C-7), 161 036 (s, C-4′ and C-9), 164 032 (s, C-2), and 182 200 (s, C-4)

Isoorientin (**3**) was identified by PC, UV, ^{1}H NMR, ^{13}C NMR and FDMS spectral studies and also by co-TLC, mp and mmp with an authentic sample

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FLAVONOIDS AND A COUMARIN FROM *GUTIERREZIA SPHAEROCEPHALA*

RHONGZHI LI,* NIANBAI FANG† and TOM J MABRY

The Department of Botany, The University of Texas at Austin, Austin, TX 78713-7640, U S A

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Key Word Index—*Gutierrezia sphaerocephala*; Compositae; Astereae; flavones; flavonols; flavanones; 5,3′,4′,5′-tetrahydroxy-6,7-dimethoxyflavone, 5,7,4′-trihydroxy 6,3′-dimethoxyflavanone

Abstract—The ethyl acetate extract of the aerial parts of *Gutierrezia sphaerocephala* afforded, in addition to one coumarin, 10 known and two new flavonoids The structures were elucidated by spectroscopic methods. The new flavonoids are 5,3′,4′,5′-tetrahydroxy-6,7-dimethoxyflavone and 5,7,4′-trihydroxy-6,3′-dimethoxyflavanone

As a part of our chemosystematic survey of the '*Gutierrezia–Xanthocephalum* complex' [1–9], we have investigated the *Gutierrezia sphaerocephala* Gray Chromatographic separation of the ethyl acetate and dichloromethane extracts of a concentrated aqueous methanol extract of aerial parts of *G sphaerocephala* afforded one coumarin, 7,8-dihydroxy-6-methoxycoumarin (**13**) [10] and 12 flavonoids. The 10 known flavonoids are 5,7-dihydroxy-6,4′-dimethoxyflavone (**1**) [11], 5,7,3′,4′-tetrahydroxy-6-methoxyflavone (**2**) [11], 5,7,4′-trihydroxy-6,3′-dimethoxyflavone (**3**) [11], 5,7-dihydroxy-6,3′,4′-trimethoxyflavone (**4**) [11], 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**5**) [12], 3,5,7,3′,4′-pentahydroxyflavone (**6**) [11], 3,5,7,3′,4′-pentahydroxy-6-methoxyflavone (**7**) [11], 3,5,7,4′-tetrahydroxy-6,3′-dimethoxyflavone (**8**) [11], 3,5,7,3′,4′-pentahydroxyflavone 3-galactoside (**9**) and 5,7,3′,4′-tetrahydroxy-6-methoxyflavanone (**10**) [13] The new flavonoids are 5,3′,4′,5′-tetrahydroxy-6,7-dimethoxyflavone (**11**) and 5,7,4′-trihydroxy-6,3′-dimethoxyflavanone (**12**).

The ^{1}H NMR spectrum (90 MHz) of the TMSi ether derivative of **11** (Table 1) exhibited two one-proton singlets at δ6 27 and 6 51 characteristic of H-3 and H-8, respectively, and a two-proton singlet at δ6.96 typical of protons at 2′ and 6′ in a symmetrically substituted B-ring. Since the remaining signals in the ^{1}H NMR spectrum were in accord with two methoxyl groups, **11** has a 5,6,7,3′,4′,5′-oxygenation pattern The MS of **11** exhibited a molecular ion peak at *m/z* 346 (100%) in accord with an aglycone containing four hydroxyl and two methoxyl groups Compound **11** appeared as purple spot on paper under UV light and changed to yellow with ammonia, suggesting the presence of free 5 and 4′-hydroxyl groups Compound **11** also gave an orange colour with NA, which, together with the symmetrical substituted B-ring already established, indicated a B-ring with 3′,4′,5′-trihydroxyl groups. With the establishment of 5,3′,4′,5′-tetrahydroxyl groups and to accommodate the 5,6,7,3′,4′,5′-oxygenation pattern, the two methoxyl groups must be at the 6 and 7 positions These conclusions are supported by the UV spectra (Table 2) Thus, **11** is 5,3′,4′,5′-tetrahydroxy-6,7-dimethoxyflavone

The ^{1}H NMR signals at δ2 65 (1H, *d*) 2 77 (1H, *d*) and 5 20 (1H, *dd*) characteristic for H-3 *cis*, H-3 *trans* and H-2 and a molecular ion at *m/z* 332 (99%) in the MS spectrum indicated that **12** is a flavanone with three hydroxyl and two methoxyl groups The ^{1}H NMR spectrum of the

*Beijing Medical University, Beijing, China
†The Hubei College of Chinese Traditional Medicine, Wuhan, China

Table 1 ^{1}H NMR spectral data for compounds **5**, **11** and **13***

Compounds as TMSi ether†	**5**		**11**	**13**	
H	in CCl_4	in C_6D_6	in CCl_4	in CCl_4	in C_6D_6
3	6.36 (1H, *s*)	6.58	6.27	6.10 (1H, *d*)	5.97
4				7.49 (1H, *d*)	6.88
5				6.50 (1H, *s*)	6.09
8	6.58 (1H, *s*)	6.72	6.51		
2′, 6′	7.03 (2H, *s*)	6.90	6.96		
6-OMe	3.73 (3H, *s*)	3.63	3.70	3.84	3.40
7-OMe			3.86 (3H, *s*)		
3′-OMe	3.90 (3H, *s*)	3.37			
5′-OMe	3.90 (3H, *s*)	3.37			

*90 MHz, δ scale in ppm, TMS as internal standard.
†Coupling patterns are not repeated if identical with the preceding column

TMSi ether of **12** (90 MHz) suggested a 5,6,7,3′,4′,-oxygenation pattern for this flavanone: aromatic proton signals at δ6.91 (H-2′) and 6.80 (H-5′ and 6′) and a singlet at δ6.10 characteristic for H-8 [14]. This conclusion was confirmed by 1 PDFA experiment (one-pulse, Decoupler Off During Acquisition) (Table 4): a very sharp doublet at δ95.6 (d, $J_{C-8,H-8}$ = 164.0 Hz) can be assigned to C-8 not C-6 because C-6 is coupled with H-6 (J = 161.9 Hz) and C_5-OH (J = 7.3 Hz) [15] The MS fragments for $[A_1+1]^+$ at *m/z* 183 (78%), $[M-(B\text{-ring})]^+$ and $[A_1]^+$ at *m/z* 182 (100%), $[A_1-15]^+$ at *m/z* 167 (76%), $[B_3]^+$ at *m/z* 150 (48%), $[B_3-15]^+$ at *m/z* 135 (33%) and $[B_3-43]^+$ 107 (16%) indicated that the A-ring contained one methoxyl and two hydroxyl groups and that the B-ring had one of each The UV spectra of **12** ($\Delta\lambda$ Band II NaOMe–MeOH relative to Band II MeOH· +41 nm and the $\Delta\lambda$ Band II NaOAc–MeOH relative to Band II MeOH: +39 nm) and the UV spectra of the chalcone derived from **12** (Table 2) suggested the 5,7-dihydroxyl groups [14, p. 170]. Therefore, the A-ring should have 5,7-dihydroxyl and 6-methoxyl groups. The B-ring ^{1}H NMR pattern of **12** was identical to those of other flavones with a 3′-methoxy-4′-hydroxy B-ring but not those with a 3′-hydroxy-4′-methoxy system Therefore, **12** is 5,7,4′-trihydroxy-6,3′-dimethoxyflavanone [14]. This was confirmed by the UV spectra of the chalcone derived from **12**: $\Delta\lambda$ Band I NaOMe–MeOH relative to Band I MeOH: +65 with increasing intensity.

The mass spectrum of **10** exhibited a molecular ion peak at *m/z* 318/(77%) in accord with a flavanone with four hydroxyl and one methoxyl groups, which was supported by ^{13}C and ^{1}H NMR spectra (Tables 3 and 4). The same A-ring fragments in the MS of **10** as observed for **12**, namely, $[A_1+1]^+$ at *m/z* 183 (74%), $[M-(B-ring)]^+$ as well as $[A_1]^+$ at *m/z* 182 (100%) and $[A_1-15]^+$ at *m/z* 167 (84%), indicated that **10** had the same A-ring as **12**. Moreover, the $[B_3]^+$ fragment at *m/z* 136 (29%) confirmed that the B-ring contained two hydroxyl groups. The ^{1}H and ^{13}C NMR data (Tables 3 and 4) indicated that the structural differences between **10** and **12** are the presence of a hydroxyl group at C-3′ in **10** and a methoxyl group at the same position in **12**. Therefore, **10** is 5,7,3′,4′-tetrahydroxy-6-methoxyflavanone

The identities of the known compounds were determined by colour on paper under UV light, UV, ^{1}H NMR of their TMSi ethers and MS [14].

Table 2 UV spectral data for **10**, **11**, **12** and chalcone

	λ_{max} (nm)			
	10	**11**	**12**	Chalcone derived from **12**
MeOH	293, 330 sh	274, 351	289, 330 sh	288, 370
+NaOMe	247, 330	260 sh, 280 sh 417	250, 330	276, 435
+$AlCl_3$	294, 315 sh	270 sh, 310 sh 425	295, 312 sh	301, 404
+$AlCl_3$ +HCl	308	252 sh, 280 sh 310 sh, 363	290 sh, 310	307, 403
+NaOAc	295, 328	265, 410	255 sh, 280 sh 328	330, 380
+NaOAc +H_3BO_3	293, 330 sh	274, 417	285, 295 sh 325	290, 340 420

Table 3 ^{1}H NMR spectral data for compounds **10** and **12***†

	10			**12**		
		as TMSi ether		as TMSi ether		
	in DMSO-d_6‡	in CCl_4	in C_6D_6	in CCl_4	in C_6D_6	in acetone-d_6‡
2	5 33 (1H, *d*)	5 16 (1H, *dd*)	4 95	5.20	4 95	5 41
3 *cis*	2 68 (1H, *d*)	2 64	2 56	2 65	2 57	2 73 (1H, *dd*)
3 *trans*	3 19 (1H, *dd*)	2 74 (1H, *d*)	2 64	2 77	2 67	3 29 (1H, *dd*)
8	5 97 (1H, *s*)	6 08	6 30	6 10	6 38	6 01
2′	6 88 (1H, *br s*)		6 94	6 91	6 75	7 17 (1H, *d*)
5′	6 73 (2H, *br s*)	6 81 (3H, *m*)	6 79	6 80	6 83 (1H, *d*)	6 86
6′					6 64 (1H, *br d*)	6 97 (1H. *dd*)
6-OMe	3 64	3 63	3 61	3 67	3 61	3 77
3′-OMe				3 84	3 37	3 87

*90 MHz, δ-scale in ppm, TMS as internal standard
†Coupling patterns are not repeated if identical with the preceding column
‡360 MHz, δ-scale in ppm

EXPERIMENTAL

Plant material. Gutierrezia sphaerocephala was collected in Brewster County, Texas, 3 5 miles south of Ft Davis on the road to Alpine, on 7 September 1984 Voucher specimens are on deposit in the Plant Resources Center at the University of Texas at Austin (Barrie No. 971).

Isolation of the compounds. The unground aerial parts were washed with CH_2Cl_2 (twice) for 30 min and then dried and ground for further extraction The ground plant material (594 g) was exhaustively extracted with aq MeOH, first in 85% conc followed by 50% conc The extracts were combined and evapd under red pres until only H_2O remained The aq. layer was partitioned with CH_2Cl_2 and EtOAc The CH_2Cl_2 fraction yielded 10 g of residue while the EtOAc fraction afforded 11 g of concentrated material The CH_2Cl_2 and EtOAc concentrates were chromatographed over Polyclar AT (GAF Corp) Columns were packed initially with toluene–MeOH (19 1) and the eluting solvent was gradually increased to pure MeOH The material from each band, which was monitored on the column with UV light, was further separated by PC using 15% aq HOAc or aq 35% HOAc on Whatmann 3 mm paper Final purification of each compound for spectral analysis was by standard procedures [14] using 75 or 100% MeOH over Sephadex LH-20 columns

General techniques All UV spectra were recorded using standard procedures [14] ^{1}H NMR spectra of the TMSi ethers of all falvonoids were recorded in both CCl_4 and C_6D_6 at 90 MHz and those reported here are in δ values (ppm) relative to TMS as int standard [14] MS data were recorded by direct probe EIMS at 70 eV with a source temp of 250–270°

Table 4 ^{13}C NMR spectral data for **10** and **12**

	10	**12**
2	78 4 (*br d*)	80 3 (*br d*)
3	42 1 (*br t*)	43 8 (*br t*)
4	196 8 (*br s*)	198 0 (*br s*)
5	157 9 (*br s*)	156 3 (*br s*)
6	129 0 (*br s*)	130 0 (*br s*)
7	159 4 (*br s*)	159 6 (*br s*)
8	95 0 (*d*)	95 7 (*d*)
9	155 0 (*br s*)	156 0 (*br s*)
10	101 9 (*br s*)	103 5 (*br s*)
1′	129 5 (*br s*)	131 4 (*br s*)
2′	115 4 (*d*)	115.8 (*d*)
3′	145 1 (*br s*)	148.5 (*br s*)
4′	145 6 (*br s*)	148 0 (*br s*)
5′	114 2 (*br d*)	111 4 (*br d*)
6′	117 8 (*br d*)	120 5 (*br d*)
5-OMe		
6-OMe	56 9 (*q*)	56 5 (*q*)
3′OMe		60 8 (*q*)

90 8 MHz in acetone-d_6, δ-scale in ppm

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FLAVONOIDS OF *IRIS SPURIA*

ABDUL S SHAWL, N. MENGI, M. K. KAUL and VISHWAPAUL

Central Institute of Medicinal and Aromatic Plants, Regional Centre, Rawalpora, Srinagar 190005, India

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Key word Index—*Iris spuria*, Iridacae, flavonoids, 5,2′-dihydroxy-7,8-dimethoxyisoflavone; 5,8,2′-trihydroxy-7-methoxyflavanone

Abstract—Two new flavonoids were isolated from the rhizomes of *Iris spuria* and characterized as 5,2′-dihydroxy-7,8-dimethoxyisoflavone and 5,8,2′-trihydroxy-7-methoxy flavanone

INTRODUCTION

In a previous paper, we reported the isolation and structure elucidation of three isoflavones, 5,7-dihydroxy-6,2′-dimethoxyisoflavone, iristectorigenin A and iristectorin A from the rhizomes of *Iris spuria* [1] This paper deals with the isolation and characterization of a new isoflavone (**1**) and a new flavanone (**2**) from the chloroform extract of the rhizomes of *Iris spuria*

RESULTS AND DISCUSSION

Column chromatography of the chloroform extract, followed by preparative TLC and crystallization yielded yellow needles of compound **1**, mp 164–166°, M^+ at *m/z* 314 consistant with the molecular formula $C_{17}H_{14}O_6$ The IR (1660 cm^{-1}, C=O), UV (218, 262, 335 nm), and ^1H NMR (δ 8.1, 1H, C-2 proton) spectra established that compound **1** was an isoflavone. It gave a green colour with alcoholic ferric chloride solution indicating the presence of a chelated hydroxyl group Formation of a diacetate and ^1H NMR signals at δ 12 09 (1H, *s*) and 8.2 (1H, *s*) which disappeared on addition of D_2O established the presence of two phenolic hydroxyl groups. A bathochromic shift of 13 nm in the UV spectrum on addition of $AlCl_3$ and $AlCl_3$–HCl located a hydroxyl group at C-5. Absence of a shift on addition of NaOAc indicated that C-7 was substituted by a methoxyl group. The ^1H NMR spectrum showed the presence of two singlets (3H) at δ 3 80 and 3.85 attributed to the methoxyls at C-7 and C-8. The singlet (1H) at δ 6 4 was assigned to C-6 because this signal shifted to δ 6.65 with $\Delta\delta$ of 0 25 ppm characteristic of an aromatic proton with a free *ortho*-hydroxyl group [2, 3]. The mass spectrum showed $[M-Me]^+$ as the base peak and this further justified location of the methoxyl group at C-8 because in 6,7-dimethoxy-5-hydroxy flavones $[M]^+$ is the predominent peak [1, 4]. RDA fragmentation ions at *m/z* 196 and 118 suggested the presence of one hydroxyl and two methoxyl groups in ring A and one hydroxyl group in ring B. More important was the $[M-17]^+$ peak which is characteristic of 2′-hydroxylated flavones [5, 6]. ^{13}C NMR chemical shifts for C-2 and C-3 were in agreement with the values reported for isoflavones and were in accord with the known substituent effects [7, 8]. Thus from the above data compound **1** was characterized as 5,2′-dihydroxy-7,8 dimethoxyisoflavone.

Compound **2**, mp 204–205°, $C_{16}H_{14}O_6$ [M^+ at *m/z* 302], gave IR (1650 cm^{-1}, C=O) and UV (216, 290, 330 nm) spectra indicating that it was a flavanone This was further substantiated by the ^1H NMR spectrum with an ABX system centred at δ 2.8, 3.05 and 5 75 for the H-2