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FLAVONOIDS FROM *GUTIERREZIA TEXANA* VAR. *TEXANA*

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Key Word Index—*Gutierrezia texana* var. *texana*; Compositae; Astereae; flavonol 3-methyl ether; acetoxyflavonol 3-methyl ether.

Abstract—Eleven flavonoids, including two new compounds, were isolated from *Gutierrezia texana*: the structures of the new compounds are 5,7,2′,5′-tetrahydroxy-3,4′-dimethoxyflavone and 5′-acetoxy-5,7,2′-trihydroxy-3,4′-dimethoxyflavone. The nine known compounds are 5,4′,5′-trihydroxy-3,6,7,8-tetramethoxyflavone, 5,7,2′,5′-tetrahydroxy-3,6,4′-trimethoxyflavone, 5,7,3′-trihydroxy-3,4′-dimethoxyflavone, 5,7,3′,4′-tetrahydroxyflavone, 3,5,7,3′,4′-pentahydroxyflavone, 5,7,3′,4′-tetrahydroxy-3-methoxyflavone, 5,6,7,3′,4′-pentahydroxy-3-methoxyflavone, 5,7,3′,4′-tetrahydroxy-3-methoxyflavone 7-*O*-glucoside and 3,5,7,3′,4′-pentahydroxyflavanone.

INTRODUCTION

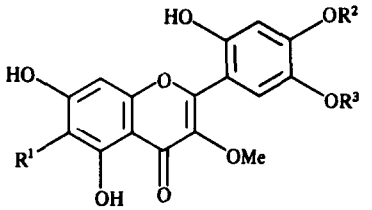
In our earlier chemotaxonomic studies in the '*Gutierrezia-Xanthocephalum* Complex' [1–6], we reported on flavonoids from *G. grandis* and *G. microcephala*, two woody species, *G. allmanii* var *megalocephala*, a perennial herbaceous species and *G. wrightii*, an annual herbaceous species. While the major flavonoids of the two woody species have 6,8-oxygenation as well as an unusual B-ring substitution pattern involving a 2′-hydroxyl group, most compounds isolated from the two herbaceous species do not have either 6,8-oxygenation or 2′-hydroxylation. The present paper describes the isolation in relatively low yields of 11 flavonoids from the annual, herbaceous species *Gutierrezia texana* (DC.) Torrey & A. Gray var. *texana*.

RESULTS AND DISCUSSION

Chromatographic separation of the material from the dichloromethane and ethyl acetate extracts of a concentrated aqueous methanol extract of *Gutierrezia texana* var. *texana* afforded two new flavonoids (**1** and **2**) and nine known flavonoids. Identification of the new compounds was based on MS, UV, ^{1}H NMR data.

5,7,2′,5′-*Tetrahydroxy*-3,4′-*dimethoxyflavone* (**1**)

The MS of **1** exhibited a molecular ion peak at *m/z* 346 (88) in accord with a flavone containing four hydroxyl and two methoxyl groups. The tetramethylsilyl ether of **1** exhibited four ^{1}H NMR one-proton signals, two of which are singlets, as well as two one-proton doublets at δ6.12 (1H, *d*, *J* = 2.5) and δ6.35 (1H, *d*, *J* = 2.5) characteristic of H-6 and H-8, respectively [7]. The two one-proton signals



	R^1	R^2	R^3
1	H	Me	H
2	H	Me	Ac
5	OMe	Me	H

at δ6.37 and 6.88, respectively, could be assigned to H-3′ and H-6′ on the basis of signals observed for other flavonol 3-methyl ethers with the 2′,4′,5′-oxygenated system [1, 2, 4]. The UV spectral data supported this conclusion: Band I for **1** occurs at 353 nm in MeOH with a relative intensity of Band II to Band I being 2.70. Moreover, the high R_f value (0.60) exhibited by **1** in 15% HOAc on a cellulose plate suggested that **1** is a flavonol 3-methyl ether with 2′,4′,5′-oxygenation in the B-ring [4]. Together, these data indicate that **1** has a 3,5,7,2′,4′,5′-oxygenation pattern. Compound **1** appeared purple on paper under UV light with and without ammonia indicating the presence of a 5-hydroxyl and a 3,4′-dimethoxyl group [7]; therefore, the remaining three hydroxyl groups must be at 7, 2′ and 5′. MS supported the 2′-OH and 3-OMe system: $[M-17]^+$ at *m/z* 329 (45), $[M-31]^+$ at *m/z* 34(100), $[B_6]^+$ at *m/z* 194(15) and $[B_6-15]^+$ at *m/z* 179(23). The unstable B-ring would account for decomposition of **1** in the sodium methoxide UV spectrum. Therefore, **1** is 5,7,2′,5′-tetrahydroxy-3,4′-dimethoxyflavone.

5′-*Acetoxy*-5,7,2′-*trihydroxy*-3,4′-*dimethoxyflavone* (**2**)

The MS of **2** (Table 1) gave $[M]^+$ at *m/z* 388 (48) for $C_{19}H_{16}O_9$ in accord with a flavonoid containing one

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Table 1. MS data compounds 1 and 2

	1	2
$[M]^+$	346 (88)	388 (45)
$[M-COCH_2]^+$		346 (94)
$[M-17]^+$	329 (45)	371 (10)
$[M-COCH_2-17]^+$		329 (49)
$[M-31]^+$	315 (100)	357 (13)
$[M-COCH_2-31]^+$		315 (100)
$[M-43]^+$	303 (29)	345 (34)
$[M-COCH_2-43]^+$		303 (27)
$[A_1+1]^+$	153 (22)	153 (15)
$[A_1]^+$	152 (7)	152 (4)
$[B_2]^+$	167 (12)	
$[B_2-COCH_2]^+$		167 (5)
$[B_6]^+$	194 (15)	
$[B_6-COCH_2]^+$		194 (8)
$[B_6-15]^+$	179 (23)	
$[B_6-COCH_2-15]^+$		179 (9)

EIMS (probe) 70 eV, *m/z* (rel. int.).

acetoxyl, two methoxyl and three hydroxyl groups. The $[M-17]^+$ (10) and $[M-31]^+$ (13) fragments, as for compound **1**, also were observed. Other fragments in the MS of **2**, formed by the loss of the acetoxyl group ($COCH_2$) were almost identical to those in the MS of **1** (Table 1), suggesting that **2** has the same 3,5,7,2′,4′,5′-oxygenation pattern as **1**. In support of this conclusion the aromatic region of the ^1H NMR of **2** (TMSi ether in CCl_4) showed signals characteristic for H-6, H-8, H-3′ and H-6′, respectively, at δ6.10 (1H, *d*, J = 2.5 Hz), 6.34 (1H, *d*, J = 2.5 Hz), 6.62 (1H, *s*) and 7.02 (1H, *s*) (Table 2). In the MS of **2** ions at *m/z* 153 (15), 152 (4), which correspond to $[A_1+1]^+$, $[A_1]^+$, indicated the presence of hydroxyl groups at 5 and 7 in the A-ring. Compound **2** appeared purple on paper under UV light with and without ammonia, indicating the absence of 3- and 4′-hydroxyl groups. After hydrolysis with 0.1 N trifluoroacetic acid **2** afforded a flavone **3**. The MS of **3** exhibited a molecular ion at *m/z* 346 ($C_{18}H_{16}O_9$) in accord with a hydroxyl group derived from loss of an acetoxyl group and UV spectra and comparison on TLC showed that **3** and **1** are the same compound, 5,7,2′,5′-tetrahydroxy-3,4′-dimethoxyflavone.

The remaining problem concerned the assigning of the acetoxyl group to two available positions in the B-ring, C-2′ or C-5′. With the assignment of the 3-OMe and to accommodate the $[M-17]^+$ at *m/z* 371 (10) and $[M-COCH_2-17]^+$ at *m/z* 329 (49), the third hydroxyl group should be at 2′ [2, 8] and second methoxyl group must be at 4′ position. In order to support the conclusion, NOE experiments (in MeOH-d_4) were conducted at 500 MHz. Irradiation on the signal at δ3.61 (OMe) did not cause any enhancement in spectrum which indicated the presence of methoxyl group at 3 position. When the second methoxyl signal at δ3.92 was irradiated, this markedly enhanced the signal at δ6.86 (H-3′) and suggested that the second methoxyl group should be at the 4′-position. Together these findings established **2** to be 5′-acetoxy-5,7,2′-trihydroxy-3,4′-dimethoxyflavone.

EXPERIMENTAL

Plant material. Gutierrezia texana (DC) T. & G. var. *texana* was collected by M. Leidig and Feng Gao on 3 September 1984 in Travis Co. TX on Highway 183 South near the bridge over Cottonmouth Creek. Voucher material (MAL 2009) is deposited in The Plant Resources Center at The University of Texas at Austin.

Extraction and isolation of flavonoids. Dried leaves and stems (1570 g) were extracted (×3) with 85 and 50% aqueous MeOH respectively. The combined extracts were concd to an aq. layer under red. pres. and the concentrate was partitioned against CH_2Cl_2 and EtOAc. In order to eliminate chlorophylls the concentrate from the CH_2Cl_2 partition (128.3 g) was passed through the cellulose column packed in 20% HOAc. The total final concentrates from the CH_2Cl_2 and EtOAc extracts (101.2 g) (51.8 g) were chromotographed, respectively, over Polyclar AT (GAT Corp.) columns initially packed in toluene MeOH (9:1); during elution this elution solvent was gradually altered in 10% increments to 100% MeOH. The columns were finally washed with acetone–water (1:1). Fractions were collected on the basis of monitoring the column with UV light. Material from each fraction was further separated by paper chromatography using 35% HOAc and TBA (3:1:1) on Whatman 3MM paper. After purification over Sephadex LH-20 (MeOH) all compound were identified by UV, ^1HNMR, MS and colour reactions. 5,7,2′,5′-Tetrahydroxy-3,4′-dimethoxyflavone, 5′-acetoxy-5,7,2′-trihydroxy-3,4′-dimethoxyflavone, 5,4′,5′-trihydroxy-3,6,7,8-tetra-

Table 2. ^1H NMR data for compounds 1 and 2

	1		2		
	as TMSi ether in CCl_4	as TMSi ether in C_6D_6	as TMSi ether inCCl_4	in acetone-d_6	in MeOH-d_4
H-6	6.12 *d*	6.45 *d*	6.10 *d*	6.30 *d*	6.21 *d*
H-8	6.35 *d*	6.58 *d*	6.34 *d*	6.40 *d*	6.30 *d*
H-3′	6.37 *s*	6.37 *s*	6.62 *s*	6.95 *s*	6.86 *s*
H-6′	6.88 *s*	7.13 *s*	7.02 *s*	7.20 *s*	7.11 *s*
–OMe at 3	3.72 *s*	3.35 *s*	3.60 *s*	3.74 *s*	3.61 *s*
–OMe at 4′	3.80 *s*	3.83 *s*	3.84 *s*	3.94 *s*	3.92 *s*
–OCOMe at 5′			2.13 *s*	2.14 *s*	2.17 *s*

90 MHz, δ-scale in PPM.

methoxyflavone, 5,7,2′,5′-tetrahydroxy-3,6,4′-trimethoxyflavone, 5,7,3′-trihydroxy-3,4′-dimethoxyflavone and 3,5,7,3′,4′-pentahydroxyflavanone were isolated from the CH_2Cl_2 fraction while 5,7,3′,4′-tetrahydroxyflavone, 3,5,7,3′,4′-pentahydroxyflavone, 5,6,7,3′,4′-pentohydroxy-3-methoxyflavone and 5,7,3′,4′-tetrahydroxy-3-methoxyflavone were obtained from the EtOAc extract; 5,7,3′,4′-tetrahydroxy-3-methoxyflavone was isolated from both the CH_2Cl_2 and EtOAc fractions.

Hydrolysis condition. 0.5 mg dry sample was put in 10 ml 0.1 N TFA; the flask was covered with aluminium in a steam bath (60–70°) for 2 hr.

Compound **1** (5,7,2′,5′-tetrahydroxy-3,4′-dimethoxyflavone). Colour: UV purple, NH_3 purple; PC R_f (on cellulose plate): 0.96 TBA (*t*-butyl alcohol–glacial acetic–water, 3:1:1), 0.60 15% HOAc; UV λ_{max}^{MeOH} nm: 260, 300 sh, 353 (Band II/Band I: 2.70); + NaOMe: 270, 333 (dec.); + $AlCl_3$: 272, 317 sh, 395; + $AlCl_3$ + HCl: 266, 317 sh, 388; + NaOAc: 264 360; + NaOAc + H_3BO_3: 262, 355.

Compound **2** (5′-acetoxy-5,7,2′-trihydroxy-3,4′-dimethoxyflavone). Colour: UV purple, NH_3 purple; PC R_f (on cellulose plate): 0.96 TBA, 0.76 15% HOAc; UV λ_{max}^{MeOH} nm: 256, 266 sh, 337; + NaOMe: 269, 330 sh, 360; + $AlCl_3$: 267, 278 sh, 340, 385 sh; + $AlCl_3$ + HCl: 265, 332, 385 sh; NaOAc: 272, 320 sh, 355; + NaOAc + H_3BO_3: 255, 264 sh, 337.

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