

FLAVONOIDS FROM *GYMNOSPERMA GLUTINOSUM*

SANGGONG YU*, NIANBAI FANG† and TOM J. MABRY

The University of Texas at Austin, Austin, TX 78713, U.S.A.

(Received 28 August 1986)

Key Word Index—*Gymnosperma glutinosum*; Compositae; highly oxygenated flavonol 3-methyl ethers; 2'-hydroxyflavonol 3-methyl ether; 2'-methoxyflavonol 3-methyl ether.

Abstract—From *Gymnosperma glutinosum* seven new and 14 known compounds were isolated. Six of the seven new compounds have unusual B-rings with 2'-methoxyl groups. The seven new compounds are 5,7-dihydroxy-3,6,8,2',4',5'-hexamethoxyflavone, 5,4',5'-trihydroxy-3,6,7,8,2'-pentamethoxyflavone, 5,7,4'-trihydroxy-3,6,8,2',5'-pentamethoxyflavone, 5,7,4',5'-tetrahydroxy-3,6,8,2'-tetramethoxyflavone, 5,7,4',5'-tetrahydroxy-3,6,2'-trimethoxyflavone, 5,7,4',5'-tetrahydroxy-3,2'-dimethoxyflavone and 3,5,4'-trihydroxy-6,7,8,3'-tetramethoxyflavone. In addition, the following known flavonoids were isolated: 5,7,2'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone, 5,7,2',4'-tetrahydroxy-3,6,8,5'-pentamethoxyflavone, 5,3'-dihydroxy-3,6,7,8,4',5'-hexamethoxyflavone, 5,7,3'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone, 5,7,3',5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone, 5,7,3',4',5'-pentahydroxy-3,6,8,3'-trimethoxyflavone, 5,7,3',5'-tetrahydroxy-3,6,4'-trimethoxyflavone, 5,7,4'-trihydroxy-3,6,8,3'-tetramethoxyflavone, 5,7,3',4'-tetrahydroxy-3,6,8,3'-trimethoxyflavone, 3,5,7,3',4'-pentahydroxy-6,8-dimethoxyflavone, 5,7,4'-trihydroxy-3,6,8,3'-trimethoxyflavone, 5,7,3',4'-tetrahydroxyflavone, 8-C-glucosyl-5,7,4'-trihydroxyflavone and 5,7,3',4'-tetrahydroxyflavone 7-O-glucoside.

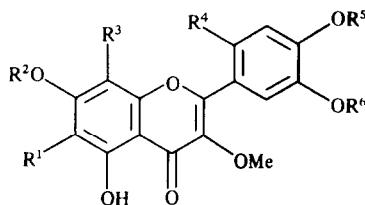
INTRODUCTION

The shrubby, monotypic *Gymnosperma glutinosum* (Spreg.) Less. is distributed from Guatemala through Mexico into the southwestern United States. It is highly variable throughout its range, and occupies a variety of habitats from extremely dry, rocky hillsides in the north to relatively humid mountain sides in the south. This species contains toxic compounds which adversely affect livestock that eat it [1], and it is noted for the sweet aroma which rises from the plants after a desert rain. *Gymnosperma* is closely related to *Gutierrezia* and presumably arose out of perennial and woody *Gutierrezia* stock. As part of a continuing chemotaxonomic study of the '*Gutierrezia*-*Xanthocephalum* complex' in North America [2-7], we report here from *Gymnosperma glutinosum* the isolation of 21 flavonoids including seven new ones. The flavonoid chemistry of this species is similar to that of woody species of *Gutierrezia*: namely, large quantities of flavonoids, mostly with 6,8-oxygenation as well as flavonol 3-methyl ethers with rare 2'-oxygenation [2-5]. The present chemical results support the close relationship of the shrubby, monotypic *Gymnosperma* to the shrubby *Gutierrezia* species. Moreover, in terms of flavonoid chemistry, the relationship is closer than that of woody *Gutierrezia* species to herbaceous *Gutierrezia* species; the latter mainly produce flavonoids without 6,8-oxygenation and these in relatively low amounts [6, 7].

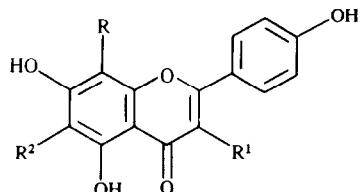
RESULTS AND DISCUSSION

Extraction of the ground air-dried leaves and stems of *Gymnosperma glutinosum* afforded 21 flavonoids, 19 agly-

cones (1-19), one C-glycosylflavonoid (20) and one O-glycoside (21). All new compounds (1-7) are aglycones including six 2'-methoxyflavonol 3-methyl ethers (1-6).



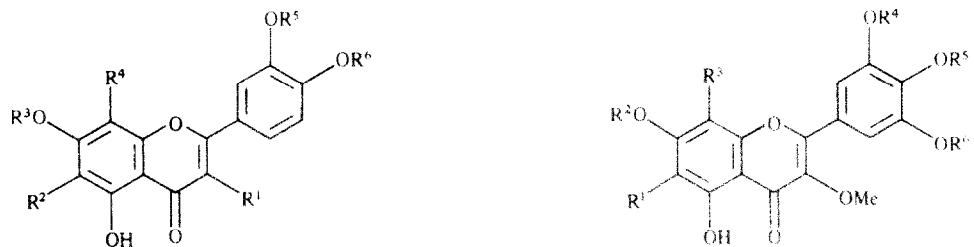
	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶
1	OMe	H	OMe	Me	Me	Me
2	OMe	Me	OMe	Me	H	H
3	OMe	H	OMe	Me	H	Me
4	OMe	H	OMe	Me	H	H
5	OMe	H	H	Me	H	H
6	H	H	H	Me	H	H
8	OMe	H	OMe	H	Me	Me
9	OMe	H	OMe	H	H	Me



	R ¹	R ²	R ³
18	OMe	OMe	OMe
20	H	H	Glu

* Permanent address: Wuhan Institute of Medical Science, Wuhan, China.

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



	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶		R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶	
7	OH	OMe	Me	OMe	Me	H								
15	OMe	OMe	H	OMe	Me	H	10	OMe	Me	OMe	H	Me	Me	
16	OMe	OMe	H	OMe	H	H	11	OMe	H	OMe	H	Me	Me	
17	OH	OMe	H	OMe	H	H	12	OMe	H	OMe	H	Me	H	
19	H	H	H	H	H	H	13	OMe	H	OMe	H	H	H	
21	H	H	Glu	H	H	H	14	OMe	H	H	H	Me	H	

Table 1-1. ^1H NMR data for flavonoids 1-3* (90 MHz, δ -scale in ppm, TMS as int. standard)

Flavonoids (underivatized)	Methoxyl groups															
	CDCl ₃				CDCl ₃					C ₆ D ₆						
	H-3'	H-6'	3	6	7	8	2'	4'	5'	3	6	7	8	2'	4'	5'
1:5,7-OH 3,6,8,2',4',5'-OMe	6.64	7.04	3.98	3.80		4.05	3.89	3.89	3.94	3.76	3.67		3.76	3.30	3.37	3.44
2:5,4',5'-OH 3,6,7,8,2'-OMe	6.68	7.16	3.95	3.68	3.91	4.11	3.81			3.80	3.68	3.61	3.83	3.29		
3:5,7,4'-OH 3,6,8,2',5'-OMe	6.61	7.06	3.99	3.80		4.04	3.84		3.93	3.79	3.67		3.83	3.23		3.36

*All signals in Table I-1 are singlets.

Table 1. 1H NMR data for TMSi ether of flavonoids 1-7 (90 MHz, δ -scale in ppm, TMS as int. standard)

CCl ₄									
Flavonoids as TMSi ether	H-6	H-8	H-2'	H-3	H-5	H-6	3	6	7
1,5,7-OH				6.55		6.96	3.83	3.74	
3,6,8,2',4',5'-OMe				(s)		(s)	(s)	(s)	
2',5,4',5'-OH				6.44		6.94	3.87	3.76	3.78
3,6,7,8,2'-OMe				(s)	(s)	(s)	(s)	(s)	
3,5,7,4'-OH				6.52		6.94	3.84	3.74	
3,6,8,2',5'-OMe				(s)		(s)	(s)	(s)	
4',5,7,4',5'-OH				6.46		6.97	3.77	3.75	
3,6,8,2'-OMe				(s)		(s)	(s)	(s)	
5,5,7,4',5'-OH	6.46			6.46		6.90	3.79	3.75	
3,6,2'-OMe		(s)		(s)		(s)	(s)	(s)	
6,5,7,4',5'-OH	6.16	6.24		6.46		6.91	3.81		
3,2'-OMe	(d)	(d)		(s)		(s)	(s)		
7',3,5,4'-OH			7.82		6.89	7.73		3.80	3.98
6,7,8,3'-OMe			(d)		(d)	(dd)		(s)	(s)

Two 2'-hydroxyflavonol 3-methyl ethers (**8**, **9**) previously known from *Gutierrezia microcephala* and *G. grandis* [2, 5] were also isolated. Detailed data are presented only for the seven new compounds **1**–**7**.

Evidence for compounds 1–6 being 2'-oxygenated flavonol 3-methyl ethers

The oxygenation patterns of **1–6** were determined from their ^1H NMR spectra (Tables 1-1 and 1-2), UV spectra (Table 2) and unusual paper chromatographic properties. High R_f values on TLC in both TBA and aq. 15% HOAc on cellulose plates (Table 5) are characteristic of 2'-oxygenated flavonol 3-methyl ethers and 2',6'-dioxy-ogenated flavones [3]. Band I in the MeOH UV spectra of the new compounds **1–6** appeared in the range 345–365 nm (345 for **1**, 358 for **2**, 352 for **3**, 355 for **4**, 346 for **5** and 347 for **6**) and the characteristic intensity ratio of Band II to Band I was between 2.47 and 2.88 to 1.0; that is, for 2'-oxygenated flavonol 3-methyl ethers Band II is about 2.5 times as intense as Band I. These data suggest that **1–6** are 2'-oxygenated flavonol 3-methyl ethers [5].

Evidence for compounds 1–4 being substituted at all positions except 3' and 6'

The two one-proton aromatic singlets ($\text{H}-3'$ and $\text{H}-6'$) in the ^1H NMR spectra (Tables 1-1 and 1-2) indicated that 1-4 have the 3,5,6,7,8,2',4',5'-oxygenation pattern, the same oxygenation pattern as two known compounds which were also isolated from this species, namely, 5,7,2'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone and 5,7,2',4'-tetrahydroxy-3,6,8,5'-tetramethoxyflavone [3].

5,7-Dihydroxy-3,6,8,2',4',5'-hexamethoxyflavone (1)

The mass spectrum of **1** exhibited a $[M]^+$ at m/z 434 (85 %) suggesting an aglycone with two hydroxyl and six methoxyl substituents. Ions at m/z 403 (12 %), 195 (3 %) and 167 (3 %), which correspond to $[M - 31]^+$, $[B_2]^+$ and $[B_2 - 28]^+$ and the absence of $[M - 17]^+$, $[B_6]^+$ and $[B_6 -$

$-15]$ ⁺ indicated that **1** is a 2'-methoxyflavonol 3-methyl ether and not a 2'-hydroxyflavonol 3-methyl ether [3]. Compound **1** appeared purple on paper under UV light with and without ammonia indicating 5-hydroxyl and 4'-methoxyl groups. In the UV spectra a free 7-hydroxyl group was indicated by the shoulder at 325 nm in sodium methoxide and was established by a bathochromic shift (+ 14 nm) of Band II in sodium acetate relative to Band II in the methanol spectrum [8]. With the two hydroxyl groups assigned to the 5 and 7 positions, the six methoxyl groups must be at the other six available positions, that is, C-3,6,8,2',4' and 5'. In the ¹H NMR spectra, the benzene-induced shifts of the methoxyl proton signals (Tables 1-1 and 1-2) supported these assignments for the six methoxyl groups. Thus, the new structure is 5,7-dihydroxy-3,6,8,2',4',5'-hexamethoxyflavone; all other spectral findings including ¹³C NMR data supported this assignment.

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

As for **1**, ions $[M - 31]^+$ at 389 (21%), $[B_2]^+$ at 167 (10%) and no $[M - 17]^+$, $[B_6]^+$ and $[B_6 - 15]^+$ fragments in the mass spectrum of **2** indicated that **2** was a 2'-methoxyflavonol 3-methyl ether. The purple colour on paper under UV light turned to yellow with ammonia indicating free 5- and 4'-hydroxyl groups. Moreover, **2** gave an orange colour when sprayed with NA reagent (Table 5) indicating a free *ortho*-dihydroxyl system in the B-ring [8]. The *ortho*-dihydroxyl group in the B-ring was confirmed by UV spectral data: in the sodium acetate + boric acid spectrum Band I appeared at 378 nm (+20 relative to Band I in methanol). The MS of **2** gave $[M]^+$ at *m/z* 420 (100%) for a flavonoid aglycone with three hydroxyl and five methoxyl groups. With the assignment of the three hydroxyl groups to the 5',4' and 5' positions and to accommodate the 3,5,6,7,8,2',4',5'-oxygenation pattern, the five methoxyl groups must be at 3,6,7,8 and 2' positions. The spectral findings (Tables 1-3) established **2** to be 5,4',5'-trihydroxy-3,6,7,8,2'-penta-methoxyflavone, a new natural product. ^{13}C NMR data were in accord with this structure.

Methoxyl groups

Table 2. UV data for flavonoids 1-7

Flavonoids	MeOH		NaOMe	AlCl ₃	AlCl ₃ -HCl	NaOAc	NaOAc-H ₃ BO ₃	$\lambda_{\text{max}}(\text{nm})$
	(Band II/Band I)*							
1:5,7-OH	262	346	276	325 sh	276	330 sh	276	325
3,6,8,2',4',5'-OMe	(2.58)		365	365	410 sh	415 sh	365	
2:5,4',5'-OH	266	358	270	397	282	425	270	382
3,6,7,8,2'-OMe	(2.47)			(dec.)		415 sh	270	378
3:5,7,4'-OH	265	352	275	345 sh	277	330 sh	276	325 sh
3,6,8,2',5'-OMe	(2.83)		375		365	365	266	356
4:5,7,4',5'-OH	265	355	275	340 sh	278	325 sh	274	335 sh
3,6,8,2'-OMe	(2.88)		395	(dec.)	410	362 415 sh	375	
5:5,7,4',5'-OH	260	346	268	337 sh	273	320	267	366
3,6,2'-OMe	(2.10)		385	(dec.)	410	380	370	
6:5,7,4',5'-OH	258	300	270	325	271	310 sh	266	365
3,2'-OMe	347 (2.58)		385	(dec.)	451	390	260	367
7:3,5,4'-OH	260	280 sh	267	447	272	385	265	400
6,7,8,3'-OMe	350 sh	380			442	442	430 sh	350 sh 380

* Numbers in parentheses represent ratio of Band II to Band I.

Table 3. Mass spectral data for flavonoids 1-7 [EIMS (probe) 70 eV, m/z (rel. int.)]

Flavonoids	[M] ⁺	[M - 15] ⁺	[M - 31] ⁺	[M - 43] ⁺	[A ₁ + 1] ⁺	[A ₁] ⁺	[A ₁ - 15] ⁺	[A ₁ - 43] ⁺	[B ₂] ⁺	[B ₂ - 28] ⁺
1:5,7-OH	434	419	403	391			197	169	195	167
3,6,8,2',4',5'-OMe	(85)	(100)	(12)	(6)			(4)	(4)	(3)	(3)
2:5,4',5'-OH	420	405	389		227		211	183	167	
3,6,7,8,2'-OMe	(100)	(97)	(21)		(3)		(16)	(12)	(10)	
3:5,7,4'-OH	420	405	389	377	213	212	197	169	181	153
3,6,8,2',5'-OMe	(75)	(100)	(14)	(3)	(4)	(3)	(19)	(13)	(13)	(3)
4:5,7,4',5'-OH	406	391	375	363	213		197	169	167	139
3,6,8,2'-OMe	(98)	(100)	(25)	(6)	(5)		(16)	(12)	(12)	(3)
5:5,7,4',5'-OH	376	361	345	333	183		167	139	167	139
3,6,2'-OMe	(100)	(49)	(45)	(20)	(10)		(8)	(3)		
6:5,7,4',5'-OH	346	331	315	303	153	152			167	139
3,2'-OMe	(97)	(12)	(100)	(12)	(58)	(8)			(9)	(6)
7:3,5,4'-OH	390	375		347				183	151	
6,7,8,3'-OMe	(92)	(100)		(25)				(3)	(10)	

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

The MS of **3** established a flavonoid with five methoxyl and three hydroxyl groups: [M]⁺ at m/z 420 (75%) corresponding to $C_{20}H_{20}O_{10}$ (Table 3). When the compound was viewed on paper under UV light it appeared as a purple fluorescent spot which changed to yellow with ammonia in accord with free 5- and 4'-hydroxyl groups. The remaining hydroxyl group was assigned to the 7 position on the basis of UV spectral data: $\Delta\lambda$ Band II sodium acetate-methanol: +11 nm and the presence of shoulders at 345 nm in sodium methoxide and 325 nm in sodium acetate. The five other oxygenated positions, 3,6,8,2' and 5', must be occupied by methoxyl groups. These assignments were further supported by MS data: an A-ring with two hydroxyl and two methoxyl groups and a B-ring with one hydroxyl and two methoxyl groups was suggested by ions [A₁ - 15]⁺ at m/z 197 (19%), [A₁

- 43]⁺ at m/z 169 (13%), [B₂]⁺ at m/z 181 (13%) and [B₂ - 28]⁺ at m/z 153 (3%). That **3** is a 2'-methoxyflavonol 3-methyl ether, as are **1** and **2**, was confirmed by MS fragments of [M - 31]⁺, [B₂]⁺ and [B₂ - 28]⁺ and the absence of ions for [M - 17]⁺, [B₆]⁺ and [B₆ - 15]⁺. All of the above results as well as the ¹³C NMR data (Table 4) indicated that **3** is the new flavonol 3-methyl ether, 5,7,4'-trihydroxy-3,6,8,2',5'-pentamethoxyflavone.

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

Compound **4** also possesses a 3,5,6,7,8,2',4',5'-oxygenation pattern as noted in the initial discussions. The MS of **4** exhibited a molecular ion at m/z 406 (98%) for $C_{19}H_{18}O_{10}$ in accord with a flavonoid containing four hydroxyl and four methoxyl groups. Colours on paper (purple without NH₃ and yellow with NH₃ under UV

Table 4. ^{13}C NMR chemical shifts for flavonoids 1-5
(90.8 MHz, δ ppm in $\text{DMSO-}d_6$)

Carbons	1:5,7-OH 3,6,8,2',4',5'- OMe	2:5,4',5'-OH 3,6,7,8,2'-OMe	3:5,7,4'-OH 3,6,8,2',5'-OMe	4:5,7,4',5'-OH 3,6,8,2'-OMe	5:5,7,4',5'-OH 3,6,2'-OMe
A- and C-ring					
2	145.06	145.00	145.90	145.73	148.98
3	138.59	138.70	139.44	137.67	138.75
4	178.34	178.68	179.15	176.73	178.35
5	147.91	148.20	148.58	147.92	152.66
6	131.30	135.52	131.92	133.80	131.25
7	156.06	157.50	156.96	160.52	157.32
8	127.66	132.54	128.42	129.54	94.02
9	152.26	151.30	151.22	154.55	156.72
10	103.87	107.19	104.73	101.38	105.21
B-ring					
1'	109.86	108.81	111.42	109.53	109.45
2'	150.59	149.26	151.22	149.02	151.25
3'	98.66	100.96	98.66	99.33	101.15
4'	152.03	152.29	152.04	150.84	152.29
5'	142.38	138.87	140.46	139.02	138.75
6'	114.41	117.07	117.34	117.17	117.18
Methoxyl groups					
3	60.92	61.69	61.45	59.82	60.03
6	60.07	61.47	60.54	59.73	60.03
7	60.92	60.01			
8	59.90	60.60	60.45	59.14	
2'	56.38	56.12	56.27	55.95	56.26
5'	56.38		56.77		

Table 5. Chromatographic data ($R_f \times 100$ and colour*) of flavonoids 1-7
(on cellulose plates)

Flavonoids	15% aq. HOAc	TBA†	UV	UV/ NH_3	NA‡
1:5,7-OH 3,6,8,2',4',5'-OMe	76	94	P	P	
2:5,4',5'-OH 3,6,7,8,2'-OMe	75	93	P	dY	Or
3:5,7,4'-OH 3,6,8,2',5'-OMe	79	92	P	dY	Y
4:5,7,4',5'-OH 3,6,8,2'-OMe	78	94	P	dY	Or
5:5,7,4',5'-OH 3,6,2'-OMe	74	91	P	dY	Or
6:5,7,4',5'-OH 3,2'-OMe	63	93	P	dY	Or
7:3,5,4'-OH 6,7,8,3'-OMe	9	89	dY	Y	Y

*P = Purple, dY = dull yellow, Or = orange.

†TBA = *t*-butyl alcohol-glacial acetic acid-water, 3:1:1.

‡NA = Dissolving 1 g of Naturstoffreagenz A (2-aminoethyl diphenylborate, Carl Roth Co., F.R.G.) in 100 ml of absolute MeOH.

light and orange with NA) indicated free 5-, 4'- and 5'-hydroxyl groups. The UV spectra (Table 2) supported these assignments and further indicated a free 7-hydroxyl group. Comparison of MS, ^1H NMR and UV data indicated that 4 and 3 have the same A-ring and 4 and 2 have the same B-ring. Thus the structure of this new compound (4) is 5,7,4',5'-tetrahydroxy-3,6,8,2'-tetramethoxyflavone.

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

The MS of 5 (Table 3) gave a molecular ion at m/z 376 (100%) in accord with a flavonoid containing four hydroxyl and three methoxyl groups. The ^1H NMR of the TMSi ether of 5 exhibited a one-proton singlet at δ 6.90 (1H, s) and a signal at δ 6.46 (2H, s) (in CCl_4) for two protons. Two of the proton signals (δ 6.90 and 6.46) are

similar to those for brickellin (22) (at δ 6.90 and 6.45 in CCl_4) [9, 10] corresponding to H-6' and H-8. The third aromatic proton signal at δ 6.46 is assigned to C-3' primarily using the same arguments as used for the other 2'-oxygenated flavonol 3-methyl ethers, namely, the high R_f values on cellulose plates in both 15% aq. HOAc (0.74) and TBA (0.91) and the high intensity of Band II to Band I in the MeOH UV spectrum as well as Band I appearing at 346 nm suggested that **5** is a 2',4',5'-oxygenated flavonol 3-methyl ether. Therefore, **5** has the same 3,5,6,7,2',4',5'-oxygenation pattern as brickellin (22). This new natural product appeared purple on paper under UV light and turned yellow with ammonia and orange with NA, indicating the presence of a free 5-hydroxyl group in the A-ring and an *ortho* 4',5'-dihydroxyl system in the B-ring. UV spectral data supported these assignments and suggested a free 7-hydroxyl group on the basis of Band III at 337 sh in NaOMe (Table 2). The structure of **5** as 5,7,4',5'-tetrahydroxy-3,6,2'-trimethoxyflavone was confirmed by ^{13}C NMR data (Table 4).

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

In addition to two one-proton singlets at δ 6.46 (H-3') and 6.91 (H-6'), the ^1H NMR spectrum of TMSi ether of **6** (in CCl_4) (Table 1) also exhibited two A-ring *meta*-coupled proton resonances at δ 6.16 and 6.24 corresponding to H-6 and H-8, thus **6** has a 3,5,7,2',4',5'-oxygenation pattern. The MS of **6** exhibited a molecular ion at m/z 346 (97%) for $\text{C}_{14}\text{H}_{14}\text{O}_8$ in accord with a flavonoid containing two methoxyl and four hydroxyl groups. Other ions were observed for $[\text{M} - 31]^\ddagger$ at m/z 315 (100%), $[\text{A}_1 + 1]^\ddagger$ at m/z 153 (58%), $[\text{B}_2]^\ddagger$ at m/z 167 (9%) and $[\text{B}_2 - 28]^\ddagger$ at m/z 139 (6%), however fragments for $[\text{M} - 17]^\ddagger$, $[\text{B}_6]^\ddagger$ and $[\text{B}_6 - 15]^\ddagger$ were not present. The UV data (Table 2) together with the characteristic R_f values [3, 5] indicated that the two methoxyl groups were located at the 3 and 2' positions. Thus, the four hydroxyl groups can be assigned to the 5,7,3' and 4' positions. The colours on the paper under UV light without and with ammonia or NA (Table 5) supported a 5-hydroxyl group in the A-ring and an *ortho* 4',5'-dihydroxyl system in the B-ring. The UV spectral data (Table 2) confirmed the free 7-hydroxyl group as well as the 5,4'- and 5'-hydroxyl groups. Thus **6** is 5,7,4',5'-tetrahydroxy-3,2'-dimethoxyflavone.

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

The dull yellow colour on paper under UV light suggested that **7** is a 5-hydroxyflavonol and not a 5-hydroxyflavonol 3-methyl ether as were the other six compounds (**1**–**6**). Since the ^1H NMR of **7** (TMSi ether in CCl_4) only showed B-ring signals characteristic for H-5', H-2' and H-6', respectively, at δ 6.89 (1H, *d*, $J = 9$ Hz), 7.73 (1H, *dd*, $J = 9$ Hz and 2.5 Hz) and 7.82 (1H, *d*, $J = 2.5$ Hz) in the aromatic region, **7** must be a flavonol with a 3,5,6,7,8,3',4'-oxygenation pattern. A free 4'-hydroxyl group is indicated by a bathochromic shift of Band I of +67 nm with an increase in intensity in the sodium methoxide spectrum relative to Band I in the methanol spectrum. The MS of **7** exhibited a molecular ion peak at m/z 390 (92%) for $\text{C}_{19}\text{H}_{18}\text{O}_9$ in accord with a flavonoid containing four methoxyl and three hydroxyl groups. With the assignment of the three hydroxyl groups to the 3,5 and 4' positions, the four methoxyl groups must

be at the 6,7,8 and 3' positions. All other spectral data supported the structure of **7** as 3,5,4'-trihydroxy-6,7,8,3'-tetramethoxyflavone.

All known compounds, except the C-glucosylflavone **20**, were identified by UV, ^1H NMR, MS and comparisons with authentic samples. The identity of **20** was determined by UV, ^1H NMR of its TMSi ether, MS of its PM derivative [11] and comparison with an authentic sample.

General comments on 2'-oxygenated flavonol 3-methyl ethers

We previously reported 11 flavonoids with 2',4',5'-oxygenation from *Gutierrezia grandis* and *G. microcephala* [2, 3, 5]. Of the 11, eight are 2'-hydroxyflavonol 3-methyl ethers and three are 2'-hydroxyflavones. In this study, we have characterized six 2'-methoxyflavonol 3-methyl ethers, in addition to two known 2'-hydroxyflavonol 3-methyl ethers [3]. 2'-Methoxyflavonol 3-methyl ethers exhibit the same characteristic UV, ^1H NMR spectral and TLC properties as 2'-hydroxyflavonol 3-methyl ethers. However, the MS of 2'-methoxyflavonol 3-methyl ethers do not yield the characteristic fragments $[\text{M} - 17]^\ddagger$, $[\text{B}_6]^\ddagger$ and $[\text{B}_6 - 15]^\ddagger$ of the 2'-hydroxyflavonol 3-methyl ethers. Instead, they give a characteristic fragment for $[\text{M} - 31]^\ddagger$. In the ^1H NMR spectra of flavonol 3-methyl ethers with 2',4',5'-oxygenated B-rings, the largest benzene-induced shifts ($\Delta_{\text{CDCl}_3-\text{C}_6\text{D}_6} = +0.61$ to +0.52 ppm) for methoxyl proton signals was noted for the 2'-methoxyl group (Tables 1-1 and 1-2). The benzene-induced shifts of 4'- and 5'-methoxyl groups are less than those of the 2'-methoxyl groups and when the 6 and 8 positions are occupied by methoxyl groups as in **2**, the methoxyl signal for the 7-methoxyl group appears in the range 3.48–3.68 ppm in C_6D_6 (Tables 1-1 and 1-2).

EXPERIMENTAL

Plant material. *Gymnosterma glutinosum* was collected on 5 June 1981 from the State of Nuevo Leon, Mexico, on Highway 40 between Monterrey and Saltillo on the road to Microondas Mariposa by Mark Leidig and Meredith Lane. A voucher specimen is on deposit in the Plant Resources Center at the University of Texas at Austin (Lane No. 2584).

Extraction and isolation. Ground, air-dried leaves and stems (1.4 kg) of *Gymnosterma glutinosum* were exhaustively extracted in aq. MeOH, first in 85% concn followed by 50% concn. These two extracts were concd, 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 fractions were evapd to dryness *in vacuo* (yield: 179 g, dry wt) and transferred to a short, large diameter column containing 700 g cellulose powder (Merck) and eluted successively with 25% aq. HOAc and 35% aq. HOAc until the chlorophylls (red colour under the UV light) moved close to the stopcock of column. All eluates were combined and evapd to dryness. The cleaned-up CH_2Cl_2 and EtOAc extracts (68.4 g and 15 g, respectively) were chromatographed over Polyclar AT (GAF Corp.) columns packed initially with toluene–MeOH (19:1) and gradually increased to pure MeOH in 10% increments and finally with Me_2CO – H_2O (1:1). The material in each band, which were monitored on the column with UV light, was collected, dried and separated by PC, using 15% HOAc or 35% HOAc on Whatman 3 MM paper. Final purification of each compound for spectral analysis was by standard procedures [8] using 75% or 100% MeOH over Sephadex LH-20 columns.

Compounds **1–3**, **7–11**, **15** and **18** were isolated from the CH_2Cl_2 fraction, compounds **5**, **6**, **13**, **16**, **17** and **19–21** from the EtOAc and compounds **4**, **12** and **14** from both the CH_2Cl_2 and EtOAc fractions. Although ground leaves and stems were used for isolation of flavonoids, the 2D-PC (15% HOAc and TBA on Whatman 3 MM) of MeOH extract of unbroken leaves and stems revealed that most of the flavonoid aglycones were on the external surface.

Derivatization. Permethylations were achieved using Methylute (Pierce) and trimethylsilylations were done as described in ref. [8].

Acknowledgements—This work was supported by the National Science Foundation (Grand BSR-8402017), the National Institutes of Health (GM-35710) and the Robert A. Welch Foundation (Grant F-130). The authors thank Mark Leidig and Meredith Lane for collecting and identifying the plant material.

REFERENCES

1. Lane, M. A. (1980) Ph.D. Dissertation, University of Texas Texas, Austin, U.S.A.
2. Fang, N., Leidig, M. and Mabry, T. J. (1985) *Phytochemistry* **24**, 2693.
3. Fang, N., Leidig, M., Mabry, T. J. and Iinuma, M. (1985) *Phytochemistry* **24**, 3029.
4. Fang, N., Mabry, T. J. and Le-Van, N. (1986) *Phytochemistry* **25**, 235.
5. Fang, N., Leidig, M. and Mabry, T. J. (1986) *Phytochemistry* **25**, 927.
6. Lenherr, A., Fang, N. and Mabry, T. J. (1986) *J. Nat. Prod.* **49**, 185.
7. Fang, N., Yu, S. and Mabry, T. J. (1987) *J. Nat. Prod.* (in press).
8. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
9. Roberts, M. F., Timmermann, B. N., Mabry, T. J., Brown, R. and Matlin, S. A. (1984) *Phytochemistry* **23**, 163.
10. Iinuma, M., Roberts, M. F., Matlin, S. A., Stacey, V. E., Timmermann, B. N., Mabry, T. J. and Brown, R. (1985) *Phytochemistry* **24**, 1367.
11. Bouillant, M. L., Jean, F. B. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.