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SIX 2′-HYDROXYFLAVONOLS FROM *GUTIERREZIA MICROCEPHALA*

NIANBAI FANG*, MARK LEIDIG, T. J. MABRY and IINUMA MUNEKAZU†

The Department of Botany, The University of Texas at Austin, TX 78712, U.S.A.; †Gifu Pharmaceutical University, 6-1 Mitahora-higashi 5 chome, Gifu 502, Japan

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Key Word Index—*Gutierrezia microcephala*; Compositae; Astereae; new flavonol 3-*O*-methyl ethers; 5,7,2′-trihydroxy-3,6,4′,5′-tetramethoxyflavone; 5,7,2′-trihydroxy-3,6,8,4′,5′-pentamethoxyflavone; 5,2′-dihydroxy-3,6,7,8,4′,5′-hexamethoxyflavone; 5,7,2′,4′-tetrahydroxy-3,8,5′-trimethoxyflavone.

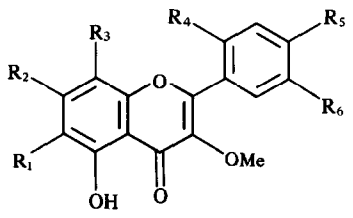
Abstract—Six 2′-hydroxyflavonols were isolated from *Gutierrezia microcephala*, including four new compounds, 5,7,2′-trihydroxy-3,6,4′,5′-tetramethoxyflavone, 5,7,2′-trihydroxy-3,6,8,4′,5′-pentamethoxyflavone, 5,2′-dihydroxy-3,6,7,8,4′,5′-hexamethoxyflavone and 5,7,2′,4′-tetrahydroxy-3,8,5′-trimethxoyflavone and two known compounds, 5,7,2′,5′-tetrahydroxy-3,6,8,4′-tetramethoxyflavone and 5,7,2′,4′-tetrahydroxy-3,6,8,5′-tetramethoxyflavone.

INTRODUCTION

As a part of a chemosystematic survey of *Gutierrezia* [1], we report here six flavonoids from *G. microcephala* (D.C.) A. Gray, all with 2′-hydroxylation, and four (**1**, **3**, **5** and **6**) of which are new. The other two compounds, 5,7,2′,5′-tetrahydroxy-3,6,8,4′-tetramethoxyflavone (**7**) and 5,7,2′,4′-tetrahydroxyflavone-3,6,8,5′-tetramethoxyflavone (**8**) were previously characterized from *G. grandis* S. F. Blake [1].

RESULTS AND DISCUSSION

Ground air-dried leaves and stems of *Gutierrezia microcephala* were extracted with aqueous methanol and after removal of the methanol the solution was partitioned between hexane, methylene dichloride and ethyl acetate. Chromatographic separation of the methylene dichloride extract afforded six 2′-hydroxyflavones (**1**, **3**, **5–8**).



	R_1	R_2	R_3	R_4	R_5	R_6
1	OMe	OH	H	OH	OMe	OMe
2	OMe	OMe	H	OH	OMe	OMe
3	OMe	OH	OMe	OH	OMe	OMe
4	OMe	OH	OMe	OMe	OMe	OH
5	OMe	OMe	OMe	OH	OMe	OMe
6	H	OH	OMe	OH	OH	OMe
7	OMe	OH	OMe	OH	OMe	OH
8	OMe	OH	OMe	OH	OH	OMe

5,7,2′-*Trihydroxy*-3,6,4′,5′-*tetramethoxyflavone* (**1**)

The MS of the PM derivative of **1** (Table 1) was identical to the MS of the PM derivative of brickellin (**2**), which was recently revised to 5,2′-dihydroxy-3,6,7,4′,5′-pentamethoxyflavone on the basis of synthesis [2]. This result established that **1** had the same oxygenation pattern as brickellin and is a flavonol not a flavone [3]. The flavone versus flavonol skeleton of brickellin can also be assigned on the basis of the ^{13}C NMR data for C-4: for all 5-hydroxyflavones the C-4 signal occurs at more than δ181.3 whereas for 5-hydroxyflavonols the C-4 signal appears at less than δ180.0 [4–7]. For example, the ^{13}C NMR signal for the C-4 of brickellin occurs at δ176.9 ppm. The ^1H NMR spectrum of **1** (Table 2) exhibited three one-proton singlets at δ6.44, 6.65 and 7.27 (in $CDCl_3$) assigned to H-8, H-3′ and H-6′, respectively, on the basis of their similarity to signals observed for brickellin. Other ^1H NMR signals were observed at δ3.90, 3.93, 3.96 and 3.98 (in $CDCl_3$) for four methoxyl groups. The MS of **1** exhibited a molecular ion at m/z 390 (100) for $C_{19}H_{18}O_9$ in accord with a flavonol containing four methoxyl and three hydroxyl groups. The assignment of three hydroxyl groups in **1** was based on the following data: **1** appeared as a purple fluorescent spot on paper under UV light (Table 5) in accord with a free 5-hydroxyl group, and the presence of Band III at 324 nm in the sodium methoxide UV spectrum and a 14 nm bathochromic shift in Band II in the sodium acetate UV spectrum relative to Band II in methanol supported a 7-hydroxyl group [8]. Ions at m/z 167 (8), 139 (10) and 181 (9), which correspond to $[A_1-15]^+$, $[A_1-43]^+$ and $[B_6]^+$ and $[B_6-15]^+$) [9, 10]. Moreover, the MS of **1** hydroxyl groups in the A-ring. A 2′-hydroxyl group was indicated by the following evidence: like brickellin, which

*Permanent address: The Hubei College of Chinese Traditional Medicine, Wuhan, The People's Republic of China.

Table 1. MS data for flavonol methyl ethers **1** and **3–6**

Flavonol methyl ethers	$[M]^+$	$[M-15]^+$	$[M-17]^+$	$[M-31]^+$	$[M-43]^+$	$[A_1-15]^+$	$[A_1-43]^+$	$[B_2]^+$	$[B_6]^+$	$[B_6-15]^+$
1: 5,7,2′-OH	390	375	373	359	347	167	139	181	208	193
3,6,4′,5′-OMe	(100)	(50)	(16)	(56)	(13)	(8)	(10)	(9)	(59)	(34)
PM-1: 3,5,6,7,	432	417		401		195		195		
2′,4′,5′-OMe	(56)	(100)		(74)		(36)				
3: 5,7,2′-OH	420	405	403	389	376	197	169	181	208	193
3,6,8,4′,5′-OMe	(100)	(46)	(9)	(21)	(6)	(41)	(15)	(10)	(68)	(53)
4: 5,7,5′-OH	420	405	403	389		197	169	181	208	193
3,6,8,2′,4′-OMe	(73)	(100)	(3)	(13)		(5)	(8)	(7)	(1)	(1)
5: 5,2′-OH	434	419	417	403	391	211	183	181	208	193
3,6,7,8,4′,5′-OMe	(100)	(35)	(5)	(15)	(3)	(37)	(11)	(4)	(35)	(17)
PM-5:* 3,5,6,7,8,	462	447		431	419		197			
2′,4′,5′-OMe	(14)	(100)		(24)	(10)		(17)			
6: 5,7,2′,4′-OH	376	361	359	345	333	167	139	167	194	179
3,8,5′-OMe	(100)	(53)	(17)	(33)	(13)	(13)	(6)		(55)	(25)

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

*3, 4 and 5 gave the same MS of PM derivatives.

Table 2. ^{1}H NMR data for flavonol methyl ethers **1–6**

Flavonol methyl ethers	$CDCl_3$			Methoxyl groups													
				$CDCl_3$							C_6D_6						
	H-8	H-3′	H-6′	3	6	7	8	2′	4′	5′	3	6	7	8	2′	4′	5′
1: 5,7,2′-OH 3,6,4′,5′-OMe	6.44	6.65	7.37	3.93	3.90				3.98	3.96	3.60	3.53				3.32	3.39
2: 5,2′-OH 3,6,7,4′,5′-OMe	6.50	6.62	7.11	3.93	3.80	3.90			3.96	3.93	3.92	3.72	3.20			3.35	3.48
3: 5,7,2′-OH 3,6,8,4′,5′-OMe		6.63	7.27	3.92	3.90		4.06		3.98	3.95	3.66	3.58		3.76		3.30	3.53
4: 5,7,5′-OH 3,6,8,2′,4′-OMe		6.61	7.06	3.93	3.84		4.04	3.81	3.99		3.77	3.63		3.77	3.13	3.31	
5: 5,2′-OH 3,6,7,8,4′,5′-OMe		6.66	7.30	3.95	3.91	3.95	4.11		3.98	3.97	3.84	3.62	3.59	3.86		3.30	3.53
				Methoxyl groups													
	CCl_4			CCl_4							C_6D_6						
	H-6	H-3	H-6′	3	6	7	8	2′	4′	5′	3	6	7	8	2′	4′	5′
6: 5,7,2′,4′-OH 3,8,5′-OMe (as TMSi ether)	6.16	6.36	6.92	3.81	3.74					3.81	3.82	3.68					3.36

90 MHz, δ-scale in ppm, TMS as int. standard, all signals in Table 1 are singlets.

has a 2'-hydroxyl group, MS of **1** exhibited B-ring fragments at *m/z* 208 (59) and 193 (34) (designated as $[B_6]^+$ and $[B_6-15]^+$) [9, 10]. Moreover, the MS of **1** exhibited an $[M-17]^+$ at *m/z* 373 (16), typical of most 2'-hydroxyflavonol 3-methyl ethers. With the assignment of the three hydroxyl groups and to accommodate the brickellin skeleton, the four methoxyl groups must be at the 3, 6, 4' and 5' positions. All other spectral data supported the assignment of **1** as the new compound, 5,7,2'-trihydroxy-3,6,4',5'-tetramethoxyflavone.

5,7,2'-Trihydroxy-3,6,8,4',5'-pentamethoxyflavone (**3**).

The MS of **3** (Table 1) gave $[M]^+$ at *m/z* 420 (100) for a flavonoid aglycone with three hydroxyl and five methoxyl substituents, i.e. one more methoxyl group than **1**. Comparison of MS, ^{1}H NMR and UV data indicated the only difference between **1** and **3** was the presence of a methoxyl group at C-8 in **3**. These data included the absence of an ^{1}H NMR singlet at 6.44 (in $CDCl_3$) for H-8. The two one-proton singlets occurring at δ6.63 (H-3') and 7.27 (H-6') indicated the same B-ring substitution as **1** and brickellin. Moreover, in support of this conclusion the MS of **3** gave the B-ring ions which correspond to $[B_6]^+$ at *m/z* 208 (68) and $[B_6-15]^+$ at *m/z* 193 (53) just as observed for **1** and brickellin. Other ions, $[A_1-15]^+$ at *m/z* 197 (41) and $[A_1-43]^+$ at *m/z* 169 (15), are in accord with **3** having a 5,7-dihydroxy-6,8-dimethoxy A-ring. The location of the three hydroxyl groups in **3** followed arguments similar to those used for **1**. Free 5 and 7 hydroxyl groups were supported by a purple color on paper under UV light and UV spectral data ($\Delta\lambda$ Band II data sodium acetate–methanol: 11 nm and the presence of Band III at 333 sh nm in sodium methoxide and 330 sh nm in sodium acetate), respectively. All the above results as well as ^{13}C NMR data (Table 4) suggested that **3** is the new flavonol, 5,7,2'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone, a structure subsequently confirmed by synthesis. Comparison of **3** with a synthetic sample of **4** (5,7,5'-trihydroxy-3,6,8,2',4'-pentamethoxyflavonol) showed that while they are difficult to distinguish by UV spectra (Table 3), R_f values and colors on paper (Table 5), they are readily distinguished by MS: **3** and **4** both gave B_2 but **3** also gave $[B_6]^+$ and $[B_6-15]^+$ as well as a more intense $[M-17]^+$ (Table 1) and by ^{1}H NMR (Table 2).

5,2'-Dihydroxy-3,6,7,8,4',5'-hexamethoxyflavone (**5**)

The PM of **5** gave the same mass spectrum as the PM of **3**, and **5** exhibited two ^{1}H NMR one-proton singlets at δ6.66 (H-3') and 7.30 (H-6') (in $CDCl_3$) similar to those for **3** (Table 2). Thus, **5** has the same 3,5,6,7,8,2',4',5'-oxygenation pattern as **3**. The MS of **5** (mp 125–127°) exhibited a molecular ion at *m/z* 434 (100) for $C_{21}H_{20}O_{10}$ in accord with a flavonol containing two hydroxyl and six methoxyl groups (Table 1). Compound **5** appeared as a purple fluorescent spot on paper under UV light indicating a free 5-hydroxyl group. The second hydroxyl group was assigned to C-2' on the basis of the characteristic B-ring MS ions: $[B_6]^+$ at *m/z* 208 (35) and $[B_6-15]^+$ at 193 (17). The spectral findings (Tables 1–3) established **5** to be 5,2'-dihydroxy-3,6,7,8,4',5'-hexamethoxyflavone, a new natural product.

5,7,2',4'-Tetrahydroxy-3,8,5'-trimethoxyflavone (**6**)

The MS of **6** exhibited a molecular ion at *m/z* 376 (100) for $C_{18}H_{16}O_9$ in accord with a flavonoid containing three methoxyl and four hydroxyl groups, which means that three positions in **6** are not substituted. The TMSi ether of **6** exhibited three ^{1}H NMR one-proton singlets at δ6.16, 6.36 and 6.92 (in CCl_4). Two of them (at δ6.36 and 6.92) are similar to those for 5,7,2',4'-tetrahydroxy-3,6,8,5'-tetramethoxyflavone (at δ6.36 and 6.91) [1] corresponding to H-3' and H-6', respectively. Band I in the aluminium chloride UV spectrum appeared at 407 nm (+53 nm relative to Band I in methanol) and in aluminium chloride/hydrochloric acid Band I appeared at 400 nm (+46 nm relative to Band I in methanol). These large shifts are in accord with an 8-methoxyl rather than a 6-methoxyl group [11]. In the ^{1}H NMR spectrum, the signal for H-6 appeared as expected at δ6.16 in CCl_4 (as TMSi ether). Thus, the above data suggested a 3,5,7,8,2',4',5'-oxygenation pattern. Since **6** appeared as a purple fluorescent spot on paper over UV light and changed to yellow with NH_3 and NA, the presence of free 5- and 4'-hydroxyl groups was indicated and confirmed that two hydroxyls were in the B-ring further suggesting a 5'-methoxyl group. A free 7-hydroxyl group was supported by UV ($\Delta\lambda$ Band II sodium acetate–methanol: 14 nm and the presence of Band III at 325 nm in sodium

Table 3. UV spectral data for flavonol methyl ethers **1** and **3–6**

Flavonol methyl ethers	λ_{max} (nm) MeOH (Band II/Band I)	NaOMe	$AlCl_3$	$AlCl_3$+HCl	NaOAc	NaOAc+H_3BO_3
1: 5,7,2'-OH	262 355	273 324	275 365 sh	272 364	276 326	262 355
3,6,4',5'-OMe	(2.97)	391	406	403 sh	374	
3: 5,7,2'-OH	262 352	273 333 sh	278 326 sh	276 367	262 330 sh	263 354
3,6,8,4',5'-OMe	(2.68)	384	380 406 sh	412 sh	372	
4: 5,7,5'-OH	265 352	275 335 sh	278 330	276 323 sh	274 335 sh	266 352
3,6,8,2',4'-OMe	(2.80)	372	390	365 412 sh	361	
5: 5,2'-OH	264 357	267 396	280 375 sh	277 370	264 357	265 360
3,6,7,8,4',5'-OMe	(2.65)		405	415 sh		
6: 5,7,2',4'-OH	262 354	275 325	275 407	275 364 sh	276 332	263 356
3,8,5'-OMe	(2.55)	411		400	395	

Table 4. ^{13}C NMR chemical shifts for flavonol methyl ethers 3 and 6

Flavonol methyl ethers	A- and C-ring carbons								
	2	3	4	5	6	7	8	9	10
3: 5,7,2'-OH 3,6,8,4',5'-OMe	145.3	138.7	178.6	148.1	131.5	156.7	127.9	152.3	104.
6: 5,7,2',5'-OH 3,6,8,4'-OMe	145.5	138.7	178.7	148.3	131.5	157.2	128.0	151.0	104.

Flavonol methyl ethers	B-ring carbons					
	1'	2'	3'	4'	5'	6'
3: 5,7,2'-OH 3,6,8,4',5'-OMe	108.3	150.6	101.5	150.8	141.9	114.
6: 5'2',5'-OH 3,6,8,4'-OMe	108.3	149.4	101.2	150.8	139.1	116.

Flavonol methyl ethers	Methoxyl carbons				
	3	6	8	4'	5'
3: 5,7,2'-OH 3,6,8,4',5'-OMe	61.0	60.1	59.9	55.6	56.5
6: 5,7,2',5'-OH 3,6,8,4'-OMe	61.3	60.3	60.1	55.6	

22.6 MHz, δ ppm in DMSO-d_6 with TMS as int. standard.

Table 5. Chromatographic data ($R_f \times 100$ and colors) of flavonol methyl ethers 1 and 3–6

Flavonol	15% HOAc	TBA		UV	UV/NH_3	UV/NA
1: 5,7,2'-OH 3,6,4',5'-OMe	66	91	(Cellulose)	P	Br P	Br P
3: 3,7,2'-OH 3,6,8,4',5'-OMe	85	89	(Whatman No. 3)	P	P	P
4: 5,7,5'-OH 3,6,8,2',4'-OMe	85	89	(Whatman No. 3)	P	P	P
5: 5,2'-OH 3,6,7,8,4',5'-OMe	65	91	(Cellulose)	P	Br P	Br P
6: 5,7,2',4'-OH 3,8,5'-OMe	73	93	(Cellulose)	P	Y	Y

P = purple, Br = bright, Y = yellow.

methoxide and 332 nm in sodium acetate). That the remaining hydroxyl group must be located at the 2'-position is supported by the notable B-ring MS ions: $[B_6]^+$ at *m/z* 194 (55), $[B_6-15]^+$ at 179 (25) and $[M-17]^+$ at 359 (17). Thus, we assign this new structure as 5,7,2',4'-tetrahydroxy-3,8,5'-trimethoxyflavone.

5,7,2',5'-Tetrahydroxy-3,6,8,4'-tetramethoxyflavone (7)

All spectral data (UV, MS, ^1H NMR) for 7 were identical with those of 5,7,2',5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone [1]. The ^{13}C NMR reported here (Table 4) confirmed this structure.

5,7,2',4'-Tetrahydroxy-3,6,8,5'-tetramethoxyflavone (8)

Compound 8 was identified by direct comparison of UV, MS, MS of PM, ^1H NMR, R_f values and colors on paper under UV light as 5,7,2',4'-tetrahydroxy-3,6,8,5'-tetramethoxyflavone [1].

EXPERIMENTAL

Plant material, Gutierrezia microcephala (D.C) A. Gray was collected on 5 June 1981 from the state of Neuvo Leon, Mexico, on Hwy. 40 between Monterrey and Saltillo on the road to Microondas Mariposa by Mark Leidig and Meredith Lane. The

plants were identified by Lane and voucher specimens are on deposit at the University of Texas Herbarium (Lane No. 2585).

Extraction and isolation. Ground, dried leaves and stems (800 g) of *G. microcephala* were extracted with 85% aq. MeOH (31 × 4) and 50% aq. MeOH (31 × 2). The extracts were combined and evapd under red. pres. until only H_2O remained. The aq. layer was partitioned with *n*-hexane, CH_2Cl_2 and EtOAc. The CH_2Cl_2 fractions were evaporated to dryness in *vacuo* and (yield 58.1 g, dt wt) transferred to a short, large diameter column bearing 500 g of cellulose powder and eluted successively with pure H_2O, 15% HOAc, 25% HOAc and 40% HOAc. All bands fluorescing under UV light were combined and evaporated to dryness and chromatographed over a Polyclar AT (GAF Corp) column packed in toluene. Elution of the 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. Compounds **1**, **3**, **5**, **6**, **7** and **8** were obtained, respectively, from fractions 10, 6, 1, 21, 16 and 18. Compounds **1**, **3**, **6**, **7** and **8** were further separated by PC using 15% HOAc on Whatman No. 3 paper, while **5** was chromatographed over a silica gel column using toluene–Me_2CO (9:1) and then separated by PC using 15% HOAc on Whatman No. 3 paper. After their purification over Sephadex LH-20 (either MeOH or 80% MeOH), all compounds were recryst. from MeOH to give yellow crystals.

Permethylation. Permethylation was achieved using Methelute (Pierce).

Synthesis of 5,7,2′-trihydroxy-3,6,8,4′,5′-pentamethoxyflavone and 5,7,5′-trihydroxy-3,6,8,2′,4′-pentamethoxyflavone. In accord with published procedures [2], 4-benzyloxy-2-hydroxy-3,5,6-trimethoxyacetophenone was condensed with 2-benzyloxy-4,5-dimethoxy- and 5-benzyloxy-2,4-dimethoxybenzaldehyde [5] in the presence of KOH to give 2,4′-dibenzyloxy-2′-hydroxy-4,5,3′,5′,6′-pentamethoxychalcone and 5,3′-dibenzyloxy-2′-hydroxy-2,4,3′,5′,6′-pentamethoxychalcone, respectively. These chalcones were subjected to the AFO reaction to give 7,2′-dibenzyloxy-3-hydroxy-5,6,8,4′,5′-pentamethoxyflavone and 7,5′-dibenzyloxy-3-hydroxy-5,6,8,2′,4′-pentamethoxyflavone in 48 and 52% yields, respectively. Standard methylation of the resulting flavonols, followed by debenzlation and partial demethylation at C-5 gave 5,7,2′-trihydroxy-3,6,8,4′,5′-pentamethoxyflavone (**3**) (mp 184–185°) and 5,7,5′-trihydroxy-3,6,8,2′,4′-pentamethoxyflavone (**4**) (mp 149–150°), respectively.

Trimethylsilylation. This was done as described in ref. [8].

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