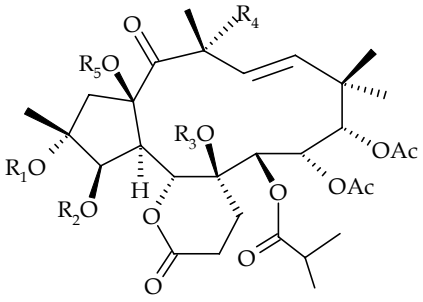
1.70



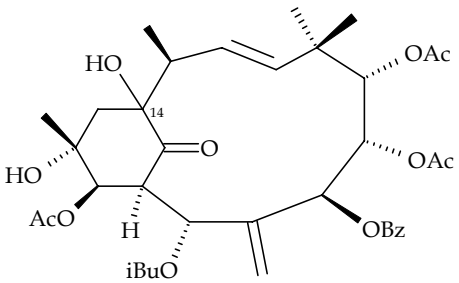
	R_1	R_2
1.71	OH	Ac
1.72	H	Nic
1.73	H	Ac



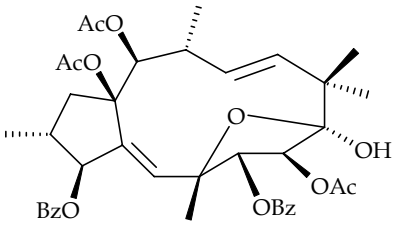
1.74



	R_1	R_2	R_3	R_4	R_5
1.75	Ac	Ac	iBu	H	H
1.76	H	Ac	Ac	H	Ac
1.77	H	Ac	iBu	H	Ac
1.78	Ac	Ac	iBu	OH	Ac



1.79: 14 β-OH
1.80: 14 α-OH



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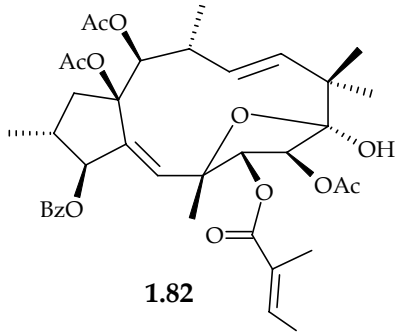
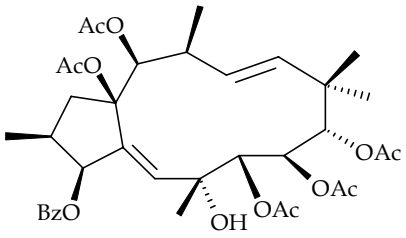
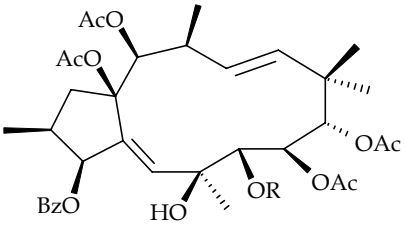


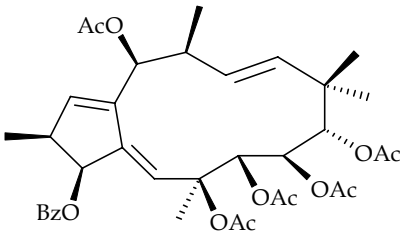
1.82



1.83



1.84 R = Ac
1.85 R = Bz



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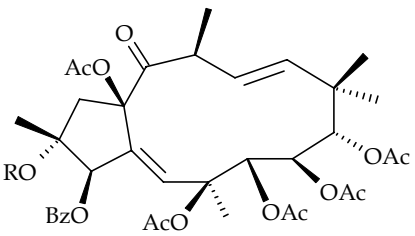
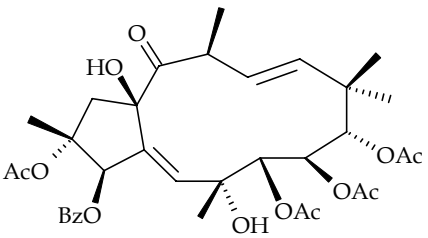
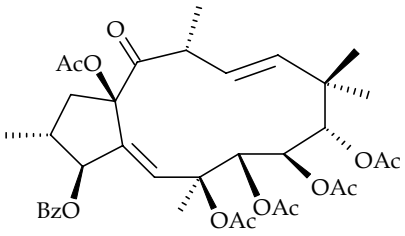


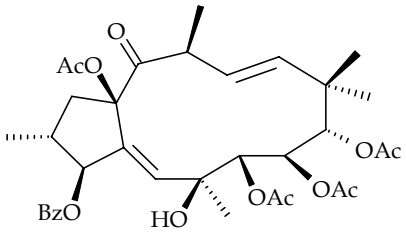
1.87 R = Ac
1.88 R = H



1.89



1.90



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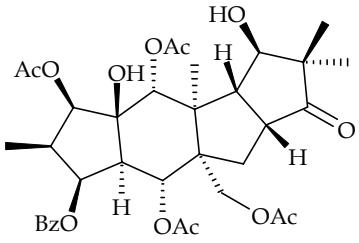

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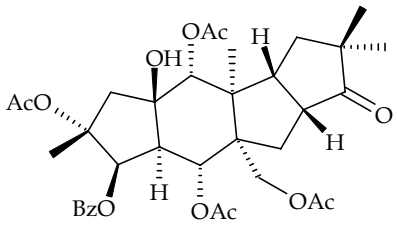

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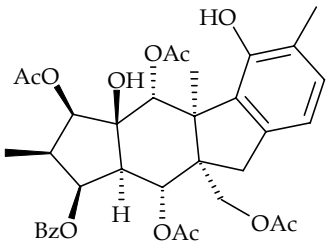

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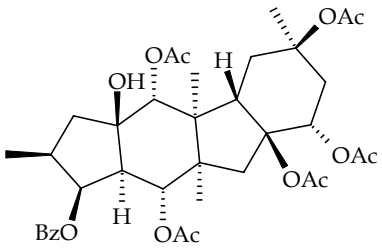

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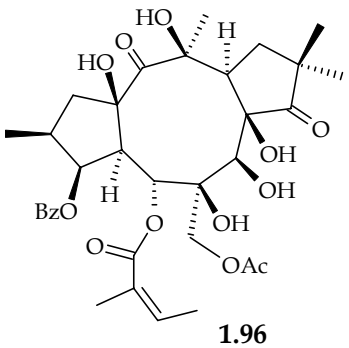

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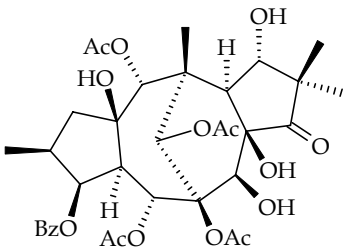

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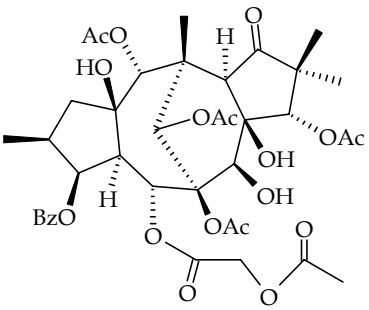

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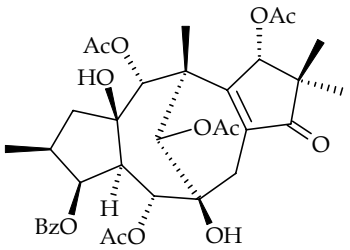

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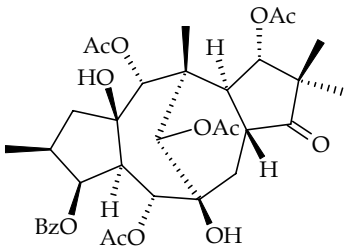
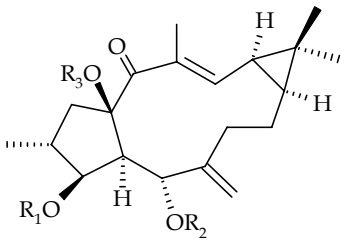


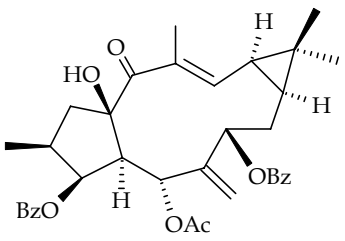
1.100

Table 1.3. New diterpenes with lathyrane and ingol skeletons and its rearranged derivatives isolated from *Euphorbia* sp. (2002 – 2007).

Euphorbia sp.	Analysed part	Extract (fraction)	Compounds	References
E. cornigera	Air-dried roots	Acetone (EtOAc fr.)	**1.115 – 1.120**	Baloch *et al*, 2006
E. hyberna	Air-dried roots	$CHCl_3$ (acetone fr.)	**1.101**, **1.102**	Ferreira *et al*, 2002
E. latazy	Latex	n.d.	**1.104**	Róndon *et al*, 2005
E. lathyris	Seeds	EtOH	**1.103**, **1.110**	Liao *et al*, 2005
	Seeds	Acetone (MeCN fr.)	**1.105**	Appendino *et al*, 2003
	Seeds	EtOH (petr. ether fr.)	**1.111**	Gao *et al*, 2007
E. nivulia	Latex	MeOH	**1.121 – 1.123**	Ravikanth *et al*, 2003
			1.124, **1.125**	Ravikanth *et al*, 2002
E. officinarum	Latex	MeOH	**1.112 – 1.114**	Daoubi *et al*, 2007
E. villosa	Air-dried whole plant	MeOH ($CHCl_3$ fr.)	**1.106 – 1.109**	Vasas *et al*, 2004



	R_1	R_2	R_3
1.101	Bz	Ac	Ac
1.102	Ac	Ac	Ac



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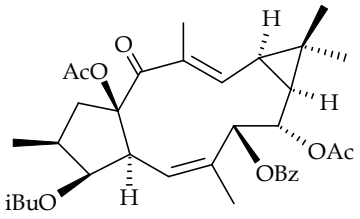

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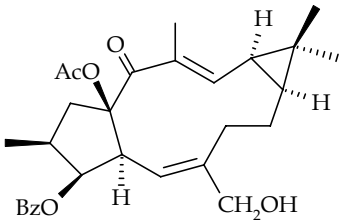

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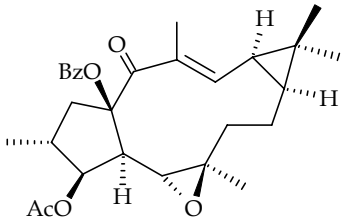
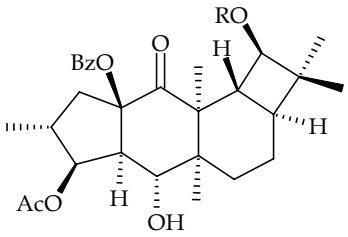
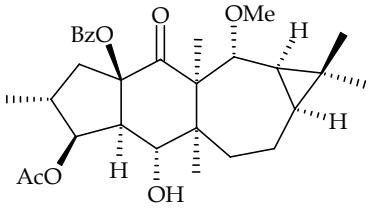


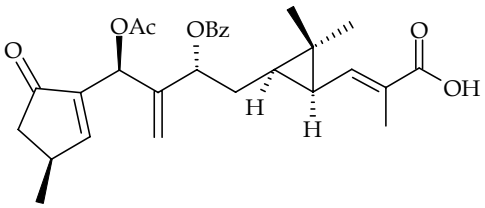
1.106



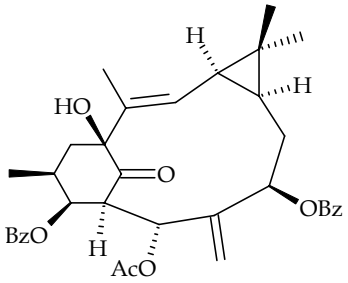
1.107: R = CH_3
1.108: R = H



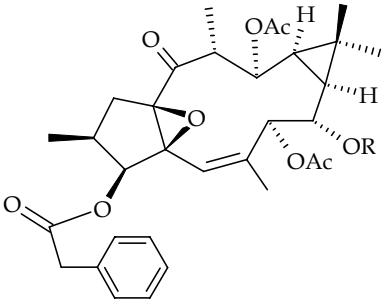
1.109



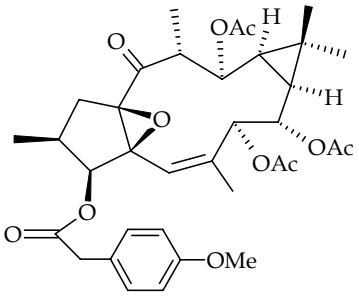
1.110



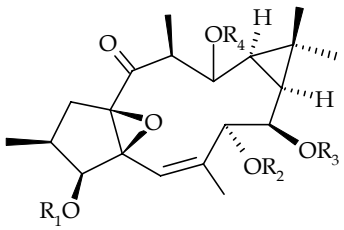
1.111



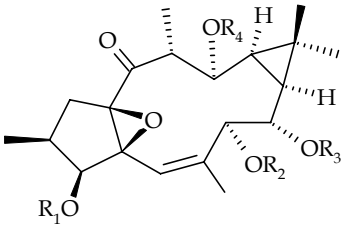
	R
1.112	Ac
1.113	Me



1.114



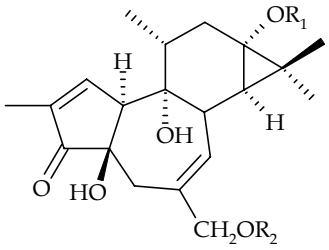
	R_1	R_2	R_3	R_4
1.115	MeBu	Ac	Ac	Ac
1.116	Ac	Ac	MeBu	Ac
1.117	Ac	Ac	Ac	MeBu
1.118	MeBu	Ac	Me	Ac
1.119	Ac	MeBu	Me	Ac
1.120	Ac	Ac	Me	MeBu



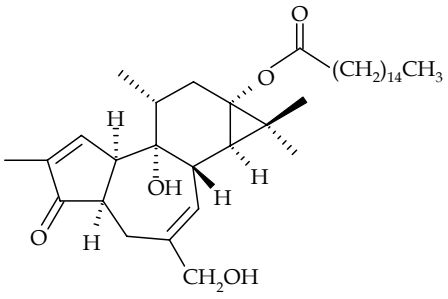
	R_1	R_2	R_3	R_4
1.121	Ac	Ang	Me	H
1.122	Ac	H	Me	Ac
1.123	Ac	Ang	H	Ac
1.124	Ac	H	Bz	Ac
1.125	Ac	Bz	Nic	Ac

Table 1.4. New diterpenes with tigliane and ingenane skeletons isolated from *Euphorbia* sp. (2002 – 2007).

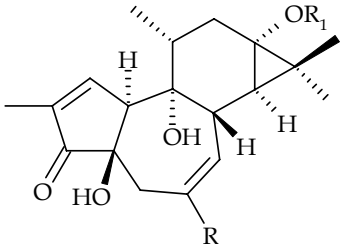
***Euphorbia* sp.**	**Analysed part**	**Extract (fraction)**	**Compounds**	**References**
E. caudocifolia	Latex	MeOH (acetone fr)	**1.140** to **1.143**	Baloch *et al*, 2005
E. cornigera	Air-dried roots	Acetone (EtOAc fr.)	**1.126 – 1.134**	Baloch *et al*, 2007
E. guyoniana	Roots	$CHCl_3$	**1.135**	Haba *et al*, 2007
E. fischeriana	Dried roots	EtOH 95% ($CHCl_3$ fr.)	**1.136– 1.138**	Wang *et al*, 2006
E. kansui	Dried roots	EtOH 60% ($CHCl_3$ fr.)	**1.144** to **1.147**	Wang *et al*, 2003 [a]
			1.148 - 1.152	Wang *et al*, 2002
	Dried roots	EtOH (*n*-hexane fr.)	**1.139**	Shi *et al*, 2007



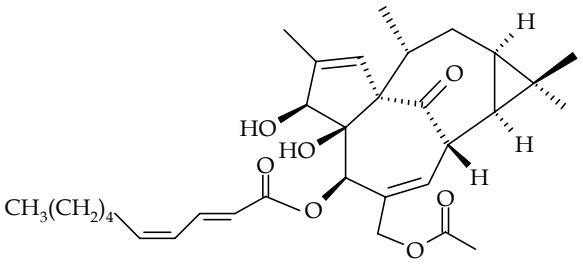
	R_1	R_2
1.126	Ac	Bz
1.127	Ac	*p*-MeOBz
1.128	Decanoyl	Ang
1.129	Decanoyl	Tig
1.130	Ac	Decanoyl
1.131	Bu	Decanoyl
1.132	Hexanoyl	Decanoyl
1.133	Octanoyl	Decanoyl
1.134	Dodecanoyl	Decanoyl



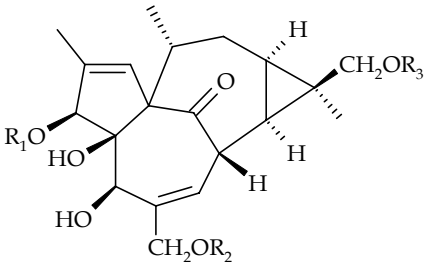
1.135



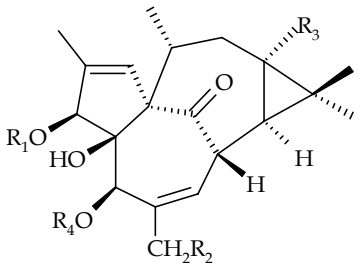
	R_1	R_2
1.136	CHO	Ac
1.137	CHO	$OCO(CH_2)_{14}CH_3$
1.138	CH_2OCOCH_3	$OCO(CH_2)_7CH{=}CH(CH_2)_7CH_3$



1.139



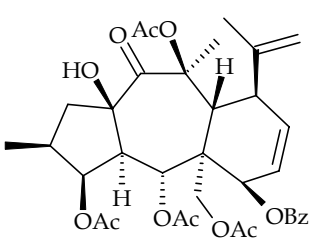
	R_1	R_2	R_3
1.140	H	H	Deca-2,4,6-trienoyl
1.141	Ang	H	Deca-2,4,6-trienoyl
1.142	Ac	Ang	H
1.143	Ang	H	Ac



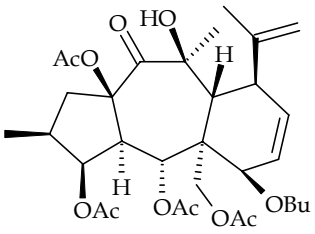
	R_1	R_2	R_3	R_4
1.144	$COCH(CH_3)CH(CH_3)_2$	$OCO(CH_2)_{14}CH_3$	$OCO(CH_2)_{10}CH_3$	H
1.145	$COCH(CH_3)CH(CH_3)_2$	OAc	$OCO(CH_2)_{10}CH_3$	H
1.146	$CO(CH{=}CH)_2(CH_2)_4CH_3$ (*E*/*Z*)	H	H	H
1.147	$CO(CH{=}CH)_2(CH_2)_4CH_3$ (*E*/*E*)	H	H	H
1.148	$COCH(CH_3)CH(CH_3)_2$	OH	$OCO(CH_2)_8CH_3$	H
1.149	H	$OCO(CH{=}CH)_2(CH_2)_4CH_3$ (*E*/*E*)	H	H
1.150	H	$OCO(CH{=}CH)_2(CH_2)_4CH_3$ (*E*/*Z*)	H	H
1.151	$CO(CH{=}CH)_2(CH_2)_4CH_3$ (*E*/*Z*)	OH	H	Ac
1.152	H	OH	H	$CO(CH{=}CH)_2(CH_2)_4CH_3$ (*E*/*E*)

Table 1.5. New diterpenes with myrsinane and casbane skeletons isolated from *Euphorbia* sp. (2002 – 2007).

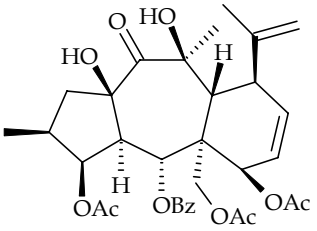
Euphorbia **sp.**	**Analysed part**	**Extract (fraction)**	**Compounds**	**References**
E. decipiens	Air-dried whole plant	Acetone ($CHCl_3$ fr.)	**1.153**, **1.154**	Ahmad *et al*, 2005 [a]
			1.155	Ahmad *et al*, 2005 [b]
			1.156 – 1.158	Ahmad *et al*, 2003 [a]
			1.159 – 1.161	Ahmad *et al*, 2003 [b]
			1.166	Ahmad *et al*, 2002
E. pekinensis	Dried roots	EtOH (petr. ether fr.)	**1.167**	Kong *et al*, 2002
E.prolifera	Dried roots	EtOH 95% (petr. ether fr.)	**1.162 – 1.165**	Zhang *et al*, 2004



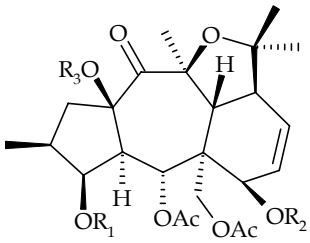
1.153



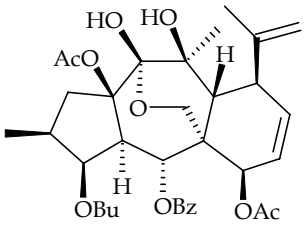
1.154



1.155



	R_1	R_2	R_3
1.156	Ac	Bz	H
1.157	Ac	Bz	Ac
1.158	Ac	Nic	Ac
1.159	Bz	Ac	H



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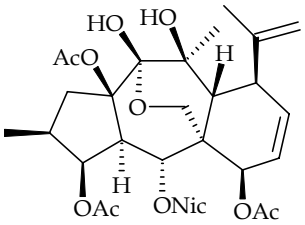

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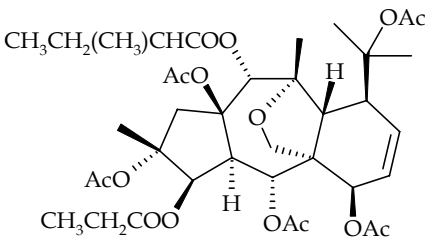

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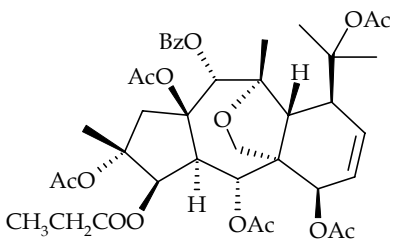

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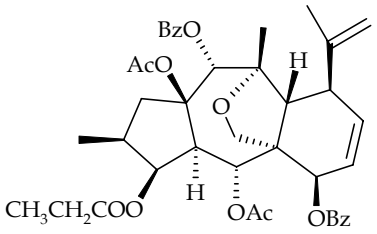

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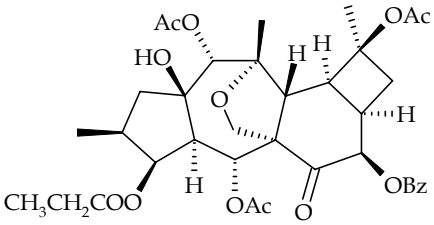

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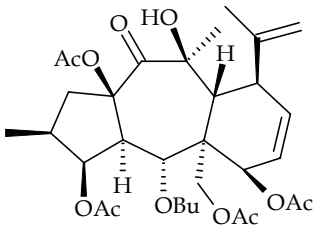
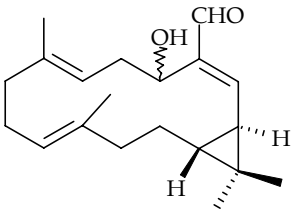


1.166



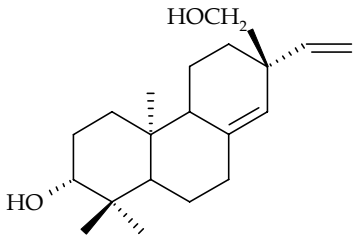
1.167

2.3. OTHER POLYCYCLIC DITERPENES

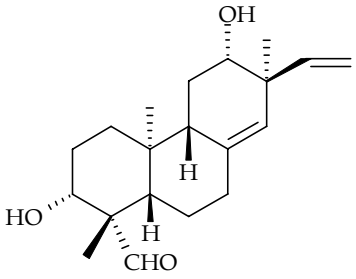
The new polycyclic diterpenes isolated from *Euphorbia* species between 2002 and 2007 are summarized in Table 1.6. They have the abietane (**1.170 - 1.178**), pimarane (**1.168**) and isopimarane skeletons (**1.169**). A rosane-type (**1.182**) and a dimeric *nor*-rosane (**1.183**) diterpenoids have been isolated from *E. ebracteolata*. A guaiane diterpene (**1.184**) has been isolated for the first time from *Euphorbia* genus. Three rearranged trachylobane diterpenes (**1.179 - 1.181**) have been reported, two of them consisting of an unprecedented pentacyclic skeleton with a cyclobutane ring. A novel symmetrical dimeric diterpenoid (**1.185**) has also been isolated from *E. quinquecostata* and is the first example of such a diterpenoid from this genus.

Table 1.6. New polycyclic diterpenes isolated from *Euphorbia* sp. (2002 – 2007).

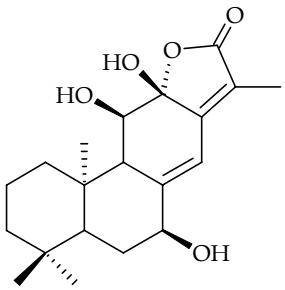
Euphorbia sp.	Analysed part	Extract (fraction)	Compounds	References
E. fischeriana	Dried roots	EtOH 95% ($CHCl_3$ fr.)	**1.168**, **1.170**, **1.171**,**1.177** **1.185**	Wang *et al*, 2006 Zhou *et al*, 2003
E. ebracteolata	Dried roots Dried roots	EtOH 95% ($CHCl_3$ fr.) EtOH 95% (EtOAc fr.)	**1.183** **1.172, 1.173, 1.182**	Fu *et al*, 2006 Shi *et al*, 2005
E. portlandica	Air-dried whole plant	Acetone (Et_2O fr.)	**1.178**	Madureira *et al*, 2004 [c]
E. quinquecostata	Dried stem wood	MeOH (EtOAc fr.)	**1.169**	Su *et al*, 2002
E. wallichii	Fresh roots Dried roots	EtOH 95% (EtOAc fr.) EtOH 95% ($CHCl_3$ fr.)	**1.179 – 1.181** **1.184** **1.174 – 1.176**	Pan *et al*, 2006 Zhang *et al*, 2006 Wang *et al*, 2004



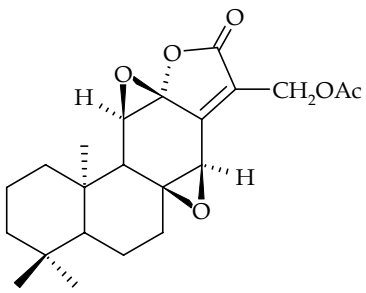
1.168



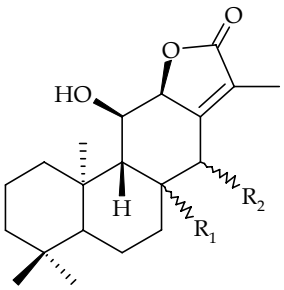
1.169



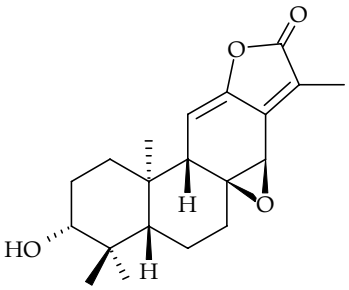
1.170



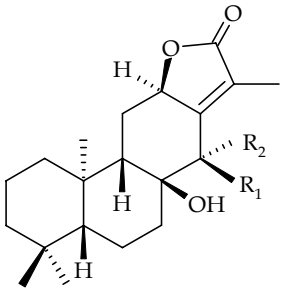
1.171



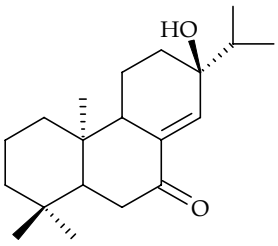
1.172 R_1 = β-OH, R_2 = α-OH
1.173 R_1 = α-OH, R_2 = β-OH



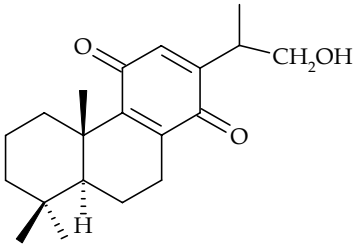
1.174



1.175 R_1 = H, R_2 = OH
1.176 R_1 = OH, R_2 = H



1.177



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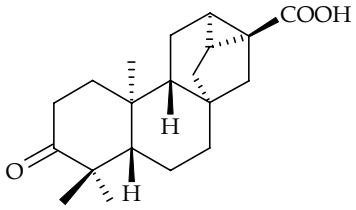
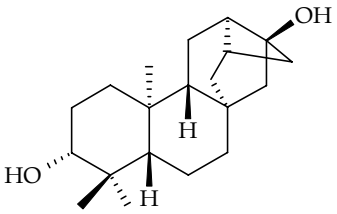
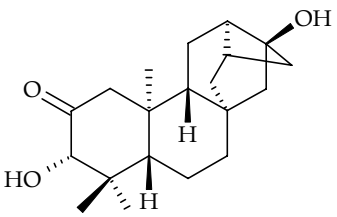


1.179



1.180



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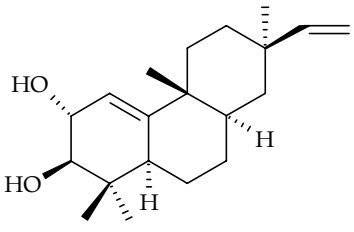

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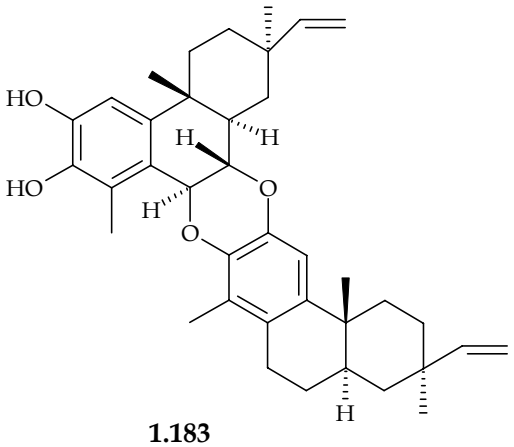

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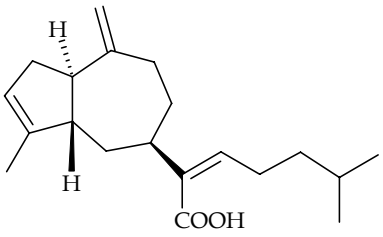

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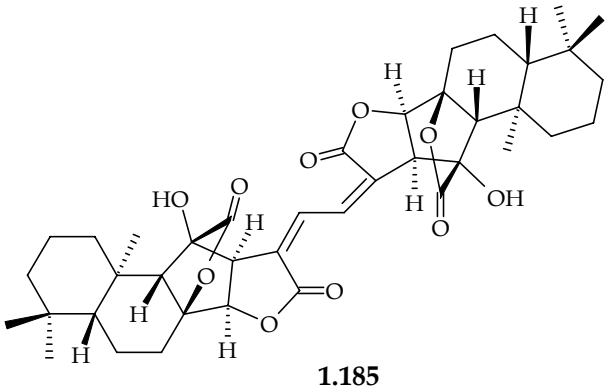


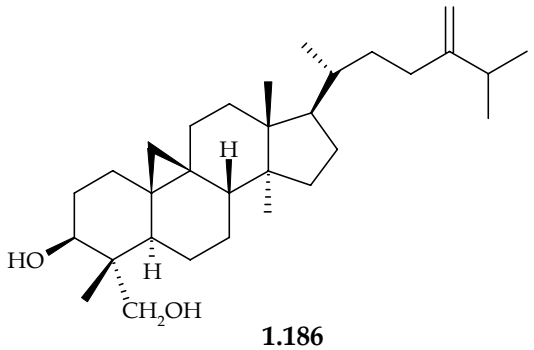
1.185

2.4. TRITERPENES AND STEROIDS

Several triterpenes (**1.186** - **1.199**) and two steroids (**1.200**, **1.201**) have been isolated from *Euphorbia* species within the referred period, as can be observed in Table 1.7. The triterpenes have the cycloartane (**1.186** and **1.187**), lupane (**1.188**), tirucallane (**1.189**), madeirane (**1.190** and **1.191**), and euphane (**1.192 – 1.198**) skeletons. A spirotriterpene (**1.199**) has been isolated from *E. guyoniana* roots.

Table 1.7. New triterpenes and steroids isolated from *Euphorbia* sp. (2002 – 2007).

***Euphorbia* sp.**	**Analysed part**	**Extract (fraction)**	**Compounds**	**References**
E. antiquorum	Fresh latex	EtOAc	**1.196 – 1.198**	Akihisa *et al*, 2002
E. guyoniana	Roots	$CHCl_3$	**1.199**	Haba *et al*, 2007
E.kansui	Dried roots	EtOH 60% ($CHCl_3$ fr.)	**1.189, 1.192 – 1.195**	Wang *et al*, 2003 [b]
E. nerifolia	Fresh latex	*n*-hexane	**1.187**	Mallavadhani *et al*, 2004
E. officinarum	Latex	MeOH	**1.186**	Daoubi *et al*, 2007
			1.200, **1.201**	Daoubi *et al*, 2004
E. portlandica	Dried whole plant	Acetone (Et_2O fr.)	**1.188**	Madureira *et al*, 2004
E. stygiana	Dried leaves	Acetone (*n*-hexane fr.)	**1.190**, **1.191**	Lima *et al*, 2003



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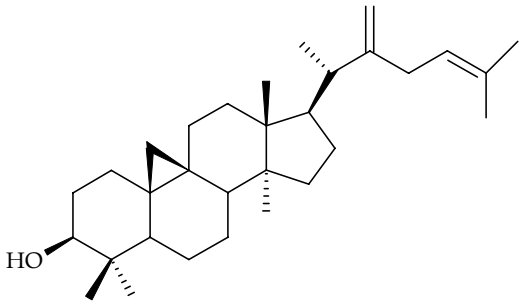

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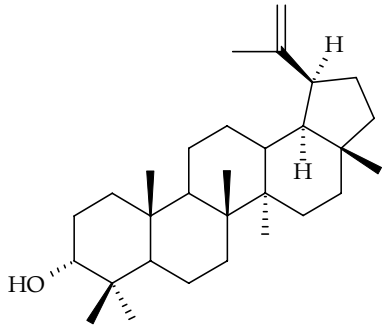

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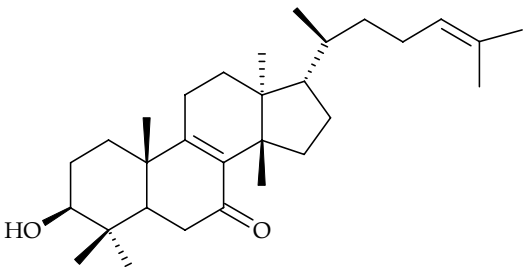

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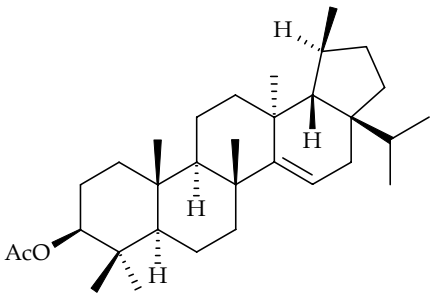

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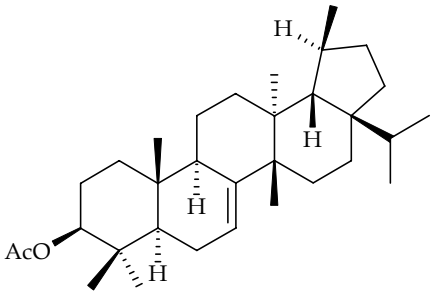

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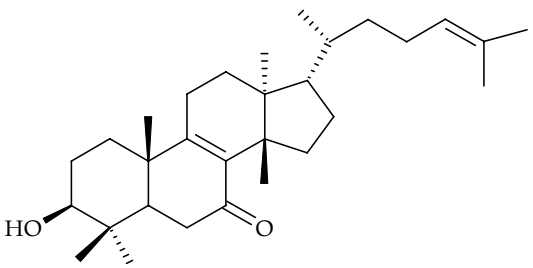

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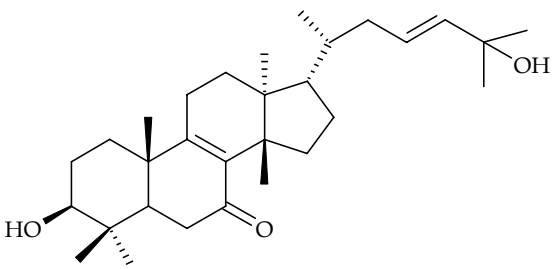

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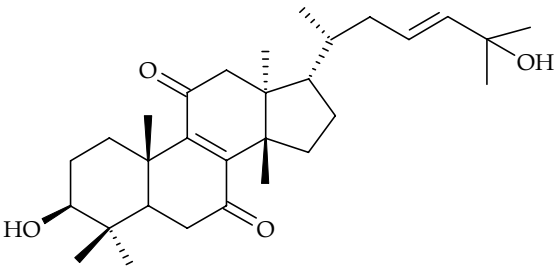

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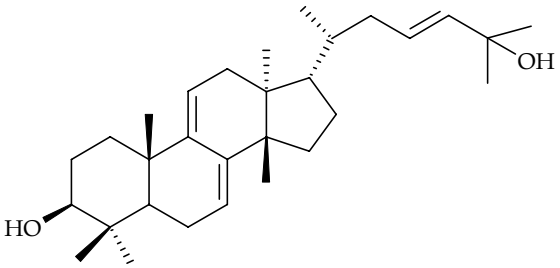

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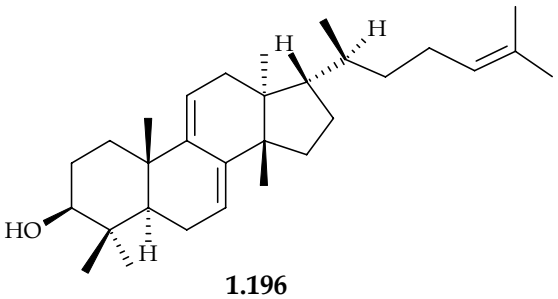

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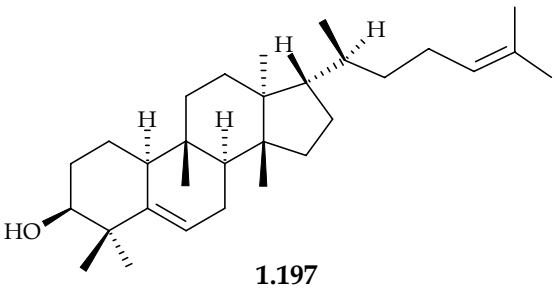

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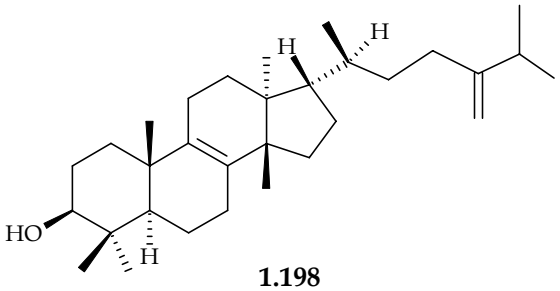

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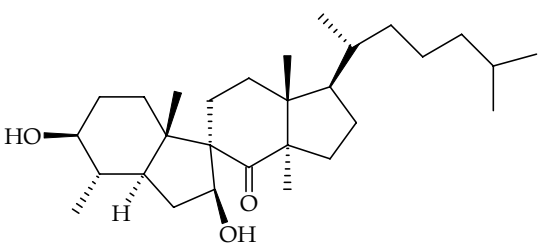

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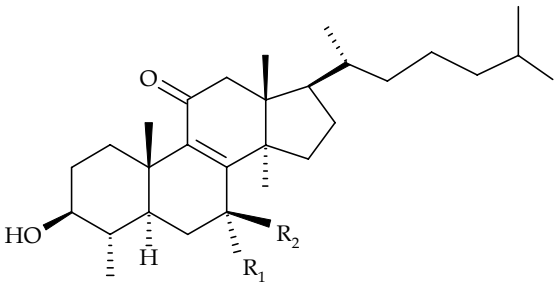


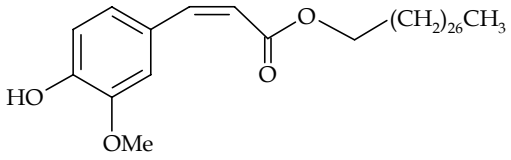
1.200: R_1 = OH, R_2 = H
1.201: R_1 = H, R_2 = OH

2.5. PHENOLIC COMPOUNDS

The new phenolic compounds (**1.202 – 1.207**) isolated from *Euphorbia* sp. between 2002 and 2007 are summarized in Table 1.8. They consist of two ferulic acid esters (**1.202** and **1.203**), three floroglucinol derivatives (**1.204 – 1.206**), and a quercetin glycoside (**1.207**).

Table 1.8. New phenolic compounds isolated from *Euphorbia* sp. (2002 – 2007).

Euphorbia sp.	Analysed part	Extract (fraction)	Compounds	References
E. ebracteolata	Dried roots	EtOH 95% ($CHCl_3$ fr.)	**1.204**, **1.205**	Fu *et al*, 2006
	Dried roots	EtOH 95% (EtOAc fr.)	**1.206**	Yin *et al*, 2005
	Dried leaves	EtOH 95% (EtOAc fr.)	**1.207**	Liu *et al*, 2004
E. hylonoma	Roots	n.d.	**1.202**, **1.203**	Ruan *et al*, 2007



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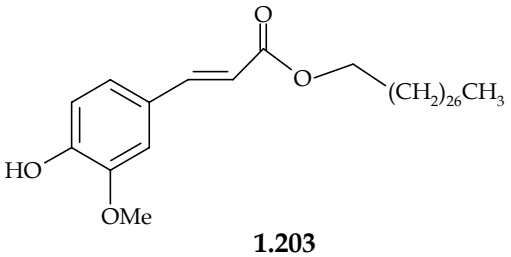

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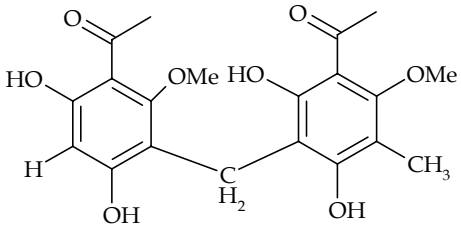

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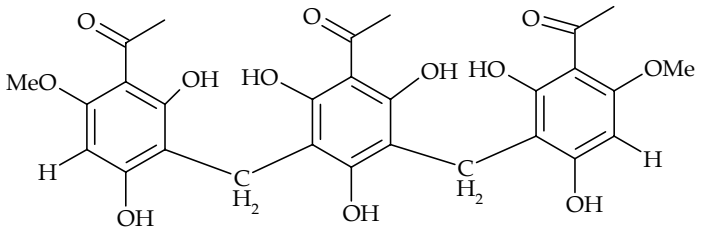
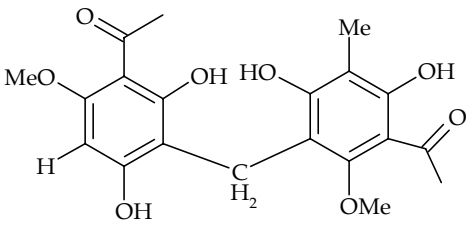
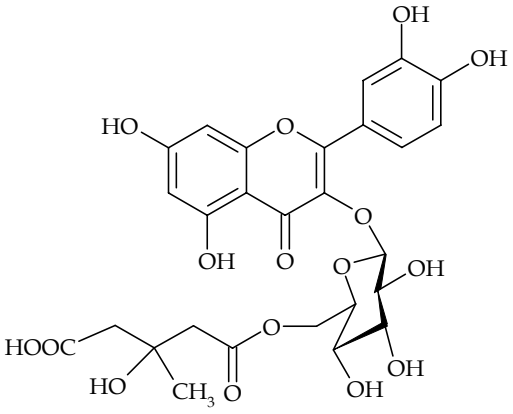


1.205



1.206



1.207

2.6. MAIN BIOLOGICAL ACTIVITIES OF MACROCYCLIC DITERPENES AND THEIR CYCLIZATION DERIVATIVES

Historically, natural products have played an important role in drug discovery and were the basis of most early medicines. Before the 20th century, medicines were based almost exclusively on multicomponent drugs obtained from natural sources. In contrast, the modern pharmaceutical industry almost uses single-ingredient drugs, known as new chemical entities (NCEs), (Schmidt *et al*, 2007). Recent analysis of natural products as source of new drugs indicates that over 60% of NCEs can be related directly or indirectly (having structures based on natural products pharmacophores) to natural products (Cragg *et al*, 2006; Newman and Cragg, 2007; Jones *et al*, 2006). Particularly in therapeutic areas such as oncology and infectious diseases over 60% and 75% of these drugs, respectively, were showed to be of natural origin (Newman and Cragg, 2007; Newman *et al*, 2003). The wide structural diversity of secondary metabolites, due to the presence of chirality and functionality represents an extremely rich biogenetic resource for the discovery of novel drugs, providing also pointers for rational drug design. Nevertheless, much of these sources still remain to be explored.

Despite its traditional medical applications, the use of *Euphorbia* species has been hampered by the occurrence of skin irritant and often tumor-promoting latex that characterize these plants. However, *Euphorbia* genus has been the subject of abundant phytochemical and pharmacological research and many biologically active natural compounds have been isolated from this genus.

The compounds responsible for the toxicity previously described are polycyclic diterpenoids, globally named "phorboids", with the tigliane, the ingenane or the daphanane skeletons, which are biogenetically related and exclusive from plants of the Euphorbiaceae and Thymelaeaceae families (Evans and Soper, 1978). Phorbol esters and related derivatives are the most power tumor promoters known, widely used in animal models for the study of carcinogenesis. Their tumor-promoting effects are due to the activation of protein kinase C (PKC) by a process that mimetize the action of the second messenger diacylglycerol (Kazanietz, 2002). Protein kinase isoforms are differently involved in the regulation of cell proliferation, differentiation, cell survival, apoptosis and carcinogenesis (Goel *et al*, 2007). Although the ability of these compounds to promote tumors is one potential limitation to their utility, it should be noted that there are many phorboids that exert biological effects without tumorigenesis. The biological activities of diterpenoids are highly structure specific. A dramatic change of the PKC agonist and antagonist activity has been observed by modification of the lipophilicity of the C-12 and C-13 ester side chains of phorbol, thus avoiding the membrane insertion of the PKC-phorbol ester complex (Wada *et al*, 2002; Bertolini *et al*, 2003).

Many biological activities have been attributed to this type of compounds. Some 13,20-diacyl derivatives of 12-deoxyphorbol (**1.126 - 1.134**) have recently been isolated from *E. cornigera* and tested for their antiproliferative activity against KB human leukemic cell line. Compounds **1.133** and **1.134** have shown significant cytotoxic activity against that cell line, without irritant activity on mouse ear assay (Balloch *et al*, 2007). Fatope *et al* (1996) have also isolated some 12-deoxyphorbol derivatives and found that 12-deoxyphorbol-20-acetate-13-phenylacetate and 12-deoxyphorbol-13-(9,10-methylene) undecanoate were selectively cytotoxic for the human kidney carcinoma (A-498) cell line, with potencies that exceeded the positive control (adriamycin) by ten thousand times. 4-deoxy-12,13-diacylphorbol derivatives have high potential as antitumour agents (Betancur-Galvis *et al*, 2003). Prostratin (12-deoxyphorbol-13-acetate), a non-tumor-promoting phorbol ester, has been considered as a new therapeutic agent capable of activate the HIV-1 replication in latently infected cell lines (Hezareh, 2005). HIV-1 reactivation is one of the major barriers preventing eradication of the virus from the infected body. However, the clinical potential of prostratin is hampered by its low potency. Bocklandt *et al* (2003) have found that 12-deoxyphorbol-13-phenylacetate, induces HIV-1 gene expression in latently infected T cells at concentrations 20 to 40 fold lower than prostatin to eliminate latent viral reservoirs. It also activates HIV expression and inhibits *de novo* infection in latently infected cells.

Another recent example of the important pharmacological potential of *Euphorbia* metabolites has been the discovery of ingenol 3-angelate as a potent antileukemic agent (Hampson *et al*, 2005). Ingenol 3-angelate has also showed to have topical antitumor activity on mouse models, being therefore considered a new chemotherapeutic agent for the treatment of skin cancer. This compound causes cell death by inducing primary necrosis instead of apoptosis but, despite its mode of action, the treatment is associated with a very favorable cosmetic outcome, a feature that was also noted after the use of *E. peplus* latex (from which it was isolated) to treat human skin lesions (Ogburne *et al*, 2004; Kedei *et al*, 2004). *In vitro* studies corroborated this mode of action, showing that ingenol 3-angelate induces apoptosis in some melanoma cell lines but the predominant form of cell death is non-apoptotic, mainly by induction of necrosis (Gillespie *et al*, 2004). Ingenol diterpenes (*e.g.* compound **1.136**) have shown anti-nematodal activity against the nematode *Bursaphelenchus xylophilus*, which causes economical important infestations of pine trees (Shi *et al*, 2007). Other studies have suggested that ingenol esters (namely compound **1.148**) act as a potent inhibitor for IgE-mediated production of inflammatory chemical mediators *in vitro* and may have a therapeutic potential for allergical diseases (Nunomura *et al*, 2006). Compounds **1.144 – 1.152** have been tested for their ability to specifically inhibit the proliferation of isolated embryonic cells of *Xenopus laevis*, which is an efficient model for predicting the response of tumor cells to potential new anticancer drugs (Wang *et al*, 2002 and 2003 [a]). Compounds **1.148 – 1.151** have shown significant anti-proliferative activity.

Besides the presence of phorboid compounds, *Euphorbia* species have also provided a wide range of structurally unique polyoxygenated macrocyclic diterpenes, as jatrophanes, lathyranes and their polycyclic derivatives. In the last years, great attention has been paid to these compounds due to their structural complexity, biogenetic relevance and noticeable biological activities.

Mysinol esters isolated from *E. decipiens* have been studied as enzyme inhibitors of prolyl endopeptidase and urease. Prolyl endopeptidase is the only serine protease which is known to cleave a peptide substrate in the C-terminal side of a proline residue. This enzyme plays an important role in the metabolism of peptide hormones and neuropeptides and was recognized to be involved in learning and memory. The enzyme urease has been implicated in a variety of pathologic conditions like pyelonephritis and hepatic encephalopathy. Urease inhibitors have recently attracted much attention as potential anti-ulcer drugs (Ahmad *et al*, 2002, 2003 [a] and 2003 [b]). Compounds **1.156**, **1.159**, **1.160** and **1.166** have shown inhibitory activity against prolyl endopeptidase, whereas compound **1.161** showed to be the first

naturally occurring urease inhibitor. Compounds **1.155** and **1.166** have also showed a strong analgesic activity in mice (Ahmad *et al*, 2005 [b] and 2002).

Ingol-type diterpenes have been tested for their cytotoxic activity against several human cancer cell lines (Ravikanth *et al*, 2003; Baloch *et al*, 2006). For example, compounds **1.115** and **1.116** have been found to be more cytotoxic against human leukemic KB cells than the positive control adryamicin (Baloch *et al*, 2006). Furthermore, compound **1.113** could also reactivate HIV-latency in the Jurkart T leukaemia cell line (Daoubi *et al*, 2007).

Jatrophane diterpenes isolated from *E. pubescens* (**1.8**, **1.9**, **1.64** and **1.67 – 1.69**) have been evaluated for their *in vitro* effect on the growth of three human cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (non small cell lung cancer) and SF-286 (CNS cancer), (Valente *et al*, 2003, 2004 [b] and 2004 [c]). Compounds **1.9** and **1.64** showed to be moderate growth inhibitors of all the tested cell lines. The remaining compounds exhibited a moderate dose-dependent growth inhibitory effect on the cancer cell-line NCI-H460, but were ineffective in inhibiting the growth of the SF-268 cell line, which could suggest a tumor-type specific sensitivity of these compounds.

Jatrophane diterpenes have also been found to have antiviral effects on the multiplication of *Herpes simplex* virus type 2 (HSV-2), although the observed activity were not associated with virucidal effects (Mucsi *et al*, 2001).

Pepluanone (**1.95**), a rearranged jatrophane diterpene with the rare pepluane skeleton has been demonstrated to possess high anti-inflamatory activity *in vivo*, because it was able to inhibit the carrageenin-induced rat paw oedema, with an activity comparable to that of the reference drug dexamethasone (Corea *et al*, 2005 [b]). In addition, pepluanone was also found to reduce the production of the inflammation signalling molecules (*e.g.* NO, PGE2 and TNF-α), (Corea *et al*, 2005 [b]). Some other rearranged diterpenes with the paraliane and pepluane skeletons have also been tested as anti-inflamatory agents *in vitro*. All of them were able to inhibit the production of NO, particularly the 11-deoxy derivative of compound **1.92**, a paraliane diterpene, which showed the highest activity, comparable to that found for pepluanone. These experiments have shown that paraliane and pepluane diterpenes are promising lead molecules for the control of inflammatory and immune reactions (Barile *et al*, 2007).

Kansuinins (**1.71 – 1.74**) have shown to enhance the survival of TrkA expressing fibroblasts. TrkA is the high-affinity receptor of the nerve growth factor (NGF) and the survival of those cells is only dependent on NGF treatment. Nerve growth factor is one of the neurotrophins that supports the survival and differentiation of a variety of neurons, such as

the cholinergic neurons of the basal forebrain, which have been reported to undergo severe degeneration in Alzheimer's disease patients. Promising results have been obtained for NGF due to its neuroprotective effects in the animal models of neurodegenerative diseases. However, the progress of clinical trials involving neurotrophic factors is hampered by the poor bioavailability at the desired target sites of these molecules. Therefore, studies on small molecules that mimic or induce NGF activity may contribute to the potential treatment of these diseases (Pan *et al*, 2004).

In the last decade, several investigations revealed that jatrophane and lathyrane diterpenes and their rearranged derivatives are promising modulators of multidrug resistance (MDR) in tumor cells, by inhibiting the efflux-pump activity, mediated by P-glycoprotein (Pgp). The first reported investigations were performed by Hohmann *et al* (2001, 2002 and 2003 [a]), who found that several jatrophanes isolated from *E. serrulata*, *E. esula*, *E. salicifolia, E. mongolica* and *E. peplus* were able to reverse the multidrug resistance in mouse lymphoma cells, some of them displaying a strong activity. The studies of Corea *et al* (2004 [a,b] and 2003 [a,b]) on *E. dendroides*, *E. peplus* and *E. characias* led to the isolation of some series of jatrophanes diterpenes, which are also strong inhibitors of Pgp-mediated daunomycin efflux. In particular, euphodendroidin D (**1.45**, Corea *et al*, 2003 [b]), pepluanin A (**1.53**, Corea *et al*, 2004 [b]) and euphocharacins C (**1.26**) and I (**1.32**, Corea *et al*, 2004 [a]), were found to be powerful inhibitors of Pgp. In further studies, jatrophane diterpenes named pubescenes A – D (**1.67 – 1.70**) have been isolated from *E. pubescens* and were also examined for MDR-reversal activity on human *MDR1* gene-transfected mouse lymphoma cells (Valente *et al*, 2004 [a]). All compounds showed to enhance the drug retention in the cells by inhibiting Pgp. This inhibition was dose-dependent and stronger than the positive control, verapamil. The MDR reversal effects of five rearranged jatrophane diterpenes, with the rare paraliane and segetane skeletons have been reported (Madureira *et al*, 2004 [b] and 2006). With the exception of portlandicine (**1.93**), all the compounds showed to be effective resistance modulators in Pgp expressing cells. However, when comparing these results with those of jatrophane diterpenes, the authors led to the conclusion that macrocyclic diterpenes are more active than their polycyclic rearranged derivatives (Madureira *et al*, 2006). A few jatrophanes, isolated from *E. semiperfoliata*, were also considered as new microtubule-interacting agents. Despite the lack of structural relationship to other known microtubule assemblers, electron microscopy studies revealed that these jatrophanes are able to stimulate purified microtubulin assembly *in vitro*, and induce paclitaxel-like microtubules, although by a different molecular mechanism (Miglietta *et al*, 2003).

3. MULTIDRUG RESISTANCE AND CANCER

Cancer, malignant tumours or neoplasms are generic terms for a group of diseases that can affect any part of the body. One defining feature of cancer is the rapid proliferation of abnormal cells, which can invade adjoining parts of the body and spread to other organs, a process called as metastasis (WHO, 2006). Chemotherapy is the main approach to the treatment of these malignant diseases. However, the resistance of cancer cells (either intrinsic or acquired) to many clinically used anticancer drugs remains a major obstacle for the successful chemotherapeutic cure (Wiese and Pajeva, 2001).

Multidrug resistance (MDR) can be defined as the intrinsic or acquired simultaneous resistance of cells to multiple classes of structurally unrelated drugs that do not have a common mechanism of action (Avendaňo and Menéndez, 2002). Intrinsic drug resistance relates to the failure of many tumours to respond to initial chemotherapy. The resistance is also frequently observed after the treatment of cancer patients with chemotherapeutic agents. These drugs are often administered in therapeutic doses in intervals of about three weeks between each treatment, in order to have an adequate repopulation from the bone-marrow stem cells. However, at the same time, repopulation of surviving tumour cells also occurs (a phenomenon called relapse), thereby increasing the number of tumour cells that must be eradicated. These cells can develop a multidrug resistance phenotype (acquired MDR), which is considered to be a significant obstacle to effective chemotherapy (Pérez-Tomás, 2006; Avendaňo and Menéndez, 2002).

The mechanisms of multidrug resistance could be related with the intracellular drug distribution through overexpression of certain transport proteins that cause an alteration in the mechanism by which the antitumour compounds accumulate inside the cells. This type of MDR is often known as classical or typical MDR. However, the overexpression of transport proteins does not completely explain the phenomenon. Alternative forms of MDR have been described, which included the change in drug targets (*e.g.* decreased activity of Topoisomerase II), better elimination of the electrophilic drugs due to the increase in the levels of glutathione and related enzymes, alterations in the repair of DNA damages and inability to initiate apoptosis (Avendaňo and Menéndez, 2002; Wiese and Pajeva, 2001; Szakács *et al*, 2006). This MDR type is known as atypical MDR. Some of these mechanisms of drug resistance may coexist, rending the tumours refractory to the treatment with drugs acting on a single target (Teodori *et al*, 2002). Moreover, drugs are usually given systemically

and are therefore subject to variations in absorption, distribution, metabolism and excretion (ADME process), which can be specific to individual patients. Tumours can be located in parts of the body which drugs do not easily penetrate, or could be protected by local environments due to increased tissue hydrostatic pressure or altered tumour vasculature (Szakács *et al*, 2006). Furthermore, specific genetic alterations are also important because they enable resistant cancer cell clones to outgrow and escape from effective treatment (Michor *et al*, 2006).

The active efflux of chemotherapeutic drugs is the most implicated and the best studied mechanism of MDR, both in tumour therapy or in bacterial and viral infections and several ABC transporters are known to be involved (Avendaňo and Menéndez, 2002).

3.1. THE ATP-BINDING CASSETTE (ABC) TRANSPORTER SUPERFAMILY

The ABC transporter superfamily is among the largest and most broadly expressed protein superfamilies known. They are present in mammals as well as in prokaryotic organisms. The vast majority of its members is responsible for the active transport of a wide variety of compounds across biological membranes (Pérez-Tomás, 2006; Teodori *et al*, 2006).

In 1976, it was discovered that the reduced drug permeation in MDR cells was associated with the presence of a cell surface glycoprotein. This glycoprotein appeared to be unique to sublines displaying altered drug permeability through the plasma membrane and was named P-glycoprotein (Pgp), (Takara *et al*, 2006). Based on the presence of specific conserved sequences, Pgp was recognized to be an ATP-binding cassette transporter protein and was proposed to function as an efflux pump. In 1988, a novel ABC transporter named MRP1 (Multidrug Resistance Associated Protein) was identified. In 1998, the use of the Pgp inhibitor verapamil, in addition with cytotoxic agent selection, resulted in the discovery of a third ABC transporter that was named Breast Cancer Resistance Protein (BCRP, ABCG2 or Mitoxantrone Resistance Protein, MXR), which has been associated with resistance to mitoxantrone and antracyclines (Avendaňo and Menéndez, 2002). This protein could efflux large, hydrophobic molecules (both positively or negatively charged) together with cytotoxic compounds (*e.g.* mitoxantrone, topotecan), fluorescent dyes and several dietary compounds (Pérez-Tomáz, 2006). Nowadays, at least 12 ABC transporters from four ABC subfamilies have been found to have a role in the drug resistance of cells maintained in tissue culture (Szakács *et al*, 2006).

3.1.1. The ABCB Subfamily: P-glycoprotein (MDR1)

The best studied form of multidrug resistance is associated with the increased expression of P-glycoprotein. Pgp expression in cells results in a broad resistance to a variety of drugs with different chemical structures and mechanisms of action (Volm, 1998). The main antitumour drugs that are subject to Pgp-mediated resistance are the anthracyclines (doxorubicine, daunorubicine), vinca alkaloids (vinblastine, vincristine), epipodophylotoxins (etoposide, teniposide) and paclitaxel (Figure 1.3, Avendaño and Menéndez, 2002).

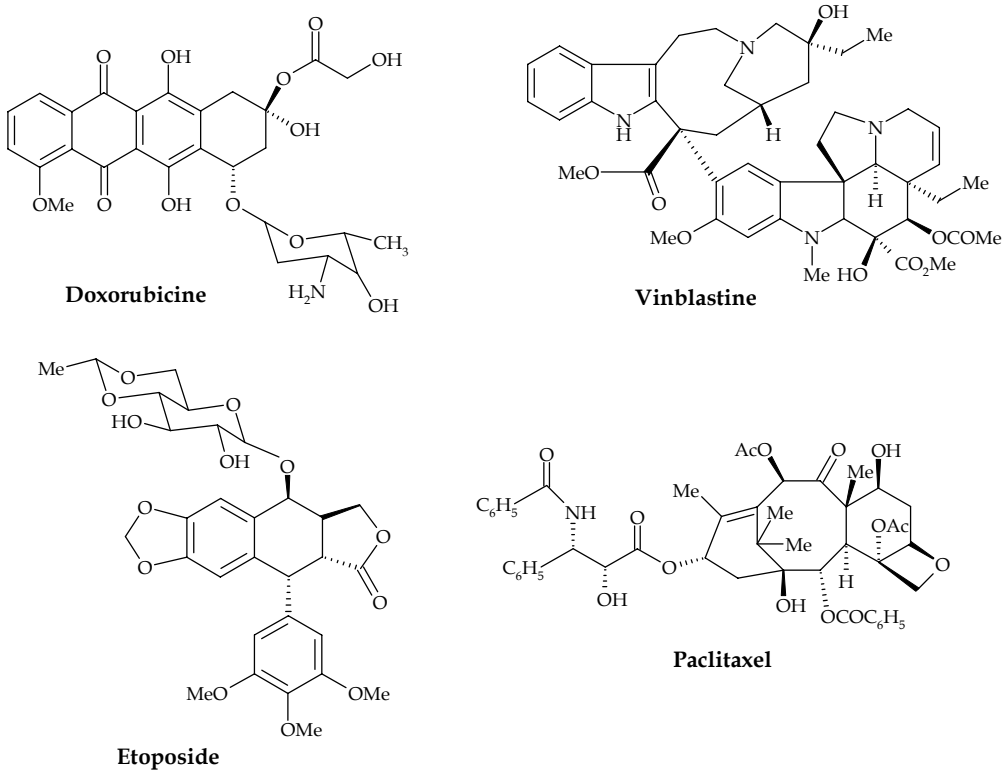


Figure 1.3. Chemical structures of the antitumour drugs doxorubicine (adryamicine), vinblastine, etoposide and paclitaxel.

Pgp is coded in humans by the *mdr1* gene (or *ABCB1* gene), which is overexpressed in cancers that exhibit acquired resistance. This gene, located in chromosome 7, was cloned and sequenced in the 80's allowing the determination of the primary structure of Pgp (Nobili *et al*, 2006). This 170 kDa membrane protein has 1280 amino acids, arranged as two homologous halves joined by a linker region (Figure 1.4).

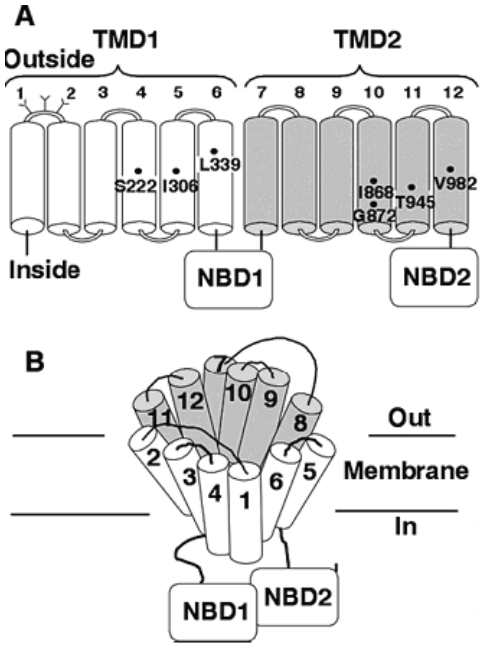


Figure 1.4. Schematic model of P-glycoprotein (Loo and Clarke, 2005).

Each of these halves has two hydrophobic transmembrane domains (TMD). Each transmembrane domain (TMD) contains six transmembrane segments (which hold the drug-binding site), followed by a large cytoplasmic domain with an ATP binding site, which is the nucleotide binding domain (Loo and Clarke, 2005; Srinivas *et al*, 2006).

Each half of the peptide shows over 65% of amino acid similarity with the other half, which are characteristic features of the most active transporter proteins. All the ABC proteins contain within each nucleotide binding domain (NBDs) at least three highly conserved sequence motifs, in contrast to the transmembrane domains (TMDs) that share little primary sequence similarity, except between closely related members of a subfamily; it is postulated that this may be due to the variety of substrates transported by different ABC proteins (Srinivas *et al*, 2006).

P-glycoprotein is present in several normal tissues and it is clear that its physiological function is to detoxify the organism from potentially toxic molecules. The presence of Pgp in the liver, kidney, small and large intestines, adrenals, pancreas and bronchus is a good indication of this function (Nobili *et al*, 2006). Moreover, Pgp is also situated in the endothelial cells of vessel present in the central nervous system, testis and placenta, with a clear role of protection of these organs (Robert and Jarry, 2003). Due to the wide and strategic physiologic distribution of Pgp, its inhibition can strongly influence the pharmacokinetics

and biodistribution of drugs, leading to increased toxicity. The role of Pgp in several diseases is very important because the protein transports a broad range of structurally unrelated compounds (antineoplasic agents, HIV protease inhibitors, prednisone, gold salts and colchicines, as well as several antibiotics), (Srinivas *et al*, 2006).

Pgp Drug Binding Sites

Although the crystallization of prokaryotic membrane transporters together with several biochemical techniques has helped in the structural design of Pgp, the determination of its three-dimensional structure to atomic resolution and the identification of its binding sites are not easy for several reasons:

1. Pgp is a membrane protein strongly embedded in the lipid phase of plasma membranes and it is difficult to overexpress in the quantity and purity, required for 3D crystallization. Even when a sufficient quantity and quality is available, producing crystals is not straightforward.
2. Pgp has a very wide specificity for the substrates transported, and is not clear whether the modulators are also substrates for transport.
3. Pgp is also an ATPase, and the molecules interfering with ATP binding and cleavage can also show modulation properties (Robert and Jarry, 2003; Srinivas *et al*, 2006).

Elucidation of the binding regions and their aminoacid residues is a key step in understanding the molecular basis of drug transport and function of Pgp as a MDR transporter. Recently, a homology model of human Pgp based on the crystal structure of Sav1866, an ABC transporter from *Staphylococcus aureus*, has been proposed by Globish *et al* (2008). The modeling results suggested that the protein has multiple binding sites and multiple pathways for drug transport (Globish *et al*, 2008).

Although the mechanism of action of Pgp is still controversial, there are several models for Pgp functioning, which are based on kinetic and mutation experiments. There are three main proposed models: "the aqueous pore", the "hydrophobic vacuum cleaner" and the "flippase" models (Teodori *et al*, 2006 and 2005; Sharom, 1997).

The "aqueous pore" model is based on the classical pump model, where the membrane protein is thought to alternate between an inward-facing conformation (with the substrate binding site accessible on the cytosolic side) and an outward-facing conformation (with the substrate binding site accessible on the extracellular side). Binding of substrate takes place on

a specific site in the transporter (on the cytosol) and due to a conformational change of the protein (probably induced by ATP hydrolysis), the substrate is released into the aqueous extracellular medium. Nevertheless, Pgp is an atypical membrane transporter because the great majority of its substrates is lypophilic and would therefore be expected to show greater solubility in the lipid bilayer than in aqueous face. This statement led to the proposal of another model, which is called "hydrophobic vacuum cleaner". This model suggests that Pgp removes drugs from the plasma membrane rather than the aqueous phase. Drugs are thought to first partition into the membrane, and then interact with the transporter within the lipid phase. The "flippase" model suggests that Pgp acts as a translocase or flippase. The drug first intercalates into the inner leaflet of the bilayer and only then interacts with the transport protein in the membrane; the transporter then flips the drug from the inner to the outer leaflet (Wiese and Pajeva, 2001; Sharom, 1997). According to Teodori *et al* (2006), the "vacuum cleaner" and the "flippase" models seem to be more realistic with respect to the aqueous pore one. Loo *et al* (2004) have also suggested a model that is a hybrid of the "aqueous pore" and the "vacuum cleaner" models, proposing that the recognition site of Pgp is accessible to the aqueous medium by the occurrence of a rehydration of the substrate within this site. Recently, it has been suggested that two ATP hydrolysis events, which do not occur simultaneously, are needed to transport one drug molecule. That is, the binding of substrate to the transmembrane regions stimulates the ATPase activity of Pgp, causing a release of substrate from the cells. Hydrolysis at the second ATP site seems to be required to re-set the transporter so that it can bind substrate again, completing one catalytic cycle (Takara *et al*, 2006; Nobili *et al*, 2006).

3.1.2 The ABCC Subfamily: Multidrug Resistance Associated Proteins (MRP)

Another subfamily of ABC transporters are the Multidrug Resistance Associated Proteins (MRP). The human MRP family consists of seven members (MRP1 to MRP7) of transporter proteins (Daoud *et al*, 2000). In addition to the Pgp-like core structure with two NBD and two TMD, they are also composed by other domains (Figure 1.5). Some MPRs (*e.g.* MRP1/ABCC1 and MRP2/ABCC2) contain an amino (N)-terminal membrane bound region (TMD0) connected to the core by a cytoplasmic linker; others (*e.g.* MRP4/ABCC4 and MRP5/ABCC5) contain the linker region, which is characteristic of the subfamily, but lack the N-terminal TMD (TMD0). They also differ in the tissue expression, number and type of

transported compounds, and in their ability to confer resistance to anticancer agents (Szakács *et al*, 2006). All members of this family are lipophilic anion pumps that could be able to confer resistance upon anticancer drugs (Pérez-Tomás, 2006). MRP1, 2 and 3 showed to confer drug resistance when transfected into drug sensitive cells. The functions of the other members are not so well studied (Daoud *et al*, 2000).

MRP1 (also known as ABCC1) is the most studied protein of this subfamily. It is a 190 kDa membrane-bound protein expressed in a wide range of tissues and clinical tumours. Sequence analysis of MRP1 has predicted the presence of three transmembrane domains (TMD0, TMD1 and TMD2) and two nucleotide binding domains (NBD1 and NBD2, Figure 1.5).

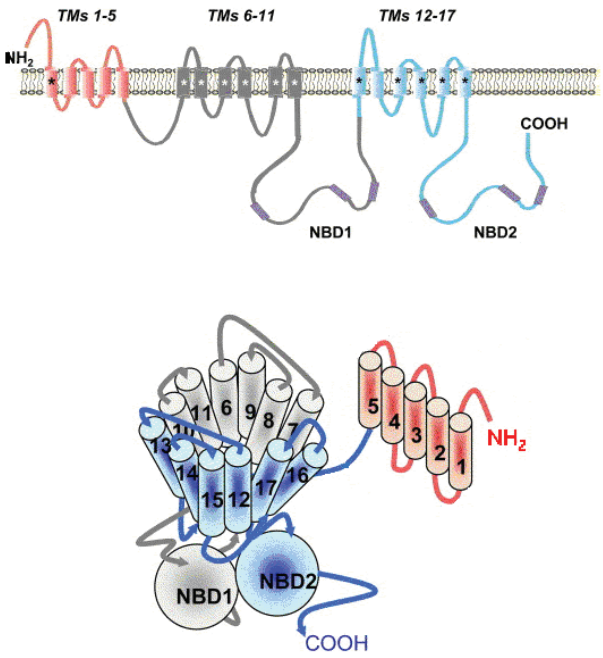


Figure 1.5. Schematic models of MRP1 (Deeley and Cole, 2006).

In normal tissues, the main physiological substractes of MRP1 are organic anions, the glutathione, glucoronate or sulphate conjugated of neutral or protonated molecules. MRP1 induces resistance to antracyclines, vinca alkaloids, epipodophylotoxins, camptothecins and methotrexate but not to taxanes, although they may require metabolism to glutathione conjugates (Avendaño and Menéndez, 2002; Pérez-Tomás, 2006). While effective inhibitors and/or modulators have been described for Pgp transport, only a few are available for MRP1. Most Pgp inhibitors showed to be inactive against MRP1 (Pgp substrates are lipophilic, MRP1 substrates are hydrophilic, Pérez-Tomás, 2006).

3.2. REVERSAL OF PGP-MEDIATED MULTIDRUG RESISTANCE

Pgp extrudes anticancer drugs from tumour cells decreasing its intracellular concentration to a level below that lethal to tumour cells. As a result, tumour cells overexpressing Pgp show resistance to anticancer drugs.

Two major ways to reverse Pgp-mediated MDR have been considered: modulation of the protein function or suppression of the protein expression (Takara *et al*, 2006). The mechanisms of modulation of Pgp-mediated multidrug resistance can be classified in two main groups: specific mechanisms or non-specific mechanisms. One of the proposed specific mechanisms is the direct interaction of the modulator agent (also called MDR reversal agents, MDR inhibitors or chemosensitizers) with one or more of the drug binding sites on Pgp, thus blocking transport by acting as a competitive or non-competitive inhibitor (Wiese and Pajeva, 2001). As previously referred, this is the most widely investigated mechanism. The non-specific mechanism suggests that the modulators can modify the physicochemical properties of the biomembranes, changing intracellular pH and/or electrical membrane potential regulated by Pgp expression (Wiese and Pajeva, 2001; Takara *et al*, 2006).

A variety of compounds have been shown to be MDR-modulators and some of them have undergone clinical trials (Takara *et al*, 2006). They are a large chemical and structural diverse group that includes natural products, semi-synthethic analogs and fully synthetic organic structures (Wiese and Pajeva, 2001). Based on their different structures and biological functions, these potential modulators include calcium channel blockers (verapamil and nifedipine), calmodulin antagonists (trifluoperazine, chlorpromazine), steroids (progesterone, tamoxifene), quinolines (chloroquine, quinidine), immunosuppressive agents (cyclosporine A) and antibiotics (rifapicine, tetracyclines), (Fu *et al*, 2001).

3.2.1. Physicochemical and Structural Characterization of MDR Modulators

They have been many structure activity relationship (SAR) studies on compounds acting on Pgp. However, these studies have been complicated by many factors. One of them is the existence of several different pharmacological screening methods. On the other hand, the compounds found active in reversing MDR have a highly heterogeneous chemical structure that makes difficult the establishment of a structure-activity relationship.

Moreover, most of studies dealt with a limited number of congeners and consequently, the resulting SAR is not always generally applicable to the structural series (Teodori *et al*, 2002). Several molecular descriptors have been applied in these studies, trying to quantify the structural and physicochemical properties of a MDR modulator. The most studied descriptor is lipophilicity, which is often measured as the partition log *P* in the octanol/water system (Srinivas *et al*, 2006; Wiese and Pajeva, 2001). Very hydrophilic compounds were found to be inactive, pointing to a required minimal lipophilic feature. In spite of that, lipophilicity is not the only parameter influent in MDR modulation. Other important molecular descriptors are the molecular weight (MW) and molecular refractivity (MR). The latter can be considered as a measure of the molecular volume and is another widely used parameter in the structural activity relationship studies of Pgp modulators (Wiese and Pajeva, 2001).

According to several authors, H-bond interactions have also been suggested to play an important role in Pgp modulation. This transport protein is considered to be an H-bond donor and the drugs should be H-bond acceptors (electron donors). Substituents with oxygen containing electron-donors functional groups (carbonyl or methoxy groups) can be involved in favourable H-bond interactions (Wiese and Pajeva, 2001). It was also postulated that the number of Pgp-interacting groups on a molecule determines the strength of the binding; a high potential to form hydrogen bonds would correspond to a high Pgp inhibitory property, while a low potential to form these bonds would correspond to a weaker inhibitory property (Robert and Jarry, 2003). In addition, the existence of weak polar interactions, including aromatic-aromatic, oxygen-aromatic, and amino-aromatic interactions between the MDR modulators and Pgp, seems to be one of the important factors for high MDR reversal activity. These weak polar interactions are believed to play an important role in stabilizing protein structures and drug-protein binding (Klopman *et al*, 1997).

Although these general physicochemical properties have been recognized to play an important role in MDR modulation, the exact structural parameters required for a molecule to be recognized and transported by Pgp are still unknown. Pajeva and Wiese (2002) have proposed a general model of Pgp modulating drugs that is based on binding data of structurally different compounds to the verapamil binding site of the protein. As can be observed in Figure 1.6 the proposed pharmacophore model consists of two hydrophobic points, three hydrogen-bond acceptor points, and one hydrogen-bond donor point. It was concluded that the binding affinity of the drugs depended on the number of pharmacophore points simultaneously involved in the interaction with the protein (Pajeva and Wiese, 2002). According to these authors, the broad structural variety of Pgp substrates and inhibitors that

bind to the verapamil binding site of Pgp can be explained by the fact that the receptor has several points able to participate in hydrophobic and hydrogen-bond mediated interactions and different drugs can interact with different receptor points in different binding modes (Pajeva and Wiese, 2002).

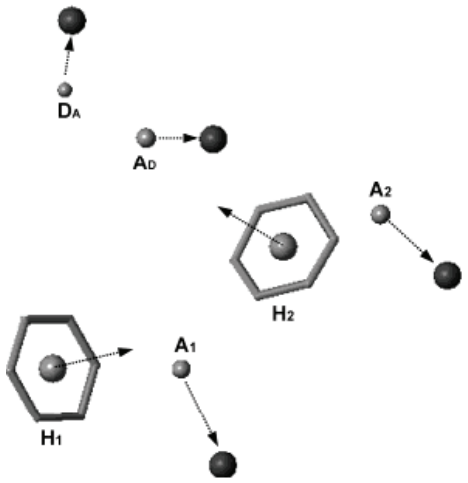


Figure 1.6. General pharmacophore model of drugs at the verapamil binding site of Pgp, as proposed by Pajeva and Wiese (2002). H_1 and H_2 are hydrophobic points; A_1, A_2 and A_D are hydrogen-bond acceptor points; D_A is a hydrogen-bond donor point; the arrows show directions of the hydrophobic and hydrogen-bond interactions (Pajeva and Wiese, 2002).

3.2.2. Reversal of MDR in Clinical Practice

Since the identification of the main mechanisms responsible for multidrug resistance, the research of molecules able to modulate these mechanisms has remained a major goal (Robert and Jarry, 2003). However, there are currently no reversal agents clinically available due to several reasons:

1. Inhibition of Pgp in tumour cells should be possible *in vivo* without side effects on normal tissues expressing the pump. Generally, the modulation of Pgp has been well documented on *in vitro* models, but these conditions do not correctly mimic the *in vivo* requirement. The absence of side effects of the reversal agent on normal tissues expressing Pgp should be showed, as well as the pharmacokinetic changes that could result from the inhibition of hepatic Pgp. Moreover, compounds used for MDR reversal should be within the limits of the toxicities acceptable for anticancer treatments;

2. Tumours treated were not truly resistant to chemotherapy or were not resistant through the mechanism targeted. The clinical trial should be performed on patients who

have a documented situation of resistance to a given protocol of chemotherapy, before the introduction of the reversal agent, to determine whether this therapy modifies tumour response. When it is clear that an MDR reversal agent is being tested on a truly-drug resistant cancer expressing the target mechanism (usually Pgp), the question arises as to whether this mechanism is unique and responsible for the drug resistance of the tumour. Therefore, defining the tumour type for drug development should be the most important step for this type of approach (Robert and Jarry, 2003).

The development of MDR modulators has been accompanied by extensive biological and pharmacological studies. Regarding the positive features and the negative side effects of these compounds, three generations of MDR modulators have been distinguished. The first generation group of compounds comprised drugs already used in clinical treatment for other pathologies. Verapamil, a calcium channel blocker, was the first compound found able to modulate Pgp and enhance the cellular concentration of anticancer drugs, such as vincristine, vinblastine, doxorubicine and daunorubicine (Nobili *et al*, 2006). Other first generation compounds were quinine, cyclosporine A, tamoxifene and nifedipine (Figure 1.7). The use of these drugs as MDR modulators required high doses, and since they are used in clinical for other indications, the resulting toxicity was unacceptable. Moreover, many of these compounds are also substrates for other ABC transporters and enzyme systems, resulting in unpredictable pharmacokinetic interactions in the presence of chemotherapeutic agents. The results of the phase I clinical trials showed that these compounds were too toxic or not active enough and were not further investigated (Nobili *et al*, 2006).

The second generation compounds were the result of the research to identify some analogues of the first-generation drugs, which were expected to be devoid of the pharmacological properties of the original molecule but able to inhibit Pgp (Nobili *et al*, 2006). In this group of drugs were included, among others, dexverapamil, valspodar, chinconine and toremifene, which are structural analogues of verapamil, cyclosporine A, quinidine and tamoxifene, respectively (Figure 1.7). Although less active on calcium channels, dexverapamil had also showed an elevated intrinsic toxicity and the clinical studies were stopped. Valspodar, an analogue of cyclosporine A, is the most studied second generation compound due to the good preclinical results. It has shown to be much more active than cyclosporine A, with lower renal toxicity and without imunossupressive activity (Nobili *et al*, 2006). Valspodar is believed to act as a non-competitive inhibitor of Pgp, interacting with the protein with high affinity and probably interfering with its ATPase activity. This compound has been extensively studied in clinical trials, including phase III

studies (Nobili *et al*, 2006). Despite their better pharmacological profile, most of these agents are of limited clinical use. Some of them are also inhibitors of other ABC transporters or substrates of cytochrome P-450 which may result in pharmacokinetic interactions and increased host toxicity from cytotoxic drug overexposure (Nobili *et al*, 2006; Pérez-Tomás, 2006).

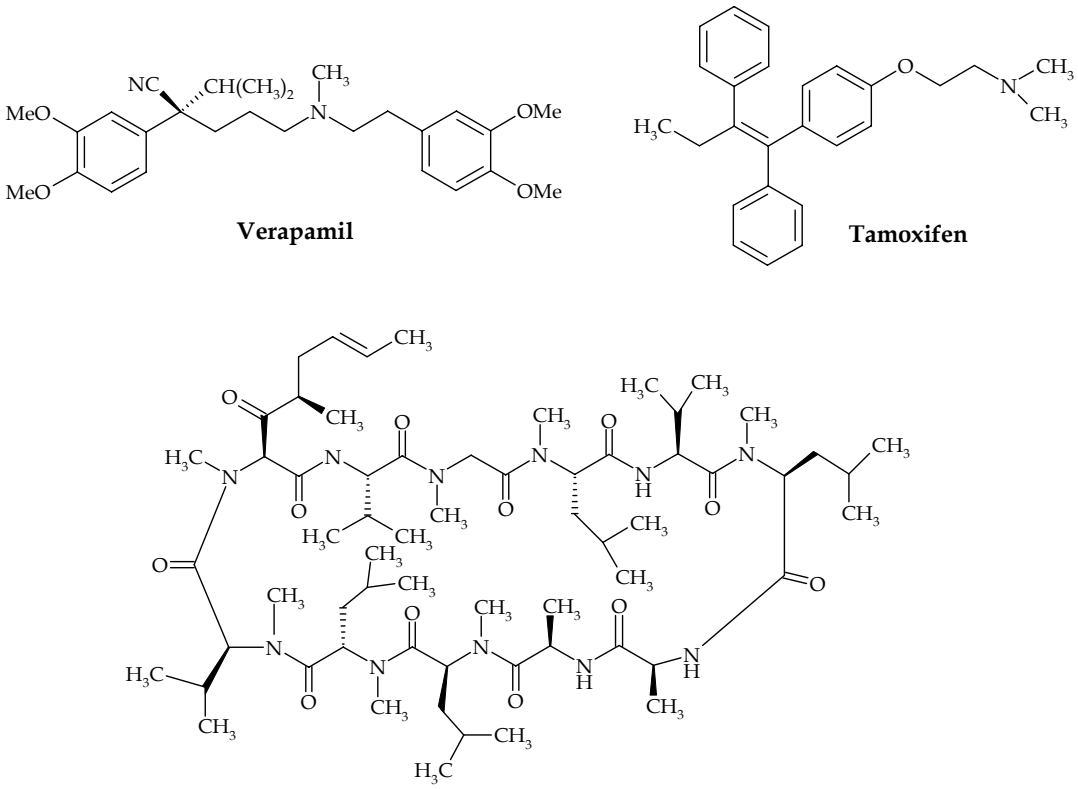
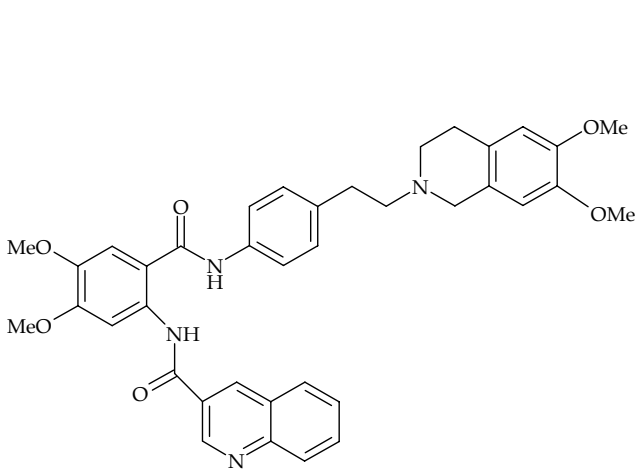


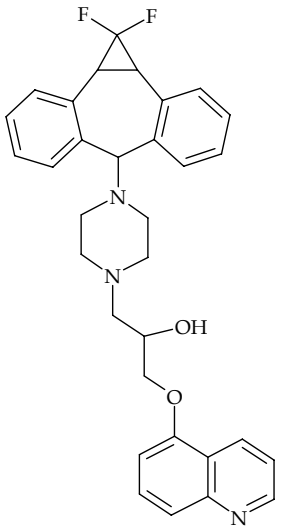
Figure 1.7. Chemical structures of verapamil, tamoxifen and valspodar.

The third generation compounds comprise molecules designed using combinatorial chemistry and quantitative structural activity relationships (QSAR), for the purpose of MDR reversal. In contrast to the second-generation modulators, these inhibitors have been designed specifically to have high transport affinity and low pharmacokinetic interactions because they are not substrates of cytochrome P_{450}. The latest generation of inhibitors includes compounds like zosuquidar, laniquidar, elacridar, tariquidar and biricodar (Muller *et al*, 2008; Szakács *et al*, 2006). Some of these compounds (*e.g.* biricodar) can act on multiple ABC transporters, which might extend the functionality of these inhibitors to Pgp negative tumours showing MDR, although the range of possible side effects also increases (Szakács *et*

al, 2006). Tariquidar and zosuquidar (Figure 1.8) are not modulators of either MRP or BCRP, but specifically inhibit the Pgp transport function with strong potency. These compounds have been tested in several clinical trials (McDevitt and Callaghan, 2007; www.clinicaltrials.gov). For example, a phase I/II clinical trial has been performed to investigate the safety and tolerance of zosuquidar in combination with the CHOP regimen [9] in patients with non-Hodgkin's lymphoma (Morschhauser *et al*, 2007). The results have shown that zosuquidar did not significantly affect the pharmacokinetics of doxorubicin and had moderate effects on the pharmacokinetics of vincristine. Moreover, at the highest dose tested, the toxicity of zosuquidar was minimal and it was not observed an increase of the CHOP-related toxicity (Morschhauser *et al*, 2007). Currently, a randomized phase III clinical trial of zosuquidar in combination with daunorubicin and cytarabine in older patients with newly diagnosed acute myeloid leukaemia is ongoing.[10]



Tariquidar



Zosuquidar

Figure 1.8. Chemical structures of tariquidar and zosuquidar.

[9] CHOP regimen: treatment of non-Hodgkin's lymphoma with cyclophosphamide, doxorubicin, vincristine and prednisolone.
[10] www.clinicaltrials.gov/ct2/show/NCT00046930 (February 2008).