

CHAPTER 2

Sesquiterpene lactones

2.1. Secondary Plant Metabolites

A characteristic feature of plants is their ability to synthesize a large variety of low molecular weight compounds, the so-called secondary metabolites. By definition secondary plant metabolites do not play a role in the primary metabolic processes essential for the maintenance of life in an individual plant, but many may be absolutely essential to the survival of the species as a whole in a given natural habitat. Secondary metabolites may function as signal molecules within the plant or between the plant producing them and other plants, microbes, herbivores, pollinating or seed-dispersing animals. More often, they serve as chemical defense compounds against herbivorous animals, microbes, viruses or competing plants.

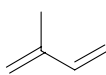
It is suggested that during the course of evolution millions of secondary products have been synthesized from time to time by different species of plants and when the presence of a particular secondary product conferred a selectionary advantage on the plant containing it, then the chances of survival of the plant, its offspring and the secondary product itself will have been enhanced.¹

Since the 1850s organic chemists have extensively investigated the chemical properties of these novel phytochemicals. Studies of natural products stimulated the development of separation techniques, spectroscopic approaches to structure elucidation, and synthetic methodologies that now constitute the foundation of contemporary organic chemistry. Interest in natural products was prompted by their immense utility as dyes, fibers, glues, polymers, oils, waxes, flavouring agents, perfumes and drugs. Recognition of the biological properties of numerous natural products has fueled the current focus of this field, namely the search for new drugs, antibiotics, insecticides and herbicides.

¹ E.A. Bell and B.V. Charlwood, 'Secondary Plant Products', Springer-Verlag, Berlin Heidelberg, 1980.

2.2. Terpenoids

The largest class of plant secondary metabolites is undoubtedly that of the terpenoids or isoprenoids. Terpenoids are not only numerous but also extremely variable in structure, exhibiting hundreds of different carbon skeletons and a large assortment of functional groups. In spite of such diversity, the simple unifying feature of all terpenoids is that these compounds are derived from the simple process of assembly of a C₅ unit *i.e.* the isoprene **(19)** unit.²



(19)

This leads to a rational classification of the terpenoids depending upon the number of such isoprene units incorporated in the molecular skeleton (Table 2.1).

Table 2.1 Classification of terpenoids

Terpenoids	Isoprene units	Carbon atoms
Monoterpenes	2	10
Sesquiterpenes	3	15
Diterpenes	4	20
Sesterterpenes	5	25
Triterpenes	6	30
Carotenoids	8	40
Rubber	>100	>500

The biosynthetic pathway to terpenoids (Figure 2.1) comprises four basic stages, the first of which involves the formation of isopentenyl pyrophosphate (IPP) **(20)**, the biological C₅ isoprene unit. IPP and its allylic isomer, dimethylallyl pyrophosphate (DMAPP) **(21)** are synthesized by plants via one of two routes: the well-established mevalonic acid pathway; or the newly-discovered glyceraldehyde phosphate/pyruvate pathway.

²A.A. Newman, 'Chemistry of Terpenes and Terpenoids', Academic Press, London and New York, 1970.

In the mevalonate pathway for IPP biosynthesis **(A)**, three acetyl coenzyme A (acetyl-CoA) **(22)** units are joined successively to form 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) **(24)**. HMG-CoA is then reduced to mevalonate (MVA) **(25)**, which is subsequently phosphorylated, decarboxylated and dehydrated to form IPP **(20)**.^{3,4} The first intermediate in the non-mevalonate pathway **(B)**, 1-desoxy-D-xylulose-5-phosphate **(28)**, is assembled by condensation of glyceraldehyde 3-phosphate (GAP) **(26)** and pyruvate **(27)**. A skeletal rearrangement coupled with a reduction step yields the branched-chain polyol, 2C-methyl-D-erythritol 4-phosphate **(29)**, which is subsequently converted into a cyclic 2,4-diphosphate **(30)**, by a series of enzymes via nucleotide diphosphate intermediates.^{5,6}

In the second stage, the basic C₅ units condense to generate geranyl pyrophosphate (GPP, C₁₀) **(31)**, farnesyl pyrophosphate (FPP, C₁₅) **(32)** and geranylgeranyl pyrophosphate (GGPP, C₂₀) **(33)**. In the third stage the C₁₀-C₂₀ pyrophosphates undergo a variety of cyclizations and rearrangements to produce the parent carbon skeletons of each terpene class. GPP is converted to the monoterpenes, FPP is converted to the sesquiterpenes and GGPP is converted to the diterpenes. FPP and GGPP can also dimerize in a head-to-head fashion to form the precursors of the C₃₀ and C₄₀ terpenoids, respectively. The fourth stage encompasses a range of oxidations, reductions, isomerizations, conjugations and other transformations by which the parent skeletons of each terpene class are converted to thousands of distinct terpene metabolites.²

There are over 30 000 terpenoid natural products known. Many of these are from plants, where they play important roles in the ecological chemistry involved in interactions with insects and pathogens. Many terpenoids have been shown to have important biological activities. There are a number of antibiotics amongst the sesquiterpenes and diterpenes. Some sesquiterpenes and diterpenes are insect and plant hormones, respectively.

³ A. L. Lehninger, 'Biochemistry', 2nd edition, Worth Publishers, New York, 1975, 681.

⁴ E.I. Wilding, J.R. Brown, A.P. Bryant, A.F. Chalker, D.J. Holmes, K.A. Ingraham, S. Iordanescu, C.Y. So, M. Rosenberg and M.N. Gwynn, *J. Bacteriol.*, 2000, **182**, 4319.

⁵ F. Rohdich, K. Kis, A. Bacher and W. Eisenreich, *Curr. Opin. Chem. Biol.*, 2001, **5**, 535.

⁶ A. Disch, J. Schwender, C. Müller, H.K. Lichtenthaler and M. Rohmer, *Biochem. J.*, 1998, **333**, 381.

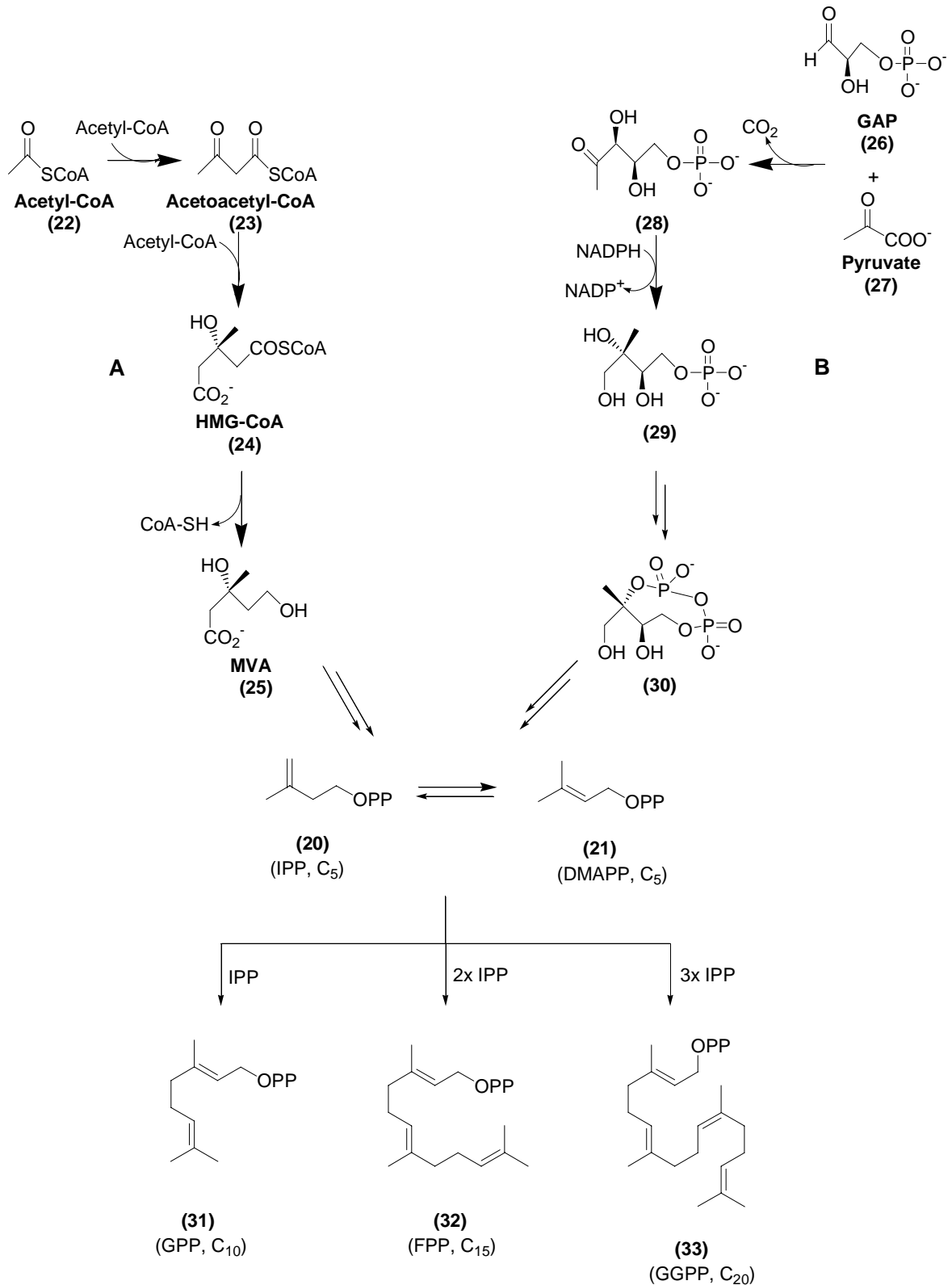


Figure 2.1 Overview of terpenoid biosynthesis in plants, showing the mevalonate (A) and GAP/pyruvate (B) pathways for the production of IPP, as well as the generation of the higher order terpenoid building blocks

Terpenoids can accumulate to high levels in some plant species and are significant components of essential oils that have found important uses in the flavour and fragrance industry. Many terpenoids find use in industry as raw materials in the manufacture of adhesives, coatings, emulsifiers and speciality chemicals, whilst others are of commercial importance as insecticides or as pharmaceuticals.

2.3. Sesquiterpene Lactone Skeleton

The sesquiterpene lactones are considered as a major class of secondary metabolites, which mainly occur in the *Asteraceae*. They are typically colourless, bitter, relatively stable, lipophilic constituents that often contain as a major structural feature an α,β -unsaturated- γ -lactone. They are biogenetically derived from *trans,trans*-farnesyl pyrophosphate (**32**) following an initial cyclisation and ensuing oxidative modifications. The typical lactones resulting from these enzyme-mediated cyclisations are primarily classified on the basis of their carbocyclic skeletons as germacranolides, guaianolides, pseudoguaianolides and eudesmanolides. However, sesquiterpene lactones exhibit a variety of other skeletal arrangements.

Generally the α,β -unsaturated lactone is either *cis*- or *trans*-fused to the C(5)-C(6), C(6)-C(7) or C(7)-C(8) positions of the carbocyclic skeleton containing, in many cases, an α -methylene group. Structural modification of the basic sesquiterpene skeleton involves incorporation of an epoxide ring, hydroxyl groups (generally esterified), and/or a C₅-acid such as tiglic or angelic acid. A few sesquiterpene lactones occur in glycosidic form and some contain halogens or sulphur.⁷

Over 4000 different sesquiterpene lactone structures are known, but the majority of them have a guaiane, eudesmane or germacrene framework (Figure 2.2). It is generally accepted that biogenetically the germacranolides represent the most primitive class and that all other sesquiterpene lactones evolved from them.⁸

⁷ A.K. Picman, *Biochem. Syst. Ecol.*, 1986, **14**, 255.

⁸ <http://www.ansci.cornell.edu/plants/toxicagents/sesqlactone/structure1.gif>

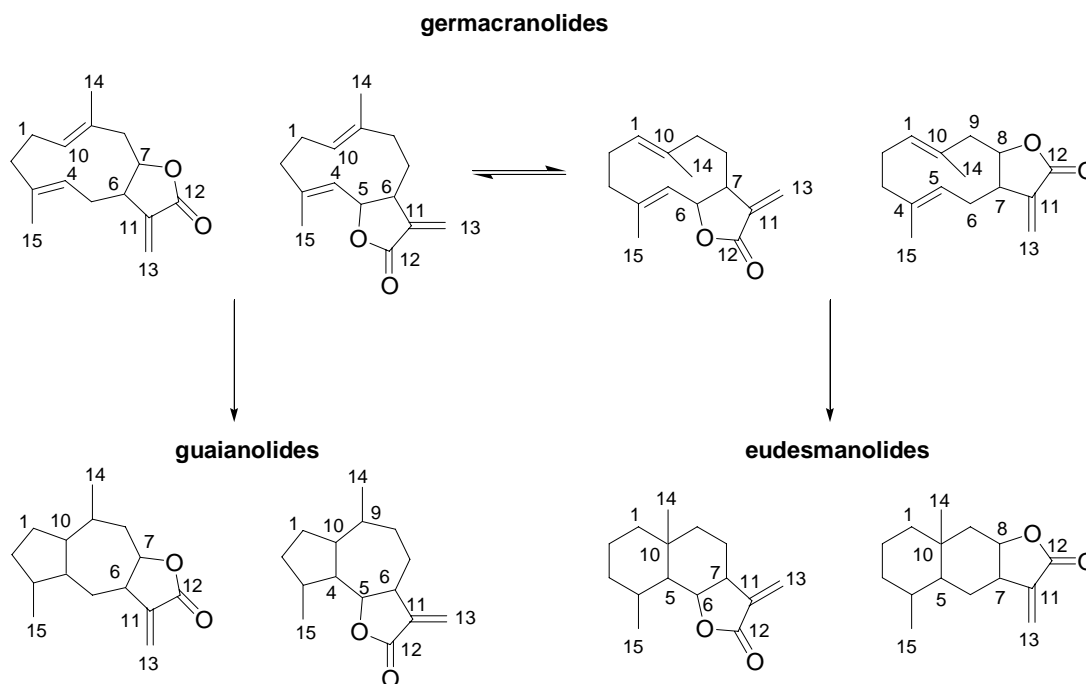


Figure 2.2 Germacranolide, guaianolide and eudesmanolide skeletal types.

2.4. Sesquiterpene Lactone Biosynthesis

By far the largest group of naturally occurring sesquiterpene lactones is the germacranolides, and the majority of sesquiterpene lactones are thought to evolve from this class. The simplest member of the germacranolides (+)-costunolide (**34**) is generally accepted as the common intermediate of all germacranolide-derived lactones. Figure 2.3 depicts the proposed biosynthetic route for the germacrene-derived sesquiterpene lactones as established in chicory (*Cichorium Intybus*) roots.⁹

The committed step in the biosynthesis of (+)-costunolide (**34**) is the cyclization of farnesyl pyrophosphate (FPP) (**32**) to (+)-germacrene A (**35**) by a sesquiterpene synthase (I). Formation of the lactone ring involves the introduction of a carboxylic acid function in the isopropenyl group of (+)-germacrene A. This process starts with the hydroxylation of (+)-germacrene A (**35**) to germacra-1(10),4,11(13)-trien-12-ol (**36**) by (+)-germacrene A hydroxylase, a cytochrome P450 enzyme (II).

⁹ J. de Kraker, M. C.R. Franssen, M.C. Dalm, A. de Groot and H.J. Bouwmeester., *Plant Physiol.*, 2001, **125**, 1930.

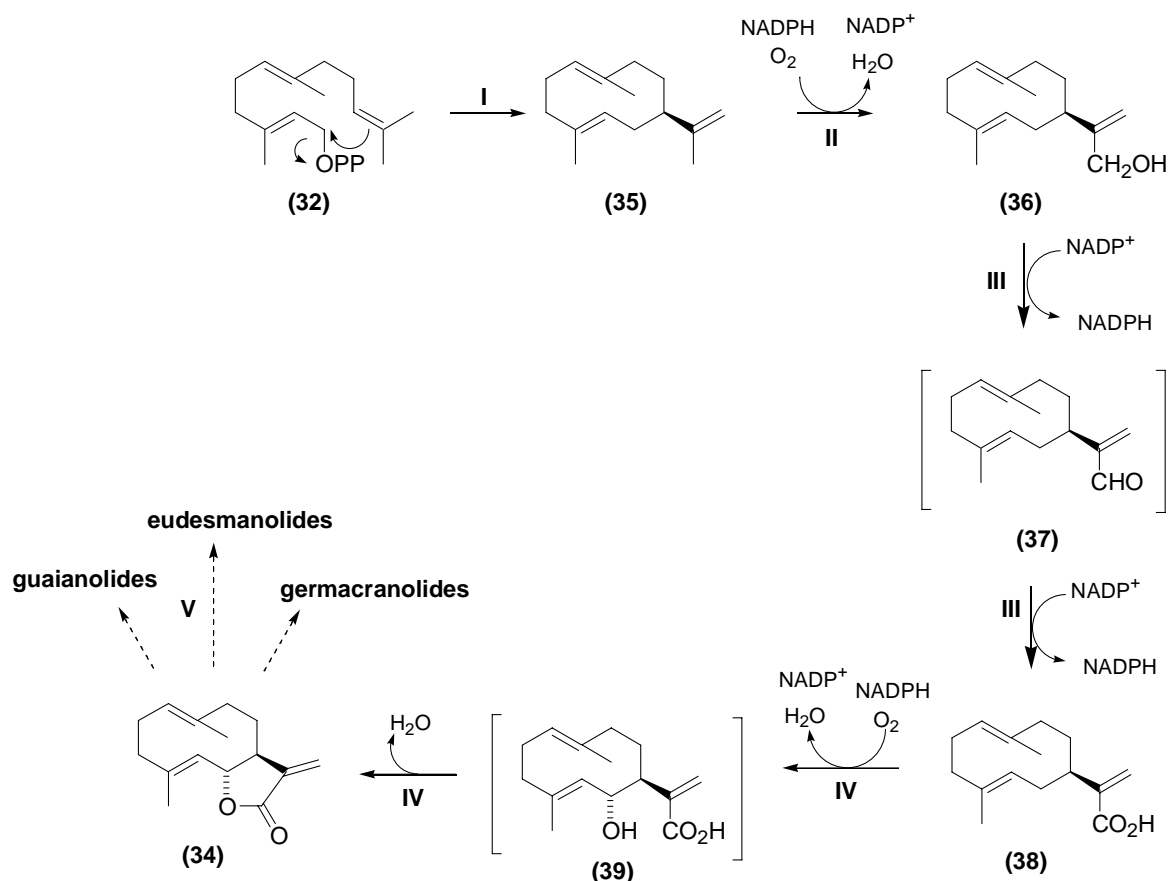


Figure 2.3 Proposed biosynthetic route for the germacrene-derived sesquiterpene lactones present in chicory.

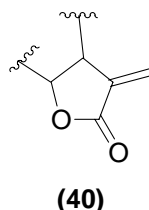
Germacra-12-ol (**36**) is subsequently oxidized to germacra-1(10),4,11(13)-trien-12-oic acid (**38**) via the germacra-1(10),4,11(13)-trien-12-al (**37**) intermediate by NADP⁺-dependent dehydrogenases (**III**). Conversion of germacra-12-oic acid (**38**) to (+)-costunolide (**34**) is proposed to proceed *via* a hydroxylation at the C(6)-position of the germacrene acid (**38**) and subsequent attack of the hydroxyl group on the carboxyl group at C(12) (**IV**). This is followed by the postulated formation of guaiane, eudesmane and germacrane lactones (**V**).

2.5. Biological Activities of Sesquiterpene Lactones

The sesquiterpene lactones from plants comprise a group of substances with a variety of biological effects. These include antibacterial, antifungal, antitumour, antiplasmodial, anthelmintic, schistosomicidal, cytotoxic, phytotoxic and

analgesic activities.¹⁰ They are also known to poison livestock, to act as insect feeding deterrents and to cause allergic contact dermatitis in humans. The variety of activities displayed by sesquiterpene lactones against numerous types of organisms suggests that the individual lactones from this group of plant secondary metabolites may play a role in the plant's defense against pathogens, herbivorous insects and mammals, and in competition with other plants.¹¹

The biological activities are generally the result of reaction of sesquiterpene lactones with the thiol groups of vitally important compounds such as enzymes. No major generalizations have emerged from various studies aimed at examining the relationship between biological activities and chemical structure of these compounds. This is because in addition to the α -methylene- γ -lactone moiety **(40)**, which has been suspected to be responsible for several activities, a number of other groups of sesquiterpene lactones as well as variation in physiology and biochemistry between diverse organisms affected by these compounds must be considered.⁷



Detailed investigations on the biological activities of sesquiterpene lactones provides useful information in our understanding of the adaptive role of these compounds in plants and contribute to a general perception of their activities in related disciplines of medicine, pharmacology and agriculture.

2.5.1. Antitumour and Cytotoxic Activity

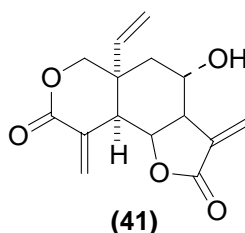
Plant extracts that exhibit anti-cancer activity have received considerable attention particularly in the last 40 years.¹¹ Sesquiterpene lactones are amongst the biggest class of cytotoxic and antitumour compounds of plant origin. Various studies on the relationship between chemical structure and cytotoxic activity of sesquiterpene lactones revealed that the presence of an exo-methylene group is an essential

¹⁰ R.V. Burim, R. Canalle, J.L.C. Lopes and C.S. Takahashi, *Genet. Mol. Biol.*, 1999, **22**, 2

¹¹ E. Rodriguez, G.H.N. Towers and J.C. Mitchell, *Phytochemistry*, 1976, **15**, 1573.

requisite for cytotoxicity. Loss of cytotoxicity and tumour inhibition was observed with changes such as saturation or addition to the methylene group. An additional conjugated ester, cyclopentenone, an epoxy group or a second α,β -unsaturated lactone appeared to enhance cytotoxicity.⁷

Studies of structure–antitumour activity established that the presence of an α -methylene- γ -lactone moiety, a β -unsaturated cyclopentenone ring or an α -epoxycyclopentenone system gives rise to significant *in vivo* antitumour activity.⁷ The reactivity of sesquiterpene lactones exhibiting antitumour activity may be associated with selective alkylation of nucleophilic groups in biological growth-regulatory macromolecules such as key enzymes which control cell division. For instance vernolepin (**41**), an eudesmanolide, has been shown to inhibit phosphofructokinase, an enzyme that has many –SH groups.¹¹



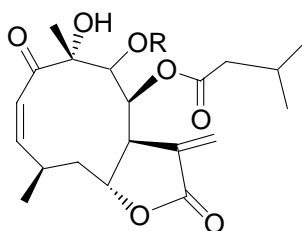
2.5.2. Antiplasmodial Activity of Sesquiterpene Lactones

After the discovery of the antiplasmodial properties of the endoperoxide sesquiterpene lactone artemisinin (**9**), many other sesquiterpene lactones have been investigated as antimalarial agents. Some examples of these are illustrated below.

Two sesquiterpene lactones of the germacranolide type neurolenin A (**42**) and neurolenin B (**43**) obtained from *Neurolaena lobata* showed interesting *in vitro* activity against *P. falciparum* (IC_{50} 0.92 μ M and 0.62 μ M, respectively) if compared with that of the reference antimalarial agents, artemisinin (**9**) (IC_{50} 0.14 μ M) and quinine (**1**) (IC_{50} 0.19 μ M).

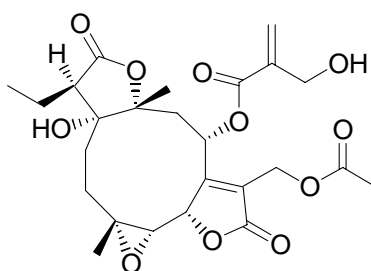
Structure-activity studies suggested that the α,β -unsaturated-keto function and a free hydroxyl function at C(8) increased the antiplasmodial activity. The

compounds were found to be cytotoxic, though their IC_{50} 's on both tumour cell lines tested were significantly higher than their IC_{50} values for activity against the parasite. The authors hypothesized that the antiplasmodial effects of the neurolenins are not due to their general cytotoxicity, caused by the alkylating properties of the exocyclic methylene group fused to the lactone ring, but are rather dependent on a more specific mechanism of action.¹² These results may explain the traditional use of decoctions of *Neurolaena lobata* in Central America to treat malaria.



(42) R = H
(43) R = Ac

The sesquiterpene dilactone 16,17-dihydrobrachycalixolide (**44**) from *Vernonia brachycalyx*, a Kenyan plant used to treat malaria, showed strong antiplasmodial activity (IC_{50} 5.9 to 32 μ M, on different *P. falciparum* strains). It was however found to strongly inhibit the proliferation of human lymphocytes at the same concentrations, so it suppresses the body's immune mechanism if administered to humans.¹³

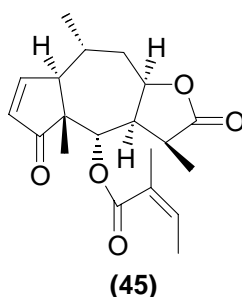


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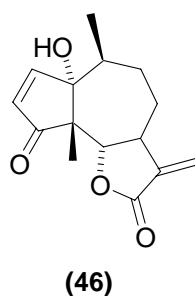
¹² G. Francois, C.M. Passreiter, H.J. Woerdenbag and M.V. Looveren, *Planta Med.*, 1996, **62**, 126.

¹³ H.A. Oketch-Rabah, S.B. Christensen, K. Frydenvang, S.F. Dossaji, T.G. Theander, C. Cornett, W.M. Watkins, A. Kharazmi and E. Lemmich, *Planta Med.*, 1998, **64**, 559.

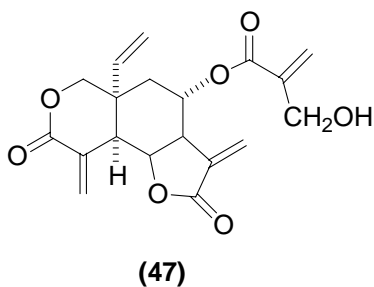
Brevilin A (**45**) which was isolated from the Chinese medicinal plant, *Centipeda minima*, was found to have activity against *P.falciparum*.¹⁴



The sesquiterpene lactone, parthenin (**46**), identified as the major active amoebicidal compound of *Parthenium hysterophorus*, was also shown to be active *in vitro* (IC₅₀ 1.29 µg/ml) against a multi-drug resistant (K1) strain of *P. falciparum*.¹⁵ A series of semi-synthetic derivatives of parthenin has been prepared which have varying activities against *P. falciparum in vitro* and it has been shown that the active moieties are the exocyclic methylene of the lactone ring and the cyclopentenone A ring.



A series of sesquiterpene lactones, including vernodalin (**47**), from *Vernonia amygdalina* is active against *P. falciparum in vitro*.¹⁶



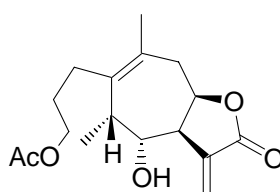
¹⁴ S. Schwikkard and F.R. van Heerden, *Nat. Prod. Rep.*, 2002, **19**, 675.

¹⁵ J.D. Phillipson and C.W. Wright, *J. Ethnopharm.*, 1991, **32**, 155.

¹⁶ W.C. Evans, 'Trease and Evans' Pharmacognosy', 14th Ed, WB Saunders, London, 1996, 429.

2.5.3. Other Pharmacological Activities of Sesquiterpene Lactones

A wide variety of sesquiterpene lactones isolated from plant extracts have been demonstrated to show various other pharmacological activities which include expectorant, blood pressure lowering, cholinergic, hypoglycaemic, anti-asthmatic and anti-inflammatory activity. Inulicin (**48**), for instance, is a sesquiterpene lactone from *Inula japonica* which acts as a stimulant of the central nervous system and smooth muscles of the intestine, has anti-ulcer activity and capillary-strengthening diuretic properties. High doses of inulicin inhibit cardiac activity but low doses have no effect.⁷



(48)

The wide variety of pharmacological activities of sesquiterpene lactones show that these compounds could have significant promise for practical utility in medicine.