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FLAVONOIDS FROM *GUTIERREZIA GRANDIS*

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Key Word Index—*Gutierrezia grandis*; Compositae; Astereae; flavonols; flavonol 3-*O*-methyl ethers; flavone 3'-*O*-glucoside; ^{1}H shift-correlated 2D-NMR.

Abstract- Thirteen flavonoids, including three new compounds, were isolated from *Gutierrezia grandis*. The structures of the new compounds were 3,5,7,3',4'-pentahydroxy-6,8-dimethoxyflavone, 5,7,3'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone and 5,7,3',5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone 3'-*O*-glucoside.

INTRODUCTION

We previously reported [1] the isolation of 21 flavonoids (**1**–**21**) from the dichloromethane extract of a concentrated aqueous methanol extract of *Gutierrezia grandis* S. P. Blake (tribe Astereae, Compositae). The present paper describes the isolation of one additional new flavonoid from this same dichloromethane extract, as well as ten known and two new flavonoids from the ethyl acetate extract of the concentrated aqueous methanol extract. The three new compounds are 3,5,7,3',4'-pentahydroxy-6,8-dimethoxyflavone (**22**), 5,7,3'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone (**23**) and 5,7,3',5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone 3'-*O*-glucoside (**24**). The ten known compounds are chrysoeriol 7-*O*-glucoside (**25**), luteolin 7-*O*-glucoside (**26**), quercetin (**27**), quercetin 7-*O*-glucoside (**28**), quercetin 3-*O*-glucoside (**29**), quercetin 3-*O*-galactoside (**30**), quercetin 3-*O*-methyl ether (**31**), vitexin (**32**), 5,7,3',4',5'-pentahydroxy-3,6-dimethoxyflavone (**33**) and isorhamnetin 3-*O*-galactoside (**34**).

RESULTS AND DISCUSSION

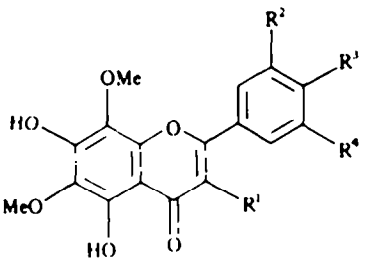
Chromatographic separation of the material from dichloromethane and ethyl acetate extracts of a concentrated aqueous methanol extract of *Gutierrezia grandis* afforded 12 new flavonoids (**1**–**9** and **22**–**24**) and 22 known flavonoids (**10**–**21** and **25**–**34**). We previously reported **1**–**21** [1]; here we present detailed data for the characterization of three new flavonoids, **22**, **23** and **24**, and also report the isolation of ten more known compounds (**25**–**34**).

3,5,7,3',4'-*Pentahydroxy*-6,8-*dimethoxyflavone* (22)

The MS of the new compound **22** exhibited a molecular ion peak at *m/z* 362 (96) in accord with a flavone containing five hydroxyl and two methoxyl groups (Table 1). Since the ^{1}H NMR of **22** (TMSi ether in CCl_4; in the aromatic region) showed only B-ring signals characteristic for H-5', H-2' and H-6' respectively [2] at δ6.84 (1H, *d*, *J* = 8.5 Hz), 7.70 (1H, *d*, *J* = 2.5 Hz) and 7.79 (1H, *dd*, *J* = 2.5 and 8.5 Hz) (Table 3), **22** must be a flavonol with a 3,5,6,7,8,3',4'-oxygenation pattern. The compound appeared as a dull yellow fluorescent spot on paper in UV light with and without ammonia indicating the presence of free 3- and 5-hydroxyl groups. Compound **22** also gave an orange colour with NA (Table 4) indicating a 3',4'-dihydroxyl group in the B-ring. The presence of B-III at 343 nm in the sodium methoxide UV spectrum (Table 2) indicated a 7-hydroxyl group [2]. Thus, the two methoxyl groups must be at the 6 and 8 positions. Therefore, the compound is 3,5,7,3',4'-pentahydroxy-6,8-dimethoxyflavone (**22**).

5,7,3'-*Trihydroxy*-3,6,8,4',5'-*pentamethoxyflavone* (23)

The MS of **23** established a flavonoid with five methoxyl and three hydroxyl groups: $[M]^+$ at *m/z* 420 (97) corresponding to $C_{20}H_{20}O_{10}$ (Table 1). Moreover, the MS of the PM derivative of **23** was identical to the MS of



	R¹	R²	R³	R⁴
22	OH	OH	OH	H
23	OMe	OH	OMe	OMe
24	OMe	OGlc	OMe	OH
35 (20)	OMe	OH	OMe	OH

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

	Flavonoids	$[M]^+$	$[M-15]^+$	$[A_1-15]^+$	$[A_1-43]^+$	$[B_2]^+$	$[B_2-28]^+$
22	3,5,7,3',4'-OH	362	347	197	169	137	109
	6,8-OMe	(96)	(100)	(3)	(4)	(12)	(6)
35*	5,7,3',5'-OH	406	391	197	169	167	139
	3,6,8,4'-OMe	(85)	(100)	(8)	(7)	(7)	(3)
PM of 35†	5,7,3',5'	462	447	225	197	195	167
	3,6,8,4'-OMe	(59)	(100)	(6)	(14)	(9)	(3)
23	5,7,3'-OH	420	405	197	169	181	153
	3,6,8,4',5'-OMe	(97)	(100)	(20)	(16)	(21)	(5)

*35 and 20 gave the same MS.
†35, 20 and 23 gave the same MS of PM derivative.

Table 2. UV data of flavonoids

		λ_{max} (nm)					
	Flavonoids	MeOH	NaOMe	$AlCl_3$	$AlCl_3+HCl$	NaOAc	$NaOAc+H_3BO_3$
22	3,5,7,3',4'-OH	260 275 sh	278 343	278 340 sh	270 308 sh	277 330	266 393
	6,8-OMe	345 sh 376	421 (dec)	465	374 438	403 (dec)	
23	5,7,3'-OH	279 335	281 313	290 312 sh	291 310 sh	282 308 sh	281 333
	3,6,8,4',5'-OMe		385	364	356	380	
24	5,7,3',5'-OH	279 330	279 305 sh	290 310 sh	292 310 sh	282 310 sh	281 330
	3,6,8,4'-OMe		382	360	351	376	
	3'-*O*-glucoside						
35*	5,7,3',5'-OH	279 334	280 312	290 310 sh	291 310 sh	282 310 sh	280 330
	3,6,8,4'-OMe		384	360	354	378	

*35 and 20 gave the same UV.

the PM derivative of 5,7,3',5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone (**20**) [1] (Table 1); therefore, the oxygenation pattern of **23** was established. The new flavonoid appeared purple with and without ammonia when viewed as a spot on paper over UV light indicating that two of the methoxyl groups are at the 3- and 4'-positions and that a hydroxyl group is at C-5. A 7-hydroxyl group was supported by the presence of B-III at 313 nm in sodium methoxide. The ^1H NMR in carbon tetrachloride of the TMSi ether of **23** indicated an unsymmetrical B-ring [3]: two doublets (J = 2.5 Hz) for H-2' and H-6' at δ 7.32 and 7.41, respectively, indicating a hydroxyl at 3' and a methoxyl at 5'. This assignment was further supported by the MS which exhibited B-ring fragments for $[B_2]^+$ at *m/z* 181 (21) and $[B_2-28]^+$ at *m/z* 153 (5). The remaining two methoxyl groups must, therefore, be at the 6 and 8 positions. These spectral findings established **23** to be 5,7,3'-trihydroxy-3,6,8,4',5'-pentamethoxyflavone.

5,7,3',5'-Tetrahydroxy-3,6,8,4'-tetramethoxyflavone 3'-O-glucoside (**24**)

Hydrolysis of **24** with 0.1 N trifluoroacetic acid afforded an aglycone (**35**) and glucose. FAB-MS of the glucoside recorded with a VG 7070E instrument using glycerol as a matrix established one glucose moiety, i.e. a strong pseudo-molecular ion was observed at 569(FAB^+) and 567 (FAB^-), suggesting a molecular weight of 568 and a $C_{25}H_{28}O_{15}$ formula. The MS of the aglycone exhibited a molecular ion peak at *m/z* 406 (85) for $C_{19}H_{18}O_{10}$ in accord with a flavone containing four hydroxyl and four methoxyl groups (Table 1). The $[M-15]$ peak appeared as the base peak in accord with the presence of either a 6- or 8-methoxyl group [4]. Fragments $[A_1-15]^+$, $[A_1-43]^+$ and $[B_2]^+$ indicated that the A-ring contained two hydroxyl and two methoxyl groups with two hydroxyl and one methoxyl group in the B-ring. Therefore, the fourth methoxyl must be at position 3. Since the aglycone appeared as a purple fluorescent spot on paper in UV light with and without ammonia 5-hydroxyl and 4'-methoxyl groups must be present. The presence of B-III at 312 nm in sodium methoxide suggested a 7-hydroxyl group [5]. Finally, the aglycone **35** was identified by direct comparison of UV, MS, MS of PM, R_f values and colours on paper under UV light as 5,7,3',5'-tetrahydroxy-3,6,8,4'-tetramethoxyflavone (**20**). The only question that remained was to assign the glucose moiety to either the 5, 7, or 3' position (the 5' being equivalent to the 3'). Since the glucoside **24** appeared as a purple fluorescent spot on paper under UV light it must contain a 5-hydroxyl group and since it exhibited B-III at 305 sh nm in the sodium methoxide UV spectrum, it should also contain a 7-hydroxyl, thus the glucose was most likely at the 3'-position. This was confirmed by ^1H NMR in methanol-d_4 of the underivatized **24** and the PM derivatized **24** both of which indicated an unsymmetrical B-ring with two doublets (J = 2.5 Hz) for

Table 3a. ^{1}H NMR data of flavonoids **22**, **23**, **24** and **29** as TMSi ethers (90 MHz, δ-scale in ppm, TMS as internal standard)

	Flavonoids (as TMSi ethers)	–OMe	CCl_4						CCl_4						C_6D_6					
			2′	5′	6′	1″	2″–7″	3	6	8	4′	5′	3	6	8	4′	5′
22	3,5,7,3′,4′-OH		7.70	6.84	7.79				3.74	3.91				3.64	3.74		
	6,8-OMe		(*d*)	(*d*)	(*dd*)				(*s*)	(*s*)				(*s*)	(*s*)		
23	5,7,3′-OH		7.32		7.41			3.90	3.73	3.93	3.83	3.87	3.86	3.71	3.82	3.51	3.66
	3,6,8,4′,5′-OMe		(*d*)		(*d*)			(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)	(*s*)
24	5,7,3′,5′-OH		7.54		7.38	5.01	3.10–3.70	3.87	3.73	3.90	3.84		3.87	3.78	3.89	3.64	
	3,6,8,4′-OMe		(*d*)		(*d*)	(*d*)	(*m*)	(*s*)	(*s*)	(*s*)	(*s*)		(*s*)	(*s*)	(*s*)	(*s*)	
	3′-*O*-glucoside																
29	5,7,3′,5′-OH		7.34		7.34			3.86	3.72	3.90	3.82		3.77	3.77	3.85	3.63	
	3,6,8,4′-OMe		(*s*)		(*s*)			(*s*)	(*s*)	(*s*)	(*s*)		(*s*)	(*s*)	(*s*)	(*s*)	

Table 3b. ^{1}H NMR data of the B-ring of **24** and PM of **24** in methanol-d_4 (90 MHz, δ-scale in ppm, TMS as internal standard)

		H-2′	H-6′
24	5,7,3′,5′-OH 3,6,8,4′-OMe 3′-*O*-glucoside	7.60 (*d*)	7.44 (*d*)
PM of **24**		7.66 (*d*)	7.74 (*d*)

H-6′ and H-2′ at δ7.44 and 7.60 and for H-2′ and H-6′ at δ7.66 and 7.74, respectively. Final proof was provided by the 2D-COSY experiment (Bruker 360 MHz) by using a delay time of 0.5 sec (D2 = 0.5) to emphasize long range coupling. An observed long range coupling between the anomeric proton of the sugar and the lower field signal for the B-ring H-2′ established the structure as 5,7,3′,5′-tetrahydroxy-3,6,8,4′-tetramethoxyflavone 3′-*O*-glucoside.

Quercetin (**27**), quercetin 3-*O*-methyl ether (**31**) and 5,7,3′,4′,5′-pentahydroxy-3,6-dimethoxyflavone (**33**) were identified by UV, ^{1}H NMR of their TMSi ethers, colour on paper under UV light and by MS. Chrysoeriol 7-*O*-glucoside (**25**), luteolin 7-*O*-glucoside (**26**) and quercetin 7-*O*-glucoside (**28**), 3-*O*-glucoside (**29**) and 3-*O*-galactoside (**30**) and isorhamnetin 3-*O*-galactoside (**34**) were identified by colour on paper under UV light, UV, ^{1}H NMR of their TMSi ethers, acid hydrolysis and, for the aglycones, colour on paper under UV light, UV and MS. The identity of vitexin (**32**) was determined by colour on paper under UV light, UV, ^{1}H NMR of its TMSi ethers and MS of its PM derivative [6].

EXPERIMENTAL

Plant material. Gutierrezia grandis S. P. Blake was collected 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. Voucher specimens are on deposit in the University of Texas and Lundell Herbarium (Lane No. 2589).

Isolation of flavonoids. Air-dried leaf material of *G. grandis* (1 kg) was exhaustively extracted in aq. MeOH, first in 85% concn followed by 50% concn. These two extracts were each evaporated *in vacuo* to a H_2O soln and the two concentrates were combined. The aq. soln was then partitioned according to standard procedures [2], and each partition was monitored for flavonoids by 2D-PC. The hexane and H_2O fractions showed no evidence of flavonoids, whereas the CH_2Cl_2 and EtOAc fractions showed several purple spots when their paper chromatograms were viewed udner UV light. When these two fractions were evaporated to dryness *in vacuo*, the CH_2Cl_2 portion yielded 61.4 g of syrup and the EtOAc fraction afforded 26.3 g.

All compounds reported here came from the EtOAc fraction except **23** which came from the CH_2Cl_2 fraction. The EtOAc syrup was chromatographed on a polyclar (Polyclar AT, GAF Corp) column packed in H_2O–MeOH–MeCOEt–Me_2CO (13:3:3:1). Elution of the column was initiated with 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 column chromato-

Table 4. Chromatographic data of flavonoids ($R_f \times 100$ and colours). (Whatman No. 3M)

	Flavonoids	15% HOAc	TBA	UV	UV/NH_3	UV/NA
22	3,5,7,3',4'-OH 6,8-OMe	11	47	dy	dy	or
23	5,7,3'-OH 3,6,8,4',5'-OMe	44	82	p	p	p
24	5,7,3',5'-OH 3,6,8,4'-OMe 3'-*O*-glucoside	65	54	p	p	p
35 (20)	5,7,3',5'-OH 3,6,8,4'-OMe	43	74	p	p	p

dy = dull yellow, or = orange, p = purple.

graphy, compounds were further separated by PC using 15% HOAc on Whatman No. 3 M paper. Sephadex LH-20 (Pharmacia) was used for the preparation of a pure compound for spectral analysis.

Hydrolysis condition. A dry sample was dissolved in 0.1 N TFA; the flask was covered with aluminum foil and heated on a steam bath for 50 minutes.

Sugar analysis. Sugar were recovered from the hydrolysed flavonoids after repeated evaporations *in vacuo* of the hydrolysis soln. The residue was taken up in H_2O, and the aqueous solution was extracted with EtOAc. Sugars present in the H_2O fraction were identified by TLC on cellulose against standard markers in pyridine-EtOAc-HOAc-H_2O (36:36:7:21). Sugars were detected by spraying plates with aniline phthalate reagent (E. Merck).

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. This was done as described in ref. [2].

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