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FLAVONOIDS FROM *AGERATINA CALOPHYLLA*

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Key Word Index—*Ageratina calophylla*; Compositae; Eupatorieae; flavonoid glycosides; 6,7-dimethoxy-3,5,3',4'-tetrahydroxyflavone 3-*O*-apioside; *C*-glycosylflavonol; 6-*C*-glucosylquercetin; attached proton test experiment (APT); chemical ionization mass spectrometry (CIMS).

Abstract—Twelve flavonoids, including one new compound, were isolated from *Ageratina calophylla*. The structure of the new flavonoid was determined by spectroscopic methods including an attached proton test experiment and CI mass spectrometry as 6,7-dimethoxy-3,5,3',4'-tetrahydroxyflavone 3-*O*-apioside. Another compound, 6-*C*-glucosylquercetin, was previously synthesized, but this is the first report of its occurrence in nature.

As part of our chemosystematic survey of the tribe Eupatorieae (Compositae) [1–3], we investigated the flavonoids of *Ageratina calophylla* (B. L. Robinson) R. M. King & H. Robinson.

Chromatographic separation of the dichloromethane and ethyl acetate extracts of a concentrated aqueous methanol extract of *Ageratina calophylla* afforded twelve flavonoids, including ten 6-methoxyflavonoids and one *C*-glycosylflavone. A new flavonol, apioside, has been identified as 6,7-dimethoxy-3,5,3',4'-tetrahydroxyflavone 3-*O*-apioside (**1**); we also report here the first occurrence of 6-*C*-glucosylquercetin (**2**) as a natural product. Five of the eleven known compounds are glycosides: 6,7-dimethoxy-3,5,3',4'-tetrahydroxyflavone 3-*O*-rhamnoside (**3**), 6,7-dimethoxy-3,5,4'-trihydroxyflavone 3-*O*-rhamnoside (**4**), 6,7-dimethoxy-3,5,3',4'-tetrahydroxyflavone 3-*O*-galactoside (**5**), 6,7-dimethoxy-3,5,4'-trihydroxyflavone 3-*O*-galactoside (**6**) and 6-methoxykaempferol 3-*O*-glucoside (**7**). The five aglycones found are 6-methoxyapigenin (**8**), 5,7-dihydroxy-6,4'-dimethoxyflavone (**9**), 5,7-dihydroxy-6,3',4'-trimethoxyflavone (**10**), patuletin (**11**) and quercetin (**12**). Detailed data for the characterizations of **1–3** and **4** are presented. The ^{1}H NMR spectrum (90 MHz) of the TMSi ether derivative of the new flavonoid **1** (Table 1) exhibited the following signals: H-6' at δ7.46 (*dd*), H-2' at

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Table 1. ^{1}H NMR spectral data for flavonoids **1–4***

H No.	**1**: 6,7-OMe-3,5,3',4'-OH 3-*O*-apioside	**2**: 6-*C*-glucosyl-quercetin	**3**: 6,7-OMe-3,5,3',4'-OH 3-*O*-rhamnoside	**4**: 6,7-OMe-3,5,4'-OH 3-*O*-rhamnoside
8	6.50 (1H, *s*)	6.40 (1H, *s*)	6.50 (1H, *s*)	6.50 (1H, *s*)
2'	7.41 (1H, *d*)	7.65 (1H, *d*)	7.30 (1H, *d*)	7.75 (2H, *d*) (H-2',6')
6'	7.46 (1H, *dd*)	7.67 (1H, *dd*)	7.36 (1H, *dd*)	
3'				6.88 (2H, *d*) (H-3',5')
5'	6.83 (1H, *d*)	6.85 (1H, *d*)	6.85 (1H, *d*)	
6 (OMe)	3.72 (3H, *s*)		3.72 (3H, *s*)	3.72 (3H, *s*)
7 (OMe)	3.90 (3H, *s*)		3.90 (3H, *s*)	3.90 (3H, *s*)
1''	5.60 (1H, *d*)	4.75 (1H, *d*)	5.16 (1H, *d*)	5.15 (1H, *d*)
2''	4.24 (1H, *d*)	4.15–4.40 (1H, *m*)	4.28 (1H, *t*)	4.30 (1H, *t*)
3''			2.90–3.80 (3H, *m*) (H-3'',4'',5'')	2.90–3.80 (3H, *m*) (H-3'',4'',5'')
4''	3.51 (4H, *s*) (H-4'',5'')	3.20–3.85 (5H, *m*) (H-3'',4'',5'',6'')		
5''				
6''			0.81 (3H, *d*)	0.81 (3H, *d*)

*90 MHz, as TMSi ethers, CCl_4, δ-scale in ppm, TMS as internal standard.

δ7.41 (*d*), H-5′ at δ6.83 (*d*), H-8 at δ6.5 (*s*), signals for two methoxyl groups at δ3.90 (3H, *s*) and 3.72 (3H, *s*) and sugar proton signals at δ5.60 (1H, *d*), 4.24 (1H, *d*) and 3.51 (4H, *s*). The CI mass spectrum of **1** exhibited a molecular ion peak at *m/z* 479 (10%) suggesting a molecular weight of 478 and a $C_{22}H_{22}O_{12}$ formula. The base peaks of the glycoside (CI mass spectrum) at *m/z* 347 (aglycone moiety) $[aglycone+H]^+$ and of the aglycone itself (EI mass spectrum) at *m/z* 346 $[M]^+$ require that the aglycone contains four hydroxyl and two methoxyl groups ($C_{17}H_{14}O_8$). Thus, the sugar moiety has a $C_5H_{10}O_5$ formula. The ^1H NMR spectrum of the TMSi ether of **1** established a 3,5,6,7,3′,4′-oxygenation pattern. Since the aglycone appeared as a dull yellow fluorescent spot on paper in UV light with and without ammonia, the presence of a 3-hydroxyl group was established and a 5-hydroxyl was indicated. When the paper was sprayed with NA, the aglycone changed to orange indicating a 3′,4′-dihydroxyl group. The UV spectral data for the aglycone clearly supported the presence of four hydroxyl groups at positions 3,5,3′ and 4′ (Table 2) [4]. With the assignment of the four hydroxyl groups and to accommodate the 3,5,6,7,3′,4′-oxygenation pattern, the two methoxyl groups must be at the 6 and 7-positions. The aglycone is therefore 6,7-dimethoxy-3,5,3′,4′-tetrahydroxyflavone. Since the glycoside **1** appeared as a purple fluorescent spot on paper under UV light the sugar moiety must be at the 3-position, a conclusion supported by UV spectra (band I in MeOH at 350 nm for the glycoside and at 368 nm for the aglycone). The only question that remained was the identity of the glycosyl group in **1**. Attached proton test experiment (125 MHz) showed that two of the five sugar carbons were methine carbons (signals at δ111.2 for C-1″ and 79.1 for C-2″) and that three other carbons were quaternary or methylene types, results accommodated by an apiosyl moiety. To confirm the identity of the sugar, **1** and authentic apiin (Pfaltz & Bauer) were hydrolysed employing identical conditions (0.1 N trifluoroacetic acid) and the hydrolysates were examined by cellulose TLC: both **1** and apiin afforded apiose. Therefore, we assign the structure of the new flavonoid as 6,7-dimethoxy-3,5,3′,4′-tetrahydroxyflavone 3-apioside.

The analysis of the ^1H NMR spectrum of the TMSi ether derivative of **2** indicated that it was a glycoside based on a quercetagetin-like skeleton (sugar proton signals at δ3–4 (6H) and 4.75 (1H), H-8 at δ6.37, H-5′ at δ6.86, H-2′ at δ7.63 and H-6′ at δ7.66). That the compound appeared as a dull yellow fluorescent spot on paper in UV light with and without ammonia established the presence of a free 3-hydroxyl group and indicated the presence of a 5-hydroxyl group. A spot of **2** on paper also gave an orange colour with NA indicating a 3′,4′-dihydroxyl group in the B-ring (Table 3). The presence of band III at 335 nm in the sodium methoxide UV spectrum (Table 2) indicated a 7-hydroxyl group [4]. Thus the sugar moiety must be at the 6-position. The mass spectrum of the PM derivative of **2** gave $[M]^+$ at *m/z* 590 for a *C*-glycosylquercetin. The glycosyl signals of the TMSi ether of **2** and isovitexin (in CCl_4) were identical, suggesting that **2** was a 6-*C*-glucosylflavonol. The mass spectrum of the permethylation product of **2** is characteristic for 6-*C*-sugars: $\mathbf{a}_2$ $[M-15]^+$ at *m/z* 575 (19%), $\mathbf{b}_3$ $[M-31]^+$ at *m/z* 427 (69%) and permethylated carbohydrate signals: **g** $[M-103]^+$ at *m/z* 487 (14%) and **h** $[M-163]^+$ at *m/z* 427 (25%) [5]. The ion $\mathbf{a}_3$ $[M-17]^+$ was absent from the mass spectrum of PM **2** supporting a glucosyl group [5].

Table 2. UV spectral data for flavonoids **1**–**4**

Flavonoids	λ_{max} (nm)					
	MeOH	NaOMe	$AlCl_3$	$AlCl_3$+HCl	NaOAc	NaOAc+H_3BO_3
1: 6,7-OMe-3,5,3′,4′-OH 3-*O*-apioside	261, 270 sh, 350	272, 389	278, 428	263, 280 sh, 369	265, 370	265, 368
Aglycone of **1**: 6,7-OMe-3,5,3′,4′-OH	258, 270 sh, 368	272, 427 (dec.)	273, 440	268, 375 sh, 425	264, 388	264, 387
2: 6-*C*-glucosylquercetin	258, 372	280, 335, 425 (dec.)	273, 455	270, 360, 433	274, 325, 395	263, 389
3: 6,7-OMe-3,5,3′,4′-OH-3-*O*-rhamnoside	258, 342	275, 394	265, 276, 405	260, 275, 360	265, 370	265, 365
4: 6,7-OMe-3,5,4′-OH 3-*O*-rhamnoside	270, 332	270, 380	255, 277 sh, 358	255, 275, 353	270, 370	268, 332

Table 3. Chromatographic data for flavonoids 1–4

Flavonoids	$R_f \times 100$				
	15% HOAc	TBA	UV	UV/NH_3	NA
1: 6,7-OMe-3,5,3',4'-OH 3-*O*-apioside	57	78	P	Y	Or
2: 6-*C*-glucosylquercetin	18	35	Y	Y	Or
3: 6,7-OMe-3,5,3',4'-OH 3-*O*-rhamnoside	84	69	P	Y	Or
4: 6,7-OMe-3,5,4'-OH 3-*O*-rhamnoside	81	72	P	Y	Y

P = purple; Y = yellow; Or = orange.

All the above results as well as the ^{13}C NMR data (Table 4) established **2** to be 6-*C*-glucosylquercetin, a compound known synthetically [6].

EXPERIMENTAL

Plant material. Leaves and heads of *Ageratina calophylla* (1 kg) were collected 6.3 miles northeast of San Antonio Penk Nevadn, Nuevo Leon, Mexico on 7 October 1984 by Tina Ayers and Randy Scott. A voucher specimen (T. J. Ayers 476) is on deposit in the Plant Resources Center at the University of Texas at Austin.

Extraction and isolation. Ground leaves and heads of *A. calophylla* were extracted repeatedly with excess vols. of 85% aq. MeOH followed by extraction with 50% aq. MeOH. The combined extracts were concentrated *in vacuo* to a thick syrup. This aq. syrup was partitioned first against CH_2Cl_2 and then against EtOAc. The conc CH_2Cl_2 extract (10 g) and conc EtOAc extract (32.5 g) were respectively chromatographed over polyclar (Polyclar AT, GTAF Corp) columns packed in toluene. Elution of each column was initiated with toluene gradually increasing in 10% increments to 100% MeOH and finally with Me_2CO–H_2O (1:1). Fractions were collected by monitoring the column with UV light. All fractions were further separated by PC using 15% HOAc on Whatman 3 MM paper. Final purification of each compound for spectral analysis was done by standard procedures [4] using 80% aq. MeOH or 100% MeOH over Sephadex LH-20 columns. Compounds **1**, **2** and **4**–**7** were isolated from the EtOAc fraction, compounds **8**–**12** from the CH_2Cl_2 fraction and **3** from both the CH_2Cl_2 and the EtOAc fractions. Compounds **1** and **2** were identified as described in the text; all other compounds were identified by UV, ^1H NMR of their TMSi ethers, colour on paper under UV light and authentic sample comparisons.

Hydrolysis conditions. A dry sample of each glycoside was dissolved in 0.1 N TFA; the flask was covered with Al foil and placed on a steam bath for 50 min.

Sugar analysis. The sugar material was recovered from the hydrolysate after repeated evaporation *in vacuo* of the hydrolysis soln and then taking up the residue in H_2O and extracting the aq. soln with EtOAc. Sugars present in the H_2O fraction were identified by TLC on cellulose against standard markers in pyridine–EtOAc–HOAc–H_2O (36:36:7:21) using aniline phthalate reagent (E. Merck).

Derivatization. Trimethylsilylation was done as in ref. [4] and permethylation was achieved using MethElute (Pierce).

Spectroscopy. UV spectroscopy was achieved as described in ref. [4]. EIMS was done by direct probe EIMS at 70 eV with a source temp. of 250–270° and CIMS employed a Finnigan MAT 4023, gas chromatograph/mass spectrometer, methane CI (0.5 Torr).

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Table 4. ^{13}C NMR spectral data for flavonoids **1** and **2***

C No.	**1**† in MeOH-d_4	**2** in DMSO-d_6	**2** in MeOH-d_4
2	153.5‡	146.5	148.0
3	135.5‡	135.5	137.3
4	179.8‡	175.9	177.5
5	159.8‡	159.7	161.3
6	133.5‡	108.1	108.2
7	160.8‡	163.0	164.5
8	92.4§	93.0	94.7
9	154.0‡	155.0	157.5
10	107.4‡	102.6	104.4
1'	123.0‡	121.8	124.0
2'	116.0§	115.0	116.0
3'	142.0‡	145.0	146.2
4'	150.0‡	147.6	148.8
5'	117.5§	115.5	116.2
6'	123.0§	120.0	121.7
6-OMe	61.5§		
7-OMe	57.6§		
1''	112.5§	73.0	75.3
2''	79.4§	70.5	72.6
3''	81.6‡	78.9	80.1
4''	77.2‡	70.3	71.8
5''	66.0‡	81.3	82.6
6''		61.4	62.9

*125 MHz, MeOH-d_4, δ-scale in ppm, TMS as internal standard.

†In attached proton test experiment.

‡Carbons are quaternary or methylene carbons.

§Carbons are methyl or methine carbons.

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