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FIFTY-ONE FLAVONOIDS FROM *GUTIERREZIA MICROCEPHALA*

NIANBAI FANG*, MARK LEIDIG 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 microcephala*; Compositae; Asteraceae; fifty one flavonoids; six 2'-hydroxyflavonol 3-methyl ethers; three 2'-hydroxyflavones.

Abstract—The isolation of forty-five of the fifty-one flavonoids from aerial parts of *Gutierrezia microcephala* are described here. These forty-five flavonoids, together with six previously reported, make fifty-one flavonoids isolated from this species. There are eight new compounds: 5,7,2',4'-tetrahydroxy-6,8,5'-trimethoxyflavone, 5,7,2',4'-tetrahydroxy-8,5'-dimethoxyflavone, 5,7,3',4'-tetrahydroxy-3,6,8,5'-tetramethoxyflavone, 5,6,3',5'-tetrahydroxy-3,7,8,4'-tetramethoxyflavone, 5-hydroxy-3,6,7,8,3',4',5'-heptamethoxyflavone, 3,5,3',4'-tetrahydroxy-6,7,8-trimethoxyflavone, 5,3'-dihydroxy-3,6,7,8,4'-pentamethoxyflavone and 5,7,8,4'-tetrahydroxy-3,6-dimethoxyflavone.

INTRODUCTION

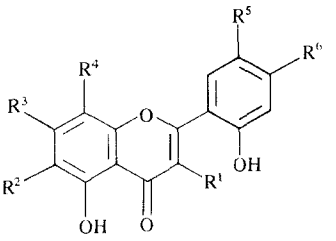
Fifty-one flavonoids, including twelve new compounds have been isolated from *Gutierrezia microcephala* (DC) A. Gray. We have already reported [1] on six of the compounds, namely: 5,7,2'-trihydroxy-3,6,4',5'-tetramethoxyflavone (**1**), 5,7,2'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone (**3**), 5,2'-dihydroxy-3,6,7,8,4',5'-hexamethoxyflavone (**5**), 5,7,2',4'-tetrahydroxy-3,8,5'-trimethoxyflavone (**6**), 5,7,2'5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone (**7**) and 5,7,2',4'-tetrahydroxy-3,6,8,5'-tetramethoxyflavone (**8**). Of these, **1**, **3**, **5** and **6** were new to the literature. In this paper, we discuss the isolation and identification of the remaining forty-five flavonoids including eight new compounds: 5,7,2',4'-tetrahydroxy-6,8,5'-trimethoxyflavone (**10**), 5,7,2',4'-tetrahydroxy-8,5'-dimethoxyflavone (**11**), 5,7,3',5'-tetrahydroxy-3,8,4'-trimethoxyflavone (**12**), 5,6,3',5'-tetrahydroxy-3,7,8,4'-tetramethoxyflavone (**13**), 5-hydroxy-3,6,7,8,3',4',5'-heptamethoxyflavone (**14**), 3,5,3',4'-tetrahydroxy-6,7,8-trimethoxyflavone (**15**), 5,3'-dihydroxy-3,6,7,8,4'-pentamethoxyflavone (**16**) and 5,7,8,4'-tetrahydroxy-3,6-dimethoxyflavone (**17**). The thirty-seven known compounds are 5,2',4'-trihydroxy-6,7,8,5'-tetramethoxyflavone (**9**) [2], 5,3'-dihydroxy-3,6,7,8,4',5'-hexamethoxyflavone (**18**) [3], 5,7,3',4'-tetrahydroxy-3,6,8,5'-tetramethoxyflavone (**19**) [4], 5,7,3',5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone (**20**) [5], 5,7-dihydroxy-3,6,8,3',4',5'-hexamethoxyflavone (**21**) [4], 5,3',5'-trihydroxy-3,6,7,8,4'-pentamethoxyflavone (**22**) [4], 5,7,3'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone (**23**) [6], 5,7,3',4',5'-pentahydroxy-3,6-dimethoxyflavone (**24**) [7], 5,7,3'-trihydroxy-3,6,4',5'-tetramethoxyflavone (**25**) [7], 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxyflavone (**26**) [4], 5,7,3',4'-tetrahydroxy-3,6,5'-trimethoxyflavone (**27**) [7], 5,7,3'-trihydroxy-3,6,8,4'-tetramethoxyflavone (**28**) [4], 5,7,4'-trihydroxy-3,6,8,3'-tetramethoxyflavone (**29**) [7], 5,3'-dihydroxy-3,6,7,4'-tetramethoxyflavone (**30**) [7], 5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone (**31**) [8], 3,5,7,3',4'-pentahydroxy-6,8-dimethoxyflavone (**32**) [6], 5,7,4'-trihydroxy-3,6,3'-trimethoxyflavone (**33**) [7], 5,7,3',4'-tetrahydroxy-3-methoxyflavone (**34**) [7], 3,5,7,4'-tetrahydroxy-6,8,3'-trimethoxyflavone (**35**) [7], 5,7,3',4'-tetrahydroxy-3,6-dimethoxyflavone (**36**) [7], 5,7,3',4'-tetrahydroxy-3,6,8-trimethoxyflavone (**37**) [7], 5,7,3',4',-tetrahydroxy-6,8-dimethoxyflavone (**38**) [7], 5,7,3',4'-tetrahydroxy-3,8-dimethoxyflavone (**39**) [4], 3,5,7,3',4'-pentahydroxyflavone (**40**), 5,4'-dihydroxy-3,6,7,8-tetramethoxyflavone (**41**) [7], 5,7,4'-trihydroxy-3,6,8-trimethoxyflavone (**42**) [9], 5,7,4'-trihydroxy-3,8-dimethoxyflavone (**43**) [7], 5,7,4'-trihydroxy-3,6-dimethoxyflavone (**44**) [7], 3,5,7,4'-tetrahydroxyflavone (**45**), quercetin 3-galactoside (**46**), isorhamnetin 3-galactoside (**47**), quercetin 7-glucoside (**48**), luteolin 7-glucoside (**49**), orientin (**50**), vitexin (**51**), violanthin (**52**) and isovitexin (**53**).

Recently, from a different population of *G. microcephala* other workers [10] isolated 20 flavonol methyl ethers including **8**, **21**, **23**, **29**, **32–34**, **37**, **38**, **42** and **43**. In contrast to the population investigated here which contained as major constituents 2'-hydroxyflavonol 3-methyl ethers, these workers reported only one minor constituent to have a 2'-hydroxyl group. We are currently examining additional populations in order to establish the extent of chemical variation in *G. microcephala*.

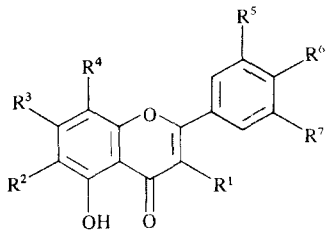
RESULTS AND DISCUSSION

Column chromatography and preparative PC of the dichloromethane and ethyl acetate extracts of a concentrated aqueous methanolic extract of the aerial parts of *G. microcephala* led to the isolation of the forty-three aglycones (**1**, **3**, **5–45**) and eight glucosides (**46–53**). All new compounds (**1**, **3**, **5**, **6**, **10–17**) are aglycones. Since we have previously reported on the new compounds **1**, **3**, **5** and **6** and the known compounds **7** and **8** [1], here we report only the isolation of thirty-seven known com-

*Permanent address: The Hubei College of Chinese Traditional Medicine, Wuhan, China.

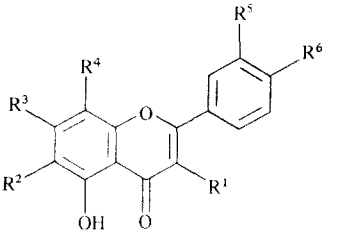


	R^1	R^2	R^3	R^4	R^5	R^6
1	OMe	OMe	OH	H	OMe	OMe
3	OMe	OMe	OH	OMe	OMe	OMe
5	OMe	OMe	OMe	OMe	OMe	OMe
6	OMe	H	OH	OMe	OH	OMe
7	OMe	OMe	OH	OMe	OMe	OH
8	OMe	OMe	OH	OMe	OH	OMe
9	H	OMe	OMe	OMe	OH	OMe
10	H	OMe	OH	OMe	OH	OMe
11	H	H	OH	OMe	OH	OMe

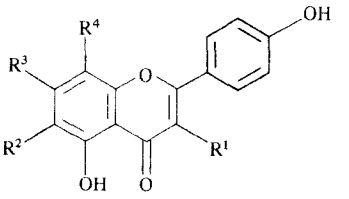


	R^1	R^2	R^3	R^4	R^5	R^6	R^7
12	OMe	H	OH	OMe	OH	OMe	OH
13	OMe	OH	OMe	OMe	OH	OMe	OH
14	OMe	OMe	OMe	OMe	OMe	OMe	OMe
18	OMe	OMe	OMe	OMe	OH	OMe	OMe
19	OMe	OMe	OH	OMe	OH	OH	OMe
20	OMe	OMe	OH	OMe	OH	OMe	OH
21	OMe	OMe	OH	OMe	OMe	OMe	OMe
22	OMe	OMe	OMe	OMe	OH	OMe	OH
23	OMe	OMe	OH	OMe	OH	OMe	OMe
24	OMe	OMe	OH	H	OH	OH	OH
25	OMe	OMe	OH	H	OH	OMe	OMe
26	OMe	OMe	OH	OMe	OH	OH	OH
27	OMe	OMe	OH	H	OH	OH	OMe

The numbering for the structures excludes compounds 2 and **4**, which were model compounds employed in the work reported in ref. [1], and were not isolated from *Gutierrezia microcephala*.



	R^1	R^2	R^3	R^4	R^5	R^6
15	OH	OMe	OMe	OMe	OH	OH
16	OMe	OMe	OMe	OMe	OH	OMe
28	OMe	OMe	OH	OMe	OH	OMe
29	OMe	OMe	OH	OMe	OMe	OH
30	OMe	OMe	OMe	H	OH	OMe
31	OMe	OMe	OMe	OMe	OH	OH
32	OH	OMe	OH	OMe	OH	OH
33	OMe	OMe	OH	H	OMe	OH
34	OMe	H	OH	H	OH	OH
35	OH	OMe	OH	OMe	OMe	OH
36	OMe	OMe	OH	H	OH	OH
37	OMe	OMe	OH	OMe	OH	OH
38	H	OMe	OH	OMe	OH	OH
39	OMe	H	OH	OMe	OH	OH
40	OH	H	OH	H	OH	OH
46	OGal	H	OH	H	OH	OH
47	OGal	H	OH	H	OMe	OH
48	OH	H	Glc	H	OH	OH
49	H	H	Glc	H	OH	OH
50	H	H	OH	Glc	OH	OH



	R^1	R^2	R^3	R^4
17	OMe	OMe	OH	OH
41	OMe	OMe	OMe	OMe
42	OMe	OMe	OH	OMe
43	OMe	H	OH	OMe
44	OMe	OMe	OH	H
45	OH	H	OH	H
51	H	H	OH	Glc
52	H	Glc	OH	Glc
53	H	Glc	OH	H

pounds (**9**, **18–53**) and present detailed data only for the characterization of eight new compounds **10–17**.

Flavones with 2′,4′,5′-oxygenated B-ring (**9**, **10** *and* **11**)

5,2′,4′-Trihydroxy-6,7,8,5′-tetramethoxyflavone (**9**). The MS of **9** exhibited a molecular ion peak at *m/z* 390 (96%) in accord with an aglycone containing three hydroxyl and four methoxyl groups (Table 1). The fragments, notably the $[A_1-15]^+$ at *m/z* 211 (56%), $[A_1-43]^+$ at *m/z* 164 (8%) and $[B_1-15]^+$ at *m/z* 149 (10%), indicated that the A-ring contained one hydroxyl and three methoxyl groups and that the B-ring had two hydroxyl and one methoxyl groups. This was confirmed by MS of the PM derivative of **9** (Table 1) [11]. The ^{1}H NMR spectrum of the TMSi ether derivative of **9** (Table 4) exhibited three one-proton singlets at δ6.36 (H-3), 6.73 (H-3′) and 7.42 (H-6′) (CCl_4), thus establishing that **9** has a 5,6,7,8,2′,4′,5′-oxygenation

Table 1. MS data of flavonoids **9**–**17** [EIMS (probe) 70 eV, *m/z* (rel. int.)]

Flavonoids	$[M]^+$	$[M-15]^+$	$[A_1-15]^+$	$[A_1-43]^+$	$[B_1]^+$	$[B_1-15]^+$	$[B_2]^+$	$[B_2-28]^+$
9 5,2′,4′-OH	390	375	211	183	164	149	—	—
6,7,8,5′-OME	(96)	(100)	(56)	(35)	(8)	(10)	—	—
PM* of **9** 5,2′,4′	432	417	225	197	192	177	—	—
6,7,8,5′-OMe	(29)	(100)	(5)	(6)	(5)	(4)	—	—
10 5,7,2′,4′-OH	376	361	97	169	—	149	—	—
6,8,5′-OMe	(70)	(100)	(27)	(14)	—	(7)	—	—
11 5,7,2′,4′-OH	346	331	167	139	164	149	—	—
8,5′-OMe	(52)	(100)	(4)	(18)	(3)	(5)	—	—
18 5,3′-OH	434	419	211	183	—	—	181	153
3,6,7,8,4′,5′-OMe	(97)	(100)	(7)	(6)	—	—	(6)	(1)
12 5,7,3′,5′-OH	376	361	167	139	—	—	167	139
3,8,4′-OMe	(74)	(100)	(5)	(5)	—	—	—	—
13 5,6,3′,5′-OH	406	391	197	169	—	—	167	139
3,7,8,4′-OMe	(100)	(90)	(16)	(7)	—	—	(18)	(4)
14 5-OH	448	433	211	183	—	—	195	167
3,6,7,8,3′,4′,5′-OMe	(60)	(79)	(20)	(12)	—	—	(73)	(10)
15 3,5,3′,4′-OH	376	361	211	183	—	—	137	109
6,7,8-OMe	(100)	(82)	(8)	(8)	—	—	(19)	(8)
16 5,3′-OH	404	389	211	183	—	—	151	123
3,6,7,8,4′-OMe	(75)	(100)	(11)	(8)	—	—	(8)	(4)
17 5,7,8,4′-OH	346	331	197	169	—	—	121	93
3,6-OMe	(94)	(100)	(4)	(9)	—	—	(49)	(13)

*Compounds 9 and 10 gave the same MS as PM derivatives.

pattern. Compound **9** appeared as a purple fluorescent spot on paper under UV light and changed to yellow with ammonia, suggesting the presence of free 5- and 4′-hydroxyl groups. When sprayed with NA, the spot was yellow suggesting that the compound did not contain a 4′,5′-dihydroxyl system and that therefore the third hydroxyl group must be at the 2′-position. With the assignment of the three hydroxyl groups and to accommodate the 5,6,7,8,2′,4′,5′-oxygenation pattern, the four methoxyl groups must be at the 6,7,8 and 5′ positions. All other spectral data supported the assignment of **9** as 5,2′,4′-trihydroxy-6,7,8,5′-tetramethoxyflavone, previously reported [2].

5,7,2′,4′-Tetrahydroxy-6,8,5′-trimethoxyflavone (**10**). The MS of the PM derivative of **10** (Table 1) was identical to the MS of the PM derivative of **9**, which established that **10** had the same oxygenation pattern as **9**. The ^{1}H NMR spectrum of the TMSi ether of **10** (in CCl_4) (Table 4) also exhibited three one-proton singlets at δ6.36 (H-3), 6.71 (H-3′) and 7.43 (H-6), confirming the 5,6,7,8,2′,4′,5′-oxygenation pattern. The MS of **10** exhibited a molecular ion at *m/z* 376 (70%) for $C_{18}H_{16}O_9$ in accord with a flavone containing four hydroxyl and three methoxyl groups. The assignment of the four hydroxyl groups in **10** was based on the following data: **10** appeared as a purple fluorescent spot on paper under UV light and turned yellow with ammonia indicating the presence of 5- and 4′-hydroxyl groups. A free 7-hydroxyl group was supported by UV ($\Delta\lambda$B-II NaOAc–MeOH: 8 nm and the presence of B-III at 330 nm in NaOMe). The methoxyl groups at the 6- and 8-positions were confirmed by the A-ring fragments, $[A_1-15]^+$ at *m/z* 197 (27%) and $[A_1-43]^+$ at *m/z* 169 (14%). When sprayed with NA, the colour of **10** under UV light changed to yellow indicating the absence of *ortho*-dihydroxyl of the B-ring. Thus we assign this new structure as 5,7,2′,4′-tetrahydroxy-6,8,5′-trimethoxyflavone.

5,7,2′,4′-Tetramethoxy-8,5′-dimethoxyflavone (**11**). Comparison of UV and ^{1}H NMR spectra of the TMSi ether (in CCl_4) of **11**, with those of **9** and **10**, provided the data concerning the structure of **11**. In addition to three one-proton singlets at 6.38, 6.73 and 7.43, which are similar to those for 5,2′,4′-trihydroxy-6,7,8,5′-tetramethoxyflavone (**9**) and 5,7,2′,4′-tetrahydroxy-6,8,5′-trimethoxyflavone (**10**) corresponding to H-3, H-3′ and H-6′, one additional one-proton singlet was observed at 6.15, typical for H-6. Thus the NMR data established the oxygenation pattern of **11** as 5,7,8,2′,4′,5′. The MS of **11** (Table 1) gave $[M]^+$ at *m/z* 346 (52%) for a flavone with four hydroxyl and two methoxyl substituents. Compound **11** appeared purple on paper under UV light and turned dull yellow with ammonia or NA, indicating the presence of 5- and 4′-hydroxyl groups and no *ortho*-dihydroxyl groups in the B-ring. The UV spectral data suggested the presence of a hydroxyl group at C-7 (Table 2). Since the fragmentation pattern $[A_1-15]^+$ at *m/z* 167 (14%), $[A_1-43]^+$ at *m/z* 139 (18%), $[B_1]^+$ at *m/z* 164 (3%) and $[B_1-15]^+$ at *m/z* 149 (5%) supported the presence of two hydroxyl and one methoxyl groups in the A-ring and two hydroxyl and one methoxyl groups in the B-ring, the remaining hydroxyl must be at C-2′. Together, the spectral findings establish that **11** is 5,7,2′,4′-tetrahydroxy-8,5′-dimethoxyflavone.

Flavonoids tri-oxygenated at C-2′, C-4′ and C-5′ in ring B are rarely found in nature. *Gutierrezia microcephala* afforded nine of this type, including **1**, **3** and **5**–**8**, all of which are 2′-hydroxyflavonol 3-methyl ethers, and the flavones **9**–**11**. The UV spectra of this type of flavonoid are characteristic (Table 3): Band I of 2′,4′,5′-substituted flavones occur in the range 370–374 nm, while Band I of 2′,4′,5′-substituted flavonol 3-methyl ethers is at a shorter wavelength (345–365 nm) with the relative intensities of Band II to Band I being more than 2.30 due to steric

Table 2. UV spectral data of flavonoids **9**–**18**

Flavonoids	λ_{max} (nm) MeOH	NaOMe	$AlCl_3$	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3
9 5,2′,4′-OH	274, 374	270, 428	281, 300 sh	280, 300 sh	272, 424	273, 376,
6,7,8,5′-OMe			415	403		
10 5,7,2′,4′-OH	268, 295 sh	276, 330 sh	276, 300 sh	255, 280 sh	276, 335 sh	277, 380
6,8,5′-OMe	370	422	412	295 sh, 390	413	
11 5,7,2′,4′-OH	266, 293 sh	275, 312 sh	264 sh, 277	263, 277 sh	278, 328 sh	268, 375
8,5′-OMe	370	421	300, 375 sh, 417	300, 375 sh, 408	410	
18 5,3′-OH	260 sh, 280	276, 285 sh	288, 310 sh	290, 310 sh	276, 334	280, 336
3,6,7,8,4′,5′-OMe	336	305, 395	363, 415 sh	351, 415 sh		
12 5,7,3′,5′-OH	275, 325	281, 318	284, 310	285, 308	283, 310 sh	275, 320
3,8,3′-OMe	355 sh	392	350, 417	348, 410	385	355 sh
13 5,6,3′,5′-OH	288, 305 sh	318 (dec.)	255, 295	254, 296	310	286, 305 sh
3,7,8,4′-OMe	333		320, 361	318, 355		333
14 5-OH	280, 334	284, 315 sh	290, 310 sh	292, 310 sh	284, 310 sh	280, 333
3,6,7,8,3′,4′,5′-OMe		382	364	358	350	
15 3,5,3′,4′-OH	262, 280 sh	270, 434 (dec.)	277, 350 sh	272, 381	267, 407	267, 394
6,7,8-OMe	350 sh, 378		462	440		
16 5,3′-OH	255, 278	281, 313	261, 287	261, 290	281, 313	280, 343
3,6,7,8,4′-OMe	344	383	370, 420 sh	363, 420 sh	382	
17 5,7,8,4′-OH	255, 275	279, 331	275, 375	260, 275 sh	280, 315	274, 340 sh
3,6-OMe	342, 363 sh	415 (dec.)	433	365, 430	394	375

Table 3. UV spectral comparison of flavonoids possessing a 2′,4′,5′-substitution pattern in methanol

	λ_{max} (nm)	Relative intensities* Band II/Band I
Flavonol		
1 5,7,2′-OH	262 355	2.97
3,6,4′,5′-OMe		
3 5,7,2′-OH	262 352	2.68
3,6,8,4′,5′-OMe		
4 5,7,5′-OH	265 352	2.80
3,6,8,2′,4′-OMe		
5 5,2′-OH	264 357	2.65
3,6,7,8,4′,5′-OMe		
6 5,7,2′,4′-OH	262 354	2.55
3,8,5′-OMe		
7 5,7,2′,5′-OH	264 350	2.88
3,6,8,4′-OMe		
8 5,7,2′,4′-OH	268 355	2.30
3,6,8,5′-OMe		
Flavone		
9 5,2′,4′-OH	274 374	0.82
6,7,8,5′-OMe		
10 5,7,2′,4′-OH	268 295 sh	0.98
6,8,5′-OMe	370	
11 5,7,2′,4′-OH	266 293 sh	1.14
8,5′-OMe	370	

*We noted that only a very few 2′,6′-dioxygenated flavones synthesized by Dr. M. Iinuma in connection with another study exhibit a similar ratio of Band II to Band I as do 2′,4′,5′-substituted flavonol 3-methyl ethers (more than 2.30); however, the 2′,6′-dioxygenated flavones appear to be clearly distinguished from the latter compounds by having Band I at less than 340 nm.

hindrance which causes non-planarity of the B-ring with ring C and thus diminishes the conjugation of the B-ring to the carbonyl group. The 2′,4′,5′-substituted flavonol 3-methyl ethers exhibit an unusual paper and cellulose TLC chromatrographic property exhibiting high mobility in both TBA and 15% acetic acid. Therefore, 2′,4′,5′-substituted flavonol 3-methyl ethers can be distinguished from other aglycones on the basis of R_f values in 15% acetic acid (Table 7); this again reflects the non-planarity of the B-ring with the rest of the molecule which diminishes bonding of the flavonoid with the cellulose.

Flavonols with a 3′,4′,5′-oxygenated B-ring (**18**, **12**–**14**)

5,3′-Dihydroxy-3,6,7,8,4′,5′-hexamethoxyflavone (**18**). The MS of **18** established a flavonoid with two hydroxyl and six methoxyl groups: $[M]^+$ at *m/z* 434 (97%) corresponding to $C_{21}H_{22}O_{10}$. Since the ^{1}H NMR of **18** exhibited, except signals for methoxyl groups, two doublets (J = 2.5 Hz) for H-2′ and H-6′ at δ7.42 and 7.60 [6], **18** must be a flavonol with four oxygen functions in the A-ring and three in the B-ring and have an asymmetrically substituted B-ring. Assignment of one of the two hydroxyl groups to C-5 was indicated by a purple colour on paper under UV light. After permethylation of **18**, the ^{1}H NMR signals for H-6′ and H-2′ appeared as one two-proton singlet at δ7.42 in CCl_4 or at 7.59 in C_6D_6, establishing that the second hydroxyl must have been at C-3′ in **18**. With the assignment of the two hydroxyl groups, the six methoxyl groups must therefore be at the 3,6,7,8,4′ and 5′ positions. All other spectral data supported the structure of **18** as 5,3′-dihydroxy-3,6,7,8,4′,5′-hexamethoxyflavone, a compound previously isolated from *Digitalis purpurea* L. [3].

5,7,3′,5′-Tetrahydroxy-3,8,4′-trimethoxyflavone (**12**). The MS of the new flavonoid **12** exhibited a molecular

Table 4. ^{1}H NMR data of flavonoids **9–12** and **15–17***

Flavonoids as TMSi ether	CCl_4						OMe											
							CCl_4						C_6D_6					
	3	6	2′	3′	5′	6′	3	6	7	8	4′	5′	3	6	7	8	4′	5′
9 5,2′,4′-OH	6.36			6.73		7.42		3.76	3.94	4.02		3.88		3.71	3.45	3.81		3.60
6,7,8,5′-OMe	(*s*)			(*s*)		(*s*)		(*s*)	(*s*)	(*s*)		(*s*)		(*s*)	(*s*)	(*s*)		(*s*)
10 5,7,2′,4′-OH	6.36			6.71		7.43		3.73		3.90		3.86		3.65		3.68		3.46
6,8,5′-OMe	(*s*)			(*s*)		(*s*)		(*s*)		(*s*)		(*s*)		(*s*)		(*s*)		(*s*)
11 5,7,2′,4′-OH	6.38	6.15		6.73		7.43				3.83		3.82						
8,5′-OMe	(*s*)	(*s*)		(*s*)		(*s*)				(*s*)		(*s*)						
12 5,7,3′,5′-OH			7.37			7.37	3.89			3.89	3.83		3.84			3.76		3.73
3,8,4′-OMe			(*s*)			(*s*)	(*s*)			(*s*)	(*s*)		(*s*)			(*s*)		(*s*)
15 3,5,3′,4′-OH			7.70		6.86	7.80		3.78	3.96	4.03				3.72	3.66	3.78		
6,7,8-OMe			(*d*)		(*d*)	(*dd*)		(*s*)	(*s*)	(*s*)				(*s*)	(*s*)	(*s*)		
16 5,3′-OH			7.71		6.90	7.73	3.91	3.75	3.91	3.91	3.88		3.86	3.68	3.44	3.73		3.57
3,6,7,8,4′-OMe			(*d*)		(*d*)	(*dd*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)		(*s*)	(*s*)	(*s*)	(*s*)		(*s*)
17 5,7,8,4′-OH			8.08	6.86	6.86	8.08		3.73		3.90				3.63		3.68		
3,6-OMe			(*d*)	(*d*)	(*d*)	(*d*)		(*s*)		(*s*)				(*s*)		(*s*)		

*90 MHz, δ-scale in ppm with TMS as internal standard.

weight of 376 (74%), corresponding to an aglycone with four hydroxyl and three methoxyl groups. The ^{1}H NMR spectrum (Table 4) showed one two-proton singlet at δ7.37 for a 3',4',5'-symmetrically substituted B-ring, a one-proton singlet at δ6.14 for H-6 and two singlets at δ3.83 (3H) and 3.89 (6H) for three methoxyl groups. The purple colour under UV with and without ammonia were indicative of one hydroxyl at C-5 and a methoxyl at C-4'. The presence of B-III at 318 nm in the NaOMe UV spectrum (Table 2) indicated a 7-hydroxyl group. Since **12** had a symmetrically substituted B-ring the two remaining hydroxyl groups must be at the 3'- and 5'-positions. The unassigned methoxyl group must therefore be at the 8-position. Thus, compound **12** is 5,7,3',5'-tetrahydroxy-3,8,4'-trimethoxyflavone.

5,6,3',5'-*Tetrahydroxy*-3,7,8,4'-*tetramethoxyflavone* (**13**). The ^{1}H NMR spectrum of **13** showed only one two-proton singlet in the aromatic region which was assigned to protons at 2' and 6' (δ7.40) in a symmetrically substituted B-ring. The mass spectrum of **13** established a flavonoid with four hydroxyl and four methoxyl groups: $[M]^+$ at *m/z* 406 (100%) corresponding to $C_{19}H_{18}O_{10}$ and a B-ring with two hydroxyl and one methoxyl groups ($[B_2]^+$ 167 (18%) and $[B_2-28]^+$ 139 (4%). Two hydroxyl groups must be at the 3'- and 5'-positions to give a symmetrically substituted B-ring. Compound **13** appeared as a purple spot on paper under UV light indicating a 5-hydroxyl group. When the UV spectra of **13** were compared with the UV spectra of 5,7,3',5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone (**20**) and 5,6,3'-trihydroxy-3,7,4',5'-tetramethoxyflavone [12], the remaining hydroxyl group could be assigned to the 6-position [a characteristic peak (IIa) at 295 nm in $AlCl_3$ and at 296 in $AlCl_3$–HCl (Table 2)] [12]. Because the 5,6,3',5'-hydroxylation results in alkali sensitivity, there was not a Band-II in NaOMe and B-I degenerated in a few minutes in NaOMe. We assign the new structure as 5,6,3',5'-tetrahydroxy-3,7,8,4'-tetramethoxyflavone.

5-*Hydroxy*-3,6,7,8,3',4',5'-*heptamethoxyflavone* (**14**). The MS of compound **14** gave a molecular ion peak at *m/z* 448 for a flavonoid containing one hydroxyl and seven methoxyl groups. The purple colour on a paper under UV light suggested that this one hydroxyl group was at the 5-position. ^{1}H NMR of **14** showed a one two-proton singlet at δ7.50 for H-2' and H-6'. Therefore, we assign this new flavonoid as 5-hydroxy-3,6,7,8,3',4',5'-heptamethoxyflavone.

Flavonols with a 3',4'-oxygenated B-ring (**15, 16**)

3,5,3',4'-*Tetrahydroxy*-6,7,8-*trimethoxyflavone* (**15**). The MS of **15** exhibited a molecular ion peak at *m/z* 376 (100%) in accord with a flavone containing four hydroxyl and three methoxyl groups (Table 1). Since the ^{1}H NMR in CCl_4 of the TMSi ether of **15** showed B-ring signals characteristic for H-5', H-2' and H-6', respectively at δ6.86 (1H, *d*, J = 9 Hz), 7.70 (1H, *d*, J = 2.5 Hz) and 7.80 (1H, *dd*, J = 2.5 Hz and 9 Hz) (Table 4), **15** was clearly a flavonoid with a 3,5,6,7,8,3',4'-oxygenation pattern. The compound appeared as a dull yellow flourescent spot on paper in UV light with and without ammonia, indicating the presence of free 3- and 5-hydroxyl groups. Compound **15** also gave an orange colour with NA, indicating a 3',4'-dihydroxyl group in the B-ring. Thus the three methoxyl groups must be at the 6-, 7- and 8-positions. Therefore, the

Table 5. ^{1}H NMR data of some flavonoids*

Flavonoids	OMe	$CDCl_3$										C_6D_6							
		2'	5'	6'	3	6	7	8	3'	4'	5'	5	3	6	7	8	3'	4'	5'	5
18 5,3'-OH		7.42		7.48	3.96	3.90	3.96	4.11		4.02	3.96		3.86	3.66	3.43	3.72		3.58	3.84	
3,6,7,8,4',5'-OMe		(*d*)		(*d*)	(*s*)	(*s*)	(*s*)	(*s*)		(*s*)	(*s*)		(*s*)	(*s*)	(*s*)	(*s*)		(*s*)	(*s*)	
PM of **18**. 3,5,6,7,8,		7.42†		7.42†	3.91†	3.81†	3.91†	4.01†	3.91†	3.95†	3.91†	3.91†	4.02	3.77	3.57	3.80	3.89	3.57	3.89	3.70
3',4',5'-OMe		(*s*)		(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)
13 5,6,3',5'-OH		7.40		7.40	3.99		3.86	4.12		4.02				3.62	3.36	3.77		3.58		
3,7,8,4'-OMe		(*s*)		(*s*)	(*s*)		(*s*)	(*s*)		(*s*)				(*s*)	(*s*)	(*s*)		(*s*)		
14 5-OH		7.50		7.50	3.90	3.90	3.90	4.04	3.90	3.99	3.90		3.85	3.76	3.53	3.76	3.76	3.53	3.76	
3,6,7,8,3',4',5'-OMe		(*s*)		(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)		(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	
16 5,3'-OH		7.80	7.03	7.86	3.98	3.90	3.98	4.04		4.00			3.77	3.73	3.38	3.77		3.53		
3,6,7,8,4'-OMe		(*d*)	(*d*)	(*dd*)	(*s*)	(*s*)	(*s*)	(*s*)		(*s*)			(*s*)	(*s*)	(*s*)	(*s*)		(*s*)		

*90 MH_3, δ-scale in ppm, TMS as internal standard.
†In CCl_4, not in $CDCl_3$.

compound is 3,5,3′,4′-tetrahydroxy-6,7,8-trimethoxyflavone, a structure supported by all other spectral data (Tables 1, 2 and 4).

5,3′-Dihydroxy-3,6,7,8,4′-pentamethoxyflavone (**16**). The MS of this new compound ($[M]^+$ at m/z 404) and ^{1}H NMR, which exhibited B-ring proton signals characteristic for H-2′, 5′ and 6′ (Tables 4 and 5), corresponded to a 3,5,6,7,8,3′,4′-oxygenation flavone with two hydroxyl and five methoxyl groups. The purple colour under UV light with and without ammonia indicated the presence of a 5-hydroxyl and 3- and 4′-methoxyl groups. The $[M-15]^+$ peak appeared as the base peak supporting the presence of both 6- and 8-methoxyl groups. The remaining problem concerned the assigning of one hydroxyl and one methoxyl to the two available positions, C-7 and C-3′. In the MS of **16**, the fragments $[A_1-15]^+$, $[A_1-43]^+$, $[B_2]^+$ and $[B_2-28]^+$ (Table 1) indicated that the A-ring contained one hydroxyl and three methoxyl groups with one hydroxyl and one methoxyl in the B-ring. Therefore, the hydroxyl group had to be at the 3′-position and the methoxyl at the 7-position. The benzene-induced shifts of the two methoxyl resonances for **16** supported the presence of one methoxyl at C-7 (δ3.98–3.38, Δ = 0.60 ppm) and one methoxyl at C-4′ (δ4.00–3.53; Δ = +0.47 ppm) (Table 5). Thus, these spectral data established **16** to be 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone.

Flavonols with 4′-oxygenated B-rings (**17**)

5,7,8,4′-Tetrahydroxy-3,6-dimethoxyflavone (**17**). The ^{1}H NMR spectrum of the TMSi ether of **17** (in CCl_4) only exhibited two two-proton doublets (J = 9 Hz) at δ6.86 and 8.08 in accord with a flavonol with a 3,5,6,7,8,4′-oxygenation pattern. The MS of **17** established a molecular weight of 346, corresponding to a flavonol with four hydroxyl and two methoxyl groups. Band I in the MeOH UV spectrum appeared at 342 nm suggesting 3-methoxyl rather than a 3-hydroxyl group. The brown colour in UV light changing to yellow with ammonia indicated free C-5 and C-4′ hydroxyl groups. A free 7-hydroxyl group was supported by UV (B-III at 331 nm in NaOMe). A large shift of 67 nm in Band I in $AlCl_3$–HCl relative to Band I in MeOH indicated 8-oxygenation [13]. All the above data suggested the structure 5,7,8,4′-tetrahydroxy-3,6-dimethoxyflavone for **17**.

5,3′-Dihydroxy-3,6,7,8,4′,5′-hexamethoxyflavone (digicitin, **18**), 5,7,3′,4′-tetrahydroxy-3,6,8,5′-tetramethoxyflavone (**19**), 5,7-dihydroxy-3,6,8,3′,4′,5′-hexamethoxyflavone (**21**), 5,3′,5′-trihydroxy-3,6,7,8,4′-pentamethoxyflavone (**22**), 5,7,3′,4′,5′-pentahydroxy-3,6-dimethoxyflavone (**24**), 5,7,4′-trihydroxy-3,6,8,3′-tetramethoxyflavone (**29**), 5,3′,4′-trihydroxy-3,6,7,8-tetramethoxyflavone (**31**), 5,7,3′,4′-tetrahydroxy-6,8-dimethoxyflavone (**38**), 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (calicopterin, **41**) and 5,7,4′-trihydroxy-3,8-dimethoxyflavone (**43**) were identified by UV, ^{1}H NMR, colour on paper under UV light and by MS. 5,7,3′,5′-Tetrahydroxy-3,6,8,4′-tetramethoxyflavone (**20**), 5,7,3′-trihydroxy-3,6,8,4′,5′-pentamethoxyflavone (**23**), 5,7,3′-trihydroxy-3,6,4′,5′-tetramethoxyflavone (**25**), 5,7,3′,4′,5′-pentahydroxy-3,6,8-trimethoxyflavone (**26**), 5,7,3′,4′-tetrahydroxy-3,6,5′-trimethoxyflavone (**27**), 5,7,3′-trihydroxy-3,6,8,4′-tetramethoxyflavone (**28**), 5,3′-dihydroxy-3,6,7,4′-

Table 6. Chromatographic data of flavonoids **9**–**18** ($R_f \times 100$ and colours) (cellulose plate)

Flavonoids	$R_f \times 100$ in 15% HOAc	TBA*	Colour in UV	UV/NH_3	UV/NA
9 5,2′,4′-OH 6,7,8,5′-OMe	4	93	P	Y	Y
10 6,7,2′,4′-OH 6,8,5′-OMe	3	82	P	Y	Y
11 5,7,2′,4′-OH 8,5′-OMe	5	92	P	dY	dY
18 5,3′-OH 3,6,7,8,4′,5′-OMe			P	P	
12 5,7,3′,5′-OH 3,8,4′-OMe	23	96	P	P	
13 5,6,3′,5′-OH 3,7,8,4′-OMe	14	87	P	P	
14 5-OH 3,6,7,8,3′,4′,5′-OMe	32	74	P	P	
15 3,5,3′,4′-OH 6,7,8-OMe	10	86	dY	dY	Or
16 5,3′-OH 3,6,7,8,4′-OMe	33	96	P	P	
17 5,7,8,4′-OH 3,6-OMe	6	84	Br	Y	Y

*TBA: *t*-BuOH–HOAc–H_2O (3:1:1).

†UV, long wavelength 366 nm; short wavelength 254 nm. Colours are: P = purple; Y = yellow; Br = brown; Or = orange; dY = deep yellow. NA = Naturstoffreagenz A in MeOH.

Table 7. R_f value comparison of flavonoids possessing 2',4',5'-substitution pattern in methanol*

	$R_f \times 100$ in 15% HOAc	TBA
Flavonol		
1 5,7,2'-OH 3,6,4',5'-OMe	66	91
3 3,7,2'-OH 3,6,8,4',5'-OMe	85	89
4 5,7,5'-OH 3,6,8,2',4'-OMe	85	89
5 5,2'-OH 3,6,7,8,4',5'-OMe	65	91
6 5,7,2',4'-OH 3,8,5'-OMe	75	93
7 5,7,2',5'-OH 3,6,8,4'-OMe	74	85
8 5,7,2',4'-OH 3,6,8,5'-OMe	76	86
Flavone†		
9 5,2',4'-OH 6,7,8,5'-OMe	14	93
10 5,7,2',4'-OH 6,8,5'-OMe	3	82
11 5,7,2',4'-OH 8,3'-OMe	5	92

*On cellulose or paper.

†Based on several samples synthesized by Dr. M. Iinuma, 2',6'-dioxygenated flavones also exhibit high R_f values in both 15% HOAc and TBA.

tetramethoxyflavone (casticin, **30**), 3,5,7,3',4'-pentahydroxy-6,8-dimethoxyflavone (**32**), 5,7,4'-trihydroxy-3,6,3'-trimethoxyflavone (jaceidin, **33**), 5,7,3',4'-tetrahydroxy-3-methoxyflavone (**34**), 3,5,7,4'-tetrahydroxy-6,8,3'-trimethoxyflavone (limocitrol, **35**), 5,7,3',4'-tetrahydroxy-3,6-dimethoxyflavone (axillarin, **36**), 5,7,3',4'-tetrahydroxy-3,6,8-trimethoxyflavone (**37**), 5,7,3',4'-tetrahydroxy-3,8-dimethoxyflavone (**39**), quercetin (**40**), 5,7,4'-trihydroxy-3,6,8-trimethoxyflavone (**42**), 5,7,4'-trihydroxy-3,6-dimethoxyflavone (**44**), kaempferol (**45**), quercetin 3-galactoside (**46**), isorhamnetin 3-galactoside (**47**), quercetin 7-glucoside (**48**), and luteolin 7-glucoside (**49**) were identified by colour on paper under UV light, UV and ^{1}H NMR of their TMSi ethers. The identity of orientin (**50**), vitexin (**51**), isovietxin (**52**) and violanthin (**53**) were determined by colour on paper under UV-light, UV, ^{1}H NMR of their TMSi ethers and MS of their PM derivatives [14].

EXPERIMENTAL

Plant material. Gutierrezia microcephala was collected on June 5, 1981 from the state of Neuvo Leon, Mexico, on Hwy. 40 between Monterrey and Saltillo on the road to Microondas by Mark Leiding and Meredith Lane. Voucher specimens are on deposit in the University of Texas and Lundell Herbaria (Lane No. 2589).

General techniques. Flavonoids were extracted and the CH_2Cl_2 portion worked up from whole plant material (58.1 g) as described [1] previously. While whole, ground leaves and stems were used in this work, it was obvious from TLC of surface extracts that most of the aglycones were primarily on the external surface. The EtOAc fraction afforded 20 g which was chromatographed on a polyclar (Polyclar AT, GTAF, Corp.) column packed in H_2O–MeOH–MeCOEt–Me_2CO (13:3:3:1) and then with MeOH–MeCOEt–Me_2CO (3:3:1). Fractions were collected by monitoring the column with UV light. After CC, compounds were purified by PC, using 15% HOAc on Whatman No. 3 paper. Sephadex LH-20 (Pharmacia) was used for the preparation of pure compounds for spectral analysis. Chromatography and spectral analyses were made using the standard procedures described [11]. Compounds **1**, **3**, **5–25**, **27–33**, **35–39** and **41–44** were isolated from CH_2Cl_2 fraction, compounds **40**, **45**, **47–50**, **52** and **53** from EtOAc and compounds **26**, **34**, **46** and **51** from both CH_2Cl_2 and EtOAc fractions.

Derivatization. Permethylation was achieved using Methelute (Pierce) or CH_2N_2 produced by the reaction of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine with KOH; Trimethylsilylation was done as described in ref. [11].

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