New Flavonoids from the Exudate of *Baccharis bigelovii* (Asteraceae)

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Four flavonoids have been identified from the leaf and stem resin produced by *Baccharis bigelovii* (Asteraceae) in addition to twelve previously described aglycones. A flavonol (alnusin) and a dihydroflavonol (alnustinol) with 3,5,7-trihydroxy substitution had both been reported only twice before. The novel compound 2β,5,7-trihydroxyflavanone is remarkable for its 2β-substitution at the heterocyclic ring. 7-Benzoyl-chrysin is the first flavone aglycone found as ester and the first flavonoid aglycone at all that is esterified with an aromatic acid.

Introduction

In a recent publication we surveyed the known data on flavonoid aglycones in *Baccharis* species and reported the identification of a number of flavonoids from six species [1]. A dozen known flavonoids were reported from the leaf and stem resin of *B. bigelovii* A. Gray [1]. We now want to describe the structural elucidation of four additional compounds, two of which are novel natural products.

Material and Methods

Aerial parts of *Baccharis bigelovii* were collected in southern Arizona (26/12/84; Mule Mountains, Box Canyon Ranch, Cochise Co.; elev. 5600 ft). The plants form scattered muchbranched shrubs to 3 ft tall in rocky area on granitic substrate. A voucher is kept at the Herbarium of the University of Arizona in Tucson (ARIZ; G. Yatskievych 84–193). The air-dried material consisted mainly of stems, with few leaves. On rinsing with acetone, 333 g of plant material yielded 7.8 g of exudate as brown resin. The major flavonoid constituent, chrysin, crystallized from this resin (ca. 200 mg). The residue was worked up by column chromatography, monitored by TLC, as described earlier [1]. Known flavonoids were identified by direct comparisons with authentic markers. Four additional products will be discussed now. Their R_f-values are given for TLC on polyamide DC-6 with solvent toluene/petrol$_{100-140\,°C}$/MeCOEt/MeOH 60:30:10:5 [2]. Spot colours were observed in UV_{366} before and after spraying with "Naturstoffreagenz A" (C. Roth; 0.5% in MeOH). Mass spectra were recorded on a Varian MAT 311. ^{1}H NMR spectra were recorded on a Bruker WP 200 SY spectrometer at 200 MHz. The data are compiled in Table I. Melting points are uncorrected.

Compound **1**: Slightly yellow, fine crystals, m.p. 191–192 °C; R_f 0.95 (dark/dull yellow). UV λ_{max}^{MeOH} (330), 268, (227); $AlCl_3$ 375, 316, 275; NaOH 362, 275; NaOAc 268, unchanged with H_3BO_3. MS *m/z* (rel. int.) 358 (28%, M^+), 225 (3), 123 (7), 106 (12), 105 (100), 77 (56).

Compound **2**: Slightly yellow, fine crystals, m.p. 170–172 °C; R_f 0.40 (dark/dark brown). UV λ_{max}^{MeOH} (344), 298 (230); $AlCl_3$ 393, 316; NaOH 332 (250); NaOAc 332, unchanged with H_3BO_3. MS *m/z* (rel. int.) 302 (77%, M^+; $C_{16}H_{24}O_6$ calc. 302.0790, found 302.0789), 273 (15), 195 (9), 183 (100; $C_8H_7O_5$ calc. 183.0291, found 183.0291), 167 (63; $C_7H_3O_5$ calc. 166.9982, found 166.9982), 156 (27), 120 (12), 105 (9), 91 (31), 77 (10), 69 (57).

Compound **3**: Fine colourless crystals, m.p. 287–290 °C; R_f 0.15 (dark/dull greenish-brown). UV λ_{max}^{MeOH} (330), 289; $AlCl_3$ 375, 312; NaOH 325; NaOAc 326; NaOAc + H_3BO_3 326, 293. MS *m/z* (rel. int.) 272 (54%, M^+), 255 (26), 195 (8), 153 (58), 105 (100), 77 (73), 69 (29).

Compound **4**: Not obtained in crystalline form, due to lack of material; R_f 0.36 (brownish/dull greenish). UV λ_{max}^{MeOH} (353), 329, 273; $AlCl_3$ 415, 357, 276; NaOH 413, (350), 285. MS *m/z* (rel. int.) 300 (100%, M^+), 285 (23), 282 (33), 257 (81), 176 (10), 105 (63), 77 (46), 69 (50).

Results

The major flavonoid present in the resinous exudate of *Baccharis bigelovii* is the flavone chrysin. (Compounds **1**–**4** were cited as **3**–**6** in ref. [1].) Galangin and the new constituents **1**, **2** and **3** were also obtained in crystalline form. These flavonoids are accompanied by apigenin, kaempferol, kae-7,4′-diMe, quercetin, isorhamnetin, pinocembrin, and pinobanksin. Luteolin and its 3′- and 4′-methyl

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Table I. ^{1}H NMR chemical shifts for compounds **1**–**4**.

Assignment	Compound **1**$^+$	Chrysin*	Compound **2**$^+$	Acetate **2a**$^+$	Compound **3**§	Compound **4**$^+$
H-2β	–	–	4.56 d (12)	5.40 d (12)	–	–
H-3α	6.76 s	6.80 s	5.08 d (12)	5.73 d (12)	3.21 dd (15.5, 2)	–
H-3β			–	–	2.88 dd (15.5, 2)	
H-6	6.71 d (2)	6.28 d (2)	–	–	5.97 d (2)	–
H-8	7.00 d (2)	6.68 d (2)	6.14 s	6.75 s	6.00 d (2)	6.63 s
H-2′/H-6′	7.91 dd (8, 1.5)	8.12–8.05 m	7.58–7.43 m	7.48–7.41 m	7.71 dd (8, 1.5)	8.21 dd (8, 2)
H-3′/H-4′/H-5′	7.65–7.48 m	7.65–7.59 m			7.48–7.38 m	7.62–7.48 m
5-OH	12.79 s	12.90 s	11.36 s	–	12.04 s	11.92 s
OCH_3	–	–	3.96 s	3.79 s	–	4.06 s
Others	8.21 dd (2H, 8, 1)	–	6.63 bs	2.43 s, 2.36 s,	9.63 s, 6.54 d (2)	6.61 bs
	7.68 dt (8, 1)		(3-OH)	1.99 s	(2×OH)	
	7.65–7.48 m (2H)			(3×OAc)		

$^+$ In $CDCl_3$, * in $(CD_3)_2CO$, § in DMSO-d_6 (J in Hz, δ in ppm).

ethers (chrysoeriol and diosmetin) were observed as trace constituents.

*Structure elucidation of compounds **1**–**4***

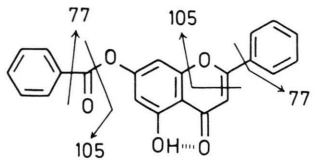
The ^{1}H NMR spectrum of compound **1** shows only aromatic protons (see Table I). The singlet at δ 6.76 ppm is assignable to H-3, while the two one-proton doublets at δ 6.71 and 7.00 are assigned to H-6 and H-8, respectively, in a 5,7-di-oxygenated flavone. The presence of two different monosubstituted benzene rings is shown by the multiplets between δ 7.48 and 8.12. The signal in the far-down low field (δ 12.79) shows the presence of a hydroxy group which is hydrogen-bonded to the carbonyl group (OH at C-5). Thus, the substituent must be located at C-7. The identification of this substituent as benzoate has been made through the NMR multiplets at δ 8.21, 7.68 and 7.65–7.48, which agree closely for H-2/H-6, H-4 and H-3/H-5, respectively, in a benzoic aromatic ester comparing with calculated values [3]. The mass spectrum supports the proposed structure by the molecular peak at m/z 358 and two unusually strong ions at m/z 105 (PhCO) and 77 (Ph), arising from the B-ring fragment as well as from the benzoic ester. Alkaline hydrolysis of **1** (KOH 8.5%, room temp., overnight) yields the flavone chrysin and benzoic acid. Both compounds are identified by their spectroscopic data and by comparisons with authentic markers. Hence the structure of **1** is undoubtedly 7-benzoyl-chrysin.

The ^{1}H NMR spectrum of compound **2** shows two one-proton doublets at δ 4.56 and 5.08 ppm, that suggest the structure of a dihydroflavonol. Like compound **1** it has an unsubstituted B-ring (multiplet between δ 7.58 and 7.43) and a OH-group at C-5 (singlet at δ 11.36), but a methoxy group (singlet at δ 3.96) and only one proton at the A-ring (singlet at δ 6.14). Acetylation of **2** affords the triacetate **2a** and confirms the dihydroflavonol structure by shifting the H-2/H-3 signals at low field and by the existence of an aliphatic acetyl (singlet at δ 1.99). In total the spectroscopic data lead us to establish the structure of compound **2** as 3,5,7-trihydroxy-6-methoxy-flavanone.

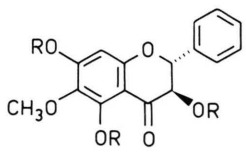
Compound **3** has a molecular formula of $C_{15}H_{12}O_5$, M^+ 272. Its chromatographic behaviour and its UV-spectrum point to a flavanone having three hydroxy groups. The singlets at δ 12.04 and δ 9.63 ppm for OH at C-5 and at C-7 as well as two doublets at about δ 6 ppm for H-6 and H-8 are easily assigned. Once again, the NMR signals indicate an unsubstituted B-ring (multiplets at δ 7.71, 2H, and at 7.42, 3H), which is confirmed by strong ions at m/z 105 and 77 in the mass spectrum. Thus, the remaining signals and the third hydroxy group must be those corresponding to the heterocyclic ring. The β-axial configuration of the OH at C-2 can be deduced from the coupling constant (2 Hz) observed between such hydroxyl (δ 6.54, d) and the transaxial H-3α (δ 3.21, dd). Compound **3** is described as 2β,5,7-trihydroxy-flavanone.

Compound **4**, M^+ 300, molecular formula $C_{16}H_{12}O_6$, also has an unsubstituted B-ring (δ 8.21 ppm, 2H, and 7.55, 3H). Further, an aromatic proton (δ 6.63, s) and a methoxy group (δ 4.06, s) can be deduced from the NMR spectrum. It is assumed to

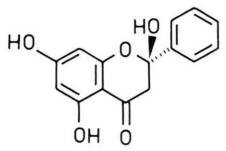
be either 6-methoxy- or 8-methoxy-galangin. Differentiation between 6- and 8-methoxy isomers is possible by the relative intensities of the peaks corresponding to M^+ and $M^+ - 15$ [4]. In this case, $M - 15 < M^+$ indicates 6-methoxy substitution. The spectroscopic properties of compound **4** are in fact identical with those reported for 3,5,7-trihydroxy-6-methoxy-flavone [5]. Direct comparison with a sample of synthetic alnusin confirms the identity.



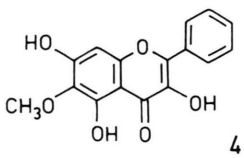
1



2 : R=H
2a: R=Ac



3



4

It is obvious that flavonoids with unsubstituted B-ring are predominant in the leaf and stem resin of *Baccharis bigelovii*. Compounds **1**–**4** are either rare or novel natural products. 3,5,7-Trihydroxy-6-methoxy-flavanone (**2**) was reported for the first time from male flowers of *Alnus sieboldiana* (Betulaceae) and was named alnustinol [6]. We assume that it there also occurs as a constituent of the resinous material, similar to the well known existence of terpenoids [7] and flavonoid aglycones [8] in bud exudates of Betulaceae. It was found a second time in roots of *Chromolaena chasleae* (Compositae) [9]. Alnustinol is still the only known dihydroflavonol with this pattern of O-substitution. 3,5,7-Trihydroxy-6-methoxy-flavone [4] which exhibits the same substitution pattern, was first reported from catkins of *Alnus sieboldiana* and named alnusin [6]. The flavonol was found in roots of *Chromolaena chasleae* as well [9].

Compounds **1** and **3** are both novel products with unusual substitution. 7-Benzoyl-chrysin (**1**) is the first flavone aglycone to be found as a natural ester, and it is the only flavonoid aglycone known that has an aromatic acid as acyl moiety (*cf.* [10]). 2β,5,7-Trihydroxyflavanone (**3**) was reported previously as a synthetic product [11], while its 7-methyl ether was found as constituent of the bud exudate of poplars (*Populus* spp., *cf.* [12]).

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