Epicuticular Leaf Flavonoids from *Eucalyptus* Species and from *Kalmia latifolia*

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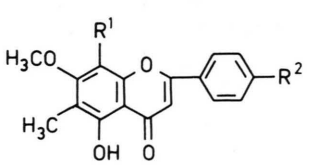
In young plants of *Eucalyptus globulus* the leaves are covered with a whitish epicuticular layer. This leaf wax contains small amounts of C-methylated flavones as aglycones. A novel flavone has now been identified as 5,4′-dihydroxy, 7-methoxy, 6-methyl-flavone (8-desmethyl-sideroxylin). Another minor external flavone is chrysin. On leaves of *E. sieberi* the flavanone pinocembrin could be detected. It is shown that the four C-methylated flavones, eucalyptin, 8-desmethyl-eucalyptin, sideroxylin, and 8-desmethyl-sideroxylin are present in the leaf wax of 8 species analyzed. The same flavones are present on leaves of *Kalmia latifolia.*

Until recently the external occurrence of flavonoid aglycones on leaves of higher plants was assumed to be a very rare phenomenon. One of the first cases in which this was reported is *Eucalyptus.* In 1964 Lamberton and coworkers described the identification of 5-hydroxy, 7,4′-dimethoxy, 6,8-demethyl-flavone (eucalyptin) from the leaf wax of various *Eucalyptus* species [1] and of 5-hydroxy, 7,4′-dimethoxy, 6-methyl-flavone (8-desmethyl-eucalyptin) from the leaf wax of *E. torelliana* and *E. urnigera* [2]. One year later Hillis and Isoi described 5,4′-dihydroxy, 7-methoxy, 6,8-dimethyl-flavone (sideroxylin) from *E. sideroxylon* [3], not from the leaf wax but from leaf extractives. We now had the opportunity to study the flavonoids of the epicuticular material on leaves of *E. globulus* Labill. In addition to the three compounds just cited we found a novel C-methylated flavone, whose structural elucidation will be reported here. We studied the distribution of these four flavonoids in the leaf wax of several species of *Eucalyptus* and rather unexpectedly we found them also in the thin epicuticular layer on leaves of *Kalmia latifolia* L.

Materials and Methods

Freshly collected leaves and twigs of some approx. 2 year old plants of *Eucalyptus globulus* grown in a greenhouse at the Botanical Garden of the TH Darmstadt were dipped in a boiling mixture of petrol ether (b.p. 40–60 °C) and acetone to dissolve the waxy material. After evaporation of the solvents the dry material was ground and treated with acetone at room temperature. A great amount of reddish-brown powder remained undissolved. The acetone solution contained the flavonoids, one of which crystallized on standing: compound **I**. The mother liquor was subject to column chromatography on silica gel, eluted with toluene and increasing quantities of methylethyl ketone and methanol. From one of the less polar fractions a second flavonoid, compound **II**, crystallized in a mixture with compound **I**. The two substances could not be separated by passage over a column of polyamide. For identification of compound **II** therefore a small amount of this was isolated by preparative TLC on polyamide, followed by purification on silica. From later more polar fractions again a crystalline material was obtained, which also seemed to consist of two flavonoids. It could be roughly fractionated by column chromatography on polyamide and yielded pure compound **III**. When we then isolated com-



I. $R^1 = CH_3$, $R^2 = OCH_3$: Eucalyptin
II. $R^1 = H$, $R^2 = OCH_3$: 8-Desmethyl-eucalyptin
III. $R^1 = CH_3$, $R^2 = OH$: Sideroxylin
IV. $R^1 = H$, $R^2 = OH$: 8-Desmethyl-sideroxylin

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pound **IV** from some of the fractions by preparative TLC on silica, it turned out that this contained a fifth substance, compound **V**. The latter was identified by direct comparison with authentic chrysin.

Dried leaf samples of 7 additional species of *Eucalyptus* were supplied by Dr. G. O. Kirst (Bonn). They had been collected and identified by Dr. P. J. Myerscough in the vicinity of Sydney, Australia. The wax was dissolved from these samples and flavonoid solution suitable for comparative studies was prepared in the same manner as described above for the bulk material of *E. globulus*. Compound **VI** was detected only in 1 out of the 8 species analyzed. It could be identified by direct comparison with an authentic sample of pinocembrin.

Fresh leaves of *Kalmia latifolia* were collected from plants growing in the Botanical Garden of the TH Darmstadt. They were rinsed with acetone to dissolve the thin epicuticular layer. The solution was subjected to column chromatography and preparative TLC. The flavonoid constituents were identified by direct comparison with those isolated from *E. globulus*.

TLC was performed either on polyamide with solvents A) toluene/petrol (b. p. 100–140 °C)/methyl ketone/methanol 30:90:2:1.5, B) the same, 60:30:15:5, C) toluene/dioxane/methanol 80:10:10 or on silica with solvent D) toluene/methylethyl ketone 9:1. "Naturstoffreagenz A" (*β*-aminoethylester of diphenyl boric acid) was used as spray reagent. – Mass spectra were recorded on a Varian MAT 311 at the Insitute of Organic Chemistry of the TH Darmstadt. ^{1}H-NMR spectra were recorded on a Brucker HFX-90 at the Institute of Organic Chemistry of the University of Heidelberg.

Results and Discussion

Leaves of *Eucalyptus* species are oftern covered with a waxy epicuticular layer which gives them a glaucous or even whitish appearance on young plants. For *E. globulus* the following composition of this wax has been reported earlier: 56% *β*-diketones, 15% esters of alkanoic acids and alcohols, 2% hydrocarbons, 1–2% sterols, 2.8% flavones [2]. The leaf wax of *Eucalyptus* hence is not a true wax according to the chemical definition (*cf.* [4]). The flavones eucalyptin, 8-desmethyl-eucalyptin and sideroxylin have already been identified from this lipophilic epicuticular material many years ago [1–3]. Their isolation on our laboratory was initially undertaken because we wanted to have these C-methylated flavones for comparative purposes. Using column chromatography we obtained a good amount of eucalyptin (**I**) together with 8-desmethyl-eucalyptin (**II**) and a much smaller amount of a mixture of sideroxylin (**III**) with two further flavones (**IV** and **V**). Compounds **I**, **II** and **III** could readily be identified by their spectral data which are in accordance with those reported in the literature [1–3] and are not repeated here. Compound **V** was found to be identical with chrysin (5,7-dihydroxy-flavone).

Compound **IV** did not match any known flavonoid. It exhibits the following spectral properties. UV λ_{max}^{EtOH}: 332,274 nm; + $AlCl_3$ 351, 301, (288) nm; + NaOEt 397, 274 nm; + NaOAc 338, 278 nm; + H_3BO_3 334, 273 nm. MS *m/e* (rel. int.): 298 (100, M^+), 297 (51, M-1), 283 (7, M-15), 280 (7, M-18), 269 (28, M-29), 268 (21), 150 (10), 121 (17). ^{1}H-NMR (90 MHz, DMSO-d_6; δ ppm/TMS): ~ 12.5 (br. sign., OH-5), ~ 11.4 (br. sign., OH-4′), 7.93 and 6.93 (AA′BB′ spin system, $J = 8$ Hz), 6.81 (1 H, s), 6.75 (1 H, s), 3.93 (3 H, s, OCH_3), 2.01 (3 H, s, CH_3).

According to its M^+, compound **IV** with the molecular formula $C_{17}H_{14}O_5$ could be a flavone with 1 OH- and 2 OCH_3-groups (*cf.* [5]). The NMR-spectrum indicates, however, that instead of 1 OCH_3 there is 1 more OH-group and 1 CH_3-group present. It further shows the presence of a H-bonded OH-group at C-5 and the presence of a *p*-substituted B-ring. A shift of 65 nm of band I in UV on addition of NaOEt points to a free OH-group at C-4′ (The same shift is observed in sideroxylin). The OCH_3-group hence must be placed at C-7. Position C-6 or C-8 are possible for the CH_3-group. Because of the weak reaction with $AlCl_3$ (20 nm also in sideroxylin) it can be located at C-6. Compound **IV** thus is 5,4′-dihydroxy, 7-methoxy, 6-methyl-flavone or 8-desmethyl-sideroxylin. This is a novel C-methylated flavone (*cf.* [6]).

In the leaf wax of *Eucalyptus globulus* both compound **IV** (8-desmethyl-sideroxylin) and compound **V** (chrysin) are present in very small amounts only. This may explain why they had not been found by the earlier workers [1–3]. Our study of seven additional species showed that in all of them eucalyptin (**I**) and 8-desmethyl-eucalyptin (**II**) are present in the leaf wax as the major flavonoid constituents. Also sideroxylin (**III**), which originally had been described as a constituent of the leaf

tissue, is found as an external leaf flavonoid in all samples analyzed. The novel flavone, 8-desmethyl-sideroxylin (**IV**) is a minor constituent. By comparative TLC on polyamide it is easily detected in the leaf wax of *E. amplifolia, E. goniocalyx, E. gunnii,* and *E. macrorhyncha.* In *E. cinerea, E. globulus, E. sieberi,* and *E. urceolaris* it is found in trace amounts only. The spot of chrysin (**V**) is hidden by compound **IV**; we therefore do not consider the distribution of this flavone. It must be mentioned, however, that in the leaf wax of *E. sieberi* we detected the corresponding flavanone, namely pinocembrin (5,7-dihydroxy-flavone). The 5,7-dimethyl ether of pinocembrin has been reported earlier for the leaf of *E. sieberi* [7]. This compound could not be detected in our material. Pinocembrin and its 5-methyl ether (alpinetin) have been reported from the leaves of several species of *Eucalyptus* [7].

In the scope of our search for flavonoid aglycones as constituents of epicuticular layers [8], a random collection of leaves of higher plants wer checked by TLC on polyamide. So we came upon *Kalmia latifolia* and found incidentally that this plant produces the same external flavonoids as do *Eucalyptus* species. There is not epicuticular layer visible on the glabrous leaves of *Kalmia* and only a very low amount of material can be dissolved with organic solvents. Most of this probably consists of terpenoid compounds and the flavonoids are present in trace amounts only. However, the 4 C-methylated flavones (**I–IV**) could be identified unambiguously by their chromatographic and spectral properties. Several further flavonoids, presumably flavanones, could not yet be elucidated due to lack of material.

C-methylated flavones are very rare natural compounds; only 4 of them have been found previously. 8-desmethyl-sideroxylin (**IV**) thus is the fifth natural representative of this group. 6-C-methyl-chrysin (strobochrysin) has been isolated from the heartwood of *Pinus strobus,* 8-desmethyl-eucalyptin, sideroxylin and eucalyptin were known from *Eucalyptus* leaf wax. Eucalyptin was also found in leaf wax of two species of *Angophora* and in leaf (leaf wax?) of *Eugenia biflora* and *Myrcia citrifolia* (*cf.* [6]). Except for *Pinus* (Pinaceae) all previous sources of such compounds are members of the Myrtaceae. With *Kalmia latifolia* we now can add a member of the Ericaceae which are not at all interrelated taxonomically.

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