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THE SYSTEMATIC AND EVOLUTIONARY SIGNIFICANCE OF EXUDATE FLAVONOIDS IN *AEONIUM*

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Key Word Index—*Aeonium*; Crassulaceae; leaf resin; exudate flavonoids; 6-*O*-methylated flavonoids; myricetin methyl ethers; chemotaxonomy; evolution; ecology.

Abstract—Leaf exudates of 32 species of *Aeonium* were examined for the presence of flavonoids. Thirty two flavonoids were detected in exudates of half of the species. The flavonoids were identified as methyl ethers of kaempferol, 6-hydroxykaempferol, quercetin, myricetin and scutellarein. The distribution of the exudate flavonoids is mostly in agreement with infrageneric sectional classifications based on morphological and molecular characters. The combination of kaempferol 3,7-dimethyl ether and quercetin 3,7-dimethyl ether is characteristic of section *Aeonium*. Myricetin methyl ethers are restricted to section *Goochia* and section *Petrothamnium*. Section *Leuconium*, section *Canariense* and section *Patinaria* are particularly poor in exudate flavonoids. 6-Hydroxyflavonoids occur in leaf waxes of six species belonging to sections *Goochia*, *Canariense*, *Chrysocome* and *Pittonium*, probably as a result of inheritance of this feature from a common ancestor. The presence of exudate flavonoids shows weak correlations with habitat and the presence of glandular hairs.

INTRODUCTION

Aeonium comprises about 37 species, 34 of which occur in Macaronesia (Canary Islands, Cape Verde Islands and Madeira), whereas the remaining three species are distributed in western Morocco (*A. korneliuslemsii*), eastern Africa and Yemen (*A. leucoblepharum* and *A. stuessyi*) [1]. The Macaronesian species are primarily single-island endemics and occupy a wide range of different ecological niches. They are found in moist and shaded as well as in exposed and arid, or (sub)alpine habitats. Natural hybridization between sympatric species with overlapping flowering times is known to occur, but is relatively rare, probably because of the absence of intermediate habitats [1, 2]. Growth forms of the perennial taxa vary from small, procumbent herbs or subshrubs to large, branched shrubs, and of the monocarpic taxa from often large rosettes to rosette trees. It is generally understood that the variation in growth forms in *Aeonium* evolved through adaptive radiation. The leaves of *Aeonium* often have a well-developed epicuticular wax layer as an adaptation to life in arid conditions. Some species secrete a sticky resin on their leaves.

Previous phytochemical investigations in *Aeonium* focused on tannins, terpenoids, leaf alkanes and flavonoids (reviewed in refs [3, 4]). The tannins of *Aeonium* have been identified as prodelphinidins and galloyl esters [5, 6]. In several species, tannins have been found in characteristic subepidermal idioblasts [1]. Baker *et al.* [7] reported labdane-8, 15-diol as the major diterpene from the leaf gum (resin) of *A. lindleyi*, and they also detected related diterpenes in *A. goochiae* and *A. spathulatum*. The leaf alkane variation within *Aeonium* was shown to be correlated with the sectional classification, but the sections could not be clearly delimited [8]. Exudate flavonoids have been reported for two species only, i.e. *A. manriqueorum* Bolle [9] and *A. saundersii* [10].

In the present study, we have examined the leaf waxes and resinous exudates of 32 species of *Aeonium* for the presence of flavonoids. In the following, we use the term 'wax' in the botanical sense, regardless of its chemical definition [10]. The exudate flavonoid variation was studied with the aim of determining its systematic and evolutionary significance. The results are discussed in relation to an infrageneric classification based on morphological and molecular characters and to the ecological preference of the species.

RESULTS

Leaf waxes of 32 *Aeonium* species were obtained by brief immersion in chloroform. Precipitation of the major part of the alkanes and other 'fatty' constituents in cold methanol, and subsequent column chromatography of the supernatant on Sephadex LH-20 yielded mixtures of flavonoid aglycones for half of the species examined. The latter purification step proved very efficient in separating

terpenoids from flavonoids. The presence of significant amounts of flavonoids showed a strong correlation with stickiness of the leaves of *Aeonium*. A similar correlation has been observed in many other angiosperms [10]. A few non-resinous *Aeonium* species also contained a rich array of flavonoids, albeit in small amounts (*A. arboreum* and *A. holochrysum*).

The flavonoid samples were analysed by a combination of co-TLC with authentic markers, colour reactions with and without Naturstoffreagenz A (NA) on polyamide 11 [11] and GC-EI-mass spectrometry after trimethylsilylation. GC-mass spectral analysis of flavonoid TMSi derivatives showed abundant $[M - 15]^+$ ions on electron impact (EI) due to loss of a methyl from a trimethylsilyl group [12]. Since all flavonoids contained at least one trimethylsilyl group on the 5-position, remarkably little fragmentation due to cleavage of the heterocyclic C-ring occurred. Only the $[B_2]^+$ ions (*m/z* 135) [13] originating from kaempferol methyl ethers with a 4′-*O*-methyl group were observed. Most of the flavonoid aglycones were separated by GC, but in cases of low resolution the mixed mass spectra generally allowed identification of the compounds.

Table 1. Flavonoids detected in leaf exudates of *Aeonium* spp.

No	Flavonoid
1	Kaempferol
2	3-methyl ether
3	7-methyl ether
4	3,7-dimethyl ether
5	3,4′-dimethyl ether
6	7,4′-dimethyl ether
7	3,7,4′-trimethyl ether
	6-hydroxykaempferol
8	6-methyl ether
9	3,6-dimethyl ether
10	6,7-dimethyl ether
11	6,4′-dimethyl ether
12	3,6,7-trimethyl ether
13	3,6,4′-trimethyl ether
14	3,6,7,4′-tetramethyl ether
15	Quercetin
16	3-methyl ether
17	7-methyl ether
18	3′-methyl ether
19	3,7-dimethyl ether
20	3,3′-dimethyl ether
21	7,3′-dimethyl ether
22	3,7,3′-trimethyl ether
23	7,3′,4′-trimethyl ether
24	3,7,3′,4′-tetramethyl ether
	Myricetin
25	3,7-3′-trimethyl ether
26	3,7,4′-trimethyl ether
27	7,3′,4′-trimethyl ether
28	3,7,3′,4′-tetramethyl ether
29	3,7,3′,4′,5′-pentamethyl ether
	Scutellarein
30	6,7-dimethyl ether
31	6,4′-dimethyl ether
32	Luteolin 4′-methyl ether

Thirty two flavonoids were identified as methyl ethers of kaempferol, 6-hydroxykaempferol, quercetin, myricetin and scutellarein (6-hydroxyapigenin), as well as traces of kaempferol, quercetin and luteolin 4′-methyl ether (Tables 1 and 2). Furthermore, *A. smithii* contained two as yet unidentified flavones or flavonols in addition to 6-hydroxykaempferol 3,6,7-trimethyl ether. Colour reaction with and without NA and GC-EI-mass spectrometry of this sample points to highly methylated derivatives of 6-hydroxymyricetin. Altogether these flavonoids provide a fairly complete picture of the species' flavonol and flavone chemistry. A few additional flavanones and/or chalcones present in leaf resins of some species need further characterization after being isolated from bulk material.

The leaf wax of *A. arboreum* contained quercetin 3,7-dimethyl ether as the principal flavonol along with several other quercetin methyl ethers. This is in agreement with a previous report of quercetin 3,7-dimethyl ether from leaf waxes of *A. manriqueorum* [9], which is conspecific with *A. arboreum* [1]. Furthermore, we were able to confirm the presence of kaempferol 3-methyl ether and kaempferol 3,4′-dimethyl ether (ermanin) in the leaf exudate of *A. saundersii* reported by Wollenweber [10].

For seven of the species, the exudate flavonoids of two or three plants were examined to determine the intraspecific variation. The waxes from different plants of the same species contained the same principal flavonols, the intraspecific variation being limited to the minor flavonoids. The two plants examined of *A. spathulatum* had identical exudate flavonoid profiles (cf. Table 2). These results indicate that exudate flavonoid chemistry has potential taxonomic significance at the infrageneric and species level in *Aeonium*.

DISCUSSION

The exudate flavonoid patterns within *Aeonium* agree mostly with an infrageneric sectional classification primarily based on morphological characters [1, 14] and modified on the basis of molecular systematic studies [15]. Taxonomically the occurrence of myricetin methyl ethers and 6-hydroxykaempferol methyl ethers is most significant (Table 2). Especially since extra hydroxylation at the 6-position is generally regarded as an advanced feature and is uncommon in the Crassulaceae. However 6-oxygenated flavonoids have been reported from *Kalanchoe spathulata* [16] and *K. gracilis* (Crassulaceae) [17, 18], which are both endemic to southern Africa and not directly related to *Aeonium*. On the other hand, the Eurasian Sedoideae, which are more closely related to *Aeonium* [15], are characterized by 8-hydroxy and 8-methoxyflavonols [4].

The species of section *Aeonium* contain kaempferol 3,7-dimethyl ether and quercetin 3,7-dimethyl ether as the major flavonoids, except for *A. gorgoneum* and *A. simsii* which lack exudate flavonoids. Liu [1] placed *A. leucoblepharum* and *A. gorgonium* in section *Pittonium*.

However, the flavonoid profile of *A. leucoblepharum* strongly supports its classification with the large woody taxa in section *Aeonium* (= section *Holochrysa* (Christ) Praeger) as has previously been suggested by Praeger [14]. Molecular characters also support the classification of the large woody species in a single section [15], and further indicate that the rosulate *A. simsii* with axial inflorescences should be included in section *Aeonium*. Praeger [14] and Liu [1] placed the latter species among the small herbaceous or shrubby taxa of section *Goochia* and section *Chrysocome*, respectively. However, the absence of exudate flavonoids from *A. simsii* neither favours or disfavours its inclusion in section *Aeonium*.

Myricetin methyl ethers are restricted to section *Goochia* and section *Petrothamnium*. Praeger [14] and Liu [1] placed the five species of both sections in a single section, and our results support the latter treatment rather than the classification based on molecular characters [15], in which the relationships between section *Goochia* and section *Petrothamnium* were not very well resolved. Both sections hold a position at a basal polychotomy of the phylogeny together with other sections lacking myricetin methyl ethers. This may indicate that the production of leaf surface myricetin methyl ethers originated only once in the evolution of the genus, and that these two sections belong to a single lineage. *Aeonium goochiae* does not produce myricetin derivatives. This may be either because it has lost the ability to produce these compounds or because it holds a more distant evolutionary position.

Aeonium goochiae and *A. viscatum* also contain a number of 6-hydroxykaempferol methyl ethers. However, the occurrence of 6-oxygenated flavonoids is not restricted to species of section *Goochia*. *Aeonium palmense* a member of section *Canariense*, which is otherwise devoid of exudate flavonoids, *A. smithii* and *A. spathulatum* (section *Chrysocome*) and *A. glutinosum* (section *Pittonium*) also contain these compounds. As hydroxylation at the 6-position is a special feature and rare in the Crassulaceae, it could indicate a close relationship between these taxa. However, this is not in agreement with the morphology of the species and the molecular phylogeny. Alternatively, the scattered distribution of 6-oxygenated flavonoids could be the result of parallel evolution, but this is also not very likely in view of the rare occurrence of these flavonoids in the Crassulaceae. The most plausible explanation for the distribution of 6-oxygenated flavonoids in *Aeonium* seems to be a unique gain of this feature early in the evolution of the genus and subsequent loss of it in various lineages. Molecular studies [15] support this hypothesis, because *A. glutinosum*, which contains the widest variety of 6-hydroxykaempferol and scutellarein methyl ethers, holds a basal position in the molecular phylogeny of the genus.

Aeonium section *Leuconium*, which comprises the white-flowered shrubby species, is particularly poor in exudate flavonoids. Exceptions are *A. decorum* and *A. nobile* which contain quercetin methyl ethers. On the island of Gomera, *A. decorum*, *A. gomerense* and *A. urbicum* grow intermingled. Liu [1] suggested a hybrid origin for *A. gomerense*, which he considered to be morphologically intermediate between *A. decorum*, and *A. urbicum*. However, if *A. gomerense* is indeed of hybrid origin, *A. decorum* is less likely to be involved as one of the parental species, because *A. gomerense* lacks exudate flavonoids completely.

In the molecular phylogeny the two species of *Aeonium* from Madeira, viz. *A. glandulosum* of section *Patinaria* and *A. glutinouum* of section *Pittonium*, each represent a single, independent lineage [15]. *Aeonium glandulosum* is usually classified in section *Canariense* which comprises all the rosulate, hapaxanth species [1, 14]. The lack of exudate flavonoids precludes any further speculation about the systematic position of this species. *Aeonium glutinosum* often forms large (sub)shrubs, and consequently Liu [1] considered it to be affiliated with the woody taxa of section *Aeonium*. However, the exudate flavonoid spectrum of *A. glutinosum* excludes a relationship with section *Aeonium*. On the contrary, the presence of a variety of 6-hydroxykaempferol and especially of scutellarein methyl ethers may indicate a closer link with *A. spathulatum* of section *Chrysocome*.

Accumulation of surface flavonoids is very often found in plants living in or originating from (semi-)arid habitats [19]. The correlation between the presence of exudate flavonoids and habitat is not obvious in *Aeonium*, however. Plants from laurel forests (moist, cool) are indeed poor in exudate flavonoids, but the plants from dry habitats (south-facing slopes and (sub)alpine regions) as well as the more humid north-facing cliffs either contain significant deposits of flavonoids on their leaves or lack exudate flavonoids completely (cf. Table 2). Apparently, the other wax constituents play a more important role in the protection of plants against desiccation and irradiation. The large deposits of leaf surface alkanes present in members of section *Leuconium* (unpublished results) merit further attention in this respect.

In *Aeonium*, the presence of large deposits of resinous material containing significant amounts of flavonoids is weakly correlated with the occurrence of secretory multicellular trichomes (cf. Table 2). The sticky leaves of *A. spathulatum*, *A. palmense*, *A. goochiae*, *A. lindleyi*, *A. viscatum* and *A. sedifolium* are variously covered with glandular hairs [1], but the exceedingly sticky leaves of *A. glutinosum*, which is also a rich source of exudate flavonoids, are glabrous. On the other hand, *A. simsii*, *A. valverdence*, *A. glandulosum* and most species of section *Canariense* also have glandular pubescent leaves, but are devoid of exudate flavonoids. Using histochemical methods, Liu [1] revealed tannin (not further characterized) in the heads of glandular hairs of *A. palmense*, *A. lindleyi*, *A. sedifolium* and *A. smithii*, and he surmised that tannin is secreted from the heads of these glandular hairs. Although monomeric flavans and biflavanoids (condensed tannins) have been reported from leaf resins [20], we have not been able to confirm the presence of flavans in leaf resins of *Aeonium* by TLC. In contrast, our TLC and GC-EI-mass spectral examinations point to the abundant presence of diterpenes in leaf resins in accordance with the results of Baker *et al.* [7], who found diterpenes

Table 2. Classification,* origin, habitat, indument

Taxon	Origin (Acc. no.)	Habitat†	Indument‡	Flavonoids§ 1	2	3	4	5	6	7
Aeonium Webb & Berth.										
A. sect. Aeonium Webb & Berth.										
arboreum (L.) Webb & Berth.	Cultivated (31850)	S	–	–	–	–	–	–	–	–
gorgoneum J. A. Schmidt.	Cape Verde (31888)	A	–	–	–	–	–	–	–	–
leucoblepharum Webb ex A. Rich.	Yemen (30669)	A	–	–	–	–	+	–	–	–
leucoblepharum Webb ex A. Rich.	Yemen (31898)	A	–	+	–	+	+	–	–	–
holochrysum Webb & Berth.	Tenerife (30467)	N	–	–	–	–	+	–	–	+
holochrysum Webb & Berth.	Tenerife (31615)	N	–	–	–	–	+	–	–	–
holochrysum Webb & Berth.	Tenerife (31852)	N	–	–	–	–	+	–	–	–
rubrolineatum Sventenius	Gomera (31856)	S	–	–	–	–	+	–	–	–
simsii (Sweet) Stearn	G. Canaria (30440)	A	G	–	–	–	–	–	–	–
A. sect. *Goochia* (Christ) Praeger										
goochiae Webb & Berth.	La Palma (31885)	N	G	–	–	–	+	+	–	+
lindleyi Webb & Berth.	Tenerife (31617)	N	G	–	+	–	+	+	–	–
lindleyi Webb & Berth.	Tenerife (31901)	N	G	–	–	–	+	+	–	+
viscatum Bolle	Gomera (30479)	N	G	–	+	–	–	–	–	–
A. sect. *Petrothamnium* (Webb) Liu										
saundersii Bolle	Gomera (31478)	N	G	–	+	–	–	+	–	–
saundersii Bolle	*Gomera* (31906)	N	G	–	–	–	–	+	–	–
sedifolium (Webb) Pit. & Proust	Tenerife (31479)	S	G	–	–	–	–	–	–	–
sedifolium (Webb) Pit. & Proust	Tenerife (31602)	S	G	–	–	–	+	–	+	–
sedifolium (Webb) Pit. & Proust	Tenerife (31907)	S	G	–	–	–	+	–	–	–
A. sect. *Canariense* (Christ) Praeger										
canariense (L.) Webb & Berth.	Tenerife (31857)	L	G	–	–	–	–	–	–	–
canariense (L.) Webb & Berth.	Tenerife (31858)	L	G	–	–	–	–	–	–	–
cuneatum Webb & Berth.	Tenerife (31873)	L	–	–	–	–	–	–	–	–
palmense Webb ex Christ	Hierro (30491)	L	G	–	+	–	+	+	–	–
palmense Webb ex Christ	Hierro (31859)	L	G	–	+	–	–	–	–	–
subplanum Praeger	Gomera (30483)	L	G	–	–	–	–	–	–	–
tabulaeforme (Haw.) Webb & Berth.	Tenerife (31918)	N	G	–	–	–	–	–	–	–
virgineum Webb ex Christ	G. Canaria (31619)	N	G	–	–	–	–	–	–	–

and exudate flavonoids of *Aeonium* spp.

Flavonoids§																										
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32		
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Table 2.

(*Continued*)

Flavonoids§ 8 9 10 11 12 13 14	15 16 17 18 19 20 21 22 23 24	25 26 27 28 29	30 31	32
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+ + − + − + −	− − − − − − − − − −	− − − − −	− +	−
− − − − − − −	− − − − − − − − − −	− − − − −	− −	−
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− − − − − − −	+ + − + − − + + − −	− − − − −	− −	−
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cooled to − 18° to ppt. the major part of the alkanes and other 'fatty' constituents. After centrifuging the mixts, the supernatants were concd and chromatographed directly over Sephadex LH-20 (column 35 × 1 cm) with MeOH as eluent. Frs. of *ca* 5 ml were collected and monitored with TLC. The flavonoid-containing frs (predominantly free of terpenoids) were combined and concd.

Analysis and identification of flavonoids. Identification was achieved by co-TLC with authentic markers available in E.W.s lab, and GC/GC-EIMS of the flavonoid TMSi ethers. Thin-layer chromatogrammes were run on polyamide DC-11 with (a) toluene–petrol (100°–140°)–MeCOEt–MeOH (12:6:2:1), (b) toluene–dioxane–MeOH (8:1:1) and (c) toluene–MeCOEt–MeOH (12:5:3), and on silica gel with (a) toluene–MeCOEt (9:1) and (b) toluene–dioxane–HOAc (18:5:1). Developed plates were examined under UV (350 nm) before and after spraying with NA. For GC/GC-EIMS analysis, portions of the flavonoid mixts were evapd *in vacuo* at 70° to remove traces of MeOH

and redissolved in 100 μl of pyridine. These solns were mixed with equal vols of bistrimethylsilyl acetamide, and kept at 70° overnight prior to GC analysis. GC was performed under the following conditions: capillary column, WCOT fused silica CP Sil 5 CB, 10 m × 0.32 mm i.d., film thickness of the stationary phase: 0.12 μm; temp. programme: 125–325° at 4° min^{-1}, 325° maintained for 5 min.; injector temp.: 250°; FID temp.: 300°. Carrier gas and flow: N_2 at 34 cm^{-1} s. Injection vol. 1.0–3.0 μl; split ratio 1:60. GC-EIMS (70 eV) data were obtained under similar conditions: capillary column, WCOT fused silica CP Sil 5 CB, 10 m × 0.25 mm i.d., film thickness of the stationary phase: 0.12 μm; temp. programme: 125–325° at 4° min^{-1}; injection temp. 250°. Carrier gas and flow: He at 1 ml min^{-1}. Injection vol.: 1.0 μl; split ratio 1:20.

Isolation of kaempferol 3,7-dimethyl ether from the leaf wax of A. holochrysum (acc. no. 30467). The wax from fr. leaves (135 g) was extracted with 25 ml boiling MeOH; the mixt. was left standing at 4° for 30 min and then filtered. After evapn of the filtrate, the residue was fractionated on a Sephadex LH-20 column with increasing proportions of MeOH in H_2O. The flavonoid-containing frs were combined and subjected to prep. silica gel TLC with toluene–HCOOEt–HCOOH (5:4:1) as eluent. Kaempferol 3,7-dimethyl ether was scraped off and recovered from the stationary phase with MeOH containing 1% TFA. TLC on polyamide 11 (toluene–MeCOEt–MeOH, 13:5:3) R_f 0.51, spot colour with NA: yellow. GC of TMSi ether: RR_t (relative to fisetin) 0.951. GC-EIMS of TMSi ether, *m/z* (rel. int.): 458 $[M]^+$ (3), 443 $[M - 15]^+$ (54), 73 (100). UV λ_{max} (MeOH) nm: 266, 295 (sh), 351. ^{1}H NMR (300 MHz, DMSO-d_6): δ12.68 (1H, *s*, OH-5), 7.98 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 6.94 (2H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.75 (1H, *d*, *J* = 2.0 Hz, H-8), 6.37 (1H, *d*, *J* = 2.0 Hz, H-6), 3.86 and 3.80 (2 × O-Me).

Isolation of the principal exudate flavonols from the leaf wax of A. goochiae (acc. no. 31885). The wax from fr. leaves (100 g) was dissolved in 25 ml boiling MeOH. The mixt. was left standing at 4° for 30 min and then filtered. After evapn of the filtrate, the residue was chromatographed on silica gel (pre-washed with conc. HCl) with increasing proportions of Et_2O in hexane, and finally with MeOH. The fr. containing mainly kaempferol 3,7,4′-trimethyl ether and 6-hydroxykaempferol 3,6,4′-trimethyl ether was evaporated. Both flavonoids were purified on prep. silica gel plates with toluene–HCOOEt–HCOOH (5:4:1) as eluent, and recovered from the stationary phase with MeOH containing 1%TFA.

Kaempferol 3,7,4′-trimethyl ether. TLC on polyamide 11 (toluene–MeCOEt–MeOH, 13:5:3). R_f 0.83, spot colour with NA: yellow. GC of TMSi ether: RR_t (relative to fisetin) 0.885. GC-EIMS of TMSi ether, *m/z* (rel. int.): 400 $[M]^+$ (4), 385 $[M - 15]^+$ (100), 135 $[B_2]^+$ (34) (cf. ref. [13]), 73 (47). UV λ_{max} (MeOH) nm: 267, 300 (sh), 325 (sh), 343. ^{1}H NMR (300 MHz, DMSO-d_6): δ12.62 (1H, *s*, OH-5), 8.06 (2H, *d*, *J* = 9.2 Hz, H-2′ and H-6′), 7.15 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 6.77 (1H, *d*, *J* = 2.2 Hz, H-8), 6.39 (1H, *d*, *J* = 2.2 Hz, H-6), 3.87, 3.86, and 3.81 (3 × O-Me).

6-Hydroxykaempferol 3,6,4′-trimethyl ether. TLC on polyamide 11 (toluene–MeCOEt–MeOH, 13:5:3). R_f 0.60, spot colour with NA: brown. GC of TMSi ether: RR_t (relative to fisetin) 0.995. GC-EIMS of TMSi ether, *m/z* (rel. int.): 488 $[M]^+$ (1), 473 $[M - 15]^+$ (66), 135 $[B_2]^+$ (14) (cf. ref. [13]), 73 (100). UV λ_{max} (MeOH) nm: 272, 295 (sh), 335. ^{1}H NMR (300 MHz, DMSO-d_6): δ12.73 (1H, *s*, OH-5), 8.02 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 7.13 (2H, *d*, *J* = 9.2 Hz, H-3′ and H-5′), 6.57 (1H, *s*, H-8), 3.86, 3.79, and 3.76 (3 × O-Me).

Detection of other resin constituents. Frs from Sephadex CC (see above) containing sticky material were also analysed by TLC and GC/GC-MS. Thin-layer chromatogrammes were run on cellulose with 15% HOAc and on silica gel with toluene–HCOOEt–HCOOH (5:4:1). Developed cellulose plates were sprayed with vanillin–conc. HCl to detect flavanoids and silica gel plates with anisaldehyde–H_2SO_4 for detection of terpenoids.

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