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LEAF WAX CONSTITUENTS OF SOME MYRTACEOUS SPECIES

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Abstract—Eucalyptin and 8-desmethyleucalyptin have been isolated from the leaf wax coatings of several species of *Eucalyptus*, from *Syncarpia glomulifera*, *Lophostemon confertus* and an *Angophora* hybrid indicating that *C*-methylated flavones may be fairly distinctive of the family Myrtaceae. Ursolic acid has been isolated from the leaf waxes of *E. youmanii*, *S. glomulifera*, *Leptospermum petersonii* and *Melaleuca quinquenervia*, friedelin from *Lophostemon confertus* and 4,6-dimethoxy-2-hydroxyacetophenone from *E. michaeliana*.

Earlier reports on the occurrence of two unusual *C*-methylflavones, eucalyptin (4′,7-dimethoxy-6,8-dimethyl-5-hydroxyflavone) and 8-desmethyleucalyptin in the leaf waxes of several species of *Eucalyptus* and of the botanically very closely related *Angophora* [1, 2] prompted us to extend the investigation to additional species of *Eucalyptus* spread widely throughout the genus (according to Pryor and Johnson's classification [3]) as well as to other myrtaceous genera.

Eucalyptin was obtained, albeit in vastly varying amounts (Table 1), from *E. eximia* Schau. (but not from the flower bud wax coating), *E. gummifera* (Gaertn.) Hochr. and *E. maculata* Hook. (subgenus *Corymbia*), *E. elata* Dehnh., *E. haemastoma* Sm., *E. luehmanniana* F. Muell. and *E. youmanii* Blakely and McKie (subgenus *Monocalyptus*), *E. michaeliana* Blakely (subgenus *Symphyomyrtus*) as well as from a hybrid *Angophora hispida* (Sm.) Blaxell × *A. bakeri* C. Hall (= '*A. clelandii*' Maiden). The less common 8-desmethyleucalyptin accompanied eucalyptin in the leaf waxes of *E. michaeliana* and *E. youmanii*. Neither flavone was present in sufficient quantity for isolation in either *E. peltata* Benth. (subgenus *Corymbia*) or *E. staigeriana* F. Muell. ex F. M. Bail. (subgenus *Symphyomyrtus*) although TLC showed in both cases very faint spots of R_f values identical with those of eucalyptin.

Among other myrtaceous species eucalyptin has been isolated from *Syncarpia glomulifera* (Sm.) Niedenzu and 8-desmethyleucalyptin from *Lophostemon confertus* (R. Br.) Peter Wilson and J. T. Waterhouse (syn. *Tristania conferta* R. Br. [4]). Neither flavone appeared to be present in the leaf waxes of *Melaleuca quinquenervia* (Cav.) S. T. Blake, *M. leucadendron* (L.) L. or *Leptospermum petersonii* F. M. Bail.

The ubiquitous ursolic acid has been isolated from the leaf waxes of *E. youmanii*, *S. glomulifera*, *M. quinquenervia* and *Leptospermum petersonii*, whilst friedelin and 4,6-dimethoxy-2-hydroxyacetophenone were obtained from *Lophostemon confertus* and *E. michaeliana* waxes, respectively.

In addition to our results eucalyptin has also been reported from the foliage of *E. citriodora* F. Muell. [5] as well as from the two myrtaceous species *Myrcia citrifolia* (Aubl.) Urb. and *Eugenia biflora* (L.) DC. [6], although in neither case was any mention made of whether it was present in the leaf wax coating. More recently eucalyptin, 8-desmethyleucalyptin, sideroxylin (4′,5-dihydroxy-6,8-dimethyl-7-methoxyflavone) and 8-desmethylsideroxylin have been reported from the leaf coating of several additional species of *Eucalyptus* [7]. Sideroxylin has been originally obtained from the leaf parenchyma of *E. sideroxylon* A. Cunn. ex Woolls [8] and *E. dundasii* Maiden [9].

It thus appears that *C*-methylflavones are distributed throughout both subfamilies of the Myrtaceae: the Myrtoideae (*Eugenia*, *Myrcia*) and Leptospermoideae (*Angophora*, *Eucalyptus*, *Lophostemon*, *Syncarpia*) and that they are probably quite distinctive of the whole family. However, their chemotaxonomic significance is somewhat diminished owing to the occurrence of all four earlier mentioned flavones in the epicuticular leaf coating of *Kalmia latifolia* L. (Ericaceae) [7] and of strobochrysin (5,7-dihydroxy-6-methylflavone) in the heartwood of *Pinus strobus* (Pinaceae) [10]. Of the 15 related *C*-methylflavonols [11] two occur in the family Polypodiaceae (Pteridophyta), three in the Pinaceae (Gymnospermae) and 10 in the Didieraceae, an angiosperm family botanically far removed from both Myrtaceae and Ericaceae.

It is interesting to note that 4,6-dimethoxy-3,5-dimethyl-2-hydroxyacetophenone, a putative precursor of some of these flavones, has been reported from the volatile leaf oil of *Melaleuca cajuputi* Powell [12]. Also a compound (mp 184–185°) named 'eucalyptin', isolated independently by Elkeiy *et al.* [13] from the foliage of *Eucalyptus citriodora* and *E. globulus* at about the same time as the investigation of Lamberton *et al.* [1, 2], is not, according to the authors, a flavonoid but may be a coumarin.

EXPERIMENTAL

Origin of plant material. Foliage from single trees was collected near Sydney, New South Wales, at the following locations: *Eucalyptus eximia*, *E. gummifera*, *E. haemastoma* and *Angophora*

Table 1. Percentage composition of myrtaceous leaf waxes*

Species	Total leaf wax	Eucalyptin	8-Desmethyleucalyptin	Ursolic acid	Friedelin	4,6-Dimethoxy-2-hydroxyacetophenone
Eucalyptus elata	0.18	0.0010	—	—	—	—
E. eximia	0.13	0.0008	—	—	—	—
E. gummifera	0.14	0.0060	—	—	—	—
E. haemastoma	0.15	0.0040	—	—	—	—
E. luehmanniana	0.23	0.0033	—	—	—	—
E. maculata	0.03	0.0013	—	—	—	—
E. michaeliana	0.20	0.0012	0.0008	—	—	0.0009
E. youmanii	0.28	0.0006	0.0006	0.0092	—	—
Angophora hispida × *A. bakeri*	0.28	0.0002	—	—	—	—
Syncarpia glomulifera	0.23	0.0008	—	0.0074	—	—
Lophostemon confertus	0.66	—	0.0003	—	0.0154	—
Leptospermum petersonii	0.16	—	—	0.0091	—	—
Melaleuca quinquenervia	0.18	—	—	0.0926	—	—

*All percentage values refer to fr. wt of foliage.

hispida × *A. bakeri* at Berowra Heights, *Eucalyptus luehmanniana* near Waterfall, *Syncarpia glomulifera* and *Lophostemon confertus* at Parramatta and of all remaining species from cultivated trees growing at the Museum of Applied Arts and Sciences Experimental Plantation at Castle Hill. Voucher specimens are held at the Biological and Chemical Research Institute's Herbarium.

Isolation of cuticular leaf wax and of leaf wax constituents. Fresh foliage (1 kg) was dewaxed by dipping for 5 min into warm petrol bp 60–80° (5 l. × 2). The filtered petrol washings were concd to 5% of their original vol. and any ppt filtered off. Evaporation of the filtrates to dryness yielded pale yellow waxy solids, which were redissolved in a slight excess of 95% aq. EtOH and refrigerated overnight at −10°. The ppted waxes were filtered off in the cold through a sintered glass filter, the filtrates concd to *ca* 50% their original vol. and the cooling process repeated in order to remove any residual waxy material. Further concn of the dewaxed filtrates, followed by fractional crystallization yielded, depending on the species, eucalyptin, 8-desmethyleucalyptin and friedelin. Crystallization of the ppts obtained by concn of the original leaf washings yielded ursolic acid, some eucalyptin and in one case 4,6-dimethoxy-2-hydroxyacetophenone. All compounds (and where appropriate their derivatives) were identified by their mp, mmp, IR, UV, ^{1}H NMR and, in the case of friedelin, ^{13}C NMR spectra. Yields of all products are summarized in Table 1.

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