Flavonoid Aglycones in the Leaf Resin of Some *Cistus* Species

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Members of the genus *Cistus* excrete a leaf resin that consists mainly of terpenoids. This resin also contains methylated flavonoids as aglycones. Rare methyl derivatives of myricetin have been isolated from *C. monspeliensis*, along with uncommon methyl derivatives of quercetin. Further flavonoids encountered in the leaf resin of this and six further species are methyl derivatives of kaempferol and quercetin, some of apigenin and in one species also of luteolin. The results indicate that there might exist species-specific flavonoid patterns.

Introduction

Production of a leaf resin is a well-known feature of some species of *Cistus*. It is especially obvious in *Cistus ladanifer* L. The resin exuded by this species during the summer ("Gumma Labdanum") is collected and distilled to produce a heavily scented perfume ("Labdanum") [1]. The diterpene labdanic acid was isolated and identified from this material in 1956 [2, 3]. The existence of a leaf resin is less obvious in species like *C. salvifolius* L. which is only a little bit sticky, and it is rather unexpected in *C. albidus* L. with its pilous leaves.

Our attention was drawn to the genus *Cistus* by a hint from a gardener who knows about our interest in flavonoid excretion in general [4–6]. Some preliminary tests revealed that *Cistus* was indeed interesting enough to be studied in the scope of our continuing research on this subject. It is surprising that as yet only a few reports on flavonoid aglycones from this genus have appeared. There is a report on the rare flavonol myricetin-3,7,3′,4′-tetramethyl ether from *C. monspeliensis* [7] and there is a report on kaempferol-3,7-dimethyl ether from *C. ladanifer*, in which the authors gave this flavonol the trivial name "jaranol" (from "droga de jara") [8]. Only in the latter paper was it pointed out that the flavonoid aglycone was found in the exudate. In a report on flavonoid patterns of several *Cistus* species [9] only those aglycones have been considered that came from tissue glycosides after hydrolysis. A series of papers has been published recently on the composition of cuticular waxes in the genus *Cistus* [10a–f], but epicuticular flavonoid aglycones have not been taken into account in these publications. We have now reinvestigated *C. monspeliensis*, expecting to detect minor flavonoid components in the exudate, and have also examined material obtained from *C. ladanifer* and *C. laurifolius* L. Further *C. albidus*, *C. creticus* L., *C. psilosepalus* Sweet and *C. salvifolius* L. were studied by TLC and their exudate flavonoids identified.

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Materials and Methods

Cistus albidus, *C. ladanifer*, *C. laurifolius*, *c. monspeliensis* and *C. salvifolius* are cultivated at the Botanischer Garten der TH Darmstadt. Bulk material could be collected of *C. ladanifer*, *C. laurifolius* and *C. monspeliensis*, but only samples, *i.e.* single twigs, could be taken of *C. albidus* and *C. salvifolius*. Part of the material analyzed in *C. monspeliensis* came from the Palmengarten in Frankfurt/Main, where we also obtained twigs of *C. creticus* and *C. psilosepalus*. The leaf exudate was collected in autumn 1982 by rinsing the leaves and twigs with acetone. Concentration of the solutions obtained yielded resinous crude material.

Bulk material was processed in the usual manner (see *e.g.* [11, 12]) by CC on silica of the crude materials, on polyamide (SC-6) for some mixed fractions. With *C. ladanifer* and *C. laurifolius* we thus obtained fractions enriched in flavonoids, which could then be identified by direct comparison with authentic markers on polyamide-TLC in our standard solvents [13]. Crystalline flavonoids were isolated from *C. monspeliensis*, sometimes as mixtures only, but identifications were unambiguous. Quercetin-3,7,3′-triMe could not be separated from myricetin-3,7,3′,4′-tetraMe, even by preparative TLC on silica or polyamide. It is also very hard to distinguish from quercetin-3,7,4′-triMe on TLC, but this can be achieved when both markers are compared simultaneously.

Most of the myricetin-3,7,3′,4′-tetraMe was obtained from the mixed fraction with quercetin-3,7,3′-triMe by fractionated crystallization from

ethanol. Myricetin-3,3′,4′-triMe and quercetin-3,3′-diMe were also obtained in a mixed fraction. The parallel in their chromatographic behaviour to the first mentioned pair of higher methylated products gave the first hint as to the identity of the myricetin trimethyl ether. This product could be obtained only by repeated fractionated crystallization from methanol. It was not absolutely pure, but was good enough for analysis. The very faint amount of quercetin dimethyl ether still present could be detected only on TLC (polyamide), but did not show up in the MS. The terpenoids cover a broad region of polarity and therefore most flavonoids were accompanied by these inconvenient compounds. They could be eliminated, though, by a passage over Sephadex LH-20, eluted with methanol.

Results and Discussion

In all species analyzed most of the leaf resins consist of terpenoid material which we did not investigate further.

Among the resin flavonoids of *C. ladanifer*, kaempferol-3-Me and kae-3,7-diMe are the major components; kae-3,4′-diMe, kae-3,7,4′-triMe and apigenin-7-Me occur in lesser amounts. Apigenin, ap-4′-Me and ap-7,4′-diMe are minor constituents. One or two trace flavonoids could not be identified.

In *C. laurifolius*, kaempferol-3-Me, quercetin-3-Me, qu-3,7-diMe and qu-3,3′-diMe were found as major flavonoid components of the resin. Further flavonols in this exudate are kae-3-Me, kae-3,7-diMe, kae-3,4′-diMe, kae-3,7,4′-triMe; quercetin-7-Me, qu-3′-Me, qu-7,3′-diMe, qu-3,7,3′-triMe, qu-7,3′,4′-triMe, qu-3,7,3′,4′-tetraMe, and apigenin-7-Me. Apigenin, ap-7,4′-diMe, luteolin, lut-7,3′-diMe and the flavanone pinocembrin were encountered in trace amounts.

On TLC we further observed two spots with blue fluorescence in UV_{366}. The more polar one on chromatograms of crude material conceals the spot of apigenin-7-Me, the second one has about the same R_f as apigenin-7,4′-Me.

In *C. monspeliensis* the ratio flavonoids/terpenoids is more in favour of the flavonoids. We were able to isolate several constituents from this species, some in rather good amounts. By far the dominant flavonol is myricetin-3,7,3′,4′-tetramethyl ether, which is probably why this flavonol had been found by the earlier authors as well [7]. Lower amounts could be isolated of myricetin-3,3′,4′-trimethyl ether, quercetin-3,3′-dimethyl ether, kaempferol-3,7-dimethyl ether, and ap-7-Me. In addition we identified kaempferol, kae-3-Me, kae-7-Me, kae-3,4′-diMe; quercetin, qu-3-Me, qu-3′-Me, qu-7-Me, qu-3,7-diMe, qu-7,3′-diMe. Traces of myricetin-7,3′,4′-triMe could also be detected.

Since some of the flavonols encountered in this species are very rare natural products, we want to give their analytical data and information on their distribution in plants.

Myricetin-3,7,3′,4′-tetraMe crystallized from ethanol in fine yellow needles, m.p. 154–155° (Lit. [7] 149–151°). UV: λ_{max} (nm) in MeOH 350, 270; $+AlCl_3$ 400, 352 (308), 278, unchanged with HCl; +NaOH 364, 265; +NaOAc 347, 265, unchanged with H_3BO_3. These data are in accordance with those cited in literature [14]. The MS as to our knowledge has not been published as yet. MS: *m/z* (rel. int.) 374 (100, M^+), 373 (43), 359 (49), 345 (5), 331 (18), 173 (12), 167 (7).

Myricetin-3,7,3′,4′-tetramethyl ether was first described from leaves of *Cistus monspeliensis* [7] and later from leaves of *Doliocarpus amazonicus* (Dilleniaceae) [14]. We also detected it as a trace constituent in the bud exudate of *Betula nigra* (Betulaceae) [Wollenweber, unpubl., *c.f.* 15] and as a typical farina component on the Mexican fern *Notholaena candida* var. *candida* [Wollenweber, unpubl., mentioned in 16].

Myricetin-3,3′,4′-trimethyl ether crystallized from methanol as fine light yellow needles, m.p. 251–253° (m.p. not reported in literature previously). Its identity could be confirmed by direct comparison with an authentic sample [17]. UV: λ_{max} (nm) in MeOH 348, 268; $+AlCl_3$ 398, 349, 305, 275, unchanged with HCl; +NaOH 382, 317, 275; +NaOAc 375, 305, 278; $+H_3BO_3$ 352 (307), 274. These data are more or less in accordance with those reported in [17], except for band I with NaOAc. MS *m/z* (rel. int.): 360 (100, M^+), 359 (40), 345 (72), 331 (17), 330 (16), 317 (37), 153 (17). The fragments are the same as in [17], but not the intensities.

Myricetin-3,3′,4′-trimethyl ether had been cited erroneously in [4] as a constituent of *Decarya madagascariensis* (*c.f.* [18]). It was in fact described for the first time as a natural product from leaves of *Haplopappus integerrimus* (Asteraceae; exudate

component?) [17]. So this is only the second report of its occurrence in plants.

Myricetin-7,3′,4′-trimethyl ether has also been found only once before, namely in the bud exudate of *Aesculus* [19, 20]. Quercetin-3,7,3′-trimethyl ether is also a rather rare flavonol [*c.f.* 21].

One further, as yet unknown, flavonoid in *C. monspeliensis* appears as a spot concealed by quercetin-3,7,3′-triMe only after spraying with "Naturstoff-reagenz A". This constituent could not be separated by any method. A product causing a blue spot on TLC in UV_{366} could be isolated in a very low amount, but has not yet been identified. It is a compound with considerably lower molecular weight than flavonoids. MS *m/z* (rel. int.): 190 (100, M^+), 162 (85), 161 (58), 104 (9), 76 (22). UV: λ_{max} in MeOH 350 (300), 241 nm. Maybe one of our readers can tell us what this might be.

Only small samples were available of *C. albidus*, *C. creticus*, *C. psilosepalus* and *C. salvifolius*. Therefore the exudate flavonoids were determined by direct comparison of crude exudates with markers in different solvents.

In *C. albidus* we thus identified kaempferol, kae-3-Me, kae-7-Me, kae-4′-Me, kae-3,7-diMe, kae-3,4′-diMe, kae-7,4′-diMe, kae-3,7,4′-triMe; quercetin-3-Me, qu-3′-Me, qu-7,3′-diMe, qu-3,3′-diMe, qu-3,7,3′-triMe, and qu-3,7,3′,4′-tetraMe. Among these, kae-3,7-diMe, kae-7,4′-diMe, kae-3,7,4′-triMe and qu-3,7,3′,4′-tetraMe are minor and/or trace constituents only.

In *C. creticus* the flavonoids in total are trace components in the terpenoid resin. The products we identified were kaempferol, kae-7-Me, kae-4′-Me, and kae-3,4′-diMe; quercetin-3′-Me, and qu-7,3′-diMe. The latter seems to be the major flavonol in this species.

In *C. psilosepalus* the flavonoid content of the resin is a little bit higher. We found kaempferol-3-Me, kae-3,4′-diMe, quercetin-3,3′-diMe and qu-3,7,3′-triMe as major flavonoid components, kae-7,4′-diMe, apigenin, ap-7-Me and luteolin-7,3′-diMe as minor constituents, and trace amounts of kaempferol, kae-3,7-diMe, kae-3,7,4′-triMe, qu-3,7,3′,4′-tetraMe and apigenin-7,4′-diMe.

In *C. salvifolius* the flavonoid content of the resin is very low again. We identified kaempferol, kae-7-Me, kae-4′-Me and quercetin-7,3′-diMe as major flavonoids, kae-7,4′-diMe, kae-3,7,4′-triMe, qu-3′-Me, apigenin-7-Me and ap-7,4′-diMe as minor flavonoids. It should be stressed that the results reported here are preliminary in so far as only single plants or a maximum of three plants were studied. We therefore do not claim at present that these flavonoid patterns are characteristic for the individual species. For this reason the flavonoid patterns are not presented in a Table. We assume, however, that there exist species-specific flavonoid patterns in the leaf resin of members of the genus *Cistus*. Further studies on this subject would be desirable.

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Note added in proof: After submission of the manuscript a relevant paper appeared that reports on the identification of diterpenes and flavonoid aglycones from hexane extract of *Cistus palinhae* [22]. We assume that these compounds also are constituents of a leaf resin. Further a paper is in press (P. Proksch, pers. comm.) that reports on flavonoid aglycones from the leaf resin of *C. ladanifer* and *C. palinhae* [23].