Current Trends in Medicinal Chemistry
Vol. 7, 2013

Review

Secondary metabolites isolated from the genus *Eucalyptus*

Viliam Brezáni and Karel Šmejkal*

University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1/3, CZ-612 42, Brno, Czech Republic

ABSTRACT

The genus *Eucalyptus* is a unique source of a wide spectrum of secondary metabolites. In addition to the volatile terpenic compounds characteristic of *Eucalyptus* trees, many other interesting chemical substances are biosynthesized via different metabolic pathways. The essential oils comprise terpenes, β-triketones, and simple acylphloroglucinols. Acylphloroglucinol-terpene adducts, acylphloroglucinol dimers, flavonoids, tannins, triterpenes, and glucose esters of oleuropeic acid are non-volatile *Eucalyptus* compounds. Secondary metabolites isolated from *Eucalyptus* trees have been tested for their antitumor, anti-inflammatory, and antioxidative activities, and they have also shown antibacterial, antifungal, antileishmanial, and antiviral effects.

KEYWORDS: *Eucalyptus*, terpenes, acylphloroglucinols, euglobals, macrocarpals, sideroxylonals, flavonoids, oleuropeic acid, β-triketones, triterpenes

INTRODUCTION

In botanical taxonomy *Eucalyptus* L'Hèritier belongs to the *Myrtaceae* family. *Eucalyptus* species occur naturally on the Australian continent, where they represent the second most widely spread plant genus, after *Acacia*. There are about 800 known species of *Eucalyptus* [1]. Charles Louis L'Héritier de Brutelle was the first to describe *Eucalyptus* as an individual genus. The name *Eucalyptus*, derived from the Greek words *eu* and *calyptos*, which mean "well covered", was chosen because of the operculum that covers the stamens in the bud before anthesis. The whole genus is divided into 13 subgenera. There are five major polytypic subgenera: *Corymbia, Blakella, Eudesmia, Symphyomyrtus,* and *Monocalyptus* [2]. Although *Eucalyptus* is grown all over the world, Australia is the only place where *Eucalyptus* trees dominate the landscape [3]. *Eucalyptus* has been introduced into other regions where the climate is favorable, such as California and the Mediterranean region and has become a significant part of the vegetation in these areas [4].

The *Eucalyptus* is a large tree with a trunk that exfoliates long shreds. Its leaves are characterized by a typical dimorphism. The leaves on young shoots are horizontaly opposite, sessile, and have a rounded blade, whereas the leaves on older branches dangle vertically, alternate, are shortly petiolate, and have a scythe-shaped blade. The flower bud has four rough and waxy sepals fused into a four-sided urn with a lid formed by four fused petals. Upon anthesis this lid becomes detached and opens, allowing numerous stamens to appear [5]. *Eucalyptus* is suited to subtropical climates with winter rainfall and to cool, high elevations in tropical climates. *Eucalyptus* trees are used to dry out marshy areas and other plots of land where the water table is high. The trees dry the land by absorbing water and allowing it to evaporate through their leaves [6].

The Aborigines traditionally used *Eucalyptus* leaves and bark to treat colds, influenza, toothaches,

*Corresponding author: karel.mejkal@post.cz

snakebites, fevers, diarrhoea, and other complaints. Kino, the astringent exudation produced following pathological or mechanical injury to the wood, has been used as a powder or paste to treat open sores and as an aqueous solution for external or internal complaints [7]. Many species of the genus *Eucalyptus* are used in Chinese folk medicine for a variety of medical conditions. Hot-water extracts of the dried leaves of *Eucalyptus* species are traditionally used as analgesic, anti-inflammatory, and antipyretic remedies. They are used, for example, to treat the symptoms of respiratory infections, such as cold, tonsillitis, influenza, and sinus congestion, and also for treatment of dysentery and articular pain [8, 9, 10]. *Eucalyptus* leaf extract has been approved as a natural food additive, and is included among the antioxidants in Japan [11]. The powdered bark has been used as an insecticide in Africa. *Eucalyptus* oil is listed in the pharmacopeas of many countries, such as the UK, France, Germany, Belgium, the Netherlands, the EU, the USA, and Japan [8].

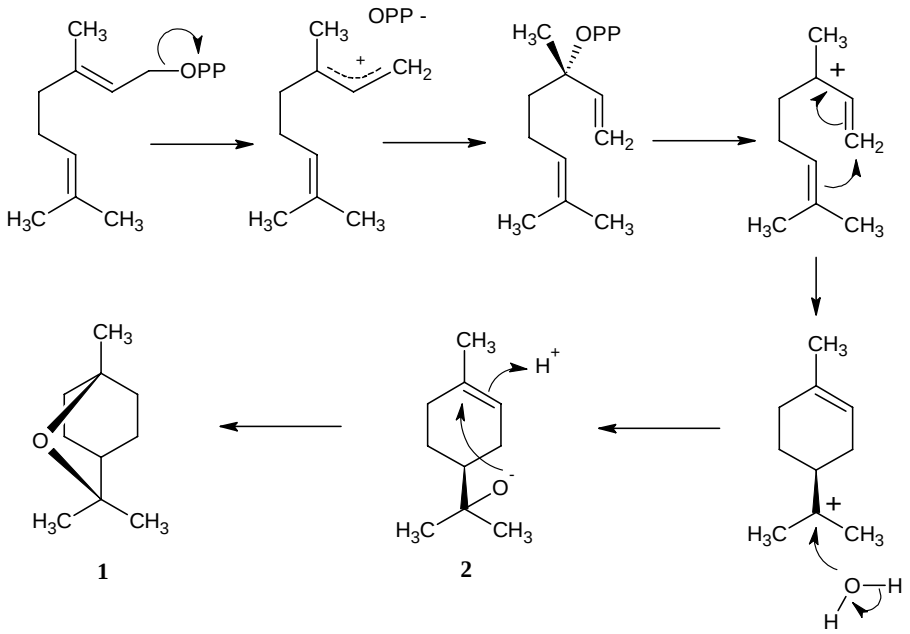
Eucalyptus secondary metabolites

Eucalyptus is known to be a rich source of biologically active terpenic and polyphenolic compounds. The biologically active components of its essential oils are monoterpenes, sesquiterpenes, β-triketones, and acylphloroglucinols. β-diterpenes, triterpenes, and steroidal compounds are among the non-volatile compounds biosynthesized from mevalonic acid. Non-volatile polyphenolic compounds are derived via shikimic or acetate pathways. Gallic acid and ellagic acid are two of the constituents derived from shikimic acid, while phloroglucinol derivatives originate in the acetate pathway. Many of these products are biosynthesized by the crossing of several metabolic pathways. Flavonoids and catechins are created by combining shikimic and acetate metabolic pathways, and euglobals and macrocarpals are biosynthesized by combining acetate and mevalonate pathways. The constituents of *Eucalpytus* species have been isolated, and their pharmacological activities and biological effects studied intensively. The following sections focus on the most significant constituents of *Eucalyptus* species and their biological activities.

Monoterpenes

Eucalyptus essential oils are volatile odorous products of the secondary metabolism that display different medicinal activities according to their qualitative and quantitative chemical compositions and therefore to the presence of terpenoid and phenolic compounds [3]. The essential oil extracts of *Eucalyptus* trees possess a wide range of biological activities such as antimicrobial, fungicidal, insecticidal, herbicidal, acaricidal, and nematicidal [6]. *Eucalyptus* essential oils have been used in traditional medicine as a secretolytic remedy for bronchitis, sinusitis, and colds [12]. The major compound in *E. globulus* essential oil is the cyclic ether 1,8-cineole (**1**) also known as eucalyptol [11]. It is derived biogenenetically from geranyl pyrophosphate [1]. A heterocyclic ring is formed by the cyclization of α-terpineol (**2**) (Fig. 1) [13]. Compound **1** has a significant anti-inflammatory activity. It suppresses arachidonic acid metabolism and cytokine production in human monocytes. Long-term systemic therapy with **1** has a significant steroid-saving effect in steroid-dependent asthma [12]. Compound **1** has also shown myorelaxant properties, reducing the contractile response induced by K^+ ions in guinea pig airway smooth muscle [9].

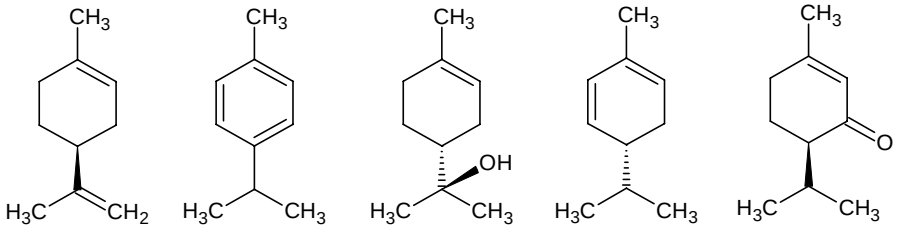
Eucalyptus essential oils have demonstrated antimicrobial activity against a wide range of pathogenic microorganisms [11]. In general, G+ bacteria are more sensitive to essential oils than G- bacteria. *Staphylococcus aureus* is the strain most sensitive to *Eucalyptus* essential oil, while *Pseudomonas aeruginosa* is the most resistant [3]. The essential oil from *E. globulus* shows different degrees of antibacterial activity against 14 clinical strains of methicillin-resistant *S. aureus* [14]. Oil in the vapour phase inhibited microbial growth more effectively than the liquid oil. The higher percentage of monoterpenes in the vapour and its direct absorption into the microorganisms are two factors contributing to this effect [11]. *E. globulus* essential oil possesses dose-dependent antifungal activity. Compound **1** is not the only component that limits fungal growth. The growth of *Aspergillus flavus* and *A. parasiticus* is more sensitive to a mixture of two or more compounds than to a single monoterpene therapy. The synergistic effect of minor components represents



1 **2**

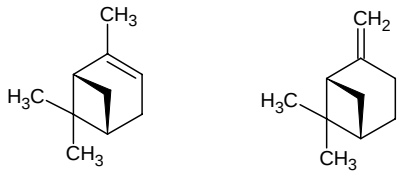
Fig. 1. Biosynthesis of 1,8-cineole (1).

an important part of the antimicrobial activity of essential oils [15]. Further, monoterpenes in *E. globulus* essential oil are limonene (**3**), p-cymene (**4**), α-terpineol (**5**), α-phellandrene (**6**), and piperitone (**7**). α-terpineol (**5**) shows greater activity against *S. aureus* than 1,8-cineole (**1**) [11]. Compounds **3**, **4**, **5**, and **6**, isolated from the essential oil of *E. camaldulensis* leaves, have shown strong larvicidal effects against *Aedes aegypti* and *A. albopticus* [10].



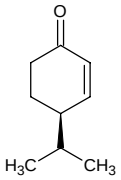
3 **4** **5** **6** **7**

The bicyclic monoterpenes α-pinene (**8**) and β-pinene (**9**) have also shown larvicidal activity against *A. aegypti.* Extracts of *E. globulus* seeds and leaves are effective against *Culex pipiens* [6]. The effects of **8** and **9**, isolated from the essential oil of *E. tereticornis*, on rat trachea *in vitro* have been studied. They potentiated the contractions induced in the trachea rings by acetylcholine. Monoterpenes **8** and **9** are involved in potentiating actions, and are therefore not responsible for the myorelaxant effects of the *E. tereticornis* essential oil [9].

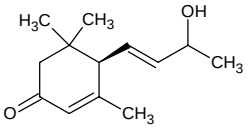


8 **9**

Cryptone (**10**) is a monoterpenic ketone present in the essential oils of *E. grandis, E. cladocalyx, E. botryoides, E. tereticornis, E. odorata,* and *E. diversicolor* [16]. Vomifoliol (**11**) has been isolated from the benzene-soluble part of the acetone extract obtained from the bark of *E. globulus* [17].

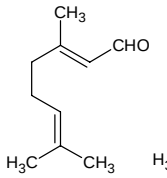


10

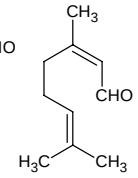


11

E. staigeriana essential oil exhibits anthelmintic activity *in vitro* and *in vivo*. Geranial (citral A) (**12**) and neral (citral B) (**13**) are major constituents of this essential oil. These compounds display insecticidal properties. They also possess antifungal, antibacterial, and antihelmintic activities [18].



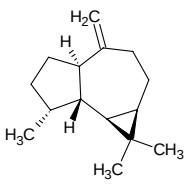
12



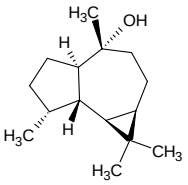
13

Sesquiterpenes

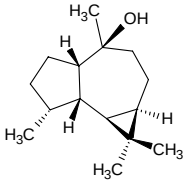
The *E. globulus* essential oil components aromadendrene (**14**) and globulol (**15**) have been tested against antibiotic-resistant microorganisms [19]. Aromadendrene (**14**) apparently contributes to the antimicrobial activity of the essential oil; it exhibits stronger antimicrobial properties than compound **15**. Its combination with 1,8-cineol (**1**) showed a synergistic effect, and the combination reduces the minimum inhibitory concentration (MIC) [19]. Viridiflorol (**16**) is a sesquiterpene isolated from the essential oils of *E. macrorrhyncha, E. exserta, E. puciflora,* and *E. gunii* [16].



14

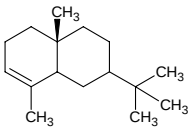


15

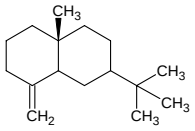


16

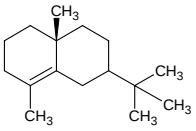
The essential oils of *Eucalyptus* species are excellent sources of α-eudesmol (**17**), β-eudesmol (**18**), γ-eudesmol (**19**), isobicyclogermacral (**20**), and spathulenol (**21**) [1, 14].



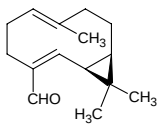
17



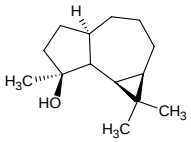
18



19



20

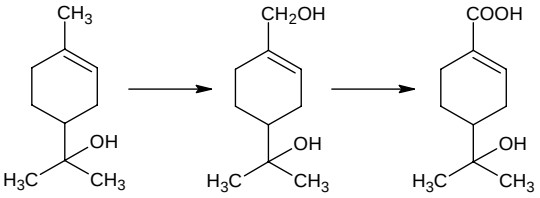


21

Oleuropeic acid glucosides

Several glucose esters of oleuropeic acid have been isolated from *Eucalyptus* extracts. Some of these compounds contain aglycones of the phenolic type [20]. Oleuropeic acid can be considered to be derived from α-terpineol (**2**), which commonly occurs in *Eucalyptus* essential oils. The C-7 position of **2** is preferentially oxidized to create 7-hydroxy-α-terpineol, which then forms oleuropeic acid (**22**) (Fig. 2) [21].

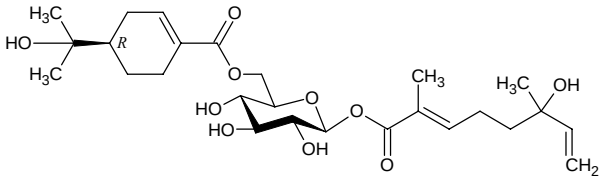
The role of these non-volatile compounds in *Eucalyptus* secretory cavities is not clear. It has been suggested that they may be involved in the biosynthesis of monoterpenes and their subsequent mobilization to the extracellular lumen [22]. They may form a buffer zone between the potentially autotoxic, lipophilic monoterpenes housed within the lumen interior and the hydrophilic epithelial cells lining the lumen of the cavity [23]. The reported bitterness, repellency, and cytotoxicity suggest a role played in the plant defence against pathogens and herbivores. The localization of some of the compounds on the exterior of the lumina of the secretory cavity suggests that they may also play a role in protecting secretory cells from the toxic terpenes housed in these structures [20]. Oleuropeic acid glucosides can be divided into two groups. Compounds which contain a terpene moiety other than oleuropeic acid belong to the first group. Compounds with a polyphenolic moiety are in the second group.



22

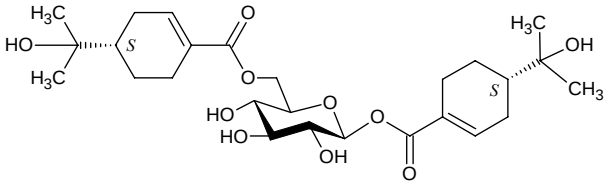
Fig. 2. Biosynthesis of oleuropeic acid (**22**).

Froggattiside A (**23**) is a glucose ester of oleuropeic acid (**22**) and mentiafolic acid. It has been isolated from the leaves of *E. cypellocarpa, E. froggattii, E. globulus, E. polybractea,* and *E. behriana* [20, 23].



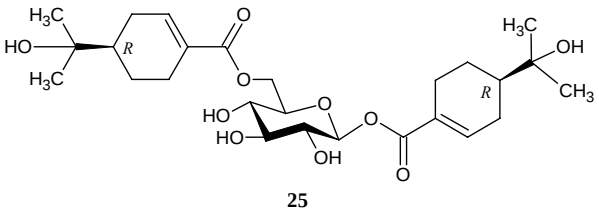
23

Cuniloside A (**24**) and cuniloside B (**25**) are glucose esters with two molecules of oleuropeic acid. These molecules differ in their optical rotation at two carbon atoms of the molecule. The absolute configuration of cuniloside A (**24**) at C-4 and C-4´´ is *S* while the absolute configuration of cuniloside B (**25**) is *R*. Compound **24** is chemically1,6-di-*O*-((*S*)-oleuropeyl)-β-D-glucopyranoside. In addition to the leaves of *E. globulus*, it has also been isolated from *Cunila spicata* (*Lamiaceae*). In the literature, **24** has sometimes been designatted simply as cuniloside [20, 22, 23, 24].



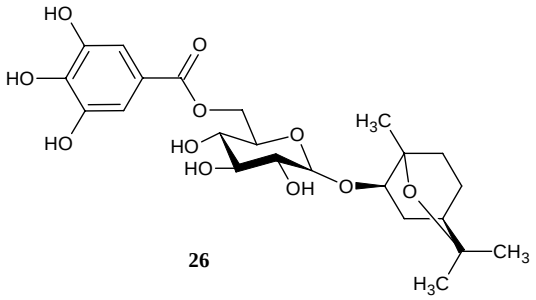
24

Cuniloside B (**25**) is a substance identical with eucalmaidin E. Chemically it is a 1,6-di-*O*-((*R*)-oleuropeyl)-β-D-glucopyranoside. It has been isolated from *E. globulus* and other *Eucalyptus* species, and it has also been identificated in the fruits of *E. maidenii* [20, 22, 23, 24].



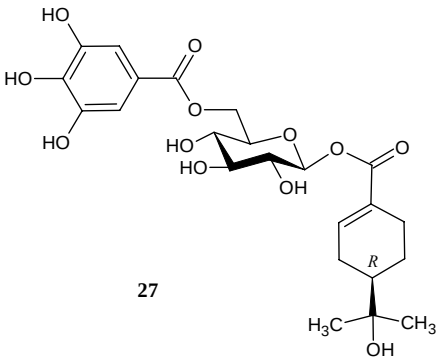
25

Globulusin A (**26**) and globulusin B (**27**) have been detected in the leaves and fruits of *E. globulus* [25]. Compound **26** is a β-glucopyranose ester of 2-hydroxy-1,8-cineol and gallic acid [26].



26

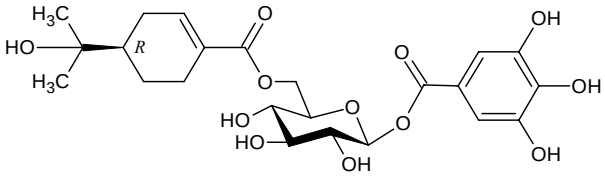
Globulusin B (**27**) is a glucose ester of oleuropeic acid and gallic acid, isolated from the leaves and fruits of *E. globulus* [20]. The globulusins are potent scavangers of free radicals. Their scavenger activity is more potent than that of ascorbic acid. Globulusin A (**26**) suppresses *in vitro* inflammatory cytokine production (TNF-α, IL-1β) in THP-1 cells. Furthermore, antimelanogenic activity as tested on B16F1 mice melanoma cells has also been observed [26].



27

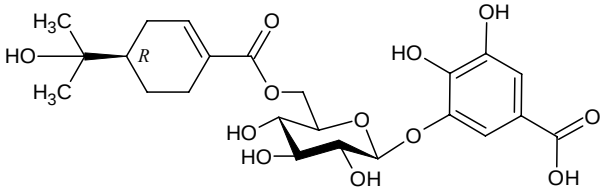
Eucaglobulin (**28**) is a regioisomer of globulusin B (**27**). The absolute configuration of the oleuropeic acid group is *R* [23]. Compound **28** has been isolated from the leaves and fruits of *E. globulus*

and from the fruits of *E. maidenii* [20, 25]. It possesses potent antioxidant and anti-inflammatory activities and inhibits melanogenesis [26]. When compound **28** was evaluated *in vitro* for activity against the herpes simplex virus type 1, no inhibition of HSV was observed [20].



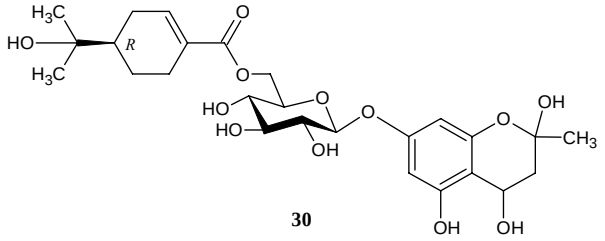
28

Cypellocarpines are a group of oleuropeic acid glucosides differing only in their phenolic moiety. Cypellocarpine A (**29**) incorporates gallic acid, cypellocarpine B (**30**) has a chromane moiety, and cypellocarpine C (**31**) has noreugenine [20, 21]. Cypellocarpine A has been detected in the leaves of *E. globulus* and *E. cypellocarpa* and in the leaves and fruits of *E. maidenii* [20, 27].



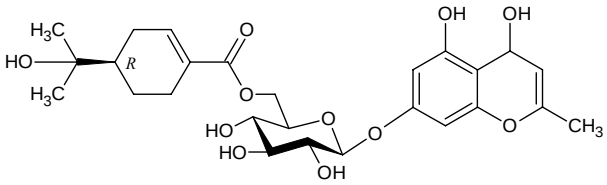
29

Cypellocarpine B (**30**) has been obtained from the ethyl acetate fraction of the acetone extract of the leaves of *E. cypellocarpa* and *E. maidenii* [20, 27]. Cypellocarpine C (**31**) was first isolated from the leaves of *E. cypellocarpa*, and has also been reported in the fruits of *E. globulus* and *E. camaldulensis* var. *pendula* [21, 28].



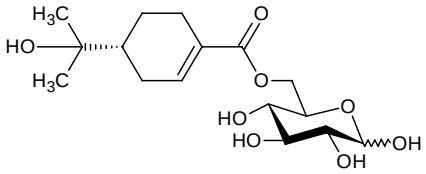
30

These compounds possess an inhibitory effect on the early antigen activation of Epstein-Barr virus comparable to that of (-)-epigalloylcatechingallate. Cypellocarpines also suppress the two-step cancerogenesis induced in mouse skin cells by TPA *in vitro* [27]. Cypellocarpine C (**31**) has shown potent antitumor-promoting activity *in vitro* [21, 28].



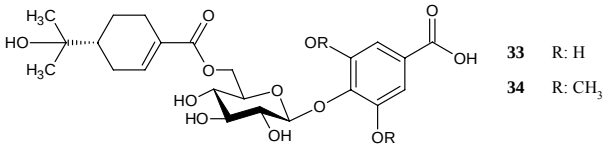
31

Five derivatives of (+)-oleuropeic acid, named eucalmaidins, have been isolated from acetone extracts of the fresh leaves of *E. maidenii*. The chemical structure of eucalmaidin A (**32**) is 6-*O*-oleuropeoyl-D-glucopyranoside [24].



32

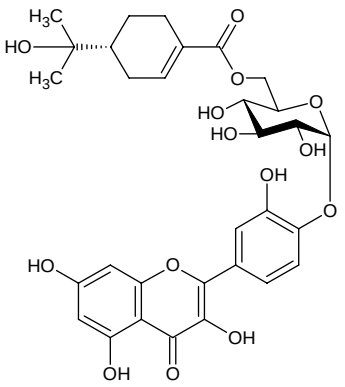
Eucalmaidin B (**33**) is formed by combining single oleuropeoyl, glucosyl, and gallic acid moieties. The structure of eucalmaidin C (**34**) has been determined to be 3,5-dimethyleucalmaidin B [24].



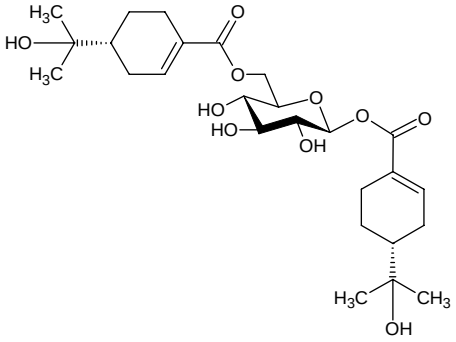
33 R: H

34 R: CH_3

The structure of eucalmaidin D (**35**) is quercetin-4′-*O*-(6-*O*-oleuropeoyl)-*R*-D-glucopyranoside. It represents a rare example of an *R*-configured glucoside found in nature. The structure of eucalmaidin E (**36**) was confirmed by enzymatic hydrolysis, which yielded (+)-oleuropeic acid (**22**) and eucalmaidin A (**32**) [24].



35

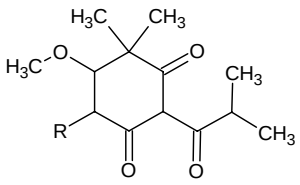


36

Eucalmaidins B, C, and E (**33**, **34,** and **35**) have shown strong cytotoxicity. All three compounds have been evaluated for their *in vitro* effect against herpes simplex virus type 1, but no inhibitory effect has been observed [24].

Cyclic polyketones

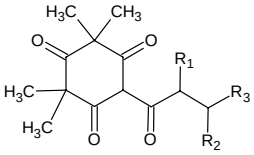
Essential oils from *Eucalyptus* species also contain β-triketones. These cyclic polyketones differ in the side chain, in the number of nuclear methyl groups, and in the level of oxygenation. They are commonly found in *Eucalyptus* species and in related members of the *Myrtaceae* family such as in *Leptospermum, Xanthostemon, Darwinia, Backhousia, Calytrix, Baeckea,* and *Melaleuca* [7].



37 R: CH_3

38 R: H

Robinson and Smith isolated the first β-triketone in 1914 from a plant which they thought to be *E. risdonii* or *E. linearis* but was probably *E. tasmanica* [7]. They considered this compound to be a phenol and named it tasmanol. Birch and Elliot later showed it to be a β-triketone and renamed it tasmanone (**37**). Its occurrence is limited, but it can constitute up to 40% of the essential oil of *E. camfieldii*, and its synthesis has been described [7]. Agglomerone (**38**) is an optically inactive solid, first isolated from *E. agglomerate* and *E. mckieana,* in which it is the major component of the essential oil. Agglomerone (**38**) exists in solution as a mixture of tautomers, and the chemical synthesis of this β-triketone has been described [7].



39 R_1: H R_2: CH_3 R_3: CH_3

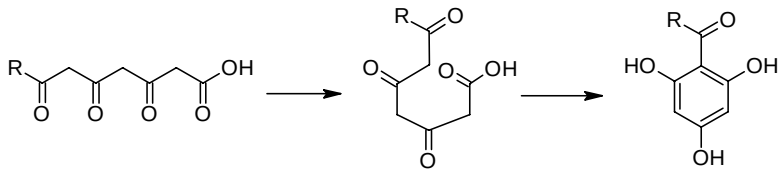
40 R_1: CH_3 R_2: CH_3 R_3: H

41 R_1: CH_3 R_2: H R_3: H

Leptospermone (**39**) was first isolated from *Leptospermum* species growing in Australia and New Zealand. It occurs along with its homologue flavesone (**41**) in *Eucalyptus* species. These compounds exist as tautomers in solutions. Isoleptospermone (**40**) was detected in the essential oil of *E. grandis* where it co-occurs with flavesone (**41**). Both of these compounds belong to the group of potent germination regulators which were named G-regulators [7].

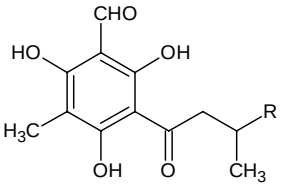
Acylphloroglucinols

Acylphloroglucinols (**43**) are phenolic compounds biosynthesized by intramolecular Claisen-type acylation between atoms C-1 and C-6 of the corresponding acetogenin (**42**) (Fig. 3) [29]. A wide range of acylphloroglucinols with different levels of methylation are biosynthetized in *Eucalyptus* species. Grandinol (**44**) and homograndinol (**45**) are known as growth regulators. Of the two compounds, grandinol (**44**), first isolated from *E. grandis*, shows the greater activity. Both of these compounds also inhibit Epstein-Barr virus (EBV) activation [7].



42 **43**

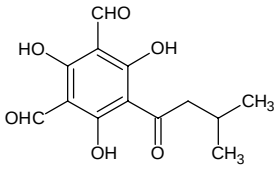
Fig. 3. Scheme of acylphloroglucinol biosynthesis.



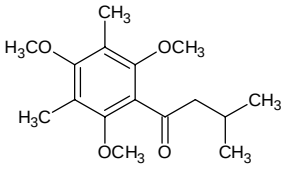
44 R: CH_3

45 R: CH_2CH_3

Jensenone (**46**) is a structurally very interesting acylphloroglucinol that occurs naturally in different *Eucalyptus* foliage. It was first obtained from the essential oil of *E. jensenii* leaves and was found to be a precursor of the sideroxylonals, euglobals, and macrocarpals. Jensenone (**46**) acts as an anti-feedant against vertebrate herbivores [7, 30, 31]. Torquatone (**47**) is the most widespread acylphloroglucinol in *Eucalyptus* species [7].

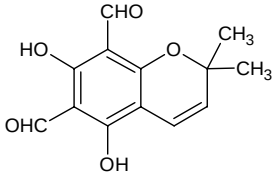


46



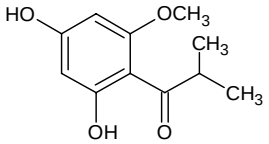
47

Loxophlebene (**48**), obtained from the leaves of *E. loxophleba,* is a typical diformyl monomeric phloroglucinol. Biogenetically, it is formed via a polyketide pathway, but after prenylation of the core phloroglucinol moiety, direct cyclization between the prenyl group and the ortho hydroxyl functionality forms a benzopyran ring [30].



48

Robustaol B (**49**) has been isolated from the leaves of *E. robusta* [7].



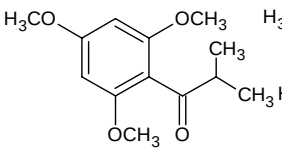
49

Many other phloroglucinol derivatives have been isolated from *Eucalyptus* species, namely conglomerone (**50**), pulverulentone A (**51**), pulverulentone B (**52**), baeckeol (**53**), isobaeckeol (**54**) and homoisobaeckeol (**55**) [7]. 2,6-dimethoxy-p-benzoquinone (**56**), 3,4,5-trimethoxyphenol acetate (**57**), and 2,4,6-trimethoxyphenol acetate (**58**) have been isolated from the benzene-soluble part of the acetone extract of the bark of *E. globulus* [17].

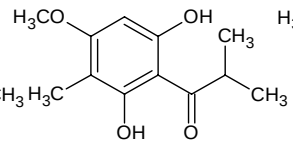
Phloroglucinol glycosides

Five phloroglucinol glycosides, the eucalmainosides A-E, have been isolated from the fresh fruits of *E. maideni*. The chemical structure of eucalmainoside A (**59**) was determined to be 2-methylphloroglucinol-O-β-D-glucopyranoside. Eucalmainoside B (**60**) is 2,4-dimethyl-phloroglucinol-*O*-*β*-D-glucopyranoside. 2,4,6-tri-hydroxy-3-methylbenzaldehyde-2-*O*-*β*-D-gluco-pyranoside is the structure of eucalmainoside C (**61**) [32]. Eucalmainoside D (**62**) and eucalmainoside E (**63**) are isomers. Acidic hydrolysis of eucalmainoside D leads to D-xylose and D-glucose [32].

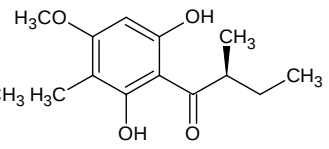
Isobiflorin (**64**) and chromene glycoside (**65**) have been isolated from the acetone extracts of the dried leaves of *E. globulus* [27].



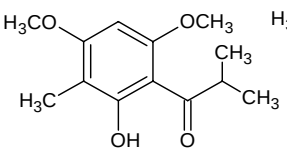
50



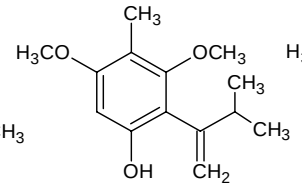
51



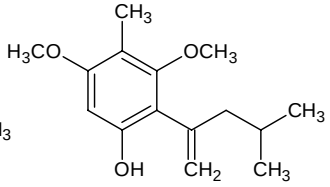
52



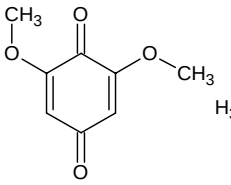
53



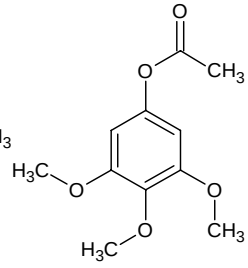
54



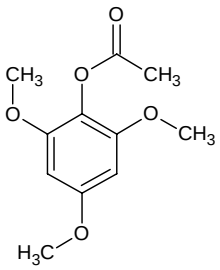
55



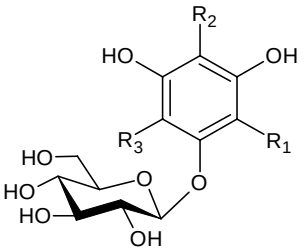
56



57



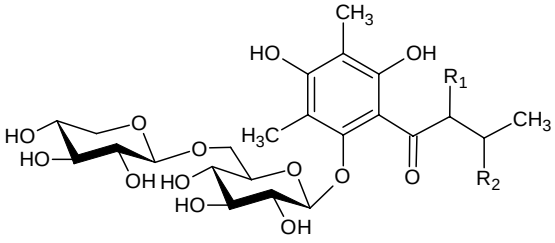
58



59 R_1: CH_3 R_2: H R_3: H

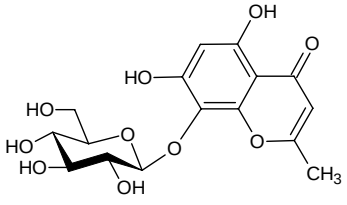
60 R_1: CH_3 R_2: CH_3 R_3: H

61 R_1: CHO R_2: H R_3: CH_3

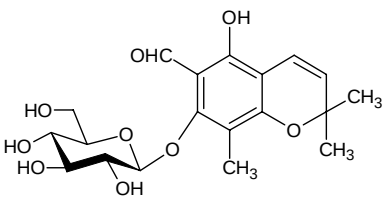


62 R_1: CH_3 R_2: H

63 R_1: H R_2: CH_3



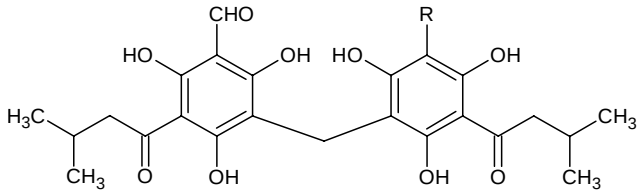
64



65

Dimeric acylphloroglucinols

Two types of phloroglucinol dimers in *Eucalyptus* species have been described. The benzylbenzene group is represented by two compounds: robustaol A (**66**) and jensenone dimer (**67**). These compounds contain the benzene cores of two monomeric acylphloroglucinols connected by a methylene bridge. The acyl chains do not participate in the dimerisation process. Jensenone dimer (**67**) has been obtained from the leaves of *E. saligna* [33]. Robustaol A (**66**) contains only one formyl substituent and has been isolated from the leaves of *E. robusta*. In China these leaves are used to prepare an antimalarial medicine. Although robustaol A (**66**) possesses antimalaric activity against *Plasmodium berghei*, the main compounds of *E. robusta* responsible for its antimalaric activity are robustadials [7].



66 R: CH_3

67 R: CHO

The second type of dimeric acylphloroglucinols is represented by the 2-phenylchromane group. Although these compounds resemble the flavonoid skeleton, their biosynthetic pathway is different. In contrast to flavonoids, the phenyl ring is substituted with hydroxyl groups at the *meta* positions and there is no oxo substituent at C-4. 2-phenylchromane dimeric acylphloroglucinols are biogenetically formed from isopentenyl-phloroglucinol precursors by a hetero-Diels-Alder coupling process (Fig. 4) [34]. The acyl side chains of both monomers take part in the formation of the heterocyclic ring of sideroxylonals (**68**-**70**) and loxophlebal A (**71**), but loxophlebal B (**72**) and grandinal (**73**) are formed by a cyclization involving the acyl side chain of only one of the monomers [35].

Sideroxylonals are compounds with a 2-phenylchromane skeleton. The numbering of their skeleton is different from the numbering of flavan compounds. Some typical structural characteristics of the syderoxylonals are the four formyl groups located at positions C-3, C-5, C-3′, and C-5′, the isobutyl substituent at C-7; and the isopropyl substituent at position C-10′. The differences between individual sideroxylonals appear in the stereochemistry at C-7 and C-10′ [35]. Loxophlebal A differs from the sideroxylonals only in the replacement of one formyl group with a methyl group.

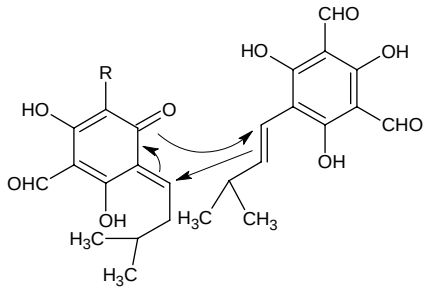
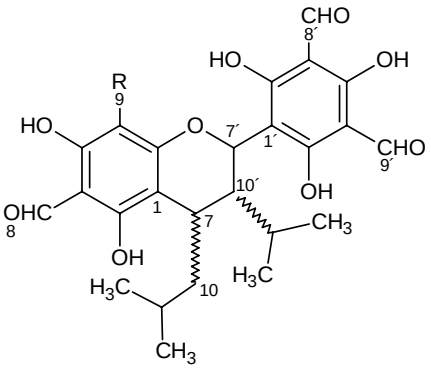


Fig. 4. Biosynthesis of sideroxylonals.

Sideroxylonals have been found in the leaves and flower buds of some *Eucalyptus* species. Sideroxylonal A (**68**) and sideroxylonal B (**69**) have been isolated from extracts of *E. sideroxylon* [35]. Sideroxylonal C (**70**) has been obtained from the leaves of *E. melliodora* [36]. Loxophlebal A (**71**) and loxophlebal B (**72**) have been isolated from the leaves of *E. loxophleba* [18], and grandinal (**73**) has been obtained from *E. grandis* [2].

Sideroxylonals exhibit activity against the G+ bacteria *S. aureus* and *Bacillus subtilis,* and they strongly inhibit the activation of human plasminogen. Various microorganisms (e.g., *Streptococcus pneumonia*) activate human plasminogen in order to promote their own penetration through the reconstituted basal membranes [7, 35, 36]. Sideroxylonal A (**68**) is

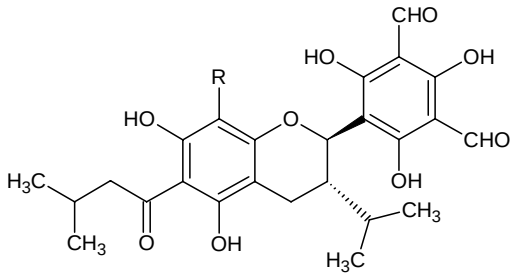


68 R: CHO 7α–isobutyl 10′α–isopropyl

69 R: CHO 7α–isobutyl 10′β–isopropyl

70 R: CHO 7β–isobutyl 10′α–isopropyl

71 R: CH_3 7α–isobutyl 10′α–isopropyl



72 R: H

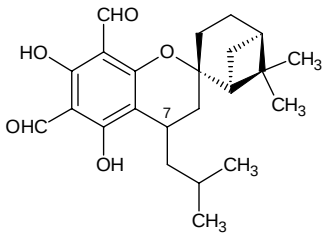
73 R: CHO

also a potent inhibitor of the attachment of the blue mussel (*Mytilus edulis*), suggesting that it could be a potent marine anti-fouling agent, with activity comparable to the most active anti-fouling compound, 2,5,6-tribromo-1-methyl-gramine. The sideroxylonals are potent mammalian antifeedants. They inhibit aldol reductase and the growth of HeLa cells [7, 35, 36]. Loxophlebal A (**71**) possesses greater activity against *E. coli, P. aeruginosa*, *S. epidermis*, and *S. aureus* than any of the sideroxylonals. Removal of the formyl group from position C-3 of the sideroxylonal molecule increases the antibacterial activity against both G+ and G- bacterial strains [35].

Sideroxylonals A-C (**68-70**), loxophlebal A (**71**), and grandinal (**73**) have been tested for their anti-leishmanial activity against *Leishmania donovani in vitro*. Sideroxylonals with four formyl groups, are more active than loxophlebal A or grandinal, which have only three each. This suggests a plausible role for the formyl functionality in the bioactivity of these compounds [30].

Robustadials

Robustadials are acylphloroglucinol-monoterpene adducts containing a pinane structure in their molecule. Robustadial A (**74**) and robustadial B (**75**) have been isolated from the most potent fraction of the leaves of *E. robusta*. These leaves have traditionaly been used in China as a medicine for the treatment of malaria. Some fractions of the ethanol extract of the leaves exhibit inhibitory effects against *Plasmodium berghei.* Robustadials are considered to be the active components responsible for the antimalarial activity [2].



74 7β-isobutyl

75 7α-isobutyl

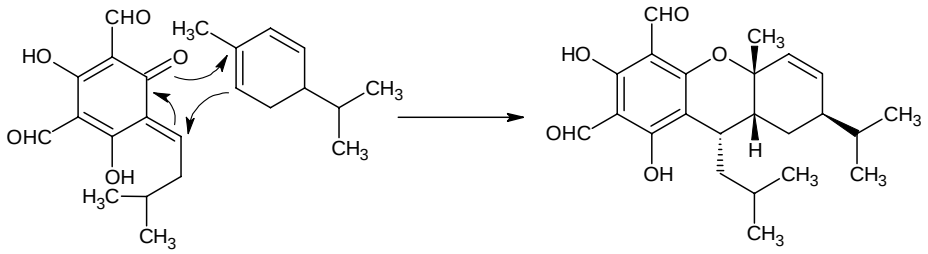
Euglobals

Euglobals are formyl-isovaleryl or diformyl phloroglucinol-terpene adducts occurring widely in *Eucalyptus* species [37, 38]. Two groups can be distinguished, depending on whether the terpenoid component is a mono- or a sesquiterpene [7]. These compounds are biosynthesized by hetero-Diels–Alder reactions between the various terpenes and *O*-quinone methides derived from oxidation of the phloroglucinol derivatives jensenone or grandinol [37, 38]. A number of

euglobals have been isolated from various species of *Eucalyptus*. The yields of the compounds isolated from the leaves were around 0.03-0.40% [2, 30].

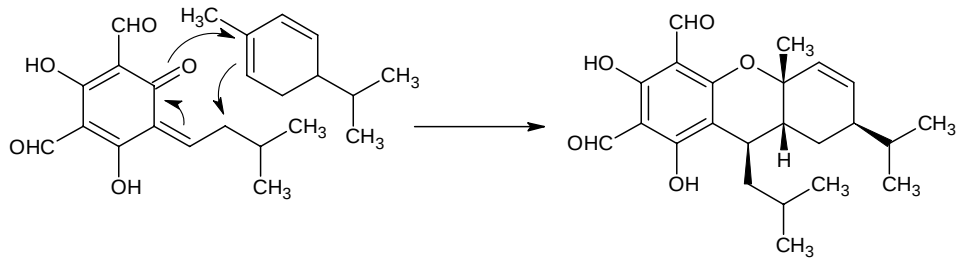
Monoterpenic euglobals can be divided into two classes. In the first, the alkanoyl side chain of the acylphloroglucinol component is involved in the formation of the chromane ring (Figs. 5, 6), and in the second it is not (Figs. 7, 8, 9) [7].

The terpenic component of euglobals -G1, -G2, -G3, -G4, and -G5 (**76**, **77**, **78**, **79,** and **80**) is a pinane moiety. Euglobal-G1 (**76**) and euglobal-G2 (**77**) contain α-pinene fused to a dihydropyran ring, while euglobal-G3 (**78**) and euglobal-G4 (**79**) incorporate β-pinene connected to the pyran ring to form spiro compounds. These constituents have been isolated from *E. grandis* leaves and their structures are similar to robustadials. Euglobals -G3 and -G4 (**78** and **79**) differ from robustadials in the position of the isobutyl chain, which is present on the aromatic ring of the euglobals and on the pyran ring of the robustadials [39]. Euglobal-G5 (**80**) has also been isolated from the leaves of *E. grandis* [2].



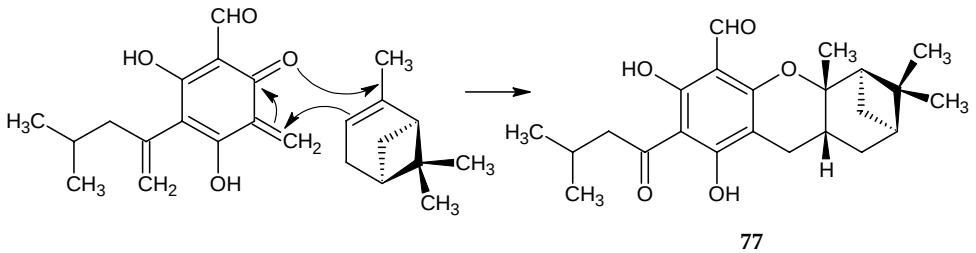
95

Fig. 5. Biosynthesis of euglobal Ia_1 (**95**).



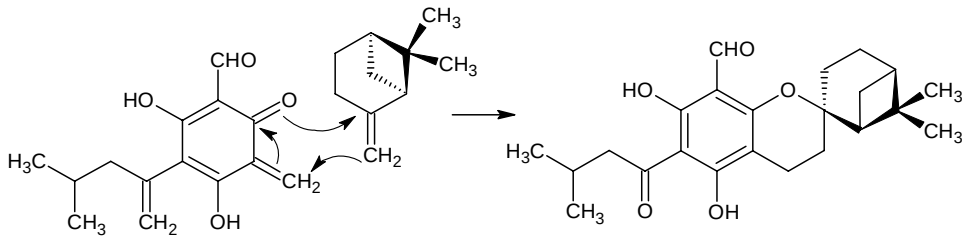
96

Fig. 6. Biosynthesis of euglobal Ia_2 (**96**).



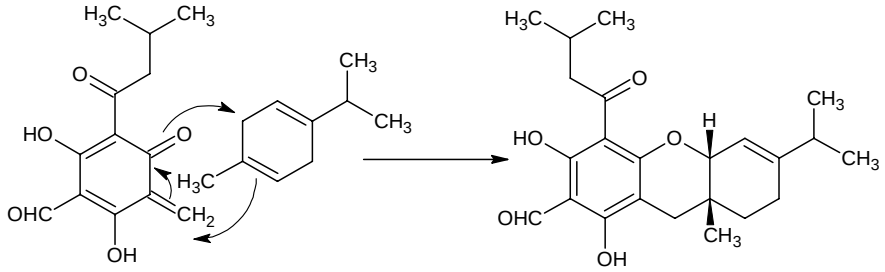
77

Fig. 7. Biosynthesis of euglobal-G2 (**77**).



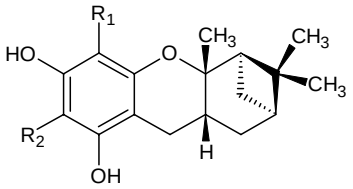
78

Fig. 8. Biosynthesis of euglobal-G3 (**78**).



84

Fig. 9. Biosynthesis of euglobal-G10 (**84**).

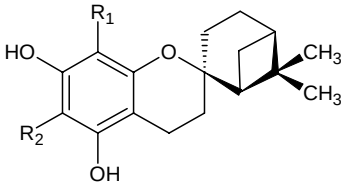


76 R_1: $COCH_2CH(CH_3)_2$

R_2: CHO

77 R_1: CHO

R_2: $COCH_2CH(CH_3)_2$

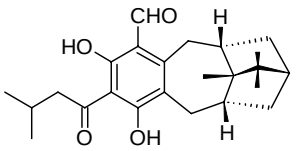


78 R_1: CHO

R_2: $COCH_2CH(CH_3)_2$

79 R_1: $COCH_2CH(CH_3)_2$

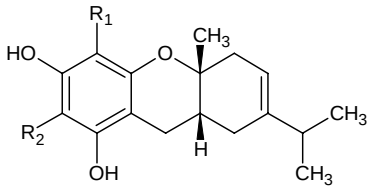
R_2: CHO



80

Biogenetically, euglobal-G6 (**81**) and euglobal-G7 (**82**) are formed by a Diels-Alder type cycloaddition combining γ-terpinene with an *O*-quinone methide derived from grandinol. These compounds have been isolated from *E. grandis* [40]. Euglobal-G9 (**83**) and euglobal-G10 (**84**) differ from each other in the attachment of the formyl and isovaleroyl groups on the phloroglucinol ring. They are derived from α-terpinene with the participation of the 1, 2 double bond [41].

Euglobal-G8 (**85**), isolated from *E. grandis*, differs from euglobal-G6 (**81**) in the positions at which the isopropyl and methyl group are attached to the monoterpene part of the molecule. Euglobal-G8 (**85**) is derived by Diels-Alder cycloaddition of the appropriate *O*-quinone

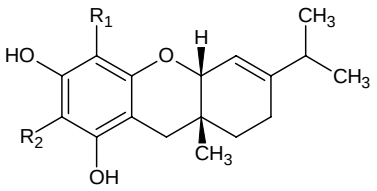


81 R_1: CHO

R_2: $COCH_2CH(CH_3)_2$

82 R_1: $COCH_2CH(CH_3)_2$

R_2: CHO

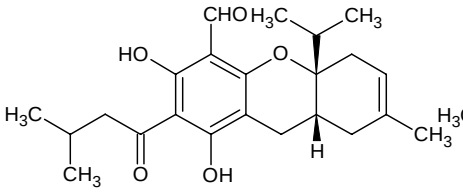


83 R_1: CHO

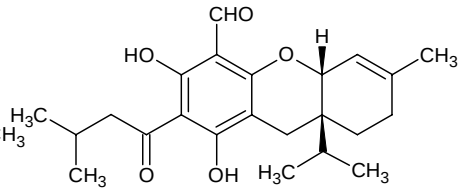
R_2: $COCH_2CH(CH_3)_2$

84 R_1: $COCH_2CH(CH_3)_2$

R_2: CHO



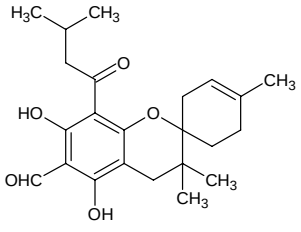
85



86

methide to γ-terpinene, the double bond at position 4 and 5 being involved in this cycloaddition reaction. Euglobal-G11 (**86**) differs from euglobal-G9 (**83**) in the positions of attachment of the isopropyl and methyl groups to the monoterpene moiety. Compound **86** has the isopropyl group attached to C-6′ and the methyl at C-3′. Euglobal-G11 (**86**) is derived from α-terpinene with the participation of the 3,4 double bond [41].

Euglobal-G12 (**87**) is derived by the cycloaddition of terpinolene to the *O*-quinone methide. It has been isolated from the leaves of *E. grandis* [41].



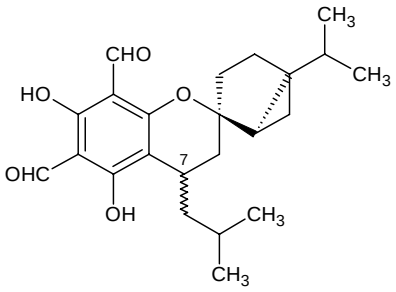
87

The monoterpene part of the structure of euglobal-Ib (**88**), euglobal-B1-1 (**89**), euglobal-Ic (**90**), and euglobal-IIa (**91**) is sabinene. Euglobal-IIb (**92**) has a β-phellandrene skeleton as the terpenic component [7]. Euglobal-Am-1 (**93**) and euglobal-Am-2 (**94**) have been isolated from *E. amplifolia* [2].

The monoterpene moiety of euglobal Ia_1 (**95**), euglobal Ia_2 (**96**), euglobal T1 (**97**), and euglobal IIc (**98**) is α-phellandrene [7, 37].

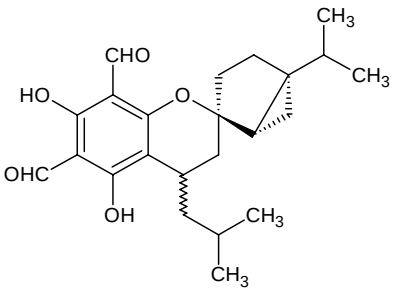
The sesquiterpene bicyclogermacrane is the terpene moiety of euglobal-III (**99**), euglobal-IVa (**100**), euglobal IVb (**101**), and euglobal-VII (**102**) [7]. Euglobal V (**103**), originally classified as euglobal, should be considered as a macrocarpal [7]. Euglobal-III (**99**) was first isolated from the buds of *E. globulus* [2]. Euglobal-In-1 (**104)** and euglobal-In-2 (**105**) have been isolated from the leaves of *E. incrassata* [2].

Euglobals exhibit a number of pharmacological activities, such as anti-inflammatory, anti-tumor promoting, antimicrobial, and antileishmanial, and they inhibit the activation of Epstein–Barr virus (EBV) [2, 30]. From the results of a two-stage carcinogenesis test, it has been concluded that euglobal-G1 (**76**) inhibits both the promotion stages of two-stage carcinogenesis and pulmonary tumorigenesis. Compound **76** might be valuable as a chemopreventive agent in chemical carcinogenesis [8]. Euglobal-G4 (**79**) exhibits good antibacterial activity against methicillin-resistant *S. aureus* [39]. Euglobal-G6 (**81**) and



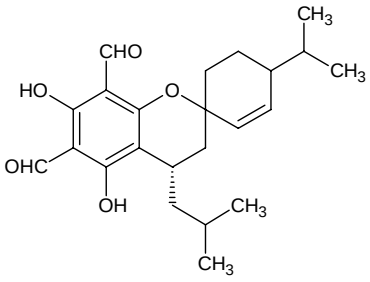
88 7α-isobutyl

89 7β-isobutyl

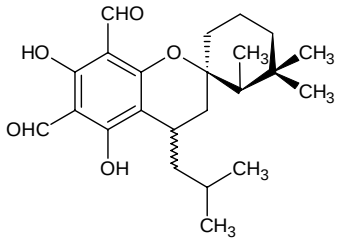


90 7β-isobutyl

91 7α-isobutyl

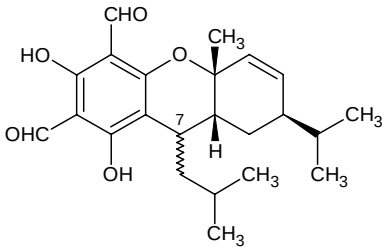


92



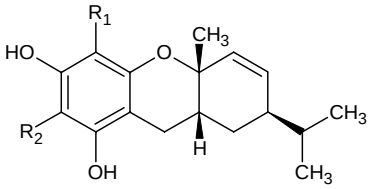
93 7α-isobutyl

94 7β-isobutyl



95 7β-isobutyl

96 7α-isobutyl

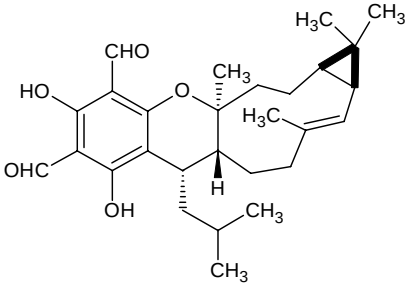


97 R_1: CHO

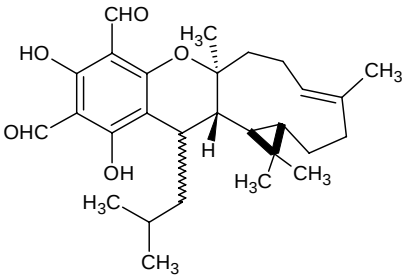
R_2: $COCH_2CH(CH_3)_2$

98 R_1: $COCH_2CH(CH_3)_2$

R_2: CHO

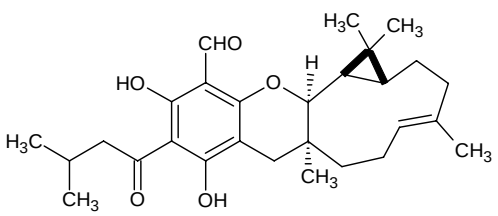


99

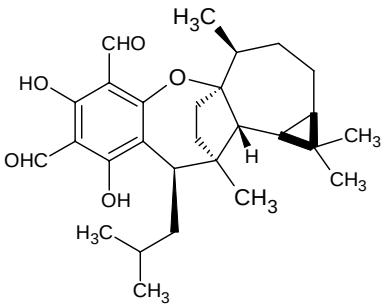


100 7a-isobutyl

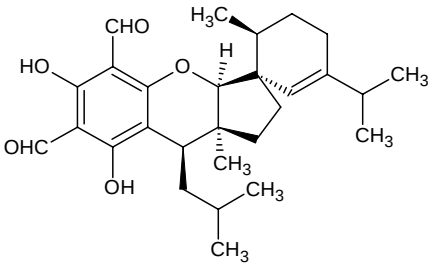
101 7b-isobutyl



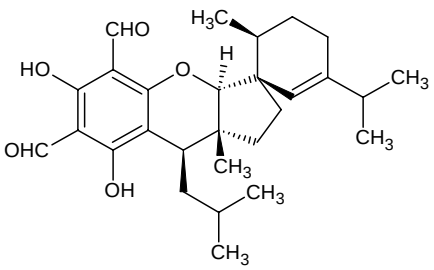
102



103



104



105

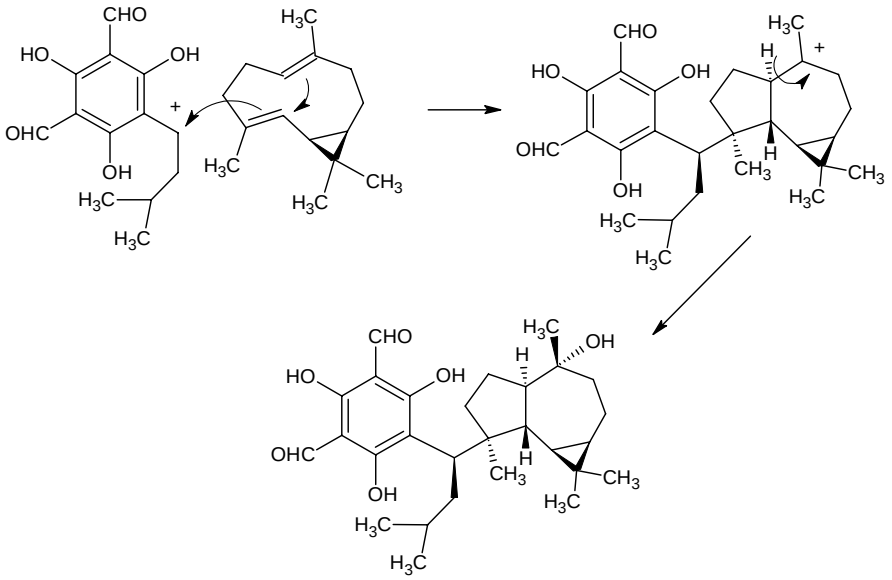
euglobal-G7 (**82**) inhibit early antigen activation of Epstein-Barr virus [40]. Euglobal T1 (**97**) and euglobal IIc (**98**) only weakly inhibit Epstein-Barr virus activation [42]. Euglobal-III (**99**) inhibits granulation [2].

Macrocarpals

Macrocarpals are phloroglucinol-sesquiterpene adducts. Macrocarpal A (**106**) was the first macrocarpal to be isolated and has its structure elucidated. Many other macrocarpals have since been isolated from various *Eucalyptus* species. The macrocarpals possess structures composed of isopentenylphloroglucinol and a sesquiterpene moiety [2]. The simplest proposal for the biogenetic generation of the macrocarpals involves a carbocationic species or a stabilized equivalent. Such a species could replace a proton as the cationic initiator of the cyclization of a sesquiterpene precursor. For macrocarpals A, B, C, and D the sesquiterpene bicyclogermacrene can be considered to be cyclised to form the globulol or aromadendrene skeleton (Fig. 10) [7]. Macrocarpals exhibit antibacterial activity against cariogenic and periodontopathic bacteria and potent inhibitory activity against HIV-RTase (HIV-reverse transcriptase) [28].

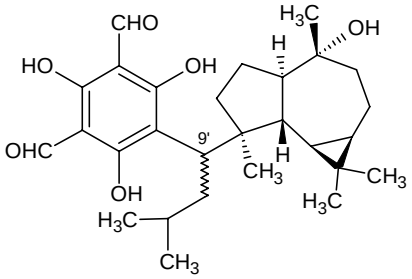
Macrocarpal A (**106**) was isolated for the first time as the principle antibacterial component of the leaves of *E. macrocarpa*. The stereochemistry of macrocarpal B (**107**) is the opposite of that of macrocarpal A (**106**) at the position C-9′ [43]. Compounds **106** and **107** possess strong antibacterial activity against cariogenic and periodontopathic bacteria with MIC values of 0.39-1.56 and 0.39-3.23 μg/mL, respectively. These two compounds inhibit the HIV-RTase activity with IC_{50} 10 μM and 5.3 μM. Anti-HIV-RTase activity of macrocarpal B (**107**) is the more significant. Compounds **106** and **107** selectively accumulate in the epithelial cells of the digestive tract, where their cancer preventing effects are displayed [28].

Macrocarpal C (**108**) has been isolated from *E. globulus* as an amorphous solid [43]. A potential source of confusion exists in the literature because macrocarpal G has the same structure as that established for macrocarpal C (**108**) [2, 7]. Compound **108** exhibits inhibitory activity against HIV-RTase, with an IC_{50} value of 8.4 μM [43]. Macrocarpal D (**109**) has been obtained from *E. globulus*. It has a guaiane-type of skeleton and does not contain a cyclopropane ring. Its IC_{50} value against HIV-RTase is 12 μM [43].



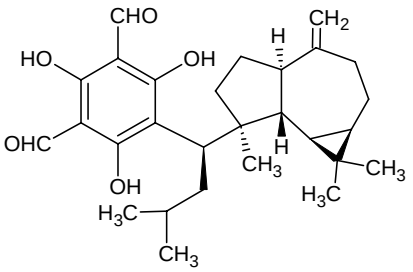
106

Fig. 10. Biosynthesis of macrocarpal A.

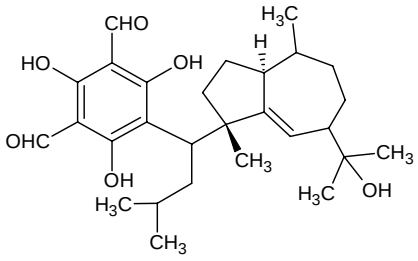


106 9′β-isobutyl

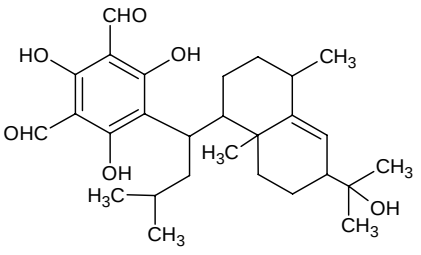
107 9′α-isobutyl



108



109

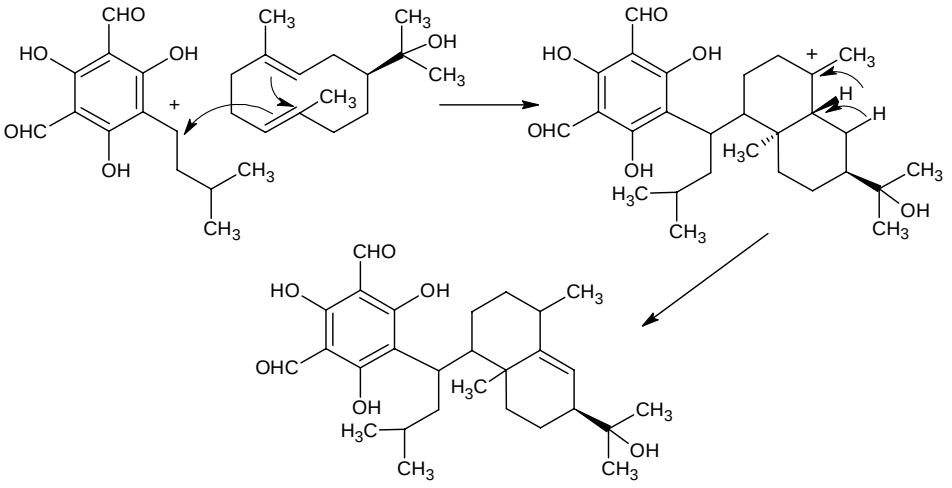


110

The formation of macrocarpals E, H, I, and J involves the cyclization of germacrol (Fig. 11) [7].

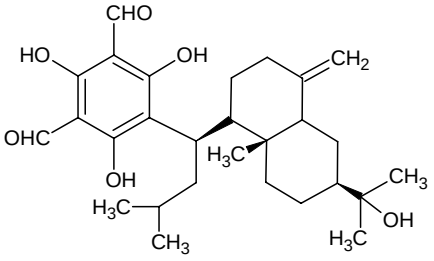
Macrocarpal E (**110**) has been isolated from *E. globulus*. Its IC_{50} towards HIV-RTase is 8.1 μM [43]. Macrocarpal H (**111**), macrocarpal I (**112**), macrocarpal J (**113**), and macrocarpal am-1 (**114**) isolated from the extract of the dried leaves of *E. globulus*, inhibit the action of the enzyme glucosyltransferase (GT) and also exhibit appreciable antibacterial activity against cariogenic bacteria [2].

Eucalyptone (macrocarpal am-1) (**114**) has a unique sesquiterpene moiety comprising a cyclopropane

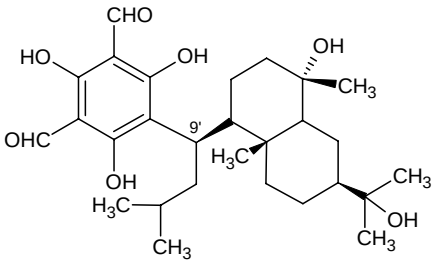


110

Fig. 11. Biosynthesis of macrocarpal E.

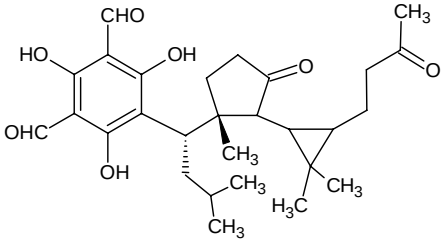


111



112 9'β-isobutyl

113 9'α-isobutyl



114

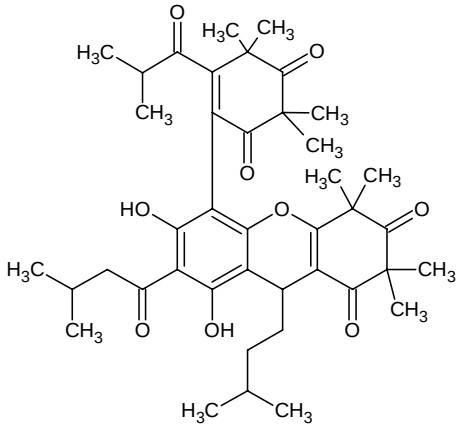
ring and a five-membered ring system. Compound **114** has an MIC of 12.5 μg/mL against *Streptococcus muttans* and towards *S. sorbinus* the MIC is 6.25 μg/mL [2, 44].

Eucalyptone G and rhodomyrtone

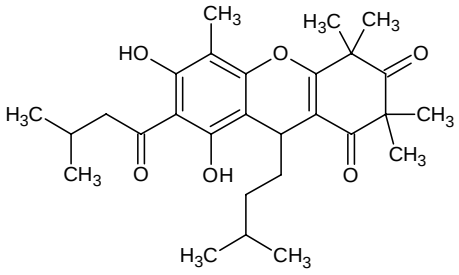
Eucalyptone G (**115**) and rhodomyrtone (**116**) have been isolated from the ethyl acetate fraction of the methanolic extract of the powdered bark of *E. globulus*. Compound **115** is active against *B. subtilis* and *S. aureus and* also displays activity against *E. coli* [2].

Flavonoids

Flavonoids are a class of natural compounds that possess 2-phenylchromane as their basic structural element [5]. The flavonoid molecule is a product of two biosynthesis pathways. The bridge and the aromatic B-ring constitute a phenylpropanoid unit that is synthesized from cinnamoyl-CoA precursor (**117**) from the shikimate pathway, this acts as a starter group (Fig. 12). The six carbons of ring-A result from the condensation of three malonyl-CoA units (**118**), each contributing two carbon atoms to the ring. The fusion of these two parts leads to a poly-β-keto chain (**119**) that is folded, allowing aldol-type cyclization catalysed by



115



116

chalcone synthase. The product of this reaction is naringenin chalcone (**120**). The six-member heterocyclic ring characteristic of most flavonoids is formed by the chalcone-isomerase-catalyed nucleophilic attack of a phenol group of the acetate-derived ring on the α,β-unsaturated ketone. The product of this reaction is naringenin (**134**), the central intermediate of flavonoid biosynthesis. From this point the pathway diverges into several side branches, each resulting in the production of a different class of flavonoids, including isoflavones, flavanones, such as pinocembrin (**121**), flavones, such as chrysin (**122**), flavanols, flavan-3-ols, and anthocyanins [13, 45]. Flavonoids can occur in two forms: as glycosides or as free aglycones. The glycoside forms are water-soluble and accumulate in vacuoles. Free aglycones, found in the leaf cuticle, are made more lipophilic by methylation of some or all of their hydroxyl groups [5]. Flavonoids display a remarkable spectrum of biological activities [46]. These phenylbenzo-pyrones possess direct antibacterial activity, work synergistically with antibiotics, and suppress bacterial virulence [47]. They also display antitumor, antioxidative, antiallergic, antiplatelet, anti-ischemic, and anti-inflammatory activities [48].

Rutin (**123**) was at one time extracted from the leaves of *Eucalyptus* species, particularly from *E. macroryncha* and *E. youmanii*, on a commercial scale. It is used in the treatment of fragile capillaries, especially varicose veins, hemorrhoids, and frostbite. But extracting rutin from *Eucalyptus* could not compete economically with obtaining it from *Sophora japonica* [2].

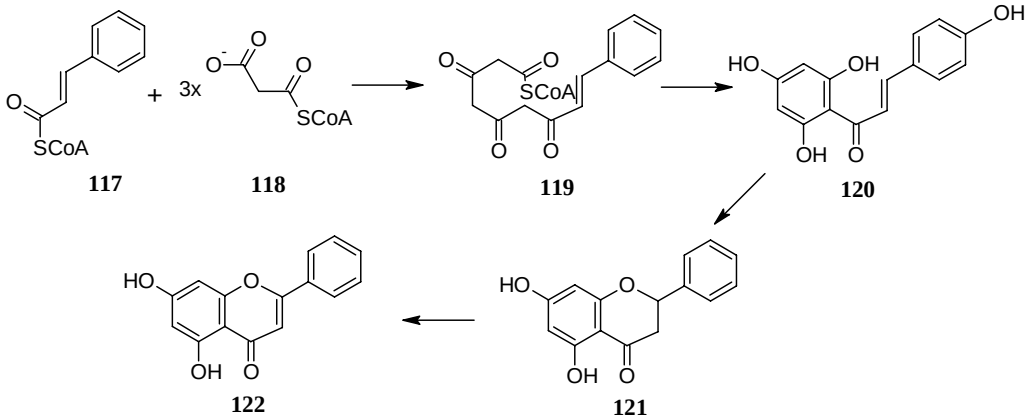
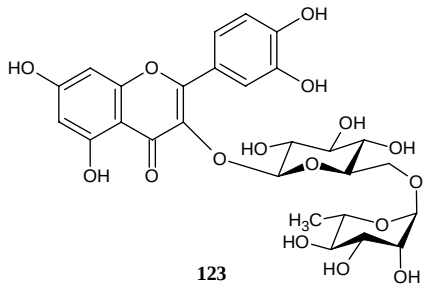
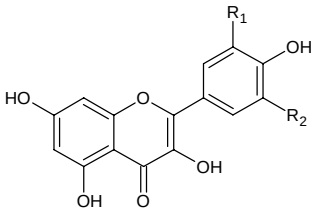


Fig. 12. Biosynthesis pathway of flavonoids.



123

Quercetin-3-*O*-D-glucopyranoside, a glycoside of quercetin (**124**) has been identified in the fruits of *E. camaldulensis*, and *E. rudis* and in both the fruits and the bark of *E. globulus*. Quercetin-3-*O*-glucuronide has been detected in various species of *Eucalyptus* [25]. The aqueous part of the acetone extract of the fresh leaves of *E. maidenii* contains quercetin (**124**) and its glycosides quercetin-3-*O*-α-L-rhamnopyranoside, quercetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-β-D-(6´´-feruloyl) galactopyranoside, and a glycoside syringetin-3-*O*-β-D-glucopyranoside of syringetin (**125**) [32]. Four flavonoids have been isolated from the leaves of *E. ovata*, namely, quercetin-3-*O*-β-arabinoside, quercetin-3-*O*-α-rhamnoside, quercetin-3-*O*-2-(β-xylosyl)-α-rhamnoside, and quercetin-3-*O*-β-galactoside-6´´-O-gallate [49]. Flavonol glycosides have also been isolated from the leaves of *E. consideniana* and *E. iminalis* [50]. Quercetin (**124**) isolated from *E. maidenii* exhibits a slight activity against herpes simplex virus type 1 *in vitro* [32].



124 R1: OH R2: H

125 R1: OCH_3 R2: OCH_3

3,4´-dimethoxy-6,8-dimethylkaemferol (**126**) and 3-methoxy-6,8-dimethylkaemferol (**127**) have been isolated from the aerial parts of *E. occidentalis.* These derivatives of kaempferol (**128**) induce apoptosis mediated by the activation of caspase-8/caspase-3 and the release of the cytochrome c. They also inhibit the proliferation of human HL-60 myeloid leukemia cells in a dose-dependent manner [46]. Eucalyptin (**128**) and 8-desmethyleucalyptin (**129**) have been isolated from the leaf waxes of several species of *Eucalyptus* [51].

Several flavonoids, namely kaempferol (**130**), myricetin (**131**), luteolin (**132**), tricetin (**133**), and quercetin (**124**) have been detected in *Eucalyptus* honeys. These honeys also contain the propolis-derived flavonoids pinobanksin (3,5,7-trihydroxyflavanone), pinocembrin (**121**) (5,7-dihydroxyflavanone), and chrysin (**122**) (5,7-dihydroxyflavone) [4, 52].

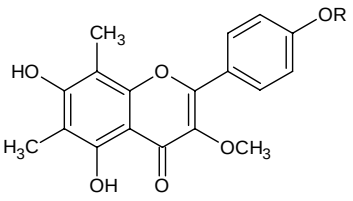
Catechins

Catechins belong to the group of flavan-3-ols. They are derived from flavanones by several biosynthetic steps (Fig. 13). Naringenin (**134**), an intermediate in flavonoid biosynthesis, is converted to dihydrokaempferol (**135**) by flavanone-3-hydroxylase. In the following step, flavonol-3´-hydroxylase is responsible for the synthesis of dihydroquercetin (**136**). Dihydroflavanols are converted to leucoanthocyanidins, such as leucoanto-cyanidine (**137**), by dihydroflavonol-reductase. Leucoanthocyanidins are key intermediates in the formation of flavan-3-ols, proanthocyanidins, and anthocyanidins. The enzyme leucocyanidin-4-reductase converts leucocyanidin to the flavan-3-ol (+)-catechin (**138**) [45].

The compound (+)-catechin (**138**), (-)-catechin (**139**), (-)-epicatechin (**140**), (+)-epicatechin (**141**), and (+)-gallocatechin (epigallocatechin) (**142**) are major catechin tannins. Nine tannins: catechin, gallocatechin, catechin-3-*O*-gallate, epicatechin-(4*β*-8)-catechin, catechin-(4*β*-8)-catechin, gallocatechin-(4β-8)-catechin, gallocatechin-(4*β*-8)-gallocatechin, gallocatechin-(4*β*-6)-catechin, and gallocatechin-(4*β*-8)-gallocatechin-(4*β*-8)-catechin have been isolated from *E. ovata*. Gallocatechin shows antitumor, anti-inflammatory, and antimutagenic activity [49].

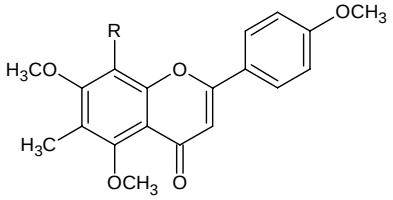
Hydrolysable tannins

Gallic acid (**148**), ellagic acid (**155**), and their derivatives are hydrolysable tannins that have been detected in methanolic extracts of the bark of *E. globulus*. Condensed and hydrolysable tannins and their degradation products are potent antioxidants [53]. Oxidative stress is known to



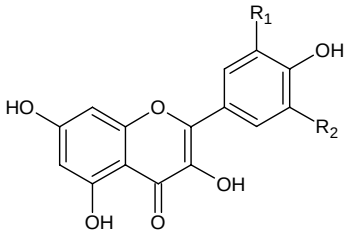
126 R: CH_3

127 R: H



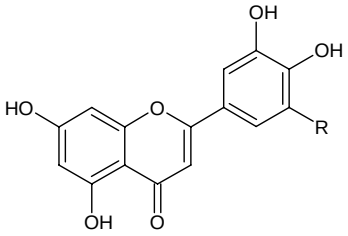
128 R: CH_3

129 R: H



130 R1: H R2: H

131 R1: OH R2: OH



132 R: H

133 R: OH

induce the production of reactive oxygen species (ROS) that are toxic to pancreatic cells and reduce the synthesis and release of insulin. Extracts of *E. globulus* possess antioxidant properties and play a potential role in defence against an excess of free radicals. *E. globulus* constituents can be considered to offer a valuable additional therapy for diabetic patients, especially for alleviating the renal complications caused by this disease [53, 54].

Gallic acid (**148**), 3,4,5-trihydroxybenzoic acid, is a product of the shikimate pathway that begins with the coupling of D-erythrose-4-phosphate (**143**) and phosphoenolpyruvic acid (**144**) to give the seven-carbon 3-deoxy-D-arabino-heptulosonic

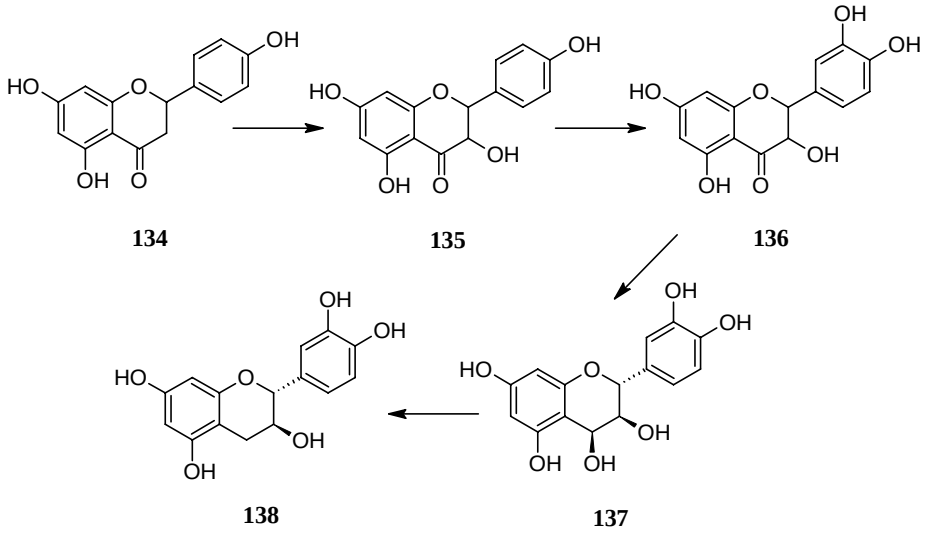
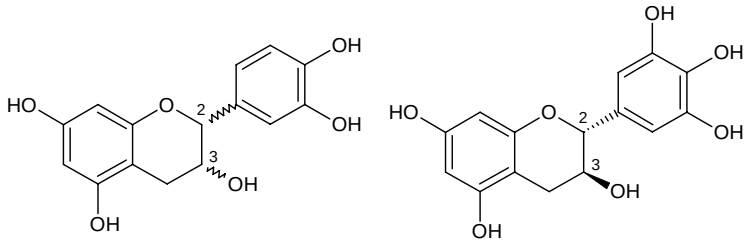


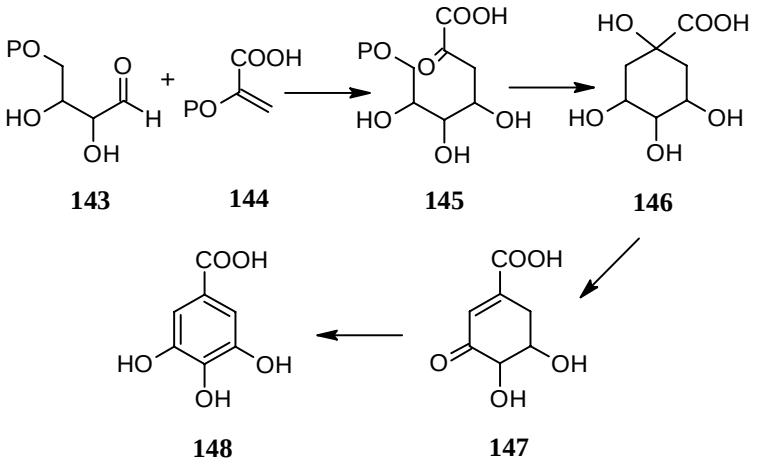
Fig. 13. Biosynthesis of catechins.



139 (2*S*,2*R*)
140 (2*R*,2*R*)
141 (2*S*,2*S*)
142

acid 7-phosphate (DAHP) (**145**) (Fig. 14). The elimination of phosphoric acid from DAHP followed by an intramolecular aldol reaction generates 3-dehydroquinic acid (**146**). 3-dehydroshikimic acid (**147**) is formed by dehydration and reduction of 3-dehydroquinic acid. Gallic acid is biosyntesized from 3-dehydroshikimic acid by dehydration and enolization [13].

Gallic acid (**148**) has been detected in the fruits, leaves, and wood of *E. globulus*, in the leaves of *E. camaldulensis* and *E. rudis*, and in the bark and wood of *E. regnans* [25]. In addition to gallic acid, other gallotannins have been isolated from various *Eucalyptus* species. Digalloylglucose and the isomers of trigalloylglucose have been detected in the fruits of *E. globulus*, in the wood of *E. nitens*, and in the leaves of *E. consideniana*. Tetragalloylglucose and its derivates have been identified in the wood of *E. nitens* and in the leaves of *E. viminalis*. Pentagalloylglucose has been isolated from the wood of *E. nitens* and the fruits of *E. alba.* Methyldigalloyldiglucose has also been detected in the fruit of *E. globulus*. Methylated tetragalloylglucose and trigalloyl-glucose were detected in the fruits of *E. globulus* and in the wood of *E. nitens*. Some monoterpene glycosides, eucaglobulin and the globulusins, contain a galloylglucose moiety in their molecules [25]. Hexahydroxydiphenoyl-galloylglucose (HHDP-galloylglucose) has been isolated from the fruits of *E. globulus* and the wood of *E. nitens*. The presence of 2,3-(S)-HHDP-D-glucose has been described in the fruits of *E. alba*. Isomers of HHDP-galloylglucose have been identified in the fruits of *E. globulus*, the wood of *E. nitens*, and the leaves of *E. consideniana* [25]. Tellimagrandin I (**149**) has been identified in the leaves and fruits of *E. globulus*, the wood of *E. nitens*, the fruits of *E. alba*, and the leaves of *E. rostra* and



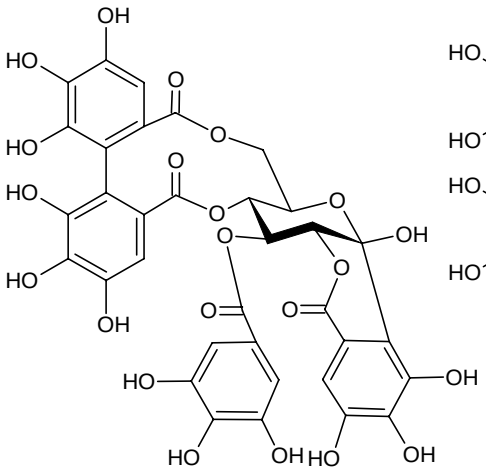
143 **144** **145** **146**
148 **147**

Fig. 14. Biosyntesis of gallic acid.

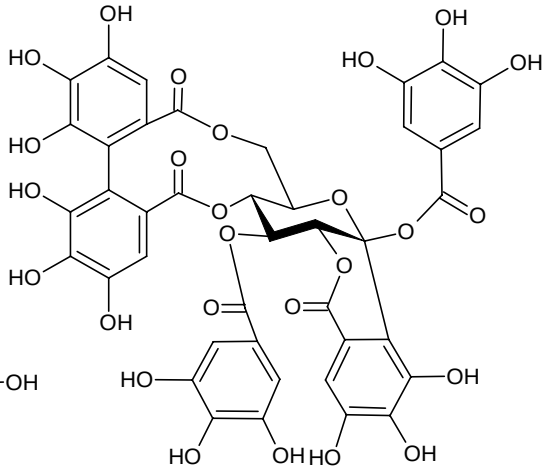
E. consideniana. Tellimagrandin II (**150**) has been detected in the fruits of *E. globulus*, the wood of *E. nitens*, and the leaves of *E. viminalis*. This compound differs from tellimagrandin I (**149**) by having one additional galloyl unit attached to the glucopyranosyl skeleton. Tellimagrandin II (**150**) is a product of the enzymatic transformation of pentagalloylglucose [25].

Three isomers of pedunculagin (**151**) have been detected in the wood of *E. nitens*, but only one in *E. consideniana* leaves. Casuarictin (**152**) has been identified in the wood of *E. nitens* [25].

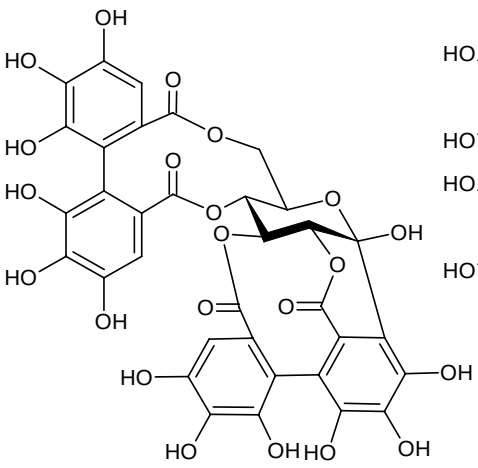
Casuariin (**153**) has been identified in the fruits of *E. globulus* and *E. alba*, and in the wood of *E. nitens*. Casuarinin (**154**) has been detected in the wood of *E. nitens* [25]. Galloylation of a glucose molecule leads to the formation of pentagalloyl-glucose. Oxidative coupling between the galloyl groups at C-4 and C-6 produces tellimagrandin II (**150**), which in turn yields casuarictin (**152**) upon oxidative coupling between the galloyl groups at C-2 and C-3. Cleavage of the galloyl group at C-1 of this compound leads to the formation of casuarinin (**154**). It has been suggested that C-glycosidic ellagitannins are formed from pedunculagin (**151**) through casuariin (**152**) as an intermediate [25]. Oenothein B, the dimer of tellimagrandin I (**149**), has been isolated from the leaves of *E. consideniana, E. viminalis*, and *E. alba*. It exhibits antiviral and antitumour activities [25, 50].



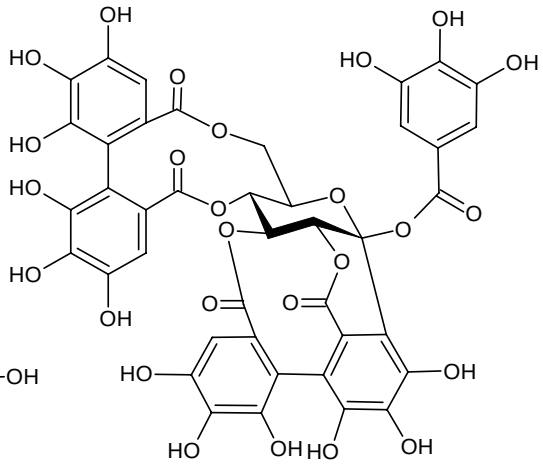
149



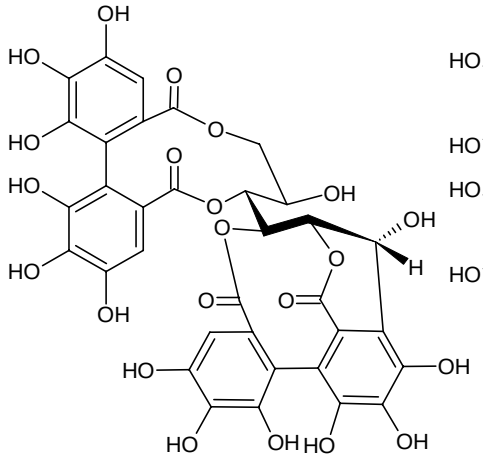
150



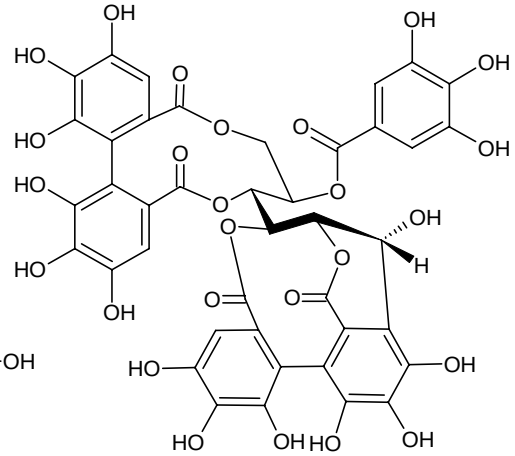
151



152

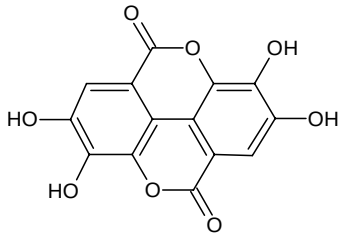


153



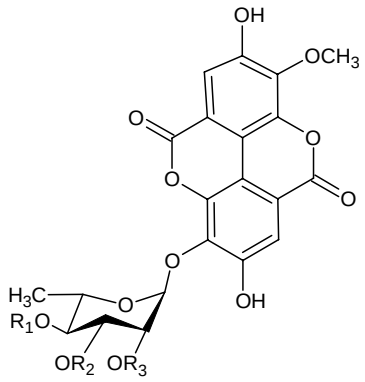
154

Isomers of trigalloyl HHDP-glucose have been detected in the wood of *E. globulus* and *E. nitens*. These compounds consist of HHDP-glucose and a trisgalloyl group such as valoneic acid dilactone, targallic acid, sanguisorbic acid, or flavogallic dilactone. Eucalbanin A and its isomer cornusiin B have been detected in fruit extracts of *E. alba*. Valoneic acid dilactone is producted by the hydrolysis of cornusiin B or oenothein B whereas the hydrolysis of tergallic acid dilactone leads to the eucalbanin A isomer [25]. Grandinin has been isolated from the leaves and pterocarinin from the bark of *E. consideniana* and *E. viminalis* [50]. Gallic acid (**148**) and its derivates exhibit considerable inhibitory activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl radical, and superoxide anion radical scavenging assays, an indication that most of these compounds exhibit strong antioxidant activity [55]. Ellagic acid (**155**) is the dilactone of hexahydroxydiphenic acid. It is a dimer composed of two molecules of gallic acid (**148**) bound with a C-C bond. Ellagic acid (**155**) is the basic structure of the hydrolysable ellagotannins. It is isolated from *Eucalyptus* bark as a by-product of the pulp industry. Free ellagic acid has been detected in the fruits of *E. globulus*. Ethyl acetate fractions obtained from acidic hydrolysates of *E. globulus* wood show potent DPPH radical scavenging activity, especially the fractions which contain ellagic acid (**155**) as the main phenolic component. The antioxidative activity of ellagic acid (**155**) is stronger than that of α-tocopherol. It is an effective inhibitor of *in vitro* lipid peroxidation and a potent inhibitor of the perferryl-dependent initiation step of NADPH-dependent microsomal lipid peroxidation. Tested *in vitro*, compound (**155**) inhibit the mutagenicity and carcinogenicity of the potent carcinogen benz[α]pyrene-7,8-diol-9,10-epoxide [2, 25, 56]. It significantly potentiated the effects of quercetin (**125**) in reducing both the proliferation and the viability and in inducing apoptosis (at 5 and at 10 μmol/mL) [57].



155

Four rhamnosides of ellagic acid (**155**) determined to be 3-*O*-methylellagic acid 3′-*O*-α-rhamnopyranoside (**156**), 3-*O*-methylellagic acid 3′-*O*-α-3′′-O-acetylrhamnopyranoside (**157**), 3-*O*-methylellagic acid 3′-*O*-α-2′′-*O*-acetylrhamnopyranoside (**158**), and 3-*O*-methylellagic acid 3′-*O*-α-4′′-*O*-acetylrhamnopyranoside (**159**) have been isolated from the bark of the stem of *E. globulus*. Derivates of methylellagic acid have been detected in the leaves and fruits of *E. globulus* and in the leaves of *E. camaldulensis* and *E. rudis*. The antioxidant activity of these compounds was evaluated by measuring the



156 R_1: H R_2: H R_3: H

157 R_1: H R_2: $COCH_3$ R_3: H

158 R_1: H R_2: H R_3: $COCH_3$

159 R_1: $COCH_3$ R_2: H R_3: H

inhibition of lipid peroxidation via a thiobarbituric acid method using rat liver microsomes, with IC_{50} values of 0.010-0.014 mg/mL [25, 58].

β-diketones

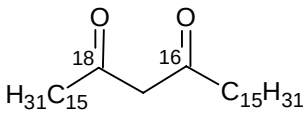
The waxy layer that covers the surface of *Eucalyptus* leaves makes up the cuticle of the epidermal cells and provides them with antioxidative protection. The waxes of several *Eucalyptus* species show strong antioxidative effects [2]. Plants produce waxes *via* different metabolic pathways. Among the common components of plant cuticular waxes are n-alkanes, iso-alkanes, alkenes, monoketones, β-diketones, primary and secondary alcohols, wax esters, normal fatty acids, and ω-hydroxy acids [59]. In the leaf waxes of *Eucalyptus* and *Acacia* species and also in many graminaceous species, β-diketones are particularly dominant [7]. The content of leaf wax in *E. globulus* accounts for more than 0.3% of the weight of the fresh leaves [2]. Long chain β-diketones are the main components of most leaf waxes from *Eucalyptus* species [7]. β-diketones with long alkyl side chains show significantly more antioxidative activity than other β-diketone analogues. Most β-diketones that have no hydrophobic side chains can also act as chelating agents, but they show no antioxidative activity [59]. The Japanese scientists Osawa and Namiki have isolated and identified the major β-diketone homologue n-tritriacontane-16,18-dione (**160**) in leaf wax extracted from *E. globulus* [59]. A correlation between the concentration of n-tritriacontane-16,18-dione and antioxidative activity has been observed for almost all of the *Eucalyptus* species tested [2]. n-hentriacontane-14,16-dione (**161**) and n-pentatriacontane-16,18-dione (**162**) are the minor homologues in *Eucalyptus* species. Diketones from the leaf waxes of *E. ovata* tend to contain a higher proportion of the C35 homologue, while on the contrary *E. viminalis* leaf waxes include the C31 homologue. The C37 homologue occurs in sufficient proportion in *E. rodwayi* and *E. beriberi*. A significant proportion of the C29 homologue has been detected in the waxes of *E. gunnii, E. archeriand*, and *E. urnigera* [7].

4-hydroxy-tritriacontane-16, 18-dione (**163**), obtained from the leaf wax of *E. globulus*, shows considerable antioxidative activity in an aqueous/alcohol system, when measured by thiocyanate and thiobarbituric acid methods [59]. The use of β-diketones as antioxidative agents in food systems has been studied by Osawa and Namiki. β-diketones show strong antioxidative activity in an oil/aqueous food system, such as mayonnaise or salad dressing [59].

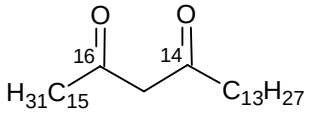
Triterpenes

The lipophilic extracts of the bark and fruits of *E. globulus* are mainly composed of triterpenoids, followed by smaller amounts of fatty acids, fatty alcohols, and aromatic compounds [60]. Triterpenoids have also been isolated from the leaves and fruits of *E. camaldulensis*, *E. grandis, E. urograndis*, and *E. maidenii*. The content ranges from 1.2 g/kg to 12.2 g/kg in the surface layers of the bark of the branches of *E. globulus*, where they are most highly concentrated. Triterpenic compounds include mainly triterpenic acids with lupane, ursane, and oleanane skeletons [60, 61].

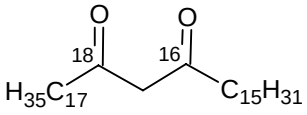
The major components with a lupane skeleton are betulonic acid (**164**), betulinic acid (**165**) 3-acetylbetulinic acid (**166**), and betulinic acid



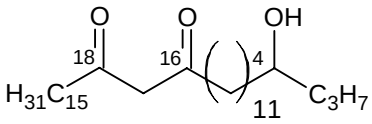
160



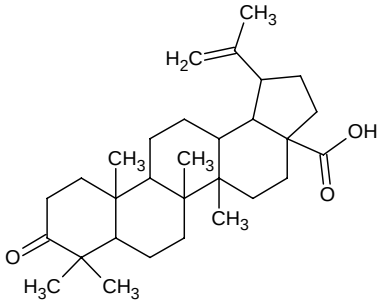
161



162



163



164

methyl ester (**167**). They have many unique and potentially usable biological effects and pharmacological activities. Betulonic acid (**164**) has been detected in amounts of 0.1-11.6 g/kg. Betulinic acid (**165**) has been detected in considerable amounts (0.2-8.1 g/kg) in the residues of extracts of *E. globulus* [60, 61].

Oleanolic acid (**168**), 3-acetyloleanolic acid (**169**), erythrodiol (**170**), β-amyrin (**171**), and cis-p-methoxy-cinnamoyloxy oleanolic acid methyl-ester (**172**) are among the compounds with an oleanane type of skeleton. Oleanolic acid (**168**) (0.2–18.8 g/kg) is the main component of all residues of *E. globulus* extracts [17, 61].

The triterpenoids with an ursane skeleton include ursolic acid (**173**), 3-acetylursolic acid (**174**), ursolic acid methyl ester (**175**), uvaol (**176**), cis-p-methoxy-cinnamoyloxyoleanolic acid methyl ester (**177**), and trans-p-methoxy-cinnamoyl-oxyoleanolic acid methyl ester (**178**) [17, 61]. Triterpenic acids, especially ursolic acid (**173**), are the major components of the epicuticular waxes of the fruits of *E. globulus* and are identified as minor components in the epicuticular wax from its leaves [61]. Ursolic acid (**173**) is also the most abundant component in extracts of the bark of several *Eucalyptus* species, varying in amount from 1.9 g/kg in *E. urograndis* up to 3.6 g/kg in *E. maidenii* [60]. 3-acetylursolic acid (**173**) is also an abundant component of *E. globulus* (0.2-6.9 g/kg) [61].

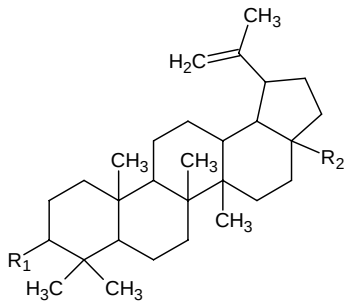
Compounds **177** and **178** have been isolated from the benzene-soluble fraction of the acetone extract of *E. globulus* wood [17].

Research on the constituents of the fresh leaves of *E. camaldulensis* var. *obtusa* led to the isolation of an ursane-type triterpene with a structure established as 2α,3β,7β-trihydroxy-11α-methoxyurs-12-en-28-oic acid (**179**). It is interesting because triterpenoids with a β-oriented hydroxyl group at C-7 are very rare in nature. This compound is easily transformed into 2α,3β,7β-trihydroxyurs-11-en-28,13β-olide in solution at room temperature [62].

3*β*-acetoxy-urs-11,13(18)-dien-28-oic acid (**180**), also named eucamaldulenoic acid, has been isolated from the fruits of *E. camaldulensis*. The desacetyl derivative of this compound has been prepared by pyrolysis of ursolic acid lactone. A similar triterpene with an oleanane skeleton that has a hydroxyl at C-3 has been isolated from the plant *Junellia tridens* [63].

3*β*-hydroxy-urs-11-en-28,13*β*-olide **(181)** has been isolated from the fruits of *E. camaldulensis*. It is also present in other *Eucalyptus* species, e.g., *E. tereticornis* [63].

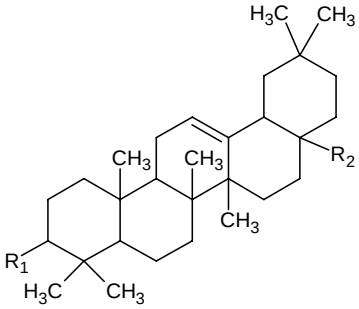
Triterpenoid components of *Eucalyptus* extracts have been investigated for various biological activities. Triterpenoids with antimicrobial activity have been isolated from *E. tereticornis* [63]. Several triterpenoid that are similar to compounds isolated from *Eucalyptus* have been obtained from *Junellia tridens*; they demonstrate



165 R_1: OH R_2: COOH

166 R_1: $OCOCH_3$ R_2: COOH

167 R_1: OH R_2: $COOCH_3$

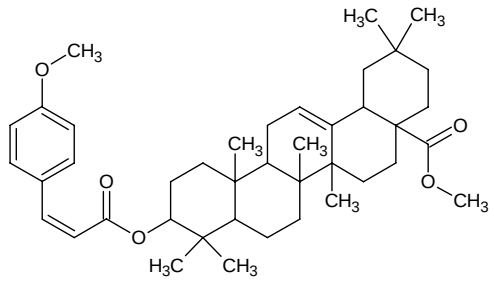


168 R_1: OH R_2: COOH

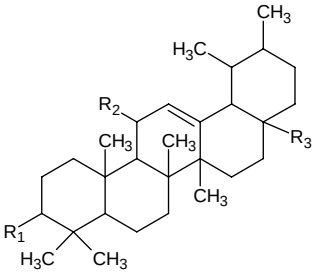
169 R_1: $OCOCH_3$ R_2: COOH

170 R_1: OH R_2: CH_2OH

171 R_1: OH R_2: CH_3



172

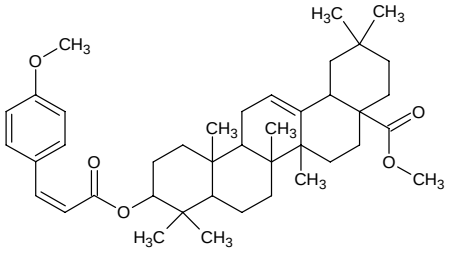


173 R_1: OH R_2: H R_3: COOH

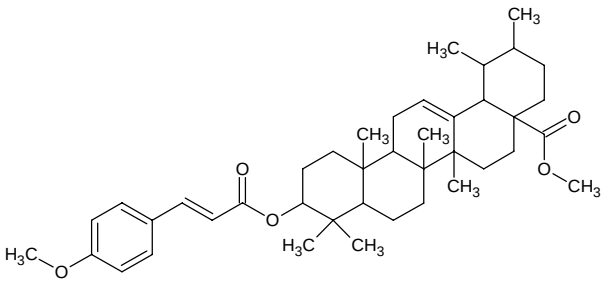
174 R_1: $OCOCH_3$ R_2: H R_3: COOH

175 R_1: $OCOCH_3$ R_2: H R_3: $COOCH_3$

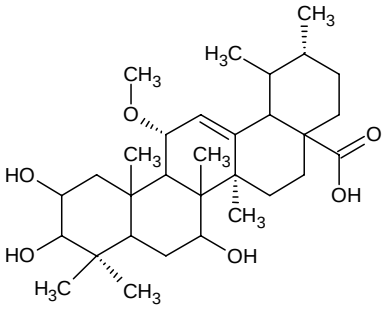
176 R_1: OH R_2: H R_3: CH_2OH



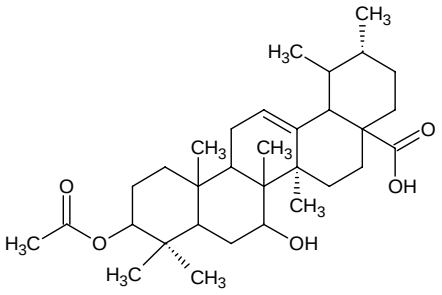
177



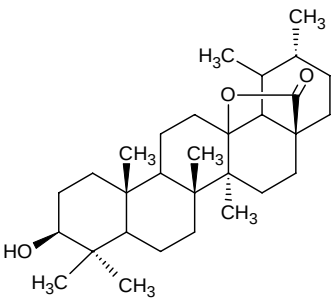
178



179



180



181

antitubercular activity against *Mycobacterium tuberculosis* [63]. The most active are oleanonic acid, 3-epi-oleanonic acid, and oleanolic acid (**168**). Triterpenoids with spasmolytic activity have been isolated from the leaves of *E. camaldulensis* var. *obtusa* [63]. Triterpenoids obtained from *E. globulus* have been evaluated for their antiproliferative activity against ovarian cancer cell lines. The greatest activities are shown by ursolic acid (**173**) and oleanolic acid (**168**) [63].

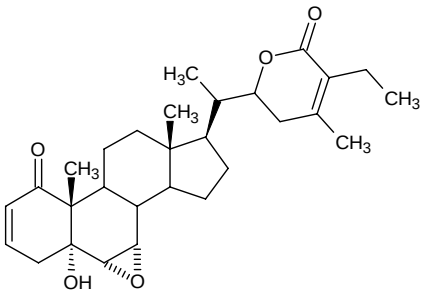
Steroidal compounds

Withanolides are a group of natural C-28 steroidal lactones with an ergostane type of skeleton, isolated from various genera of *Solanaceae*. Compounds highly modified in both the steroid nucleus and the side chain can be produced by biogenetic transformations. These compounds exhibit diverse biological activities, such an antiproliferative, antifeedants, and cancer-chemoprotective activity [64]. Two withanolides A have been isolated from the bark of *E.globulus* by supercritical fluid extraction. The major compound (**182**) is the steroidal lactone (+)-6*α*, 7*α*-epoxy-5*α*-hydroxy-1-oxowitha-2, 24-dienolide. The minor compound (**183**) has an ethyl group at C-25 and a hydroxyl group at C-17 [65].

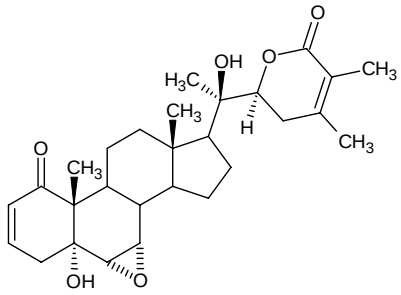
β-sitosterol and 3-*O*-*β*-D-glucopyranosyl-β-sitosterol have been isolated from the cyclohexane fraction of the ethanolic extract of *E. globulus* fruits [21]. Tetraacetyldaucosterol has been isolated from the acetylated benzene-soluble fraction of an acetone extract of the wood of *E. globulus* [17].

Cyanogenic glycosides

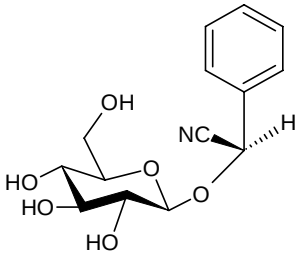
Many *Eucalyptus* species contain cyanogenic compounds capable of producing toxic cyanide when the leaf tissue is disrupted by chewing. There have been numerous reports of livestock poisoned by eating the leaves of *E. cladocalyx*, and there have been some cases of koalas dying after eating cyanogenic *E. viminalis*. (*R*)-prunasin (**184**) has been identified in 12 of the 18 *Eucalyptus* species found to be cyanogenic. Sambunigrin (**185**) has also been found in some *Eucalyptus* species. In addition to (*R*)-prunasin (**184**) and sambunigrin (**185**), *E. camphora* contains the diglycoside amygdalin (**186**). With only a few exeptions, the taxonomic distribution of cyanogenic species within this genus is restricted to the subgenus *Symphyomyrtus* [66].



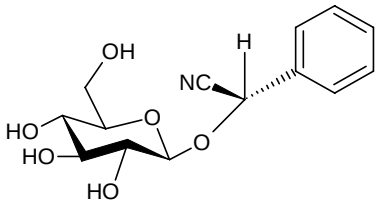
182



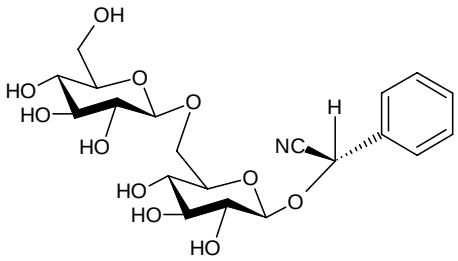
183



184



185



186

CONCLUSION

Eucalyptus plants can be used as a source of broad spectrum of secondary metabolites. In addition to the well-known volatile terpenoids, many other interesting chemical substances are present. The essential oils comprise terpenes, β-triketones, and simple acylphloroglucinols. Acylphloroglucinol-terpene adducts, acylphloroglucinol dimers, flavonoids, tannins, triterpenes, and glucose esters of oleuropeic acid are non-volatile *Eucalyptus* compounds. As visible from our review, different secondary metabolites isolated from *Eucalyptus* trees have been tested for their antitumor, anti-inflammatory, and antioxidative activities, and they have also shown antibacterial, antifungal, antileishmanial, and antiviral effects. We hope that this review would inspire further research touching isolation of *Eucalyptus* content compounds and elucidating their bioactivities.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

REFERENCES

1. Mohamed, G. A. and Ibrahim, S. R. M. 2007, AKRIVOC, 15, 281.
2. Coppen, J. J. W. 2002, Eucalyptus: The Genus Eucalyptus. Taylor & Francis, London, UK.
3. Gilles, M., Zhao, J., An, M. and Agboola, S. 2010, Food Chemistry, 119, 731.
4. Yao, L., Jiang, Y., D´Arcy, B., Singanusong, R., Datta, N., Caffin, N. and Raymont, K. 2004, J. Agric. Food Chem., 52, 210.
5. Bruneton, J. 1999, Pharmacognosy, Phytochemistry, Medicincal Plants. 2nd Edition. Lavoisier Publishing Inc., Paris, 263.
6. Medhi, S. M., Reza, S., Mahnaz, K., Reza, A. M., Abbas, H., Fatemeh, M. and Hassan, V. 2010, Asian. Pac. J. Trop. Med., 3, 841.
7. Ghisalberti, E. L. 1996, Phytochemistry, 41, 7.
8. Takasaki, M., Konoshima, T., Etoh, H., Singh, I. P., Tokuda, H. and Nishino, H. 2000, Cancer Letters, 155, 61.

9. Lima, F. J. B., Brito, T. S., Freire, W. B. S., Costa, R. C., Linhares, M. I., Sousa, F. C. F., Lahlou, S., Leal-Cardoso, J. H., Santos, A. A. and Magalhães, P. J. C. 2010, Fitoterapia, 81, 649.
10. Cheng, S.-S., Huang, C.-G., Chen, Y.-J., Ju, J.-J., Chen, W.-J. and Chang, S.-T. 2009, Bioresource Technology, 100, 452.
11. Tyagi, A. K. and Malik, A. 2011, Food Chemistry, 126, 228.
12. Jurgens, U. R., Dethlefsen, U., Steinkamp, G., Gillisen, A., Repges, R. and Vetter, H. 2003, Respiratory Medicine, 97, 250.
13. Dewick, P. M. 2002, Medicinal Natural Products. A biosynthetic approach. John Wiley & Sons, Inc., Chichester, UK.
14. Tohidpour, A., Sattari, M., Omidbaigi, R., Yadegar, A. and Nazemi, J., 2010, Phytomedicine, 17, 142.
15. Vilela, G. R., de Almeida, G. S., D´Arce, M. A. B. R., Moraes, M. H. D., Brito, J. O., da Silva, M. F. das G. F., Silva, S. C., Piedade, S. M. de S., Calori-Domingues, M. A. and da Gloria, E. M. 2009, J. Stored Products Res., 45, 108.
16. Elaissi, A., Salah, K. H., Mabrouk, S., Larbi, K. M., Chemli, R. and Harzallah-Skhiri, F. 2011, Food Chemistry, 129, 1427.
17. Santos, G. G., Alves, J. C. N., Rodilla, J. M. L., Duarte, A. P., Lithgow, A. M. and Urones, J. G. 1997, Phytochemistry, 44, 1309.
18. Macedo, I. T. F., Bevilaqua, C. M. L., de Oliveira, L. M. B., Camurça-Vasconcelos, A. L. F., Vieira, L. da S., Oliveira, F. R., Queiroz-Junior, E. M., Tomé, A. da R. and Nascimento, N. R. F. 2010, Veterinary Parasitology, 173, 93.
19. Mulyaningsih, S., Sporer, F., Zimmermann, S., Reichling, J. and Wink, M. 2010, Phytomedicine, 17, 1061.
20. Goodger, J. Q. D. and Woodrow, I. E, 2011, Phytochemistry, 72, 2259.
21. Guo, Q.-M. and Yang, X.-W. 2006, Biochemical Systematics and Ecology, 34, 543.
22. Hakki, Z., Cao, B., Heskes, A. M., Goodger, J. Q. D., Woodrow, I. E. and Williams, S. J. 2010, Carbohydrate Research, 345, 2079.
23. Goodger, J. Q. D., Cao, B., Jayadi, I., Williams, S. J. and Woodrow, I. E. 2009, Phytochemistry, 70, 1187.
24. Tian, L. W., Zhang, Y.-J., Wang, Y.-F., Lai, Ch.-Ch. and Yang Ch.-R. 2009, J. Nat. Prod., 72, 1608.
25. Boulekbache-Makhlouf, L., Meudec, E., Chibane, M., Mazauric, J.-P., Slimani, S., Henry, M., Cheynier, V. and Madani, K. 2010, J. Agric. Food Chem., 58, 12615.
26. Hasegawa, T., Takano, F., Takata, T., Niiyama, M. and Ohta, T. 2007, Phytochemistry, 69, 747.
27. Ito, H., Koreishi, M., Tokuda, H., Nishino, H. and Yoshida, T. 2000, J. Nat. Prod., 63, 1253.
28. Yang, X.-W., Guo, Q.-M., Wang, Y., Xu, W., Tian, L. and Tian, X.-J. 2007, Bioorganic & Medicinal Chemistry Letters, 17, 1107.
29. Tomko, J. 1999, Farmakognózia 2. Opravené vydanie, Osveta, Martin, Slovak Republic.
30. Sidana, J., Singh, S, Arora, S. K., Foley, W. J. and Singh, I. P. 2011, Fitoterapia, 82, 1118.
31. Lawler, I. R., Eschler, B. M., Schliebs, D. M. and Foley, E. J. 1999, J. Chem. Ecology, 25, 2561.
32. Tian, L. W., Zhang, Y.-J., Qu, Ch., Wang, Y.-F. and Yang, Ch.-R. 2010, J. Nat. Prod., 73, 160.
33. Mitaine-Offer, A. C., Djoukeng, J. D., Tapondjou, L. A., Bouda, H., Lerche, H., Lontsi, D. and Lacaille-Dubois, M.-A. 2003, Biochemical Systematics and Ecology, 31, 1457.
34. Foley, W. J. 2009, Sideroxylonal: A Potentially Valuable Extract from Oil-Mallee Crops. Union Offset Printing., Canberra, Australia.
35. Sidana, J., Rohilla, R. K., Roy, N., Barrow, R. A., Foley, W. J. and Singh, I. P. 2010 Fitoterapia, 81, 878.
36. Eschler, B. M. and Foley, W. J. 1999, Aust. J. Chem., 52, 157.
37. Chiba, K., Arakawa, T. and Tada, M. 1998, J. Chem. Soc., Perkin Trans 1, 2939.

38. Bharate, S. B., Khan, S. I., Tekwani, B. L., Jacob, M., Khan, I. A. and Singh, I. P. 2008, Bioorganic & Medicinal Chemistry, 16, 1328.
39. Bharate, S. B., Bhutani, K. K., Khan, S. I., Tekwani, B. L., Jacob, M. R., Khan, I. A. and Singh, I. P. 2006, Bioorganic & Medicinal Chemistry, 14, 1750.
40. Singh, I. P., Umehara, K., Asai, T., Etoh, H., Takasaki, M. and Konoshima, T. 1998, Phytochemistry, 47, 1157.
41. Umehara, K., Singh, I. P., Etoh, H., Takasaki, M., and Konoshima, T. 1998, Phytochemistry, 49, 1699.
42. Kokumai, M., Haruna, M and Ito, K. 1991, J. Nat. Prod., 54, 1082.
43. Nishizawa, M., Emura, M., Kan, Y., Yamada, H., Ogawa, K. and Hamanaka, N. 1992, Tetrahedron Letters, 33, 2983.
44. Osawa, K., Yasuda, H., Morita, H., Takeya, K. and Itokawa, H. 1995, Phytochemistry, 40, 183.
45. Croizer, A., Clifford, M. N. and Ashihara, H. 2006, Plant secondary metabolites: Occurrence, Structure and Role in the Human Diet. Blackwell Publishing Ltd, Oxford, UK.
46. Benyahia, S., Banayache, S., Banayache, F., Quintana, J., López León, F., Hernández, J. C., Estévez, F. and Bermejo, J. 2004, J. Nat. Prod., 67, 527.
47. Cushnie, T. P. T. and Lamb, A. J. 2011, Int. J. Antimicrob. Agents, 38, 99.
48. Vasquez, G., Fontenla, E., Santos, J., Freire, M. S., González-Álvarez, J. and Antorrena, G. 2008, Industrial Crops and Products, 28, 279.
49. Santos, S. C. and Waterman, P. G. 2001, Fitoterapia, 72, 316.
50. Santos, S. C. and Waterman, P. G. 2001, Fitoterapia, 72, 95.
51. Courtney, J. L., Lassak, E. V. and Speirs, G. B. 1983, Phytochemistry, 22, 947.
52. Martos, I., Ferreres, F. and Tomás-Berberán, F. A. 2000, J. Agric. Food Chem., 48, 1498.
53. Ahlem, S., Khaled, H., Wafa, M., Sofiane, B., Mohamed, D., Jean-Claude, M. and Abdelfattah, E. F. 2009, Chemico-Biological Interactions, 181, 71.
54. Amakura, Y., Umino, Y., Tsuji, S., Ito, H., Hatano, T., Yoshida, T. and Tonogai, Y. 2002, Food Chemistry, 77, 47.
55. Singab, A. N., Ayoub, N., Al-Sayed, E., Martiskainen, O., Sinkkonen, J. and Pihlaja, K. 2011, Rec. Nat. Prod., 5(4), 271.
56. Cruz, J. M., Domínguez, H. and Parajó, J. C. 2006, Food chemistry, 90, 503.
57. Cseke, L. J., Kirakosyan, A., Kaufman, P. B., Warber, S. L., Duke, J. A. and Brielmann, H. L., 2006, Natural Products from Plants, Second Edition, Taylor & Francis Group, Boca Raton, USA.
58. Kim, J. P., Lee, I.-K., Yun, B.-S., Chung, S.-H., Shim, G.-S., Koshino, H. and Yoo, I.-D. 2001, Phytochemistry, 57, 587.
59. Osawa, T. and Namiki, M. 1985, J. Agric. Food Chem., 33, 777.
60. Domingues, R. M. A., Sousa, G. D. A., Silva, C. M., Freire, C. S. R., Silvestre, A. J. D. and Neto, C. P. 2011, Industrial Crops and Products, 33, 158.
61. Domingues, R. M. A., Sousa, G. D. A., Freire, C. S. R., Silvestre, A. J. D. and Neto, C. P. 2010, Industrial Crops and Products, 31, 65.
62. Siddiqui, B. S., Sultana, I. and Begum, S. 2000, Phytochemistry, 54, 861.
63. Topçu, G., Yapar, G., Türkmen, Z., Gören, A. C., Öksüz, S., Schilling, J. K. and Kington, D. G. I. 2011, Phytochemistry Letters, 4, 421.
64. Garcia, M. E., García, M. E., Barboza, G. E., Oberti, J. C, Ríos-Luci, C., Padrón, J. M., Nictora, V. E., Estévez-Braun, A. and Ravelo, A. G. 2012, Phytochemistry, 76, 150.
65. Vankar, P. S., Srivastava, J., Molčanov, K and Kojic-Prodić, B. 2009, Phytochemistry Letters, 2, 67.
66. Gleadow, R. M., Haburjak, J., Dunn, J. E., Conn, M. E. and Conn, E. E. 2008, Phytochemistry, 69, 1870.