Novel Epicuticular Leaf Flavonoids from *Kalmia* and *Gaultheria* (Ericaceae)

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Leaves of a number of Ericaceous plants have a thin epicuticular layer that consists mostly of triterpenes. In *Kalmia angustifolia, K. latifolia* and *K. polifolia* as well as in *Gaultheria procumbens* and *G. shallon* this material also contains trace amounts of flavonoid aglycones. In *Kalmia* they are the 3-OMe-derivatives of four C-methyl flavones reported previously as the typical leaf-wax flavonoids of *Eucalyptus*. The new compounds, 5-OH-3,7,4′-triOMe-6,8-diCH_3-flavone (kalmiatin), 5-OH-3,7,4′-triOMe-6-CH_3-flavone (8-desmethyl-kalmiatin), 5,4′-diOH-3,7-diOMe-6,8-diCH_3-flavone (latifolin) and 5,4′-diOH-3,7-diOMe-6-CH_3-flavone (8-desmethyl-latifolin) are novel natural products. They could not be detected in eight species of *Eucalyptus*. A screening of 15 species of Ericaceae revealed that 8-desmethyl-sideroxylin and 8-desmethyl-latifolin are present also in the epicuticular layer on leaves of *Gaultheria procumbens*, while on *G. shallon* and on *Andromeda polifolia* traces of galangin-3-methyl ether were found. In the other species checked no external flavonoid aglycones could be detected.

Introduction

Only recently the mountain laurel, *Kalmia latifolia* L. (Ericaceae; E. N-America), was found to contain some rare flavonoid aglycones in an epicuticular layer on its leaves [1]. They are the same four C-methyl flavones that are present in the leaf-wax of *Eucalyptus* species (Myrtaceae; Australia): eucalyptin (5-OH-7-OMe-6,8-diCH_3-flavone), sideroxylin (5,4′-diOH-7-OMe-6,8-diCH_3-flavone) and the respective 8-desmethyl compounds. We now report on the identification of additional trace constituents with corresponding substitution patterns and their occurrence in other species.

Materials and Methods

Fresh leaves of *Kalmia latifolia*, collected from shrubs growing in the Darmstadt Botanical Garden, were rinsed with acetone and the solution was evaporated to dryness. The crude material was powdered and stirred with toluene to dissolve most of the flavonoids present, leaving a relatively large sludge of undissolved material. The toluene solution was subjected to column chromatography on silica to get rid of some waxy material and to yield fractions enriched in flavonoids. Fractions containing the non-polar flavonoids as well as those containing the relatively more polar flavonoids were combined to form portions A and B. Close examination by polyamide TLC revealed the presence of two new flavonoids in each, A and B. By repeated preparative TLC on silica these flavonoids were separated and freed of accompanying terpenoids. This finally lead to the isolation of the flavonoid aglycones **2**–**4**, while compound **1** could be obtained only in a mixture with compound **2**. (It may appear odd to give no. **1** to the one product that could not be isolated, but by this numbering we want to make the structural relation to the previously described flavones [1] evident.)

This study was performed with material from plants with light green foliage and pale pink flowers and with material from plants with dark green foliage and more intense pink flowers. Once the flavonoid aglycones in the epicuticular layer of *K. latifolia* had been isolated and identified, reliable TLC-comparisons on polyamide could also be made with samples of *K. angustifolia* L., *K. polifolia* Wangenh. and other Ericaceae available in the Darmstadt Botanical Garden.

TLC was either on polyamide DC-11 with solvents A) toluene/petrol$_{100-140°}$/methylethylketone/methanol 30:90:2:1.5 and B) the same, 60:30:10:5, or on silica with solvents C) toluene/methylethylketone 9:1 and D) petrol$_{100-140°}$/toluene/methylethylketone 18:1:1. As spray reagent for flavonoids on polyamide and on silica we used Naturstoffrea-

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genz A. Terpenoids were visualized by spraying with $MnCl_2$-solution, followed by heating to 110° [2]. Mass spectra were recorded on a Varian MAT 311 at the Institute of Organic Chemistry of the TH Darmstadt, the ^{1}H-NMR spectra of compound **2** and of phloridizin were recorded on a Brucker HFX-90 at the Institute of Organic Chemistry of the University of Heidelberg. Authentic samples of eucalyptin, 8-desmethyleucalyptin, sideroxylin, and 8-desmethylsideroxylin were isolated from *Eucalyptus globulus* leaf wax [1]. β-amyrin and ursolic acid were purchased from C. Roth, Karlsruhe, and β-amyrin acetate was prepared from β-amyrin by acetylation (acetanhydride/pyridine). δ-amyrenon was isolated from the bud excretion of *Alnus japonica* [3].

Results and Discussion

The epicuticular layer on leaves of *Kalmia latifolia* is barely visible with the naked eye since the amount of "leaf wax" is extremely small and the portion of flavonoid aglycones in this material is minute. Most of the exudate material consists of triterpenes, especially ursolic acid, which forms the residuum from the crude extract (identified by co-chromatography, m.p., MS). TLC of *K. angustifolia* "leaf wax" with markers (silica, solvents C and D) further suggests the presence of β-amyrin, δ-amyrenon, and β-amyrin acetate. Several additional spots showing terpenoid reaction could not be identified.

Among the epicuticular flavonoids the compounds now studied are trace constituents only. We were able to isolate minute amounts each of compounds **2–4** and to obtain comp. **1** in a mixture with comp. **2.** We thus could run UV- and mass spectra of compounds **2–4**, a MS of the mixture **1/2**, and in comp. **2** even a ^{1}H-NMR spectrum.

In the following we report the spectral data of the new compounds as far as they could be determined.

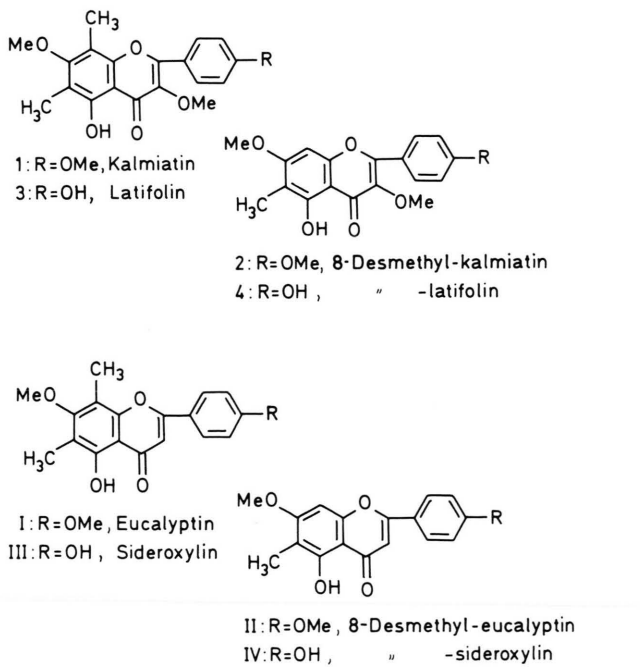
Comp. **1**: MS *m/z* (rel. int.): 356 (42, M^+), 355 (23), 337 (7), 313 (26), 232 (6), 217 (6). (Spectrum of mixture with comp. **2**, where M^+ of the latter appears as 100%.)

Comp. **2**: Light yellow crystals, m.p. 178–180°. UV λ_{max}^{MeOH} (nm): 332, 272; + $AlCl_3$ 357, 283; $AlCl_3$ + HCl 352, 284; + NaOH 333, 274; + NaOAc 335, 274; NaOAc + H_3BO_3 335, 274. MS *m/z* (rel. int.): 342 (100, M^+), 341 (87), 327 (7), 323 (43), 299 (31), 218 (63), 203 (17). ^{1}H-NMR (δ ppm/TMS): 8.03 and 7.10 (AA'BB' spin system, J = 8 Hz), 6.82 (1 H, s; H-8), 3.91, 3.87, 3.79 (3 H each, s; 3 OCH_3 groups), 2.01 (3 H, s; C–CH_3), (OH-5-proton exchanged).

Comp. **3**: UV λ_{max}^{MeOH} (nm): 315, 282; + $AlCl_3$ 310, 283; $AlCl_3$ + HCl 309, 283; + NaOH 366 (307), (272); + NaOAc 313, 280; NaOAc + H_3BO_3 317, 283. MS *m/z* (rel. int.): 342 (30, M^+), 341 (18), 327 (6), 323 (7), 299 (8).

Comp. **4**: UV λ_{max}^{MeOH} (nm): 332, 271; + $AlCl_3$ 359, 281; $AlCl_3$ + HCl 356, 282; + NaOH 388, 270; + NaOAc 366, 273; NaOAc + H_3BO_3 344, 272. MS *m/z* (rel. int.): 328 (78, M^+), 327 (54), 309 (20), 285 (15).

The conclusive hints as to structure elucidation of these flavonoids came from detailed evaluation of their chromatographic behaviour on polyamide DC-11 in our standard solvent systems [4]. Spectral data confirmed these results. The crucial point was the parallel in R_f-differences and in colour reactions with Naturstoffreagenz A between the new compounds **1–4** and the previously reported compounds **I–IV**. We tried to make these relations clear by the arrangement of the structural formulae presented here, following decreasing R_f from top to bottom of the figure. The tetrade **1, 2, I, II** is found in the medium R_f region on polyamide plates



developed with solvent A, while the tetrade **3, 4, III, IV** appears in the medium R_f region when solvent B is used. Products with 6-CH_3 appear as greenish-yellow (**II, IV, 4**) or yellow (**2**) spots in UV_{366} after spraying with Naturstoffreagenz A, such with 6,8-diCH_3 appear as brownish (**III**) or reddish-brown (**1, 3, I**) spots. Among the more trivial flavonoids the pair corresponding structurally and chromatographically to **1/I** and **2/II** is kaempferol-3,7,4′-trimethyl ether/apigenin-7,4′-dimethyl ether, the pair corresponding to **3/III** and **4/IV** is kaempferol-3,7-diMe/apigenin-7-Me.

These R_f-differences and spot colours, combined with the M^+-values, suggest that compound **1** is the 3-O-methyl flavonol corresponding to eucalyptin, comp. **3** is the 3-O-methyl flavonol corresponding to sideroxylin, and compounds **2** and **4** are their relative 8-desmethyl derivatives. The UV-spectral data are indeed in accordance with this suggestion and so are the mass spectra and, for comp. **2**, the NMR spectrum. These four compounds are to our knowledge novel flavonols [*cf.* 5], not found in nature previously. In analogy to the trivial names given to the *Eucalyptus* flavones, we refer to them by the trivial names of kalmiatin (5-OH-3,7,4′-triOMe-6,8-diCH_3-flavone), 8-desmethyl-kalmiatin, latifolin (5,4′-diOH-3,7-diOMe,6,8-diCH_3-flavone), and 8-desmethyl-latifolin.

As mentioned in the Experimental section, column chromatography of epicuticular material from plants of *Kalmia latifolia* yielded two portions, A and B, containing the non-polar compounds and the relatively more polar compounds, respectively. It was observed that the composition of these two portions varied between material obtained from plants with light green leaves and pale pink flowers and plants with dark green leaves and more intense pink flowers. These plants are not recognized as taxonomic categories of any kind. It is remarkable, however, that they differ in the pattern of the epicuticular leaf flavonoids. Dark green plants exhibit eucalyptin and 8-desmethyl-eucalyptin as major flavonoids, 8-desmethyl-kalmiatin, sideroxylin and 8-desmethyl-sideroxylin as minor flavonoids, and kalmiatin and latifolin as trace flavonoids. Light green plants exhibit a little more sideroxylin and especially more 8-desmethyl-kalmiatin, while eucalyptin and kalmiatin can not be detected. The terpenoid composition also appears somewhat different. *Kalmia angustifolia* and *K. polifolia* show only 8-desmethyl-eucalyptin and 8-desmethyl-sideroxylin. It is noteworthy that in these species the acetone-"washing" also contains flavonol glycosides, presumably quercetin glycosides, and the dihydrochalcone phloridzin (6′-glucoside of the 2′,4′,6′,4-tetra-OH-dihydrochalcone, phloretin). The latter was identified after isolation by co-TLC with authentic sample, UV, MS and ^{1}H-NMR. We have no explanation at present for the strange occurrence of glycosidic flavonoids in such material. We are reasonably certain, though, that they were not extracted from the tissue while the leaves were rinsed with acetone. It should be mentioned that phloretin, phloridzin, quercetin, and other flavonoids were reported recently as leaf constituents of *K. latifolia* [6].

We also checked a series of a further Ericaceae plants for the presence of external flavonoid aglycones and terpenoids: *Agapetes buxifolia* Nutt., *Andromeda polifolia* L., *Arbutus unedo* L., *Arctostaphylos uva-ursi* (L.) Spreng., *Bruckenthalia spiculifolia* (Salisb.) Rchb., *Calluna vulgaris* (L.) Hull, *Erica carnea* L., *Gaultheria procumbens* L., *G. shallon* Pursh., *Ledum groenlandicum* Oed., *L. palustre* L., *Leucothoe fontanesiana* (Stud.) Sleum, *Pernettya mucronata* Gaudich ex Spreng., *Pieris floribunda* (Pursh. ex Sims) Benth. ex Hook., and *Pieris japonica* (Thunb.) D. Don ex G. Don. This screening showed that on all the species studied the epicuticular layer consists mostly of several triterpenes. Ursolic acid always occurs as a prominent spot, and so does α/β-amyrin. One spot occurs rather regularly but could not be attributed to any of the markers available In some cases a phytosterol-acetate seems to be present. *Erica carnea* might have δ-amyrenon. These observations are in accordance with the data tabulated in [7]. The Ericaceae are well known for the production of ursolic acid and a variety of other triterpenes.

External flavonoid aglycones were observed in this screening only on *Andromeda polifolia, Gaultheria procumbens* and *G. shallon*, while glycosidic flavonoids seem to be present also on *Andromeda polifolia, Calluna vulgaris* and *Ledum palustre*. As on *Kalmia angustifolia*, they are also supposed to be quercetin glycosides. *Gaultheria procumbens* is exceptional insofar as it exhibits 8-desmethyl-sideroxylin and a little more 8-desmethyl-latifolin. A sample of the same species, obtained from the Heidelberg Botanical Garden, shows a considerably

lower content of flavonoids in the epicuticular layer. In addition to the C-methyl flavonoids just mentioned, this specimen shows galangin-3-methyl ether, overlapping the spot of 8-desmethyl-latifolin. On *Gaultheria shallon* as well as on *Andromeda polifolia*, galangin-3-methyl ether is the only flavonoid aglycone we could detect.

It will be interesting to search for external flavonoids and especially for the C-methylated compounds in further members of the family Ericaceae not now available to us. Also, further samples of the species reported here to produce these flavonoids have to be studied to find out if the flavonoid patterns observed are constant. At any rate, our present results stress once more that C-methyl-flavonoids are more abundant in the plant kingdom than thy had previously [8] been believed to be [*cf.* 9, 10]. Finally it should be mentioned that we checked *Eucalyptus* samples at our disposal (for sources see [1]) for the presence of the newly described flavonoids: *E. amplifolia*, *E. cinerea*, *E. globulus*, *E. goniocalyx*, *E. lehmannii*, *E. macrorhyncha*, *E. sieberi* and *E. urceolaris*. In none of these eight species could we detect any of the novel C-methylflavonols, although they all produce the corresponding C-methylflavones.

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