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BIOACTIVE ACYLPHLOROGLUCINOL DERIVATIVES FROM *EUCALYPTUS* SPECIES

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Key Word Index—*Eucalyptus*; Myrtaceae; acylphloroglucinols; G-regulators; sideroxylonals; euglobals; macrocarpals.

Abstract—The acylphloroglucinol derivatives produced by *Eucalyptus* species are reviewed. Aspects of their chemistry, stereochemistry, biological activity and biogenesis are discussed.

INTRODUCTION

Eucalyptus L'Héritier (Myrtaceae) is one of the world's most important and most widely planted genera. In Australia, this genus is the second largest, after *Acacia*, and contains about 750 species [1]. All are native to Australia and some islands to the north of it. *Eucalyptus* species grow in a wide range of climatic conditions and are widely distributed throughout Australia, except for the arid zone of central Australia and in regions of dense rainforests. Since the 1850s, it has been successfully introduced into 90 countries worldwide [1].

The main uses of *Eucalyptus* species are in forestry (timber, fuel, paper pulp), environmental planting (water and wind erosion control), amenity planting, as sources of essential oil (medicinal, perfumery oils), in floriculture and, to a lesser extent, for art and crafts. Only about 20 *Eucalyptus* species have been exploited commercially, mostly for their essential oils [2, 3]. Because of their fast growth *Eucalyptus* trees, e.g. *E. globulus* Labill., and *E. grandis* W. Hill ex Maiden, are being established in plantations in many countries, mainly for pulp and paper production. As a consequence, there is potential for development of secondary industries to recover not only the essential oils as a by-product, but also other potentially useful compounds available from the leaves and bark. The sesquiterpene aromadendrene, the main component in a commercially available distillation tail of the oil of *E. globulus*, has been exploited as a chiral pool synthon for the synthesis of several sesquiterpene derivatives [4–7].

The sustained interest in *Eucalyptus* is evidenced by two recent publications. One details the history, research and commercial aspects of *Eucalyptus* leaf oil and contains a compilation of the composition of the essential oils from 111 species [2]. Another is an indexed bibliography on oil-bearing eucalypts and their multipurpose use [3]. For those interested in an overview of the phytochemistry of *Eucalyptus* species in particular, and the Myrtaceae family in general, the extensive compilations by Hegnauer [8, 9] are recommended.

In this review, attention is focused on a group of secondary metabolites, which, for the most part, are unique to *Eucalyptus* species. This group includes cyclic polyketones, simple acylphloroglucinols, and complex acylphloroglucinol derivatives originating from mixed biogenesis involving the terpene pathways. Aspects of their chemistry, stereochemistry, biogenesis and biological activity are discussed.

HISTORICAL PERSPECTIVE

Eucalyptus species have been utilized for cultural and medicinal purposes and as a food source possibly ever since Aboriginal people first occupied Australia some 40 000 years ago. Spears were fashioned from the slender stems of some species, roots were dug out and cut up as a source of water, and the root bark was eaten. Seeds of some species were eaten raw or after drying and manna was collected from a number of species [1]. The bark and leaves of selected species were used to treat colds, influenza, toothaches, snakebites, fevers, diarrhoea and other complaints [1, 10–15]. Kino, the astringent exudation produced after pathological or mechanical injury to the wood, was used in a powdered form or paste on open sores and as an aqueous solution for external or internal complaints [10, 11].

William Dampier in 1688 referred to the eucalypts along the north-western coast of Australia as Dragon-trees and likened the kino to dragon's blood [16]. Joseph

Banks in 1770 drew the same analogy (*sanguis draconis*) and mistakenly gave the name gum to these phenolic exudates of eucalypts [16]; hence, the popular name 'gum' for several *Eucalyptus* species, e.g. sugar gum for *E. cladocalyx* F. Muell. Botanically, the genus was first named *Aromadendron* by Dr Anderson, the surgeon of Captain Cook's second and third expeditions. *Eucalyptus* (Greek: *eu*, well; *calyptos*, I cover; in reference to the flower bud which has an operculum) was coined by L'Héritier in 1788 [17]. The alternative names, *Eudesmia* proposed by Robert Brown in 1814 and *Symphyomyrtus* by Schauer in 1844 [17], have been retained for two of the subgenera.

Following European settlement in Australia in 1788, the medicinal value of *Eucalyptus* oil was soon recognized and several efforts were made to exploit this. Interest in the chemistry of the essential oils began with Cloez in 1870, who named the principal component of *E. globulus* oil eucalyptol. It was identified as cineole by Jahns in 1884 [18]. Essential oils from different species are rich sources of many terpenes, particularly citronellal, α-phellandrene, α-terpineol, piperitone, α-and β-eudesmols, aromadendrene, globulol and spathulenol [2]. *Eucalyptus* species also produce a range of polyphenols, flavonoids, gallo- and ellagi-tannins and triterpenes [8, 9]. In recent years, a number of biologically active secondary metabolites have been isolated from *Eucalyptus* species, sparking renewed interest in the phytochemistry of this genus.

CYCLIC POLYKETONES

The essential oils from *Eucalyptus* species are well known because of their terpene content. Less recognized is the fact that these essential oils, and those from other myrtaceous plants, contain an array of cyclic polyketones, 'β-triketones', which are of some historical, structural and chemotaxonomic interest. They differ in the nature of the side chain, the number of nuclear methyl groups and level of oxygenation (Fig. 1).

The first such compound was isolated by Robinson and Smith [19] in 1914 from what they thought to be *E. risdonii* Hook, f. and *E. linearis* Dehnh., but was probably *E. tasmanica* Blakely (syn. *E. risdoni* var. *elata* Bentham). Initially considered to be a phenol, and named tasmanol, it was later shown by Birch and Elliot [20] to be a β-triketone and the compound was renamed tasmanone. The structure was finally elucidated in 1963, and spectroscopic studies, including NMR, showed it to exist, in solution, as a mixture of tautomers **1a** and **2a** (7:3) in slow, but continuous equilibration (Scheme 1) [21, 22]. Tasmanone is of some historical significance since a consideration of the structure of it and related compounds first led to the hypothesis of biological *C*-methylation. In fact, labelling studies showed that [Me-^{14}C]methionine was involved in the introduction of the three nuclear methyl groups and the *O*-methyl group [23]. The occurrence of tasmanone is limited (Table 1), but it can constitute up to 40% of the essential oil of *E. camfieldii*

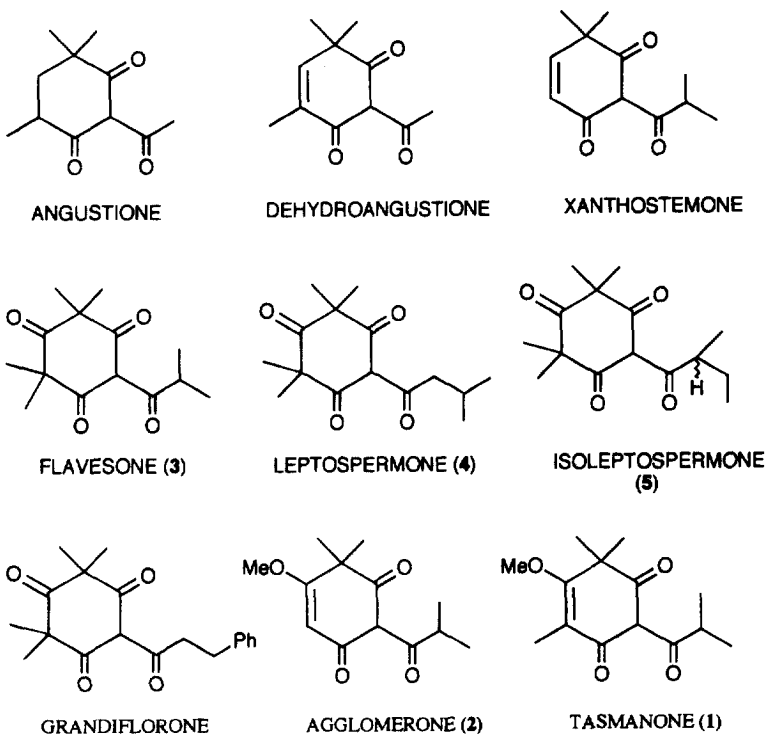


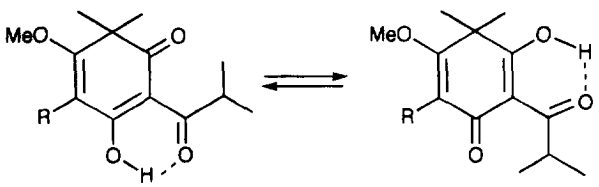
Fig. 1. Cyclic polyketones occurring in myrtaceous species. Numbered structures represent compounds occurring in *Eucalyptus* species. For the origin of the others, see refs [35–37].

Maiden [21]. Syntheses of tasmanone have been described [24].

Agglomerone (**2**) is an optically inactive solid, which was first isolated from *E. agglomerata* Maiden and *E. mckieana* Blakely where it is the major component of the steam volatile material, 80 and 50%, respectively [25]. As found for tasmanone, agglomerone exists in solution as a mixture of equilibrating tautomers (**2a**, **2b**; Scheme 1) [22, 25]. A synthesis of agglomerone has been described [26].

Leptospermone (**4**) was first isolated from *Leptospermum* species from Australia and New Zealand [27–29] and the structure was defined in 1965 [30]. Leptospermone co-occurs with the lower homologue flavesone (**3**) in *Eucalyptus* species [30] and both compounds exist as tautomers in solution. Flavesone [31, 32] and leptospermone [33, 34] have been synthesized. Interestingly, isoleptospermone (**5**) has been detected in the essential oil of *E. grandis* where it co-occurs with flavesone [2]. Both of these compounds are of some significance given the occurrence in the same species of a group of potent germination regulators which have been named G-regulators (see below).

These and other 'β-triketones' (Fig. 1) are commonly found in *Eucalyptus* species (Table 1) and related members of the Myrtaceae such as *Leptospermum*, *Xanthostemon*, *Darwinia*, *Backhousia*, *Calytrix*, *Baeckea* and *Melaleuca* [35–37].



Scheme 1. Equilibration between tautomers of tasmanone (**1**) and agglomerone (**2**).

EPIDIOXY GROWTH REGULATIONS

Species of *Eucalyptus* root easily form stem tissue provided that leafy cuttings are taken from young seedlings. With the exception of the tropical species *E. deglupta* Blume, root strike will occur only rarely if cuttings are taken from older plant material. It was suggested that this effect was due to the presence of endogenous inhibitors, which accumulate in adult leaves and in the tissue forming the base of the cuttings. The ability of seedlings of *E. deglupta* to root very easily in water provided the basis for a bioassay to monitor the presence of inhibitors in other *Eucalyptus* species [38, 39].

Subsequent chemical investigations of the leaf tissue from mature plants of *E. grandis* revealed the presence of three such inhibitors which were named 'G-inhibitors' or 'G-regulators' (G = *grandis*) and abbreviated to G-1, G-2 and G-3 (**6**–**8**) [40]. All three inhibitors were isolated as racemic mixtures. The structure and relative stereochemistry of G-1 (**6**) was secured by X-ray crystallographic methods [41]. The diastereomeric relationship between G-2 (**7**) and G-1 was established through chemical equilibration, and the structure of G-3 (**8**) was deduced by comparison of spectroscopic data with those of G-1 and G-2. Since the compounds were not optically active, the possibility that they might be produced as artefacts during isolation was considered. Supporting evidence for this came from synthetic studies in which it was shown that **9** (Scheme 2) quickly formed the epidioxide on exposure to air [42–44]. However, isolation of the inhibitors from *E. grandis* in an atmosphere of $^{18}O_2$ did not result in any detectable incorporation of isotopic oxygen in the peroxide linkage. It was concluded that, for the most part, the inhibitors are not produced as artefacts. Interestingly, it has been shown that at low concentrations (*ca* 5×10^{-6} M) they exhibit auxin-like (promotory) activity, but at levels of 5×10^{-5} M they display abscisic acid (inhibitory) activity [45]. In view of this, the compounds may be more correctly regarded as growth regulators. From the mature leaves of *E. grandis* large amounts (7500 $\mu g\,g^{-1}$, 2×10^{-2} mol kg^{-1} fresh weight) of the G-regulators can be isolated. In immature leaves of *E. grandis* and in other *Eucalyptus* and myrtaceous plants,

Table 1. Distribution of cyclic polyketones in *Eucalyptus* species

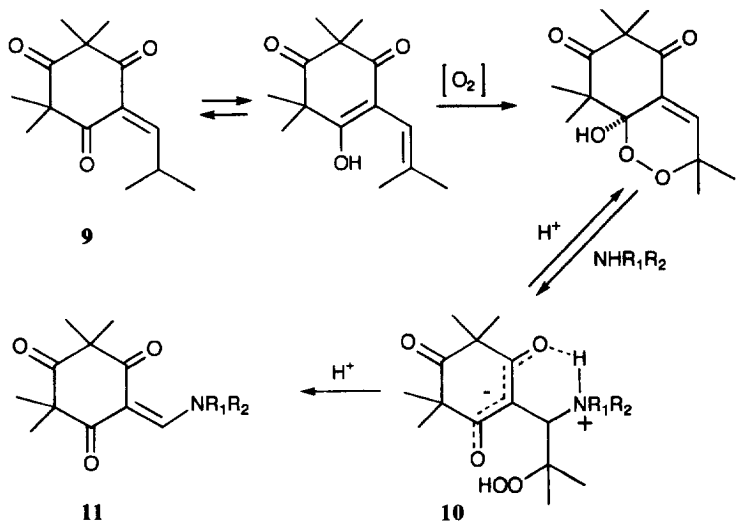
Tasmanone (**1**)
- *E. camfieldii* Maiden [35]
- *E. cloeziana* F. Muell. [2]
- *E. oblonga* D.C. [35]
- *E. tasmanica* Maiden [35]

Agglomerone (**2**)
- *E. agglomerata* Maiden [2, 35, 36]
- *E. camerooni* Blakely & McKie [35]
- *E. mckieana* Blakely [35, 36]
- *E. oblonga* D.C. [35]

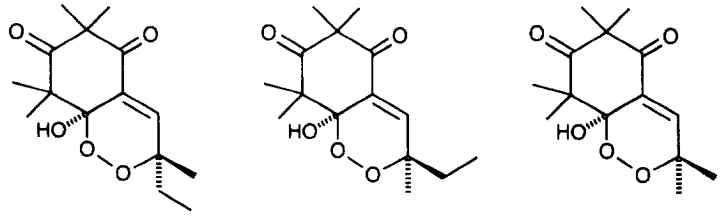
Flavesone (**3**)
- *E. decorticans* Maiden [35, 36]
- *E. grandis* W. Hill ex Maiden [2]
- *E. oblonga* D.C. [35]

Leptospermone (**4**)
- *E. caliginosa* Blakely & McKie [35]
- *E. decorticans* Maiden [35, 36]
- *E. froggatti* Blakely [35]
- *E. grandis* W. Hill ex Maiden [2]
- *E. michaeliana* Cambage [35]
- *E. nigra* R. Baker [2]
- *E. nitens* Maiden [35]
- *E. notabilis* Maiden [35]
- *E. oblonga* D.C. [35]
- *E. phaeotricha* Blakely & McKie [35]

Isoleptospermone (**5**)
- *E. grandis* W. Hill ex Maiden [2]



Scheme 2. Model *in vitro* formation of the G-inhibitors (**6**–**8**).



the levels are much lower (18.6 $\mu g\,g^{-1}$; $< 10^{-5}$ mol kg^{-1} fr wt) [46].

From a biosynthetic perspective, the likely candidates as precursors for the G-regulators would seem to be derived from leptospermone (**4**) and isoleptospermone (**5**). Formally, reduction of the acyl ketone functionality followed by dehydration could generate an hydroxybutadiene intermediate (e.g. **9**), which, given its reactivity, could interact with oxygen to produce the epidioxy functionality present in the G-regulators (Scheme 2).

It seems that in *E. grandis*, the G-regulators cannot be present in their physiologically active form since exposure of a cut twig to a 10^{-2} M solution of G-regulators results in severe damage in a very short time. One attractive hypothesis that has been advanced [45] proposes that the regulators are present in a bound (inactive) form and are readily released in response to damage to the plant or to a biological stimulus. The feasibility of such a process was explored by a study of the formation of Michael adducts between the G-regulators, or analogues, with some simple amines. The adducts thus obtained (e.g. **10**) are unstable zwitterionic compounds that are decomposed by acid into the parent G-regulators and the related 2-aminomethylene-1,3-diketone (**11**) [45]. This process is illustrated in Scheme 2 for the case of G-3 (**8**). It has also been suggested that the G-regulators reduce water loss and are involved in the frost resistance of *E. grandis* by controlling the active electron properties of membranes [47, 48]. The photosynthetic electron transport inhibitory activity originally assigned to the G-regulators [49] has been shown to be due to the two related compounds grandinol and homograndinol (**19** and **20**) [50].

ACYLPHLOROGLUCINOL DERIVATIVES

Myrtaceous plants also produce a range of acylphloroglucinols with different levels of methylation of the nuclear carbons and oxygens (Fig. 2) [2, 35–37, 51–62]. A number of these are represented in *Eucalyptus* species (numbered structures in Fig. 2; Table 2), torquatone (**18**) being the most widespread. The ^{13}C NMR spectral parameters for a representative compound (**17**) from this set are given in Fig. 3. In terms of their biological activity, the most studied so far are grandinol (**19**) and homograndinol (**20**).

Grandinol is a plant growth regulator first isolated from *E. grandis* [56]. It is present in lower amounts (0.03 mg g^{-1} fr. wt) than the G-regulators (5–10 mg g^{-1} fr. wt) in adult leaves. Both affect rooting of stem cuttings and water loss by transpiration from excised shoots; however, grandinol is more active ($> 10^{-6}$ M) than the G-regulators ($> 10^{-4}$ M).

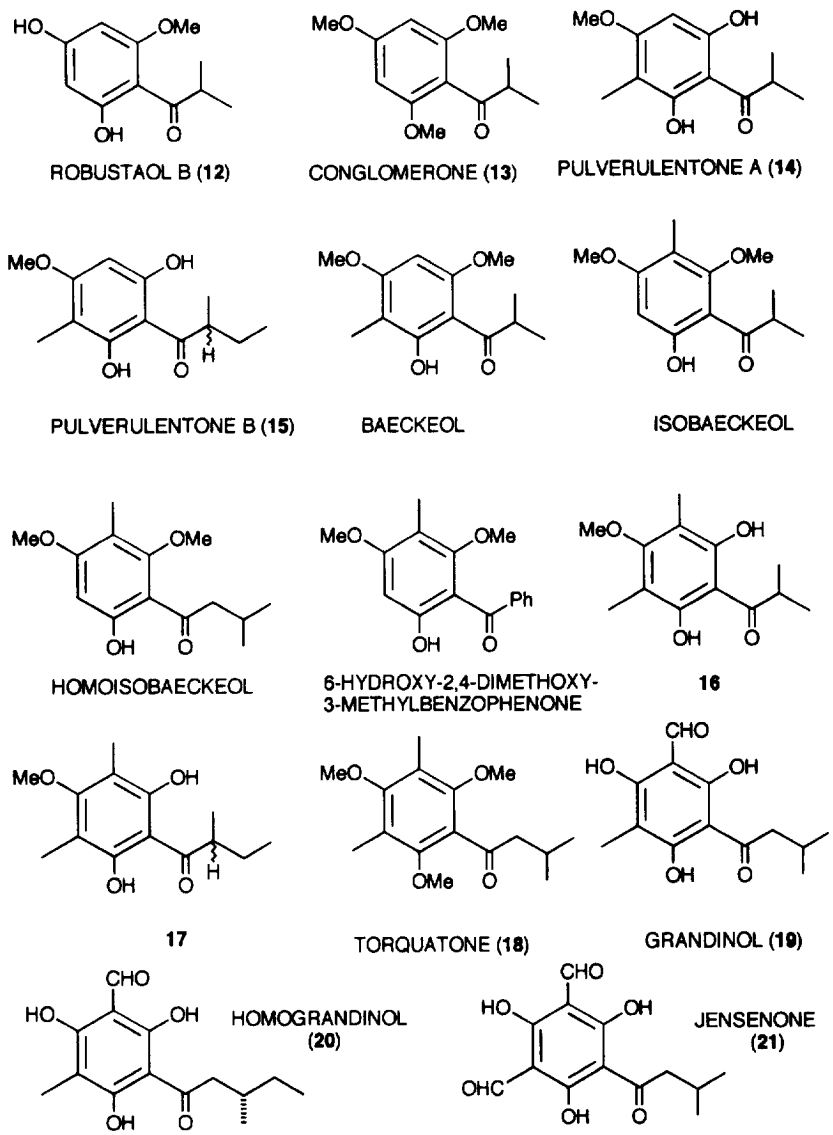


Fig. 2. Acylphloroglucinols found in myrtaceous plants. Numbered structures represent compounds occurring in *Eucalyptus* species. For the origin of others, see refs [35–37, 61].

It was found that samples of G-regulators extracted from the plant inhibited photosynthetic electron transport at the site between photosystem II and plastoquinone [49], similar to the s-triazine herbicides. However, since synthetic samples of G-regulators did not exhibit this activity, it seemed likely that the samples of natural G-regulators contained some highly active trace contaminant(s) which had escaped detection despite purification of the samples [63]. A search for the contaminants revealed that the compounds responsible for photosynthetic inhibition were grandinol and homograndinol. Moreover, it was found that the presence of 0.2% of these two metabolities explained the inhibitory activity previously attributed to the natural G-regulators [50]. This serves as a cautionary note when evaluating the activity of samples derived from natural sources.

The structure of grandinol (**19**) was secured from spectroscopic, X-ray diffraction [56] and synthetic studies [64] and that of homograndinol (**20**) by synthesis [65] of the naturally occurring *S*-enantiomer.

Structure–activity relationships of grandinol as a germination inhibitor have been investigated [66]. The essential features necessary for activity can be summarized by reference to the general structure (**22**) in which two carbonyl groups are involved in hydrogen bonding, and R^1 and R^2 must be limited in length whereas the length of R^3 is not significant; masking the phenolic hydroxyl *para* to R^1 has little effect [66]. Interestingly, the same structural requirements govern the activity of grandinol and homograndinol as inhibitors of Epstein–Barr virus activation [67]. A similar study of the photosynthetic electron transport inhibitory properties of grandinol and

Table 2. Distribution of simple acylphloroglucinols in *Eucalyptus* species

Robustaol B (**12**)
E. robusta Smith [62]
Conglomerone (**13**)
E. conglomerata Maiden & Blakely [36]
Pulverulentone A (**14**)
E. pulverulenta Sims [57]
Pulverulentone B (**15**)
E. pulverulenta Sims [57]
Compound **16**
E. robusta Smith [59]
Compound **17**
E. robusta Smith [59]
Grandinol (**19**)
E. grandis W. Hill ex Maiden [56]
E. perriniana F. Muell. ex Rodway [58]
E. pulverulenta Sims [57]
Homograndinol (**20**)
E. grandis W. Hill ex Maiden [50]
Jensenone (**21**)
E. jensenii Maiden [60]

Torquatone (**18**)
E. angulosa Schau. [54]
E. brachycalyx Maiden [55]
E. caesia Benth. [51, 52]
E. calygona Turcz. [53]
E. celastroides Turcz, spp. *celastroides* [53]
E. ceratocorys (Blakely) Johns. and Hill [54]
E. cleandii (Madien) Maiden [2, 53]
E. concinna Maiden & Blakely [55]
E. corrugata Luehm. [55]
E. erythrandra Blakely and Steedm. [54]
E. flocktoniae Maiden [52]
E. griffithsii Maiden [55]
E. incrassata Labill. [54]
E. pimpiniana Maiden [55]
E. rugosa. R. Br. ex Blakely [55]
E. salubris F. Muell. var. *glauca* Maiden [2, 53]
E. spathulata Hook ssp. *grandiflora* Benth. [52]
E. stoatei Gardner [54]
E. stricklandii Maiden [2, 53]
E. tetraptera Turcz. [54]
E. torquata Luehm. [51, 52, 55]
E. woodwardii Maiden [53]

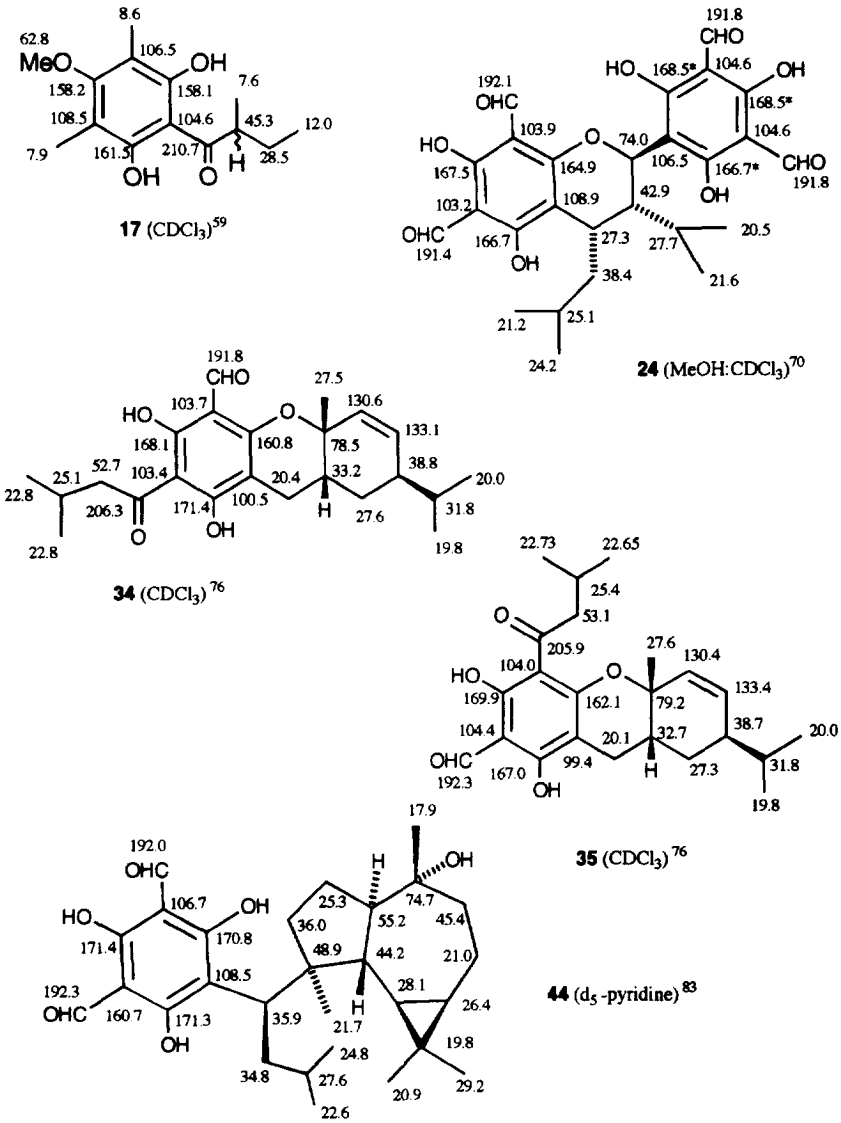
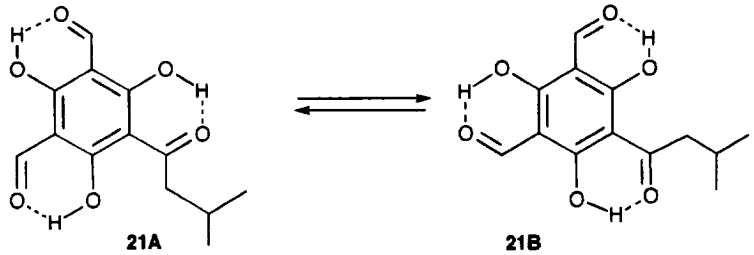
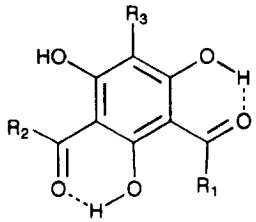


Fig. 3. ^{13}C NMR spectral parameters for selected acylphloroglucinol derivatives.



21A 21B

Scheme 3. Interconverting hydrogen bonded forms of jensenone (**21**).



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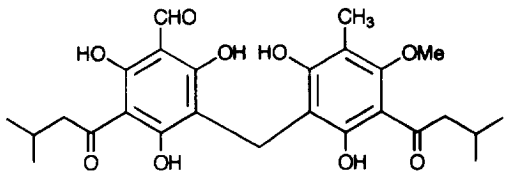

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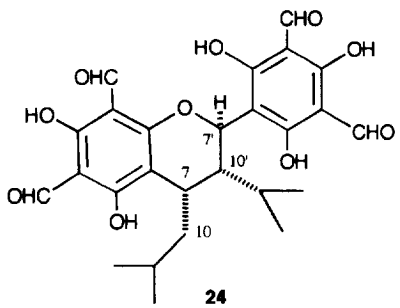

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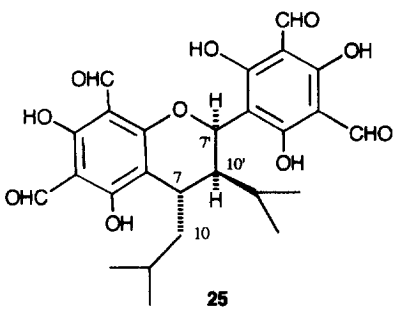


25

homograndinol suggested that at least one acyl function must be present in the phloroglucinol ring; the length of this acyl function is important, reaching a maximum activity with a hexanoyl chain. The introduction of a formyl group into a monoacyl phloroglucinol markedly enhances the activity [65, 68].

The structurally most interesting, and the most recently isolated, of these acylphloroglucinols is jensenone (**21**), which was obtained from the essential oil of the leaves of *E. jensenii* Maiden [60]. Not surprisingly, jensenone exists in Esolution as slow interconverting hydrogen bonded forms (**21A**, **21B**) (Scheme 3), thus negating the symmetry apparent in the structure. Its interest arises from the fact that it contains the level of substitution of the aromatic ring and the side chain, which suggests that it is related to the precursor of the sideroxylonals, euglobals and macrocarpals group of *Eucalyptus* metabolites, which are discussed below.

DIMERIC ACYLPHLOROGLUCINOLS

The first member of this class, robustaol A (**23**), was isolated from the leaves of *E. robusta* Smith, which are used in China to prepare the antimalarial medicine 'Da Ye An'. Although this compound shows antagonistic activity towards *Plasmodium berghei* [69], it co-occurs with the more active robustadials A and B (see section on euglobals below).

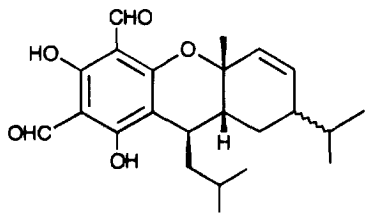
More recently, two novel dimers, sideroxylonals A and B (**24** and **25**), have been isolated from *E. sideroxylon* A. Cunn. ex Wools in 0.0012 and 0.0009% yields, respectively [70]. Both compounds were optically inactive and, although present in the crude methanol extract, it cannot be excluded that they are artefacts of the isolation procedure. The structures were elucidated by spectroscopic techniques. The relative stereochemistry of **24** was deduced from the magnitude of $J_{7'-10'}$ (11.7 Hz) and from the NOE between H-7–H-10'. Similarly for **25**, the coupling constant (1.5 Hz) between H-7' and H-10' and NOEs between H-7'–H-10' and H-7'–H-10 allows the *cis*, *trans* stereochemistry to be assigned. The ^{13}C NMR spectral parameters for **24** are given in Fig. 3. The compounds show antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (3.9 and 7.8 $\mu g\,ml^{-1}$), are aldol reductase inhibitors (IC_{50} 1.25 and 2.47 μM) and inhibit the growth of HeLa cells [70].

ACYLPHLOROGLUCINOL–TERPENE ADDUCTS

Adducts with monoterpenes

A number of novel compounds, arising from the combination of acylphloroglucinol and terpenoid residues, have been identified from *Eucalyptus* species in recent years. These compounds have been named euglobals, and two groups can be distinguished, depending on whether the terpenoid component is a mono- or a sequiterpene. Compounds of the first type were originally isolated from the buds of *E. globulus* in search for compounds responsible for the granulation-inhibiting activity of the crude extract of the plant [71, 72]. To date 13 monoterpene euglobals have been identified from *E. globulus* [72, 73], *E. grandis* [74], *E. robusta* [75] and *E. tereticornis* Smith [76]. These can be subdivided into two subclasses; eight (**26**–**33**) in which the alkanoyl side chain of the acylphloroglucinol component is involved in the formation of the chroman ring, and five (**34**–**38**) where it is not.

The structural elucidation of these compounds has, in general, been attempted by spectroscopic methods. In the case of robustadial A (**32**) and B (**33**), the structures initially assigned [75] were questioned [77] and revised on the basis of more spectroscopic data [78], synthetic studies [79, 80] and X-ray diffraction analysis [80]. Thus, the absolute configuration of **32** and **33** is that shown. Apart from these, only **29** has had its structure and relative stereochemistry confirmed from X-ray studies [72]. In the sense that the spectroscopic parameters showed **30** to be a diastereomer of **29**, the structure of the former can be taken to be that shown. For the remainder of this group, (**26**–**28**, **31**), the stereochemistry at one or more asymmetric centres remains undetermined. It is also interesting to note that it has been reported that the euglobals (**27**–**31**) are essentially racemic and that the monoterpene portion is derived from (±)-α-phellandrene (for **26**, **27**), (±)-sabinene (for **28**–**30**) and (±)-β-phellandrene (for **31**).



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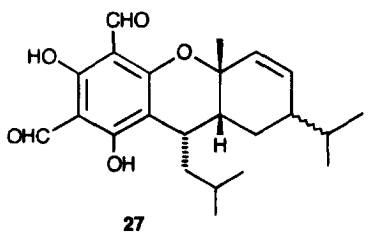

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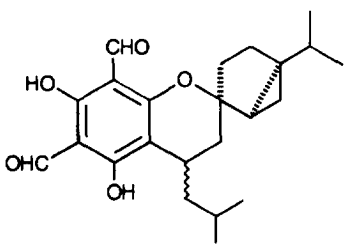

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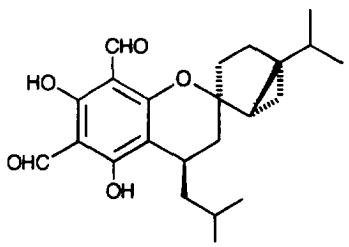

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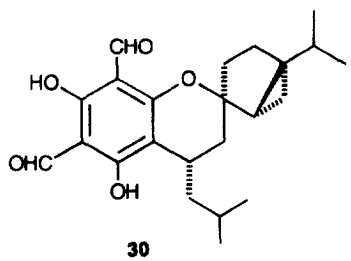

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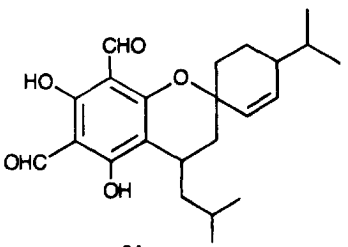

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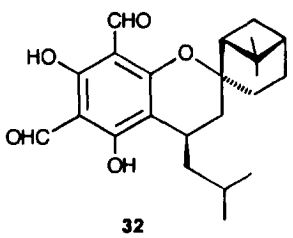

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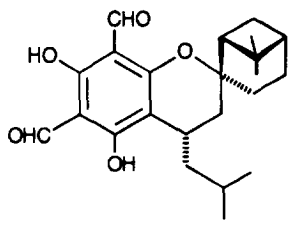

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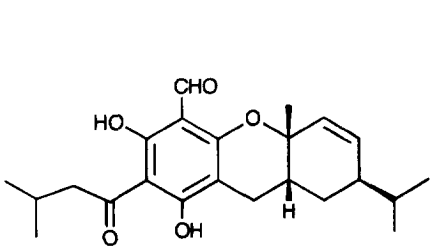

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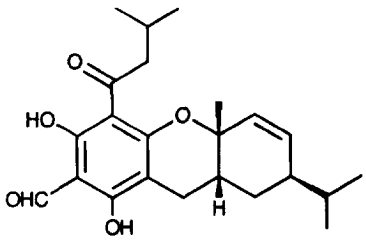

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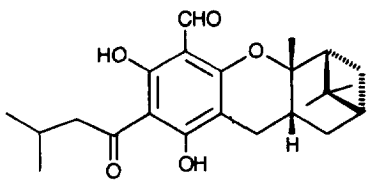

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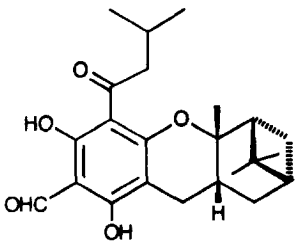

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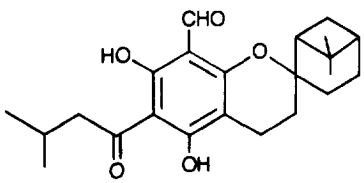

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For members of the other subclass, a number of points still require clarification. Although the relative stereochemistry of **34** and **35** has been determined from NOE studies, the absolute configuration is unknown [76]. The optical rotations measured are similar and substantial, suggesting that they are single enantiomers of identical absolute configuration. The same can be said for **36** and **37**, whereas the relative stereochemistry and the enantiomeric integrity of **38**, $[\alpha]_D$ + 11°, are undetermined [74]. The ^{13}C NMR spectral parameters for the regioisomers **34** and **35** are given in Fig. 3.

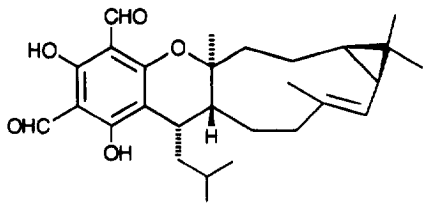
Adducts with sesquiterpenes

A group of compounds incorporating a sesquiterpene residue have been isolated from *E. globulus*. Three (**39**–**41**) represent one subclass, one (**42**) the other. Evidence for the structure and stereochemistry of these compounds rests on spectroscopic (**39**–**42**) and X-ray studies (**39** and **40**). The absolute configuration of **39**–**41** has been deduced from chiroptical measurements and that of **42** by assuming that the absolute configuration of the sesquiterpene residue (derived from bicyclogermacrene) is maintained. A fifth example (**43**), originally classified as an euglobal, should be considered as a macrocarpal (see below). A compound similar to the sesquiterpene euglobals has been isolated from *E. perriniana* F. Muell. ex Rodway, but its structure has not been defined [58].

MACROCARPALS

Bioassay-guided fractionation of the acetone extract of the leaves of *E. macrocarpa* Hook. led to the isolation of several compounds, named macrocarpals, which displayed antibacterial activity. The structure and relative stereochemistry of macrocarpal A (**44**) was established by X-ray crystallographic methods [81]. Another six macrocarpals were isolated, but only tentative planar structures could be assigned to three of them (designated macrocarpals B, D and G) [82]. At the same time, the isolation of five related compounds (M–A to M–E) from the leaves and calyces of *E. globulus* and which exhibited HIV-RTase activity was reported [83]. M–A (**44**) was obtained in 0.028% (of dry wt of calyces), M–B (**45**) in 0.018%, M–C (**46**) in 0.043%, M–D (**47**) in 0.025% and M–E (**48**) in 0.015% yields.

From X-ray diffraction studies, one of these (M–A) was shown to be identical to macrocarpal A (**44**), although the m.p. and $[\alpha]_D$ of one sample (191–192°, − 61.7°) does not correspond well with those of the other (198–200°, − 94.7°). The ^{13}C NMR spectral parameters for **44** are given in Fig. 3. The structure of M–B (**45**) was



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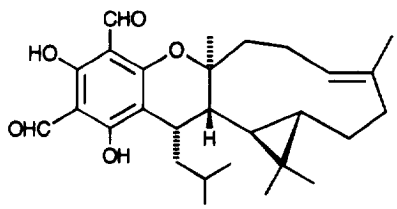

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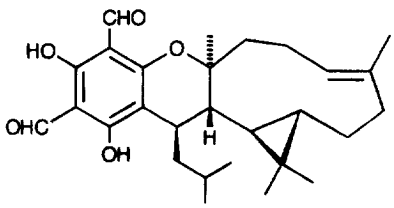

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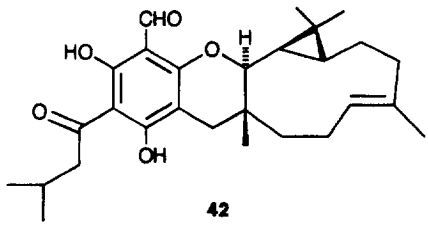

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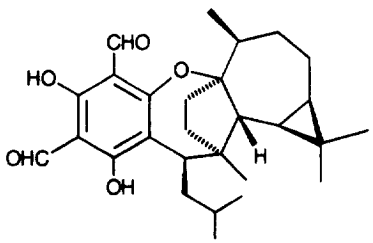

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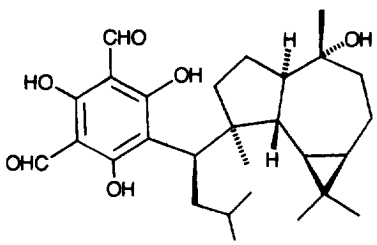

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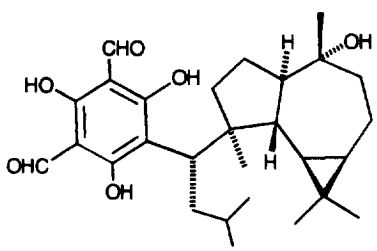

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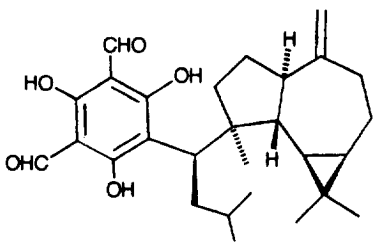

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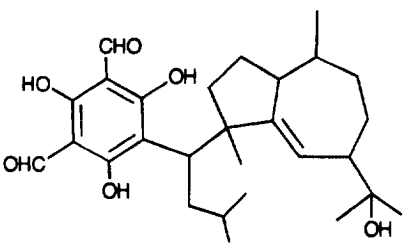

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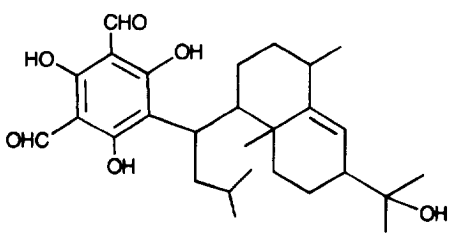

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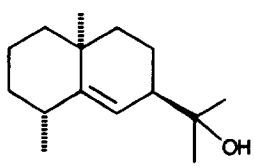

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also secured by X-ray diffraction analysis, which showed that it has the alternate stereochemistry at the benzylic carbon [83]. The parameters for M–B (198–200°, – 17.5°) are in agreement with those of macrocarpal B (196–198°, – 14.0°), suggesting the two are identical. The absolute stereochemistry of M–A and M–C (**46**), which were chemically interrelated, was determined by indirect methods [83]. It is worthwhile noting that **44** and **45** are essentially derivatives of (–)-globulol, and **46** of (+)-aromadendrene, sesquiterpenes which occur commonly in the essential oil of *Eucalyptus* species. Only planar structures were assigned to M–D and M–E (**47** and **48**). Interestingly, the sesquiterpene component of **48** has the gross structure of rosifoliol (**49**), a bicyclic sesquiterpene alcohol which occurs in some *Eucalyptus* essential oils [2].

There is a potential source of confusion in the literature on these compounds. Macrocarpal G, the structure of which is based solely on spectroscopic evidence, has been assigned the same planar structure as that established for M–C (**46**). The ^{13}C NMR spectral parameters quoted for the two compounds were obtained using different solvent making comparison difficult. However, the physico-chemical properties of the two compounds are sufficiently different to suggest that, at best, they are diastereoisomers. In the structure proposed for macrocarpal D, the sesquiterpene residue is not a regular terpene and, on biogenetic grounds, an unlikely one. Another macrocarpal, euvimal-1 from the leaves of *E. viminalis* Labill., has been assigned a structure in which the sesquiterpene portion is claimed to be a substituted ledol, but is not represented as such [84]. Compound **43** can be considered a macrocarpal on biogenetic grounds (see below).

The macrocarpals have been shown to have antibacterial activity towards Gram-positive bacteria, e.g. *S. aureus* and *B. subtilis*, but not towards Gram-negative bacteria, yeast or fungi. Typical minimum inhibitory concentrations were between 0.78 and 3.13 $\mu g\,ml^{-1}$ [82]. The macrocarpals show some activity as inhibitors of HIV-RTase. Thus, the IC_{50} for M–A (**44**) was 10 μM, M–B (**45**) 5.3 μM, M–C (**46**) 8.4 μM, M–D (**47**) 12 μM, and M–E (**48**) 8.1 μM [83]. Macrocarpal A, B, D and G had relative weak activity IC_{50} of 2.0–3.0 μM) as inhibitors of aldose reductase, an enzyme which is involved in complications such as cataract, retinopathy, neuropathy and nepharopathy arising from diabetes [85].

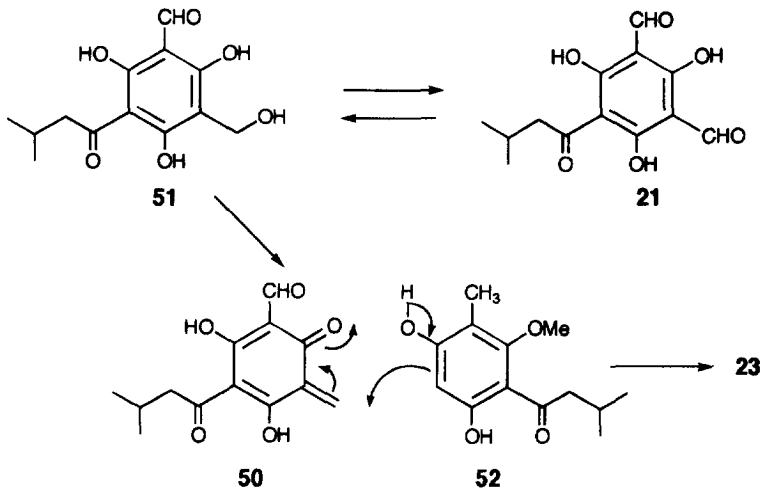
BIOGENETIC CONSIDERATIONS

In this section, an attempt is made to rationalize the formation of the metabolites considered above. It is remarkable that only one of the compounds include in this discussion has been the subject of biosynthetic studies. Incorporation of the labelled carbon from [Me-^{14}C]methionine into tasmanone (**1**) in *E. camfieldii* confirmed the origin of the nuclear methyl groups from this source. Degradation of labelled tasmanone also indicated that the *O*-methyl originated from methionine. The predicted biogenetic steps leading to the formation of the cyclic polyketones are unexceptional. Different alkanoyl coenzyme A starter units are extended by three malonyl coenzyme A units and the polyketide species thus generated undergo a Claisen-type cyclization reaction. Methylation of the doubly activated carbons by *S*-adenosylmethionine results in the introduction of the nuclear methyl groups; *gem*-dimethylation of at least one carbon prevents tautomerization to the phloroglucinol ring system. *O*-Methylation of an enolizable carbonyl group is observed in tasmanone (**1**) and agglomerone (**2**). The origin of the various acylphloroglucinols found in *Eucalyptus* species (Fig. 2) can be rationalized in a similar fashion, except that only monomethylation of the nuclear carbons occurs and *O*-methylation tends to dominate. Oxidative processes involving the nuclear methyl carbons lead to the monoaldehydic grandinol (**19**) and homograndinol (**20**) and the dialdehydic jensenone (**21**). The probable derivation of the G-inhibitors has been discussed above.

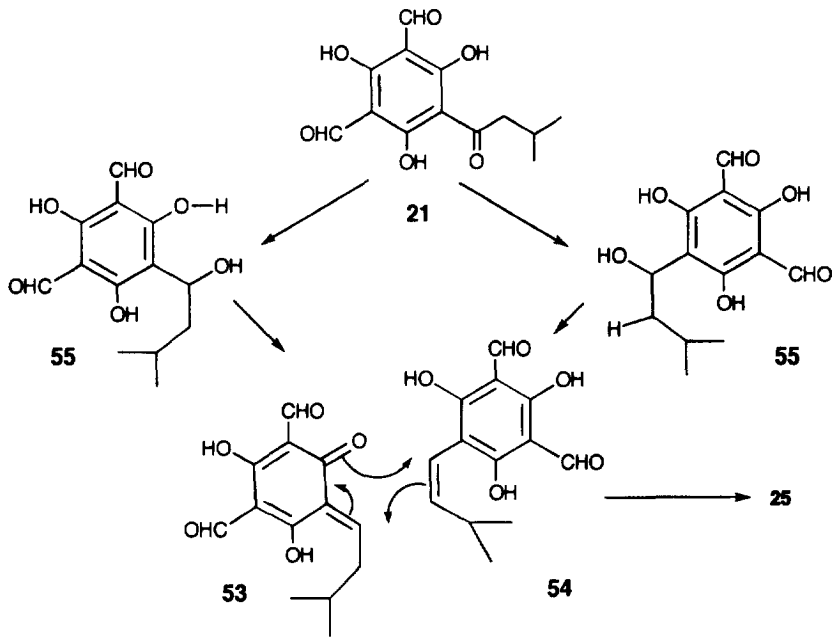
The most economical route to robustaol A (**23**) involves the intermediacy of the *o*-quinone methide (**50**), formally derivable from a precursor (**51**) of jensenone (**21**), which reacts in an aromatic electrophilic substitution reaction at the activated carbon of a demethyl analogue of homoisobaeckeol (**52**) (Scheme 4).

The sideroxylonals A and B (**24** and **25**) can be envisaged as arising from the *o*-quinone methide (**53**) and the styrene (**54**). Both of these putative precursors can be derived from the same intermediate (**55**) if it is assumed that it can undergo dehydration in two different orientations (Scheme 5). A hetero Diels–Alder reaction ([4 + 2] cycloaddition) [86] between **53** and **54** thus generates the chroman ring of the sideroxylonals. The reaction is illustrated in the scheme for one geometric isomer of **54**. It is worthwhile noting that **53** and its geometric isomer (**56**) have a tautomeric relationship because of the symmetry of substitution on the aromatic ring.

While it is convenient and satisfying to invoke a [4 + 2] cycloaddition for the formation of the chroman ring of the sideroxylonals and the the euglobals, it is clear that the same outcome could be achieved by a stepwise, rather than a concerted, process with the involvement of stabilized intermediate species. This point has already been mooted in the section on the possible biogenesis of the G-regulator metabolites. The Diels–Alder reaction type for a long time did not appear to have a biological equivalent [87]. However, the list of natural products whose parent skeleton can plausibly be constructed by assuming the biosynthetic version of this reaction is increasing [88, see also bibilography in ref. 89]. Moreover, some evidence has recently been obtained for the operation of the inter- and intra-molecular variations of this reaction in the biosynthesis of secondary metabolites [89]. In this discussion, the cycloaddition pathway has been adopted for two main reasons. First, the stereochemical outcome of a Diels–Alder reaction can be more readily predicted since the stereochemistry of the dienophile is retained. Secondly, the adoption of a



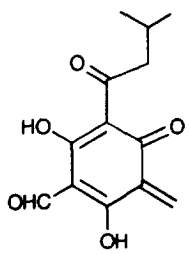
Scheme 4. Hypothetical scheme for the biosynthesis of robustaol (**23**).



Scheme 5. Hypothetical scheme for the biosynthesis of syderoxylonal (**25**).

reaction whose biological counterpart remains to be established acts as a reminder that the pathway leading to the formation of these compounds may not be exclusively under enzymic control.

The genesis of the euglobals can be rationalized by assuming the intermediacy of the *o*-quinone methide (**53**), for the subclass comprising compounds (**26–33** and **39–41**), and the equivalent regioisomeric quinone methides (**57** and **58**) for the compounds (**34–38** and **42**). In these cases the double bond of a monoterpene or sesquiterpene compound acts as the dienophile. This is illustrated in Scheme 6 for the formation of the monoterpene adducts (**26** and **27**). Thus, α-phellandrene is the terpene component for the formation of **26**, **27**, **34** and **35**;



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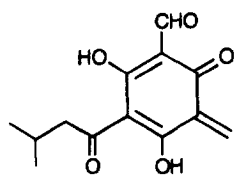

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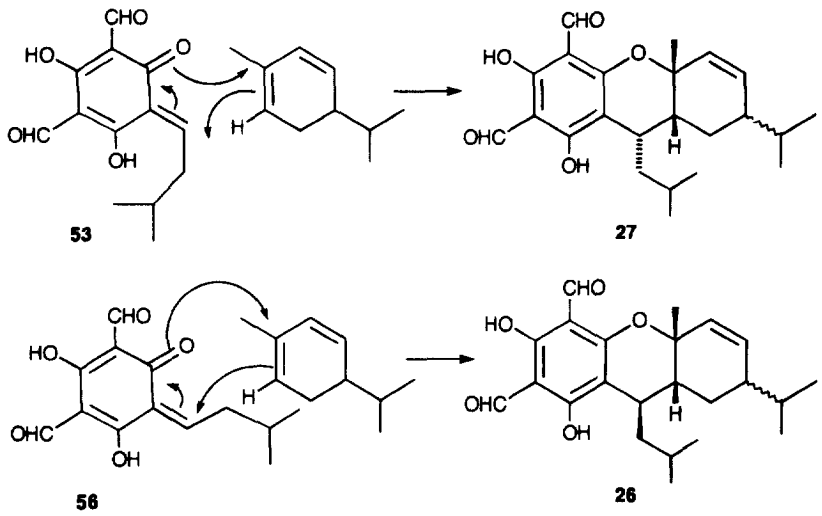
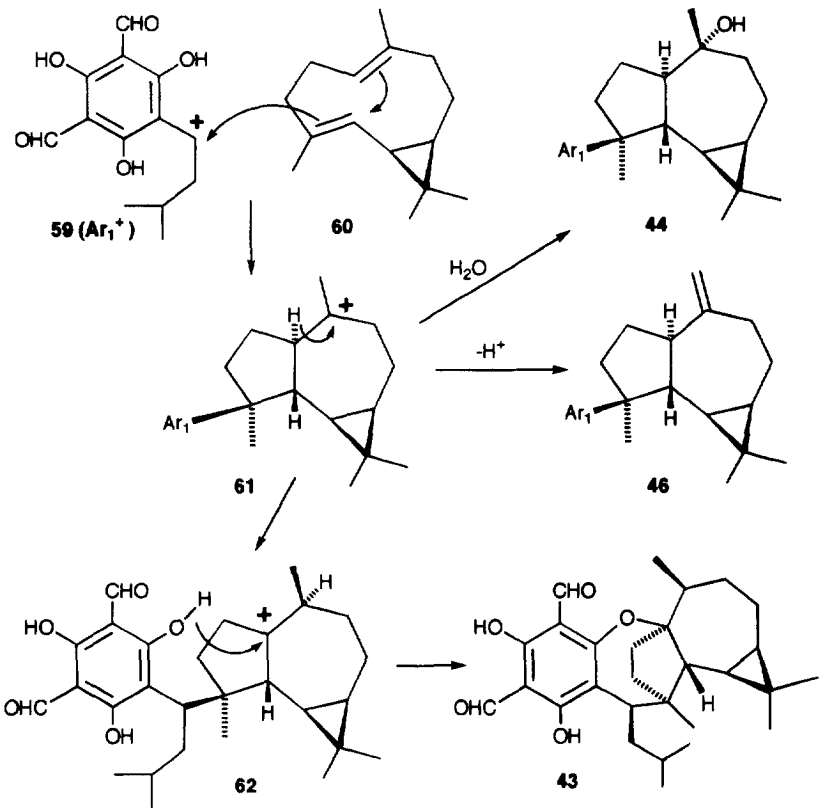
sabinene for **28–30**; β-phellandrene for **31**; α-pinene for **32**, **33** and **38**; β-pinene for **36** and **37**; and bicyclogermacrene for **39–42**.

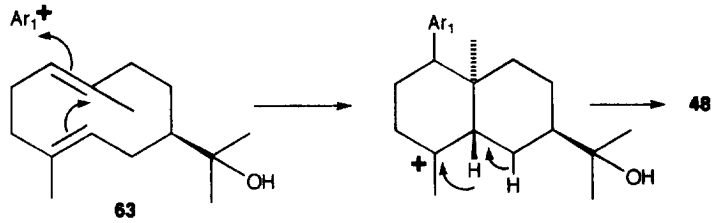


Scheme 6. Hypothetical scheme for biosynthesis of the monoterpene euglobals **26** and **27**.



Scheme 7. Hypothetical scheme for the biosynthesis of the macrocarpals (**43–46**).

The simplest biogenetic proposal for the generation of the macrocarpals involves the carbocationic species (**59**; Ar_1^+) or a stabilized equivalent. This species can replace a proton as the cationic initiator in the cyclization of a sesquiterpene precursor. For compounds **44–46**, the sesquiterpene bicyclogermacrene (**60**) can be considered to be cyclized to the globulol or aromadendrene skeleton, as shown in Scheme 7. The formation of **43** is interesting in this context since it appears to arise from trapping of the carbocationic species **62** generated by rearrangement of the ionic species **61** (Scheme 7). Further rearrangement of the carbocation **62** could be used to explain the formation of **47**, on the assumption that the sesquiterpene portion retains the predicted stereochemistry. A similar sequence involving the cyclization of germacrol (**63**) (Scheme 8) allows the formation of **48** to be rationalized.



Scheme 8. Hypothetical scheme for the biosynthesis of the macrocarpals **48**.

CONCLUDING REMARKS

A variety of structurally interesting acylphloroglucinol derivatives have been isolated from *Eucalyptus* species and many show distinctive biological activities. It is clear that for a number of compounds individuated more work is needed before the structures can be unambiguously defined. The apparent, almost racemic nature of some of the monoterpene euglobals is somewhat puzzling and warrants confirmation. The biosynthesis of these compounds, so far only a matter of conjecture, merits attention and should provide answers to what are, at present, only interesting questions.

Eucalyptus species are well known to exhibit variation at population and specific levels (polymorphism and polytypism) for attributes such as essential oil terpenoids [2], polypenols [90] and anthocyanins [91]. Differences in essential oil composition have been observed not only between 'chemical races' of plants, which occur in different geographic regions, but also between 'chemical forms', which occur sympatrically [92]. Whether this is also true for the compounds discussed in this review remains to be established. It appears that research on the phytochemistry of *Eucalyptus* species still offers much promise in areas of scientific and commercial interest.

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