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CHEMISTRY OF TOXIC RANGE PLANTS. HIGHLY OXYGENATED FLAVONOL METHYL ETHERS FROM *GUTIERREZIA MICROCEPHALA*

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Key Word Index—*Gutierrezia microcephala*; Broomweed; Compositae; Astereae; flavonol; oxygenated kaempferol; quercetin and myricetin derivatives; ^{13}C NMR.

Abstract—The perennial American desert shrub, *Gutierrezia microcephala*, contains 20 flavonol methyl ethers displaying nine different oxygenation patterns. These include 11 new flavonols: 5,7-dihydroxy-3,6,8,3′,4′,5′-hexamethoxyflavone, 5,7,4′-trihydroxy-3,6,8,3′,5′-pentamethoxyflavone, 5,7,3′-trihydroxy-3,6,8,4′,5′-pentamethoxyflavone, 5,7,2′,4′-tetrahydroxy-3,6,8,5′-tetramethoxyflavone, 5,7,3′,4′-tetrahydroxy-3,6,8-trimethoxyflavone, 5,7,8,3′,4′-pentahydroxy-3,6-dimethoxyflavone, 3,5,7,3′,4′-pentahydroxy-6,8-dimethoxyflavone, 5,7,4′-trihydroxy-3,6,8-trimethoxyflavone, 5,7,8,4′-tetrahydroxy-3,3′-dimethoxyflavone, 5,7,8,3′,4′-pentahydroxy-3-methoxyflavone and 5,7,8,4′-tetrahydroxy-3-methoxyflavone. In addition, the following known flavonols were isolated: 5,7-dihydroxy-3,8,3′,4′,5′-pentamethoxyflavone, 5,7,4′-trihydroxy-3,8-dimethoxyflavone, 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone, 5,7,3′,4′-tetrahydroxy-3,8-dimethoxyflavone, 5,7,4′-trihydroxy-3,6,3′-trimethoxyflavone, 5,7,3′,4′-tetrahydroxy-3-methoxyflavone, 5,4′-dihydroxy-3,6,7,8,3′-pentamethoxyflavone, 5,7,4′-trihydroxy-3,6,8,3′-tetramethoxyflavone and 3,5,7,4′-tetrahydroxy-6,8,3′-trimethoxyflavone.

INTRODUCTION

The names 'broomweed', 'perennial snakeweed', 'broom snakeweed', 'slinkweed' and 'turpentine weed' have all been applied to species of the genus *Gutierrezia* (Compositae, Astereae), as well as to the related genus *Xanthocephalum* in which *Gutierrezia* was formerly included. Broomweeds grow commonly in the arid regions of the American south-west from Texas to California but range as far as Idaho to the north and Mexico to the south [1]. They have been implicated in serious losses of range cattle due to abortion characterized by placental retention, haemorrhage and weak offspring which often do not survive [2–4]. Based on animal experiments, a partially identified saponin has been suggested as the abortifacient [5, 6]. As part of a continuing study of the chemical constituents of toxic range plants we have recently examined *Gutierrezia microcephala* A. Gray and report the isolation and characterization of 20 flavonol methyl ethers. In total they possess nine discrete oxygenation patterns, a rather extraordinary display of variety for a single plant. Eleven of these substances have not previously been reported.

RESULTS AND DISCUSSION

Oxygenation patterns of all of the flavonols were determined from their ^1H NMR spectra (Table 1). Spectra of 11 of the substances (**1–9, 11** and **19**) had no A-ring aromatic proton resonances indicating oxygenation at C-5, C-6, C-7 and C-8. Only one compound (**20**), the common flavonol, 3-methoxyquercetin, exhibited two A-ring *meta* coupled proton resonances corresponding to H-6 and H-8. Spectra of each of the remaining eight flavonols had a single aromatic A-ring proton absorption; in one of these (**14**) the signal occurred $\Delta\delta 0.3$ downfield from the like signal in the others indicating that the proton was attached to C-8, whereas in the remainder (**10, 12, 13** and **15–18**) the proton was at C-6.

B-ring signals were, likewise, diagnostic for B-ring substitution patterns. A two-proton singlet resonance in four of the spectra (**7–10**) arose from a pair of degenerate protons (H-2′, H-6′) indicating oxygenation at C-3′, C-4′ and C-5′. Spectra of three of the flavonols (**11–13**) each had a pair of two-proton, *ortho* coupled doublets arising from two pairs of degenerate protons (H-2, H-6) and (H-3, H-5), typical of *para*-substituted benzene rings. Most often observed (**1–6, 14–18, 20**), however, was a pattern of three one-proton signals, the multiplicity of which showed one proton coupled to the remaining two which were, in turn, not coupled to each other. The size of the coupling constants (2 and 8.5 Hz) is characteristic of *meta* and *ortho* coupling as found in 3′,4′-oxygenated flavonoids. The spectrum of one compound (**19**) contained two one proton singlets in the aromatic region indicating a pair of protons in a *para* relationship. The alternative arrangement, one proton each on otherwise fully substituted A- and B-rings, was eliminated after considering other spectral evidence.

Besides substitution patterns, ^1H NMR spectra provided the number of methoxyl groups in each flavonol and confirmed the absence of any other hydrogen-bearing substitutents (such as prenyl, *C*-methyl, etc.). The strongly chelated OH-5 present in all of the flavonols gave rise to a low-field singlet (δ12.2–12.7) in all of the spectra except that of **19** whose OH-5 was demonstrated by other spectroscopic means.

Ultra-violet spectroscopy provided a certain amount of

Table 1. ^{1}H NMR data for *Gutierrezia microcephala* flavonol methyl ethers*

Flavonol	H-6	H-8	H-2′	H-3′	H-5′	H-6′	OH-5	OMe
(1) 5,4′(OH)/3,6,7,8,3′(OMe)	—	—	7.84 *d*	—	7.05 *d*	7.80 *dd*	12.56 *s*	3.89, 3.92, 3.97, 3.98, 4.08
(2) 5,7,4′(OH)/3,6,8,3′(OMe)	—	—	7.82 *d*	—	7.05 *d*	7.79 *dd*	12.68 *s*	3.88, 3.90, 3.96, 3.96
(3) 5,7,3′,4′(OH)/3,6,8(OMe)	—	—	7.78 *d*	—	7.02 *d*	7.67 *dd*	12.70 *s*	3.88, 3.88, 3.94
(4) 3,5,7,4′(OH)/6,8,3′(OMe)	—	—	7.93 *d*	—	7.05 *d*	7.91 *dd*	12.04 *s*	3.90, 3.96, 4.00
(5)† 3,5,7,3′,4′(OH)/6,8(OMe)	—	—	7.70 *d*†	—	6.90 *d*†	7.58 *dd*†	12.31 *s*†	3.78,† 3.86†
(6) 5,7,8,3′,4′(OH)/3,6(OMe)	—	—	7.73 *d*	—	6.96 *d*	7.67 *dd*	12.43 *s*	3.83, 3.85
(7) 5,7(OH)/3,6,8,3′,4′,5′(OMe)	—	—	7.54 *s*	—	—	7.54 *s*	12.57 *s*	3.85, 3.89, 3.94, 3.94, 3.95, 3.99
(8) 5,7,4′(OH)/3,6,8,3′,5′(OMe)	—	—	7.57 *s*	—	—	7.57 *s*	12.70 *s*	3.87, 3.90, 3.92, 3.92, 3.96
(9) 5,7,3′(OH)/3,6,8,4′,5′(OMe)	—	—	7.42 *s*	—	—	7.42 *s*	12.60 *s*	3.87, 3.87, 3.90, 3.93, 3.94
(10) 5,7(OH)/3,8,3′,4′,5′(OMe)	6.32 *s*	—	7.54 *s*	—	—	7.54 *s*	12.39 *s*	3.85, 3.94, 3.94, 3.95, 3.95
(11) 5,7,4′(OH)/3,6,8(OMe)	—	—	8.10 *d*	7.06 *d*	7.06 *d*	8.10 *d*	12.70 *s*	3.88, 3.88, 3.96
(12) 5,7,4′(OH)/3,8(OMe)	6.29 *s*	—	8.09 *d*	7.04 *d*	7.04 *d*	8.09 *d*	12.50 *s*	3.87, 3.91
(13) 5,7,8,4′(OH)/3(OMe)	6.32 *s*	—	8.11 *d*	7.02 *d*	7.02 *d*	8.11 *d*	12.26 *s*	3.97
(14) 5,7,4′(OH)/3,6,3′(OMe)	—	6.62 *s*	7.80 *d*	—	7.02 *d*	7.71 *dd*	12.98 *s*	3.88, 3.89, 3.95
(15) 5,7,8,3′,4′(OH)/3(OMe)	6.31 *s*	—	7.75 *d*	—	6.99 *d*	7.68 *dd*	12.26 *s*	3.84
(16) 5,7,3′,4′(OH)/3,8(OMe)	6.27 *s*†	—	7.59 *d*†	—	6.92 *d*†	7.50 *dd*†	12.40 *s*†	3.78†, 3.81†
(17) 5,7,8,4′(OH)/3,3′(OMe)	6.30 *s*	—	7.82 *d*	—	6.98 *d*	7.75 *dd*	12.24 *s*	3.87, 3.93
(18) 5,7,4′(OH)/3,8,3′(OMe)	6.31 *s*	—	7.83 *d*	—	7.05 *d*	7.78 *dd*	12.48 *s*	3.91, 3.95, 3.97
(19) 5,7,2′,4′(OH)/3,6,8,5′(OMe)	—	—	—	6.56 *s*	—	7.18 *s*	—	3.79, 3.80, 3.84
(20) 5,7,3′,4′(OH)/3(OMe)	6.25 *d*	6.48 *d*	7.72 *d*	—	7.00 *d*	7.59 *dd*	12.81 *s*	3.87

*Spectra were obtained in acetone-d_6 solution unless otherwise indicated. Chemical shift values are reported in δ-values downfield from TMS as int. standard. Coupling constants for multiple signals where applicable, are: $J_{6,8} = 2$ Hz; $J_{2',3'} = 8.5$ Hz; $J_{2',6'} = 2$ Hz; $J_{5',6'} = 8.5$ Hz.

†Spectrum obtained in dimethylsulphoxide-d_6.

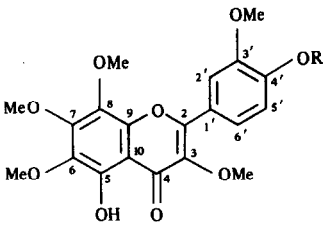
useful information. When the spectra of **3**, **5**, **6**, **13**, **15–17** and **20** were determined in methanol containing boric acid and sodium acetate, bathochromic shifts of 14–30 nm for band I were observed indicating the presence of *ortho* dihydroxy groups in these compounds [7]. This was confirmed by addition of hydrochloric acid to methanolic aluminum chloride solutions causing hypsochromic shifts of 23–44 nm of band I in these flavonols only [7]. Moreover, thin layer chromatograms sprayed with ammoniacal silver nitrate rapidly produced brown to black spots for these flavonols alone. Addition of sodium methoxide to methanolic solutions caused bathochromic shifts in band I of 38–70 nm with an increase in intensity for all of the flavonols, except **7**, **9** and **10**, indicating the presence of OH-4′ groups in all but these three [7]. The alkali spectra* of several of the flavonols changed rapidly after a few minutes, or less, indicating that hydroxyls were present at C-3 and C-4′ (**4**, **5**), *ortho* to one another in the A-ring (**6**, **13**, **15**, **17**), or that a pyrogallol type B-ring was present [7]. Moreover, the sodium methoxide spectra of all the compounds except **1**, exhibited a peak or shoulder between 315 and 340 nm suggesting an OH-7 [7]. In the presence of aluminum chloride and hydrochloric acid, band I shifted by 40–90 nm to a higher wavelength in the spectra of all of the flavonols, a characteristic of C-5 hydroxylation.

The mass spectra of all of the flavonols provided intense molecular ions, accurate mass measurement of which provided elemental formulae confirming numbers of oxygens and methyl groups. The spectra of all of the C-8 methoxylated flavonols (**1–5**, **7–12**, **16**, **18**, **19**) had $[M-15]^+$ as base peak, except for **19**. (Goudard *et al.* [8] have proposed $[M]^+$–$[M-15]^+$ ratios as a method to distinguish OMe-6 from OMe-8 flavonoids.) Furthermore, we have observed that spectra of those compounds lacking A-ring methoxylation but with a methoxyl in the B-ring (**17**) or lacking A- and B-ring methoxylation (**13**, **15**, **20**) altogether, had $[M-15]^+$ peaks of low, or very low (< 5%), intensity, respectively, indicating that loss of a methyl from the heterocyclic C-ring is an unfavourable fragmentation pathway.

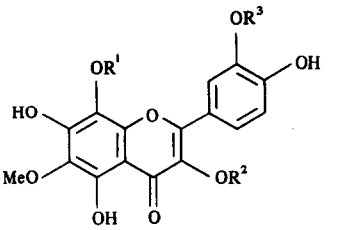
Loss of a single hydrogen from the molecular ion to produce a peak of > 20% intensity was observed in the spectra of those flavonols with OH-8 groups (**6**, **13**, **15**, **17**) or H-8 (**14**, **20**) only. Although flavonoids bearing either hydroxyl or methoxyl groups at C-2′ or methoxyl at C-5 are reported [9] to produce substantial $[M-17]^+$ (loss of hydroxyl) ions in their mass spectra, **19** has such an ion of only 6% intensity. Moreover, the spectrum exhibiting the greatest $[M-17]^+$ peak is that of **15**, which has neither substitution at C-2′ nor methoxylation at C-5.

A number of other ions formed by loss from the molecular ion of 29 (HCO), 30, 31 (OMe), 33 (Me + H_2O), 43 (MeCO), 44 (HCO + Me), 45 (CO + OH), 58 (MeCO + Me), 61 (MeCO + H_2O) and 73 were observed in many of the spectra, but little relationship between their relative abundances and the structure of the flavonoids can be made with the possible exception of the $[M-58]^+$ ion which was 20% for the two flavonols (**4**, **5**) having free OH-3 groups; the C-3 methyl ethers gave $[M-58]^+$ ions of < 11%. Ions of 4–10% intensity were observed for 14 of the flavonols at $[M]^+/2$ indicating doubly charged

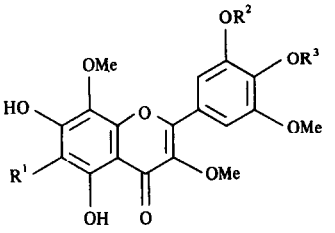
*Spectra were recorded within 4 sec after sodium methoxide addition and at various time intervals thereafter. This was accomplished by use of a diode array spectrophotometer capable of recording an entire spectrum in 1 sec.



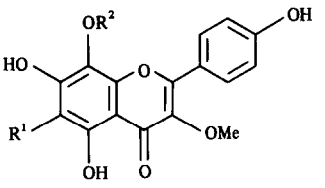
	R
1	H
21	Me



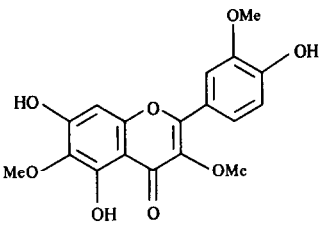
	R^1	R^2	R^3
2	Me	Me	Me
3	Me	Me	H
4	Me	H	Me
5	Me	H	H
6	H	Me	H



	R^1	R^2	R^3
7	OMe	Me	Me
8	OMe	Me	H
9	OMe	H	Me
10	H	Me	Me



	R^1	R^2
11	OMe	Me
12	H	Me
13	H	H



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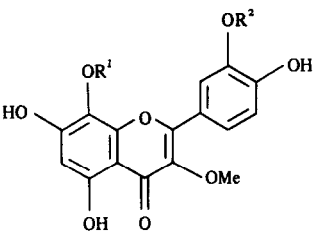
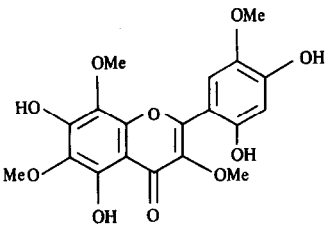
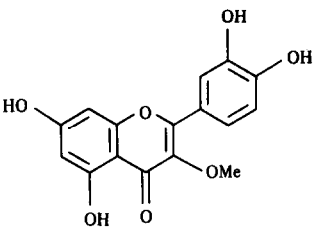


	R^1	R^2
15	H	H
16	Me	H
17	H	Me
18	Me	Me



19



20

molecular ions; however, there appears to be no correlation between the presence or absence of these ions and structures of the compounds.

Ions corresponding to $[B_2]^+$ (B-ring + CO) were observed in the mass spectra of all 20 flavonols. Moreover, other ions characteristic of retro-Diels–Alder cleavage of the C-ring [9], including $[A_1 - Me]^+$, $[A_1 - MeCO]^+$, $[B_1 - CH_2]^+$ and $[B_1 - MeCO]^+$, were observed for certain of the substances; these were usually of low intensity, however, reducing their value in structural elucidation. It was noted, nonetheless, that moderately intense ions corresponding to $[A_1]^+$ were observed for

13, **15** and **17** only and that the spectra of these three lacked $[A_1 - Me]^+$ and $[A_1 - MeCO]^+$ ions. The spectrum of **5** alone lacked any A-ring fragment ions.

Carbon NMR

Although the information gleaned from ^1H NMR, UV and mass spectra suggested the assigned structures and, in some cases, ruled out alternatives, more conclusive evidence was provided by ^{13}C NMR. The ^{13}C spectral assignments of all 20 flavonols and **21**, the trimethyl ether of **3**, are shown in Table 2.

Methoxyl resonances

It is well-known [10–14] that resonances of aromatic methoxyl groups attached to di-*ortho* substituted carbons occur considerably downfield (at *ca* δ60) from signals of those attached to carbons bearing one or no *ortho* substituents (*ca* δ55) thus providing a useful diagnostic tool for structural analysis of natural products. Methoxyl groups at the C-3 position of the quasi-aromatic heterocyclic C-ring of the flavonoids are included with the other di-*ortho* substituted methoxyls. Coupled with the knowledge of the oxygenation patterns obtained from the ^1H NMR spectra, the ^{13}C chemical shifts of and the number of methoxyl groups provided information allowing elimination of many structural isomers from consideration and in certain instances greatly simplified structural elucidation. In the case of **20** (3-*O*-methylquercetin), the single methoxyl resonance appears at δ59.7, consistent only with methoxylation at a carbon flanked by two '*ortho*' substituents, a condition met by attachment at only C-3. In a similar way, the structures of **12** and **16** (or their isomers bearing OMe-6 rather than OMe-8) are indicated by the presence of two di-*ortho*-substituted methoxyl resonances which must be assigned to C-3 and C-8 (or C-6). As a further example, the oxygenation pattern of flavonols **7**–**9** permits methoxylation at only two positions (C-3′, C-5′) *not* flanked by di-*ortho* substituents. Hence, the appearance of two methoxyl signals at δ55.9 (**7**) and 56.0 (**8**) and a single resonance at δ55.7 (**9**) allows assignment of methoxyl groups to C-3′ and C-5′ in both **7** and **8** and a single methoxyl to C-3′ (or C-5′) in **9**.

The data in Table 2 suggest that small, but measurable differences in chemical shift occur between resonances of OMe-6 and OMe-8, the former resonating $\Delta\delta$0.8–1.0 upfield from the latter. This observation was useful in the assignment of the single methoxyl group to C-6 in the A-ring of **6**.

C-ring resonances

The distinction between flavones and flavonols is easily made by the effect oxygenation at C-3 has on ^{13}C NMR resonances of the heterocyclic C-ring carbons, C-2, C-3 and C-4 [15]. Besides the large ($> \Delta\delta$30) downfield shift of the C-3 signal on oxygenation, the C-2 resonance shifts upfield by *ca* $\Delta\delta$17. The frequency of the carbonyl resonance is influenced by hydrogen bonding interactions with either OH-3 or OH-5 groups, the former causing an upfield shift of *ca* $\Delta\delta$5, the latter a downfield shift of similar magnitude. Hence, flavones lacking C-5 hydroxylation and C-5-hydroxyflavonols have carbonyl signals at the same chemical shift, the effects of each hydroxyl group nullifying the other. Methylation of an OH-5 eliminates its effect on C-4, so that flavonols with either H-5 or OMe-5 have nearly identical carbonyl resonances; methylation of the OH-3 group, however, only partially reduces its effect on the C-4 resonance, as can be seen when **4** and **5** (OH-3) are compared with **2** and **3** (OMe-3).

Although the carbonyl resonance frequency can be of important diagnostic value provided that the nature of the substituent at C-5 is known by other means, the distinction between flavonols and their 3-methyl ethers can be achieved by ^{13}C NMR if the chemical shifts of the C-2 and C-3 signals are considered. Contrasting **4** and **5** with **2** and **3** clearly shows that methylation of OH-3 causes downfield shifts of $\Delta\delta$5 and 2 to the C-2 and C-3 signals, respectively. Further small shifts caused by conversion of the hydroxyl at C-3 to a methoxyl are discernible in the signals of C-10 ($+\Delta\delta$1) and the two B-ring carbons, C-1′ ($-\Delta\delta$1) and C-4′ ($+\Delta\delta$1).

B-ring resonances

The assignment of both A- and B-ring signals was simplified greatly by: (1) use of the fact that substituent effects on aromatic nuclei are nil for carbons *meta* to the substitution site [16]; (2) separation of oxygenated from non-oxygenated carbon signals based on their characteristic (and non-overlapping) chemical shift ranges; and (3) unambiguous separation of CH signals from quaternary carbon signals by use of a pulse sequence which displays quaternary (and methylene) resonances 180° out of phase with those of methyl and methine carbons [17]. The multiplicity separation was especially useful in differentiating C-1′ from C-6′ signals, whose chemical shifts are close in flavonols with 3′,4′-dioxygenation. This is of considerable importance when questions of hydroxylation vs methoxylation on carbons *para* to C-1′ and C-6′ arise.

Mono-oxygenated B-rings. The B-ring signals of **11**–**13** were readily assigned by consideration of symmetry and standard chemical shift effects of a single oxygen on an aromatic ring. The finding of two pairs of degenerate methine carbon signals parallels the ^1H NMR data requiring the oxygen to be placed at C-4′. The lower field set of methine signals (δ130) occurs at nearly the same chemical shift as that of benzene and the absence of a sizeable oxygen-caused chemical shift indicates that those carbons are *meta* to the site of oxygenation [13], and, hence, C-2′ and C-6′. The other pair of signals (C-3′ and C-5′) exhibits typical *ortho* shielding causing the shift upfield (compared to benzene) to δ115.6. The values are also in accord with lit. [18–20] values for flavonoids similarly substituted in the B-ring.

Trioxygenated B-rings. The presence of symmetry (or lack thereof) was useful in assigning structures to the flavonols with trioxygenated B-rings (**7**–**10** and **19**). A signal corresponding to a degenerate pair of methine carbons appeared in the spectra of **7**, **8** and **10** indicating that these carbons were symmetrically disposed about the C-1′–C-4′ axis. The biogenetically unlikely arrangement with oxygens at C-2′, C-4′ and C-6′ can be ruled out by the observed chemical shift of the methine signals (δ105.7) which is considerably downfield from the position of methine resonances of carbons with two *ortho* (but no *para*) oxygens [13]. Comparison of the spectra of **7** and **10** with that of **8** shows that, although symmetry remains the same, four of the B-ring carbons of **8** resonate upfield from those in **7** and **10**. Although the symmetry require-

Table 2. ^{13}C NMR data for *Gutierrezia microcephala* flavonol methyl ethers*

Flavonol	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	OMe resonances: OMe-3	OMe-6	OMe-8	OMe-3′	OMe-5′	Other OMe
(1) 5,4′(OH)/3,6,7,8,3′(OMe)	120.6	111.7	147.5	150.1	115.8	122.3	155.9	137.7	178.5	148.1	135.4	152.3	132.4	144.3	106.7	59.6	60.5	61.4	55.5	—	61.8 (C-7)
(2) 5,7,4′(OH)/3,6,8,3′(OMe)	120.9	111.6	147.4	149.8	115.8	122.1	155.2	137.4	178.3	147.9	131.3	150.8	127.7	144.5	103.4	59.6	60.1	61.1	55.5	—	—
(3) 5,7,3′,4′(OH)/3,6,8(OMe)	120.9	115.2	145.3	148.8	115.8	120.5	155.4	137.3	178.3	147.9	131.3	150.7	127.7	144.5	103.4	59.6	60.1	61.2	—	—	—
(4) 3,5,7,4′(OH)/6,8,3′(OMe)	122.1	111.2	147.4	148.8	115.6	121.6	150.5	135.5	176.2	146.6	130.9	147.2	127.6	144.3	102.3	—	60.2	61.0	55.5	—	—
(5) 3,5,7,3′,4′(OH)/6,8(OMe)	122.1	114.9	145.1	147.8	115.7	120.0	150.4	135.5	176.2	146.9	130.9	147.2	127.7	144.4	102.3	—	60.2	61.2	—	—	—
(6) 5,7,8,3′,4′(OH)/3,6(OMe)	121.0	115.5	145.0	148.5	115.5	120.7	155.4	137.0	178.3	144.5	131.1	146.7	125.2	140.7	103.3	59.5	60.0	—	—	—	—
(7) 5,7(OH)/3,6,8,3′,4′,5′(OMe)	125.2	105.6	152.7	139.9	152.7	105.6	154.4	138.3	178.4	147.9	131.4	151.0	127.7	144.6	103.6	59.9	60.2	61.1	55.9	55.9	60.2 (C-4′)
(8) 5,7,4′(OH)/3,6,8,3′,5′(OMe)	119.7	105.8	147.8	139.0	147.8	105.8	154.9	137.6	178.3	147.9	131.3	150.8	127.7	144.5	103.4	59.6	60.1	61.1	56.0	56.0	—
(9) 5,7,3′(OH)/3,6,8,4′,5′(OMe)	125.1	109.7	150.9	138.9	153.0	103.6	154.7	138.2	178.5	147.9	131.4	150.7	127.8	144.6	103.6	59.9	60.1	61.2	—	55.7	60.1 (C-4′)
(10) 5,7(OH)/3,8,3′,4′,5′(OMe)	125.2	105.6	152.7	139.9	152.7	105.6	155.9	138.5	178.1	154.3	98.9	157.3	127.5	148.6	104.1	59.9	—	60.8	55.9	55.9	60.2 (C-4′)
(11) 5,7,4′(OH)/3,6,8(OMe)	120.7	129.9	115.7	160.2	115.7	129.9	155.5	137.3	178.3	147.9	131.3	150.7	127.7	144.2	103.5	59.6	60.2	61.2	—	—	—
(12) 5,7,4′(OH)/3,8(OMe)	120.7	129.9	115.7	160.2	115.7	129.9	155.4	137.5	178.0	155.9	98.8	156.9	127.5	148.5	104.0	59.6	—	61.0	—	—	—
(13) 5,7,8,4′(OH)/3(OMe)	120.8	130.2	115.5	160.1	115.5	130.2	155.3	137.3	178.1	152.7	98.4	153.1	124.8	144.8	103.9	59.6	—	—	—	—	—
(14) 5,7,4′(OH)/3,6,3′(OMe)	120.8	112.0	147.4	149.7	115.6	122.1	155.3	137.3	178.0	152.2	131.2	157.8	94.1	151.5	104.3	59.6	59.9	—	55.7	—	—
(15) 5,7,8,3′,4′(OH)/3(OMe)	121.2	115.7	145.2	148.7	115.7	120.9	155.5	137.5	178.2	152.8	98.4	153.1	125.0	144.9	104.0	59.7	—	—	—	—	—
(16) 5,7,3′,4′(OH)/3,8(OMe)	121.0	115.3	145.3	148.8	115.9	120.6	155.9	137.7	178.0	155.4	98.8	156.8	127.6	148.5	104.1	59.6	—	61.1	—	—	—
(17) 5,7,8,4′(OH)/3,3′(OMe)	121.1	112.3	147.4	149.7	115.6	122.4	155.2	137.5	178.1	152.8	98.5	153.3	124.7	144.9	103.9	59.7	—	—	55.8	—	—
(18) 5,7,4′(OH)/3,8,3′(OMe)	120.9	111.6	147.4	149.7	115.7	122.0	155.8	137.6	178.0	155.1	98.7	156.8	127.4	148.5	104.0	59.6	—	60.9	55.5	—	—
(19) 5,7,2′,4′(OH)/3,6,8,5′(OMe)	107.1	152.9	104.1	150.3	140.5	114.5	156.6	138.1	178.0	147.9	131.7	150.8	128.0	145.3	102.6	59.9	59.9	60.8	—	56.5	—
(20) 5,7,3′,4′(OH)/3(OMe)	120.8	115.4	145.2	148.6	115.7	120.6	155.6	137.7	177.9	161.2	98.5	164.1	93.6	156.3	104.2	59.7	—	—	—	—	—
(21) 5(OH)/3,6,7,8,3′,4′(OMe)	122.1	111.7	148.5	151.4	110.9	122.0	155.5	138.0	178.6	148.1	135.5	152.4	132.4	144.4	106.8	59.7	60.6	61.4	55.7	—	61.7 (C-7), 55.4 (C-4′)

*Measured in DMSO-d_6 at 25 MHz; assignments in δ-values downfield from tetramethylsilane.

ments would be met by altering substitution at C-4′ or at both C-3′ and C-5′, the former would leave the methine (*meta* carbon) resonances unaltered, whereas the latter would leave but one resonance, the quaternary C-1′ (*meta*) unaffected. The observed spectra clearly show that the signal of the two methine carbons is unaltered proving that the difference between the B-rings lies at C-4′, a conclusion supported by the presence of two methoxyl resonances at δ56 in all three flavonols and the UV spectral shift data in the presence of sodium methoxide which suggest a free hydroxyl at C-4′ in **8**.

When the B-ring signals of **9** were compared to those of **7** and **10** it was immediately apparent that the B-ring of **9** lacked the symmetry of the other two compounds. Since only two signals (C-1′ and C-5′) are unchanged, they were presumed to arise from carbons *meta* to the site of substitution which is, therefore, identified as C-3′. Furthermore, the spectrum of **9** has one (rather than two) methoxyl signal at *ca* δ56, further confirming that a methoxyl at C-3′ has been replaced, in this case by a hydroxyl. The methine carbon resonances were assigned after consideration of the effects of hydroxyl–methoxyl substitution on the spectra of the flavonols with dioxygenated B-rings (see below). The remaining flavonol trioxygenated in ring B (**19**) has B-ring resonances significantly different from those of **7**–**10**; discussion of the assignments is deferred until the spectra of the 3′,4′-dioxygenated flavonoids have been discussed, for **19** is best considered to be a 3′,4′-dioxygenated flavonol with an additional hydroxyl at C-6′ rather than an isomer of 3′,4′,5′-trioxygenated flavonols.

Dioxygenated B-rings. The flavonols of this group fall neatly into two groups, those with two hydroxyls (**3**, **5**, **6**, **15**, **16**, **20**) and those with one hydroxyl and one methoxyl (**1**, **2**, **4**, **14**, **17**, **18**) comparison of which provides a measure of the effect of methylation of a free hydroxyl having one *ortho* oxygen and one *ortho* hydrogen. That the two oxygens are located on adjacent carbons is apparent from the C–O resonance signals which occur below δ150 exhibiting the $> \delta$10 shielding effect each oxygen exerts on its neighbouring *ortho* carbons (cf the C-4′ resonance in the spectra of **11**–**13**). Compared with the B-ring signals of **11**–**13**, the spectra of the dioxygenated B-rings have only two resonances (C-1′, C-5′) which remain unchanged, hence *meta*, to the second oxygen thereby locating it at C-3′. Of the two C–O signals, the one at lowest field was assigned to C-4′ because of the *para* substituent effect of the C-ring on C-4′. [An estimate of this effect can be made by comparing the C–O resonance of phenol (δ154.6 [13]) with that of C-4′ in the spectra of **11**–**13** (δ160.2) indicating a deshielding of δ5.6 caused by the C-ring.]

As mentioned, assignments of C-1′ and C-5′ signals for the 3′,4′-dioxygenated flavonols were readily made by comparison with the values observed for **11**–**13**, for the effect of an additional *meta* substituent was expected to be minimal. This left two methine carbon resonances (C-2′ and C-6′) to be assigned. Both C-2′ and C-6′ are symmetrically disposed about the C-1′–C-4′ axis and, therefore, the chemical shift difference between their signals is due to the C-3′ substituent alone. Because oxygen shields *ortho* methine carbons more than *para* ones, the upfield methine signal was assigned to C-2′.

The problem of determining the point of attachment of the single methoxyl of **1**, **2**, **4**, **14**, **17** and **18** can be solved if one considers the effect of methylation on the two carbons *para* to the possible methylation sites, since these (C-1′ and C-6′) are readily distinguishable by virtue of the multiplicities of their signals. (The fact that a considerable upfield shift of one *ortho* methine carbon signal also occurs upon methylation is of no diagnostic value unless the methine carbon signal can be unambiguously assigned to a unique carbon, requiring further ^{13}C experiments such as selective proton irradiation.) Comparison of the spectra of the 3′,4′-dihydroxyflavonols with their B-ring monomethyl ethers shows that C-1′ was unaffected whereas C-6′ shifted downfield $\Delta\delta$1.5, indicating the change has occurred at C-3′, a conclusion also supported by the UV spectral shifts in sodium methoxide.

In Table 3 are shown some of the observed effects on B-ring carbon signals caused by addition of a hydroxyl or methylation of a hydroxyl flanked by one *ortho* oxygen and one *ortho* methine group. The average values for the three singly substituted (OH-4) B-ring flavonols are given in the first column, the average values for 3′4′-dihydroxy compounds in the second and for 3′-methoxy-4′-hydroxyflavonols in the third. (Values for **4** and **5** were not included in the averages given in columns II and III, respectively, because their unmethoxylated C-rings have small but noticeable effects on the resonances of C-1′ and C-4′.) In column IV the differences between columns I and II are shown, a useful measure of the effect of a second hydroxyl group on B-ring resonances. In a similar vein, the differences between 3′,4′-dihydroxy and 3′-methoxy-4′-hydroxy B-ring signals are given in column V. It should be noted that the effect of methoxylation on the *ortho*

Table 3. B-ring ^{13}C NMR resonances and substituent effects for 3-methoxy *Gutierrezia microcephala* flavonols

	Resonances			Substituent effects	
Carbon	I 4′(OH) (**11**–**13**)	II 3′,4′(OH) (**3**, **6**, **15**, **16**, **20**)	III 3′(OMe),4′(OH) (**1**, **2**, **14**, **17**, **18**)	IV Δ(3′-H→3′-OH) (Col. II − Col. I)	V Δ(3′-OH→3′-OMe) (Col. III − Col. II)
1′	120.7	121.0	120.9	+0.3 *meta*	−0.1 *meta*
2′	130.0	115.4	111.8	−14.6 *ortho*	−3.6 *ortho* CH
3′	115.6	145.3	147.4	+29.7 *ipso*	+2.1 *ipso*
4′	160.2	148.7	149.8	−11.5 *ortho*	+1.1 *ortho* CO
5′	115.6	115.7	115.7	+0.1 *meta*	0.0 *meta*
6′	130.0	120.7	122.2	−9.3 *para*	+1.5 *para*

methine carbon signal is considerably larger than that on the *ortho* hydroxyl carbon and, furthermore, the direction of the shifts are opposite.

The substituent effects shown in Table 3 were used to calculate expected resonance frequencies for the B-ring carbons of several of the other flavonols. The average values for the 3′-methoxy-4′-hydroxyflavonols (Table 3, column III) were appropriately modified by use of the substituent effects observed (Table 3, column IV) for an additional hydroxyl group placed, in this instance, at C-6′. The calculated resonances are shown in Table 4, column I. (It should be noted that addition of a hydroxyl to C-6′ of a 3′,4′-disubstituted B-ring necessitates re-numbering the ring thereby interchanging C-2′ with C-6′ and C-3′ with C-5′.) The values of the B-ring resonances of **19** are shown in Table 4, column II, and comparison with the calculated values shows rather good agreement. In order to calculate B-ring resonances for 3′,4′-dimethoxy substitution, the values for 3′-methoxy-4′-hydroxy (Table 3, column III) were adjusted by the substituent effects for methylation of a C–OH having one *ortho* methine carbon and one *ortho* C–O carbon (Table 3, column V). The results, shown in Table 4, column III, agree well with the resonances observed for the B-ring of **21** (formed by methylation of **3**) shown in the next column. The resonance values of **9** (3′-hydroxy-4,5′-dimethoxy) were used to calculate values for a 3′,4′,5′-trimethoxylated B-ring using the substituent effects (Table 3, column V) as before. The calculated results (Table 4, column V) are in good agreement with the observed B-ring resonances of **7** and **10** (Table 4, column VI). Although the effects of adding an oxygen or methylating a free hydroxyl appear consistent in the B-rings thus far discussed, the nature of steric interactions between the substituents adds considerable variation rendering broader use of substituent effects unreliable. Hence, methylation of the OH-4′ of **8**, producing a 3′,4′,5′-trimethoxy substituted B-ring (**7**, **10**), causes a rather large (δ 5) deshielding of the signals of both *ortho* carbons and the *para* carbon (C-1′) while the *ipso* carbon (C-4′) remains unaffected. The facile explanation when contrasting the signals of **8** with those of **7** and **10**, namely that the shielding effects exerted by a OH-4′ on *ortho* and *para* carbons are considerably reduced by severely hindering the oxygen (by methylating it) forcing it out of the plane of the aromatic ring, can only be partly correct, for an equally deshielded C-1′ signal occurs in the spectrum of **9** which is not as sterically compressed as **7** or **10**.

A-ring resonances

Assigning A-ring resonances is somewhat more difficult than for B-rings because of the element of asymmetry introduced by the heterocyclic ring's two points of attachment to the A-ring. The C-10 signal can be most readily identified because its multiplicity separates it from any methine carbon signals and its chemical shift is far upfield from the remaining quaternary signals which arise from oxygenated carbons. The assignment of methine carbon signals (C-6 and C-8 in the commonly-encountered phloroglucinol-type A-ring of flavonoids) is more difficult but has been accomplished for quercetin after careful consideration of one and three bond C–H coupling constants in a gated spectrum [21]. Those results showed the C-8 resonance to be δ 5 upfield from that of C-6. Based on the effects methylation of an OH-3′ had on the neighbouring methine carbon (C-2′) signal, i.e. an increase in shielding of δ 3.6 (Table 3, column V) one might expect the carbon (C-8) adjacent to the etherified carbon (C-9) to resonate upfield from the carbon (C-6) adjacent to the hydroxylated carbon (C-5).

Di- and tetraoxygenated A-rings. Signals from the A-ring of 3-*O*-methylquercetin (**20**), the only dioxygenated A-ring flavonol encountered in *Gutierrezia microcephala*, were assigned after comparison with the assignments reported for quercetin [21] and 3,4′-di-*O*-methylquercetin [15]. One can consider next the perturbations to be expected upon addition of methoxyl groups simultaneously to C-6 and C-8 to create the A-rings of **2–5**, **7–9**, **11** and **19**. Two of the carbons (C-5, C-9) have each gained an *ortho* and a *para* methoxyl group and should, thus, experience approximately the same shielding effects and retain their relative positions, C-9 resonating upfield from C-5. In fact, the two signals in question in the spectra of **2**, **3**, **7–9**, **11** and **19** occur at an average of δ 144.7 and 147.9, the $\Delta\delta$ 3.2 difference being very similar to that between the C-5 and C-9 signals ($\Delta\delta$ 3.7) of **20**. (The difference between the C-5 and C-9 signals is somewhat smaller ($\Delta\delta$ 2.4) in the spectra of the C-ring hydroxyflavonols, **4** and **5**.) The

Table 4. Calculated and observed B-ring ^{13}C NMR resonances of *Gutierrezia microcephala* flavonols

	2′4′(OH)/5′(OMe)		3′,4′(OMe)		3′,4′,5′(OMe)	
	I	II	III	IV	V	VI
Carbon	Calculated*	Observed (**19**)	Calculated†	Observed (**21**)	Calculated†	Observed (**7**, **10**)
1′	107.8	107.1	122.4	122.1	125.0	125.2
2′	151.9	152.9	111.7	111.7	106.1	105.6
3′	102.6	104.1	148.5	148.5	153.0	152.7
4′	150.0	150.3	151.9	151.4	140.0	139.9
5′	138.1	140.5	112.1	110.9	152.9	152.7
6′	112.0	114.5	122.1	122.0	105.1	105.6

*Δ(3′-H→3′-OH) values (Table 3, IV) were added to the resonances of 3′(OMe)/4′(OH) [≡ 5′(OMe)/4′(OH)] B-rings (Table 3, III) with the carbon *para* to OMe chosen *ipso*.

†Δ(3′-OH → 3′-OMe) values (Table 3, V) were added to the resonances of 3′,4′(OH) B-rings (Table 3, II).

‡Δ(3′-OH → 3′-OMe) values (Table 3, V) were added to the resonances of a 3′(OH)/4′,5′(OMe) B-ring compound (**9**, Table 2).

higher field signal was assigned to C-9 in all of the tetraoxygenated A-ring flavonols since, in a gated spectrum of **3**, the quaternary carbon resonances of C-5, C-7 and C-9 appeared as two singlets and one doublet (δ147.9, ^2J = 4.2 Hz), the latter reflecting coupling to the chelated OH-5 thereby unambiguously identifying the C-5 signal.

Reasoning analogous to that applied to the assignments of C-5 and C-9 signals was used to assign C-6 and C-8 signals. Each of these carbons has exchanged a hydrogen atom for a methoxyl group and should, thus, experience a large, but equivalent deshielding, each unaffected by the other because of their *meta* relationship. In fact, the average values for the C-6 and C-8 signals in the spectra of **2–5**, **7–9** and **19**, when compared with the C-6 and C-8 signals of **20**, show upfield shifts of $\Delta\delta$34 and 33, respectively, maintaining their relative positions and separation. Therefore, in all of these flavonols the C-8 signal was assigned upfield of the C-6 signal.

That the two methoxyl groups are attached to C-6 and C-8 and not some alternative arrangement is supported by carbon NMR and other spectral data. As mentioned earlier, C-ring chemical shifts, a very low-field proton signal, and UV shifts in the presence of aluminum chloride–hydrochloric acid confirm the presence of an OH-5 in all the flavonols. Furthermore, the absence of *ortho* dihydroxy groups in **2**, **4**, **7–9**, **11** and **19** is indicated by UV spectral data (boric acid–sodium acetate and aluminum chloride shifts) and the failure of the above to reduce ammoniacal silver nitrate solutions. The foregoing rule out all alternatives except 6,7-dimethoxylation which is, in turn, ruled out by the C-10 signal (in the ^{13}C NMR spectra of the A-ring dimethoxyflavonols) which occurs at the same chemical shift as the C-10 resonance in the spectrum of **20**, indicating that no change in substitution at C-7 (*para*) has occurred.

Addition of a third methoxyl to the A-ring can occur by methylation of either of the two free hydroxyls. As in the case of B-ring methylation, the *meta* carbon signals should be scarcely affected. When compared with the spectra of **2–5**, **7–9**, **11** and **19** the spectra of **1** and **21** show only two A-ring signals unchanged (C-5, C-9) the rest having moved downfield, in agreement with the additional methoxyl being placed at C-7; the other evidence for a free hydroxyl at C-5 (UV, ^{1}H NMR and the ^{13}C shift of C-4) supports this conclusion. The shifts of both *ortho* carbon signals are affected equally (downfield shifts of $\Delta\delta$4.1 and 4.7) and, thus, C-6 and C-8 retain their relative positions, the C-8 resonance remaining $\Delta\delta$3 upfield from that of C-6.

The monomethoxylated A-ring of **6** has two signals in common with the spectra of **2–5**, **7–9**, **11** and **19**, those of C-6 and C-10, indicating that the difference between them lies in the substitution at C-8, i.e. that the methoxyl at C-8 in the dimethoxyflavonols is no longer present, having become, in **6**, a free hydroxyl. Additional support for the structure of **6** is provided by the relative intensity of the $[M]^+$ and $[M-15]^+$ ions in its mass spectrum since, with the exception of **19**, all of the 8-methoxyflavonols had $[M-15]^+$ as base peak, whereas the remaining flavonols had $[M]^+$ as base peak. Furthermore, the methoxyl resonance at δ60.0 in the ^{13}C NMR spectrum is in better agreement with its attachment to C-6 rather than to C-8, for the OMe-8 signals appear slightly further downfield (see Table 2). That the changes in the A-ring resonances, upon methylation or demethylation of hydroxyl groups, in the tetraoxygenated A-rings are quite uniform and reasonable can be seen after discussion of the trioxygenated A-rings.

Trioxygenated A-rings. Since all of the flavonols have a hydroxyl at C-5, the other two oxygens must be at C-6 and C-7, C-7 and C-8 or C-6 and C-8. The last of these possibilities can be ruled out by the chemical shift of the C-10 resonance which is essentially the same as for the other (di- and tetraoxygenated) flavonols indicating that all have C-7 oxygenation. If one assumes that attachment of an additional oxygen to a phloroglucinol type of A-ring will have a minimal effect on the carbons *meta* to the site of substitution, then the C–H resonance of the trioxygenated A-rings should occur at the same chemical shift as one of the methine signals of **20**. In fact, seven of the flavonols (**10**, **12**, **13** and **15–18**) have a methine signal at an average frequency of δ98.6 (the same as the C-6 resonance of **20**) and one flavonol (**14**) has a methine resonance at δ94.1 (almost the same as the C-8 methine resonance of **20**). The determination of 5,7,8- vs 5,6,7-oxygenation in flavonoids by the chemical shift of the methine carbon resonance [15] is, thus, of considerable diagnostic value but, if used without additional corroboration, it can be misleading. Thus, methylation of OH-7 in a 5,7,8-trioxygenated A-ring causes an upfield shift of the *ortho* methine carbon resonance (C-6) to *ca* δ95*, where C-8 methines resonances normally appear, as in the spectra of **14** and **20**.

In all of the flavonols with trioxygenated A-rings with a single methoxyl group, location of the latter was clear because the methoxyl chemical shift (*ca* δ60) indicated di-*ortho* substitution, thereby precluding the possibility of methoxylation at carbons other than C-6 (**14**) or C-8 (**10**, **12**, **16** and **18**).

Assignments of the three downfield quaternary resonance signals in the spectra of the trioxygenated flavonols was accomplished by comparison with the spectrum of **20** and with the spectra of the 6,8-dimethoxy compounds. It was assumed that the result of substituting an oxygen for a hydrogen would cause approximately equal effects on both *ortho* carbons and a smaller effect on the single *para* carbon. In the case of O-8 vs H-8 the highest field signal (C-9) was shifted further upfield than was that of the *para* C-5, somewhat enlarging their separation. The lowest field signal (C-7) was also considerably shifted upfield (since it is *ortho* to C-8) and, hence, moved close to that of C-5. In the case of C-6 oxygenation (**14**), however, it was the upfield (C-9) signal (*para*) which was least shifted and the midfield C-5 signal moved upfield to within δ1 of the C-9 signal. In fact, the signals of C-5 and C-9 in the spectrum of **14** were too close to assign with confidence, as were those of C-5 and C-7 in the C-8 hydroxy compounds, **13**, **15** and **17**.

The observed substituent effects on A-ring signals are gathered in Table 5. The first five columns list the A-ring resonances for the variously substituted 5,7-dihydroxyflavonols. Columns VII and V show the effects of substituting OMe-8 for H-8. Although the effects are roughly comparable, as would be expected [since the only difference in the two comparisons is the presence (or

*The A-ring methine resonances of 5,8,4′-trihydroxy-3,7-dimethoxyflavone and of 5,7,4′-trihydroxy-3,7-3′-trimethoxyflavone occur at δ95.3 and 95.4, respectively (Elakovich, S., unpublished results from work carried out in this laboratory on the same instrument as used for the other flavonols).

Table 5. A-ring ^{13}C NMR resonances and substituent effects for 5,7-dihydroxy-3-methoxy *Gutierrezia microcephala* flavonols

	Resonances					Substituent effects				
	I	II	III	IV	V	VI	VII	VIII	IX	X
Carbon	5,7(OH) (20)	5,7(OH)/8(OMe) (10, 12, 16, 18)	5,7,8(OH) (13, 15, 17)	5,7(OH)/6(OMe) (14)	5,7(OH)/6,8(OMe) (2, 3, 7–9, 11, 19)	(8-OH→8-OMe) (Col. II − Col. III)	(8-H→8-OMe) (Col. II − Col. I)	(Col. V − Col. IV)	(Col. IV − Col. I)	(6-H→6-OMe) (Col. V − Col. II)
5	161.2	155.2	152.8	152.2	147.9	+2.4 *para*	−6.0 *para*	−4.3 *para*	−9.0 *ortho*	−7.3 *ortho*
6	98.5	98.8	98.4	131.2	131.4	+0.4 *meta*	+0.3 *meta*	+0.2 *meta*	+32.7 *ipso*	+32.6 *ipso*
7	164.1	157.0	153.2	157.8	150.8	+3.8 *ortho*	−7.1 *ortho*	−7.0 *ortho*	−6.3 *ortho*	−6.2 *ortho*
8	93.6	127.5	124.8	94.1	127.7	+2.7 *ipso*	+33.9 *ipso*	+33.6 *ipso*	+0.5 *meta*	+0.2 *meta*
9	156.3	148.5	144.9	151.5	144.7	+3.6 *ortho*	−7.8 *ortho*	−6.8 *ortho*	−4.8 *para*	−3.8 *para*
10	104.2	104.1	103.9	104.3	103.4	+0.2 *meta*	−0.1 *meta*	−0.9 *meta*	+0.1 *meta*	−0.7 *meta*

absence) of a *meta* methoxyl] the *para* carbon (C-5) shifted upfield more when there was no methoxyl at C-6 than when C-6 was methoxylated ($\Delta\delta - 6$ vs -4.3). Similar comparisons for H-6 vs OMe-6 are shown in Table 5 columns IX and X. The changes are again seen to be comparable with the exception of one *ortho* carbon shift which is greater in the absence of a second methoxyl at C-8 ($\Delta\delta - 9$ vs -7.3). In this case, the carbon displaying the shift variance was again C-5 which suggests that it is more sensitive to steric effects than C-7 or C-9 possibly because its hydroxyl is strongly hydrogen bonded to the carbonyl oxygen.

Comparison of the A-ring signals of the C-8 hydroxyflavonols **13**, **15** and **17** with their C-8 methoxy counterparts, **10**, **12**, **16** and **18**, provides a measure of the effect of methylation of an A-ring hydroxyl with two *ortho* oxygens (Table 5, column X). It should be noted that both *ortho* carbon signals shift downfield upon methylation of OH-8 and that the *para* and *ipso* carbon resonances also shift downfield. This is in contrast to the effects of methylation of a hydroxyl attached to a carbon with only one *ortho* oxygen (Table 3, column V), as seen in the B-ring of many of these flavonols. The closest analogy with the effect of methylation of OH-8 is the comparison (involving B-ring signals) of the spectrum of **8** with those of **7** and **10**. In this case, too, methylation caused the two *ortho* carbons signals to shift considerably downfield, but the downfield shifts of the *para* and *ipso* carbon signals were of quite different magnitude, the *para* signal having shifted further downfield than the *ortho* signals while the *ipso* carbon resonance moved only $\Delta\delta 0.9$ downfield.

In order to test whether the OH-8 → OMe-8 shifts could be applied more generally, an estimate of the A-ring resonances of a 5-hydroxy-6,7,8-trimethoxy substituted A-ring was made based on the average values for the signals of the 5,7-dihydroxy-6,8-dimethoxy A-rings of **2**, **3**, **7–9**, **11** and **19** (Table 5, column V) and the substituent effects observed for OH-8 → OMe-8 (Table 5, column VI). The resulting values (Table 6, column I) agree well with those observed for the A-ring signals of **1** and **21**. The same sets of base values and substituent effects were used to calculate (by subtraction rather than addition) expected resonance values for 5,7,8-trihydroxy-6-methoxy and 5,6,7-trihydroxy-8-methoxy A-rings. The results are shown in Table 6 columns III and V along with the observed values from the spectrum of **6** (column IV). Clearly, one set of calculated values (column III) agrees well with the observed signals for **6**, whereas the other set does not, further supporting the location of the single methoxyl group in **6** at C-6 rather than at C-8.

The ^{13}C NMR data for the *Gutierrezia microcephala* flavonols fall into consistent, logically explicable groups and illustrate the usefulness of substituent effects when applied to aromatic carbons experiencing approximately equivalent steric effects. The data also demonstrate that considerable diagnostic information useful for determining oxygenation patterns and locations of methoxyl groups on A-, B- and C-rings can be extracted from flavonol ^{13}C NMR spectra.

Gutierrezia microcephala is a rich source of highly-oxygenated flavonols. The major two flavonols, **2** and **3**, were each present in equal amounts, approximately 0.15% of the plant material dry wt. Amounts of the remaining flavonols were less and ranged from 0.04% for **11** to *ca* 0.0002% for **14**. The physical data reported for the known flavonols, 3′-methoxycalycopterin (**1**),

Table 6. Calculated and observed A-ring ^{13}C NMR resonances of *Gutierrezia microcephala* flavonols

	5(OH)/6,7,8(OMe)		5,7,8(OH)/6(OMe)		5,6,7(OH)/8(OMe)
	I	II	III	IV	V
Carbon	Calculated*	Observed (1, 21)	Calculated†	Observed (6)	Calculated‡
5	148.1	148.1	145.5	144.5	144.2
6	135.1	135.5	131.0	131.1	128.7
7	153.5	152.4	147.0	146.7	147.1
8	131.4	132.2	125.0	125.2	127.4
9	145.0	144.4	141.1	140.7	142.6
10	105.8	106.8	103.2	103.3	103.1

*Δ(8-OH → 8-OMe) values (Table 5, IV) were added to the appropriate resonances of 5,7(OH)/6,8(OMe) A-rings (Table 5, V) with C-7 chosen *ipso*.

†Δ(8-OH → 8-OMe) values (Table 4, VI) were subtracted from the appropriate resonances of 5,7(OH)/6,8(OMe) A-rings (Table 5, V) with C-6 chosen *ipso*.

‡Δ(8-OH → 8-OMe) values (Table 5, VI) were subtracted from the appropriate resonances of 5,7(OH)/6,8(OMe) A-rings (Table 5, V) with C-8 chosen *ipso*.

3-*O*-methyllimocitrol (**2**), limocitrol (**4**), conyzatin (**10**), 3,8-di-*O*-methylherbacetin (**12**), jaceidin (**14**), 3,8-di-*O*-methylgossypetin (**16**), 3,8,3′-tri-*O*-methylgossypetin (**18**) and 3-*O*-methylquercetin (**20**), agree well with our data. Although it has never appeared in the chemical lit., a flavonol isolated [23] from *Gutierrezia sarothrae* is spectroscopically (UV, ^1H NMR) identical to **11** and has the same mp.

A flavonol isolated from *Pluchea saggitalis* recently by Martino *et al.* [22] has been assigned structure **3** based on UV, ^1H NMR and mass spectral data, and conversion to a known heptamethyl ether. Although the reported ^1H NMR and mass spectral data differ somewhat from our own, the discrepancies can be due to differing experimental conditions, i.e. instrument variation and concentration effects. However, the reported UV maxima, especially of band I, are at appreciably lower wavelengths than we observed in methanol (345 vs 360 nm) and in the presence of shift reagents: sodium methoxide (385 vs 418 nm); aluminium chloride (365 sh, 435 vs 448 nm); aluminium chloride–hydrochloric acid (365 vs 376 nm); sodium acetate (380 vs 402 nm); sodium acetate–boric acid (367 vs 390 nm). Furthermore we observed a mp for **3** at 248–250°, considerably higher than the reported mp of the *Pluchea* flavonol, 167–169°. We, therefore, suggest that **3** is a new flavonol and that the flavonol described by Martino *et al.* [22] is an isomer possessing the same oxygenation pattern with a different arrangement of hydroxyl and methoxyl groups.

A new flavonol recently isolated from *Gutierrezia resinosa* (H. and A.) Blake in Chile [24, 25] has been shown to have significant activity [25] against human carcinoma of the nasopharynx and also to induce micronucleation of mouse bone marrow cells [26]. This flavonol is the 7-*O*-methyl ether of **3**, which suggests that **3** itself may exhibit some interesting physiological properties. Results of preliminary feeding trials with **2** and **3** on several insect pests of cotton indicate that **3** possesses significant anti-feeding activity (Chan, B., personal communication). Other biological testing is in progress.

EXPERIMENTAL

General. ^{13}C NMR spectra were obtained in DMSO-d_6 solns at ambient temp. at 25 MHz on a Jeol JHM PS-100 PFT spectrometer. ^1H NMR spectra were obtained in Me_2CO-d_6, unless otherwise indicated, at 200 MHz on a Nicolet NTC 200 FT spectrometer or at 90 MHz on a Varian EM 390 instrument. Electron impact MS were obtained at 70 eV on a VG Micromass 7070 spectrometer at a source temp. of 190–210°. UV spectra were measured in MeOH unless otherwise indicated. TLC was performed on pre-coated glass silica gel plates (Analtech) using $CHCl_3$–MeOH, 19:1 or 9:1 depending on the polarity of the flavonols. Prep. TLC utilized home-made 1, 2 and 4 mm thick silica gel layers on glass plates for centrifugally accelerated circular TLC employing a model 7924 Chromatotron (Harrison Research, Palo Alto, California) [27]. Flavonol spots on TLC plates were visualized by UV and by spraying with freshly mixed aq. solns of $K_3Fe(CN)_6$ and $FeCl_3$ (1:1) or dil. aq. ammoniacal $AgNO_3$ (for quinol or catechol groups). Mps are uncorrected.

Extraction and isolation of the flavonols. Plant material (aerial portions) was collected in the vicinity of Eunice, New Mexico in the Spring of 1982, air-dried and ground. A 6 kg sample, defatted by light petrol Soxhlet extraction for 3 days was extracted with Et_2O for 4 days. Evaporation of the solvent at ambient temp. gave 426 g extract containing *ca* 13% residual solvent. A portion of the crude extract (336 g) was partitioned between C_6H_6–petrol (1:1) and MeOH–H_2O (4:1) employing a procedure described [29] for purification of some terpenoid–quinone pigments. The lower layers were combined (no flavonoids were detected in the upper layers), rotory evaporated to remove most of the solvents and absolute EtOH added then removed under red. pres. to remove most of the remaining H_2O. The resulting syrup, containing *ca* 212 g solids, was diluted with absolute EtOH for chromatography.

This extract was chromatographed in 10 portions on Sephadex LH-20 (600 g) employing absolute EtOH as eluent. The various fractions were combined into 10 large fractions, the first three of which contained only non-flavonoid material (which accounted for *ca* 85% of the total partitioned extract). The fourth fraction

(53 g) was re-chromatographed in $CHCl_3$ on LH-20 (600 g) to separate non-flavonoid material from a mixture of flavonols (1.8 g) which were then separated by prep. TLC followed by recrystallization from MeOH into the pure flavonols **1** and **7**. The fifth fraction (10 g) was re-chromatographed in $CHCl_3$–MeOH (19:1) on LH-20 (500 g) in two portions to give, after recrystallization from MeOH, pure **2** (3.2 g) and **11** (1.6 g) as well as mixtures including **1**, **7** and **10**, which were isolated after further CC (LH-20) and prep. TLC. In the same way, fraction 6 (12 g) was re-chromatographed in $CHCl_3$–MeOH (19:1) on LH-20 (500 g) providing, after recrystallization from MeOH, pure **2** (2.9 g), **3** (3.0 g), **11** (0.8 g) and **18** (340 mg) as well as mixtures one of which, after repeated prep. TLC, gave small samples of pure **14** (14 mg) and **19** (12 mg).

Fraction 7 (9.8 g) recrystallized from MeOH gave pure **3** (5.8 g); repeated chromatography (LH-20 and prep. TLC) of the filtrate gave, after recrystallization from MeOH, some pure **13** (46 mg), **16** (65 mg), **20** (7 mg) and more **3** (615 mg).

Chromatography on LH-20 ($CHCl_3$–MeOH, 9:1) of fraction 8 (550 mg) followed by repeated prep. TLC, recrystallization (MeOH–H_2O) and fractional crystallization ($CHCl_3$–MeOH–HCOOH, 96:4:1) gave pure **13** (30 mg), **17** (52 mg) and **20** (23 mg), as well as 10 mg naringenin (identified after comparison of its ^{13}C NMR spectrum with a published [15] spectrum showed them to be identical). Fraction 10 recrystallized from MeOH gave pure **15** (280 mg) and a filtrate which was combined with fraction 9 and re-chromatographed several times on LH-20 ($CHCl_3$–MeOH, 7:3 and 9:1) to give pure **4** (33 mg), **5** (30 mg) and **6** (15 mg) and more **15** (250 mg). A 200 mg portion of the filtrate of the sample of **3** isolated from fraction 6 was repetitively subjected to prep. TLC to isolate, after recrystallization from MeOH–H_2O pure **12** (17 mg). From the filtrate of **2** (860 mg) were isolated, after repeated chromatography on LH-20, samples of pure **8** (173 mg) and **9** (63 mg).

5,7,3',4'-*Tetrahydroxy*-3,6,8-*trimethoxyflavone* (**3**). Obtained as bright yellow needles from MeOH, mp 248–250°; R_f silica gel, $CHCl_3$–MeOH, 9:1) 0.41. (Found $[M]^+$, 376.0779. Calc. for $C_{18}H_{16}O_9$: $[M]^+$, 376.0794.) UV λ_{max}^{MeOH} nm (rel. *A*): 264 sh (0.92), 278 (1.00), 360 (0.86); λ_{max}^{NaOMe} nm (rel. *A*): 282 (0.99), 346 (0.47), 418 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 282 (1.00), 330 (0.53), 402 (0.64); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 272 (1.00), 390 (0.86); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 284 (0.93), 314 sh (0.32), 448 (1.00); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 275 sh (0.90), 286 (0.92), 310 sh (0.61), 378 (1.00), 426 sh (0.65). MS *m/z* (rel. int.): 376 $[M]^+$ (76), 375 (11), 361 $[M-Me]^+$ (100), 345 $[M-OMe]^+$ (12), 343 $[M-Me-H_2O]^+$ (8), 333 $[M-COMe]^+$ (6), 197 $[A-15]^+$ (9), 188 $[M]^{2+}$ (6), 169 $[A-COMe]^+$ (5), 150 $[B_1-CH_2]^+$ (12), 137 $[B_2]^+$ (15), 121 $[B_1-COMe]^+$ (7). An Me_2CO soln of **3** (102 mg) and EtI (2 ml) was refluxed over K_2CO_3 for 16 hr. Separation of the products by prep. TLC ($CHCl_3$–MeOH, 99:1) gave a yellow 7,3',4'-triethyl ether (35 mg) and a colourless 5,7,3',4'-tetraethyl ether (85 mg). Triethyl ether: mp 133–134° (Et_2O); R_f (silica gel, $CHCl_3$–MeOH, 99:1) 0.31. ^1H NMR ($CDCl_3$): δ1.45 (3H, *t*, *J* = 7.5 Hz), 1.51 (6H, *t*, *J* = 7.5 Hz), 3.87 (3H, *s*), 3.94 (6H, *s*), 4.19 (2H, *q*, *J* = 7.5 Hz), 4.20 (2H, *q*, *J* = 7.5 Hz), 4.34 (2H, *q*, *J* = 7.5 Hz), 7.00 (*d*, *J* = 8.5 Hz, H-5'), 7.80 (*d*, *J* ≒ 2 Hz, H-2'), 7.82 (*dd*, *J* = 2, 8.5 Hz, H-6'), 12.40 (1H, *s*, OH-5); C_6H_6-d_6: δ1.13 (3H, *t*, *J* = 7.5 Hz), 1.24 (3H, *t*, *J* = 7.5 Hz), 1.29 (3H, *t*, *J* = 7.5 Hz), 3.66 (2H, *q*, *J* = 7.5 Hz), 3.73 (3H, *s*), 3.77 (3H, *s*), 3.89 (2H, *q*, *J* = 7.5 Hz), 3.90 (3H, *s*), 4.22 (2H, *q*, *J* = 7.5 Hz), 6.64 (*d*, *J* = 8.5 Hz, H-5'), 7.89 (*d*, *J* = 2 Hz, H-2'), 7.94 (*dd*, *J* = 2, 8.5 Hz, H-6'), 13.33 (1H, *s*, OH-5). UV λ_{max}^{MeOH} nm (rel. *A*): 262 (0.94), 282 (0.98), 348 (1.00). Tetraethyl ether: mp 123° (Et_2O); R_f (silica gel, $CHCl_3$–MeOH, 99:1) 0.14. ^1H NMR ($CDCl_3$): δ1.45 (3H, *t*, *J* = 7.5 Hz), 1.51 (6H, *t*, *J* = 7.5 Hz), 1.56 (3H, *t*, *J* = 7.5 Hz), 3.86 (3H, *s*), 3.94 (3H, *s*), 4.00 (3H, *s*), 4.14 (2H, *q*, *J* = 7.5 Hz), 4.20 (4H, *q*, *J* = 7.5 Hz), 4.31 (2H, *q*, *J* = 7.5 Hz), 7.00 (*d*, *J* = 8.5 Hz, H-5'), 7.81 (*dd*, *J* = 2, 8.5 Hz, H-6'), 7.83 (*d*, *J* = 2 Hz, H-2'); C_6H_6-d_6: δ1.15 (3H, *t*, *J* = 7.5 Hz), 1.23 (3H, *t*, *J* = 7.5 Hz), 1.26 (3H, *t*, *J* = 7.5 Hz), 1.61 (3H, *t*, *J* = 7.5 Hz), 3.69 (2H, *q*, *J* = 7.5 Hz), 3.73 (3H, *s*), 3.80 (3H, *s*), 3.89 (3H, *s*), 3.92 (2H, *q*, *J* = 7.5 Hz), 4.16 (2H, *q*, *J* = 7.5 Hz), 4.33 (2H, *q*, *J* = 7.5 Hz), 6.70 (*d*, *J* = 8.5 Hz, H-5'), 7.97 (*d*, *J* = 2 Hz, H-2'), 7.98 (*dd*, *J* = 2, 8 Hz, H-6'). UV λ_{max}^{MeOH} nm (rel. *A*): 256 (0.91), 273 sh (0.81), 344 (1.00).

5-*Hydroxy*-3,6,7,8,3',4'-*hexamethoxyflavone* (**21**). Refluxed for 1 hr with K_2CO_3, a soln of **3** (103 mg) and MeI (3 ml) in Me_2CO (10 ml) gave, after prep. TLC and recrystallization from Et_2O, fine yellow needles of a trimethyl ether of **3**, mp 112–113°. UV λ_{max}^{MeOH} (rel. *A*): 260 (0.92), 282 (0.97), 346 (1.00); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 270 (0.72), 292 (0.84), 310 sh (0.67), 372 (1.00), 432 sh (0.39). ^1H NMR ($CDCl_3$): δ3.89 (3H, *s*), 3.95 (3H, *s*), 3.96 (3H, *s*), 3.98 (3H, *s*), 3.99 (3H, *s*), 4.11 (3H, *s*), 7.03 (*d*, *J* = 8.5 Hz, H-5'), 7.80 (*d*, *J* = 2 Hz, H-2'), 7.87 (*dd*, *J* = 2, 8.5 Hz, H-6'), 12.40 (1H, *s*, OH-5).

3,5,7,3',4'-*Pentahydroxy*-6,8-*dimethoxyflavone* (**5**). Obtained as yellow needles from MeOH, mp 285–287°; R_f (silica gel, $CHCl_3$–MeOH, 9:1) 0.38. (Found $[M]^+$, 362.0646. Calc. for $C_{17}H_{14}O_9$, $[M]^+$, 362.0637.) UV λ_{max}^{MeOH} nm (rel. *A*): 264 (1.00), 280 sh (0.89), 345 sh (0.65), 382 (0.88), 452 sh (0.24); λ_{max}^{NaOMe} nm (rel. *A*): 280 (0.96), 340 (0.48), 430 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 265 sh (0.93), 280 (1.00), 329 sh (0.52), 392 (0.80); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 268 (1.00), 398 (0.80), 470 sh (0.20); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 280 (0.86), 468 (1.00); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*) 274 (1.00), 314 sh (0.28), 386 (0.64), 442 (0.93). MS *m/z* (rel. int.): 362 $[M]^+$ (91), 361 (6), 347 $[M-Me]^+$ (100), 345 $[M-OH]^+$ (5), 333 $[M-HCO]^+$ (8), 329 $[M-Me-H_2O]^+$ (9), 319 $[M-COMe]^+$ (31), 316 (7), 304 $[M-Me-COMe]^+$ (19), 301 $[M-COMe-H_2O]^+$ (7), 207 (9), 172 (7), 150 $[B_1-CH_2]^+$ (5), 137 $[B_2]^+$ (15).

5,7,8,3',4'-*Pentahydroxy*-3,6-*dimethoxyflavone* (**6**). Obtained first as a greenish yellow gum, **6** could not successfully be recrystallized even after further purification by prep TLC; R_f (silica gel, $CHCl_3$–MeOH, 9:1): 0.24. UV λ_{max}^{MeOH} nm (rel. *A*): 264 (0.77), 288 (1.00), 348 (0.82); λ_{max}^{NaOMe} nm (rel. *A*): 288 (1.00), 343 sh (0.62), 404 (0.93); λ_{max}^{NaOAc} nm (rel. *A*): 292 (0.90), 332 (0.72), 363 sh (0.64); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 274 (0.94), 290 (1.00), 370 (0.75); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 288 sh (0.98), 302 (1.00), 326 sh (0.67), 350 sh (0.47), 414 (0.91), 476 sh (0.54); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 270 (0.76), 296 (0.89), 315 sh (0.79), 376 (1.00), 438 sh (0.36). MS *m/z* (rel. int.): 362 $[M]^+$ (100), 361 (27), 347 $[M-Me]^+$ (75), 345 $[M-OH]^+$ (7), 344 $[M-H_2O]^+$ (8), 333 $[M-HCO]^+$ (6), 332 $[M-30]^+$ (20), 329 $[M-Me-H_2O]^+$ (7), 319 $[M-COMe]^+$ (11), 317 $[M-OH-CO]^+$ (8), 304 $[M-Me-COMe]^+$ (11), 301 $[M-COMe-H_2O]^+$ (11), 289 $[M-73]^+$ (13), 183 $[A_1-Me]^+$ (6), 181 $[M]^{2+}$ (7), 164 $[B_1]^+$ (7), 150 $[B_1-CH_2]^+$ (16), 147 $[B_1-OH]^+$ (8), 137 $[B_2]^+$ (12), 136 $[B_1-CO]^+$ (6), 121 $[B_1-COMe]^+$ (9).

5,7-*Dihydroxy*-3,6,8,3',4',5'-*hexamethoxyflavone* (**7**). Obtained as bright yellow fine needles from MeOH, mp 179–180°; R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.51. (Found $[M]^+$, 434.1224. Calc. for $C_{21}H_{22}O_{10}$, $[M]^+$, 434.1212.) UV λ_{max}^{MeOH} (rel. *A*): 284 (1.00), 334 (0.71); λ_{max}^{NaOMe} nm (rel. *A*): 286 (1.00), 320 sh (0.52), 386 (0.46); λ_{max}^{NaOAc} nm (rel. *A*): 286 (1.00), 320 sh (0.54), 376 (0.46); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 282 (1.00), 336 (0.73); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 292 (0.94), 319 sh (0.80), 366 (1.00), 432 sh (0.40); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 294 (0.94), 317 sh (0.80), 362 (1.00), 426 sh (0.31). MS *m/z* (rel. int.): 434 $[M]^+$ (84), 433 (6), 419 $[M-Me]^+$ (100), 404 $[M-30]^+$ (7), 401 $[M-Me-H_2O]^+$ (7), 390 $[M-44]^+$ (5), 389 $[M-OH-CO]^+$ (9), 361 $[M-73]^+$ (5), 217 $[M]^{2+}$ (10), 208 $[B_1-CH_2]^+$ (4), 197 $[A-Me]^+$ (4), 195 $[B_2]^+$ (3), 193 $[B_1-CO]^+$ (5), 179 $[B_1-43]^+$ (5).

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

from MeOH–H_2O as fine yellow needles mp 176–177°; R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.37; (silica gel, C_6H_6–pyridine–HCO_2H, 75:9:5) 0.26. (Found $[M]^+$, 420.1049. Calc. for $C_{20}H_{20}O_{10}$, $[M]^+$, 420.1054.) UV λ_{max}^{MeOH} nm (rel. *A*): 252 sh (0.76), 282 (0.86), 320 sh (0.63), 360 (1.00); λ_{max}^{NaOMe} nm (rel. *A*): 268 (0.64), 285 sh (0.59), 340 sh (0.42), 430 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 284 (1.00), 330 (0.67), 378 (0.72); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 255 sh (0.84), 282 (0.99), 315 sh (0.67), 362 (1.00); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 265 sh (0.71), 286 (0.82), 320 (0.47), 386 (1.00), 430 sh (0.66); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 265 sh (0.66), 288 (0.78), 316 (0.46), 382 (1.00), 430 sh (0.56). MS *m/z* (rel. int.): 420 $[M]^+$ (74), 405 $[M-Me]^+$ (100), 387 $[M-Me-H_2O]^+$ (7), 210 $[M]^{2+}$ (5), 197 $[A-Me]^+$ (5), 194 $[B_1-CH_2]^+$ (5), 181 $[B_2]^+$ (7), 169 $[A-COMe]^+$ (5), 165 $[B_1-COMe]^+$ (6).

5,7,3′-*Trihydroxy*-3,6,8,4′,5′-*pentamethoxyflavone* (**9**). Recrystallized from MeOH as fine yellow needles, mp 207–208°; R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.40; (silica gel, C_6H_6–pyridine–HCO_2H, 75:9:5) 0.31. (Found $[M]^+$, 420.1045. Calc. for $C_{20}H_{20}O_{10}$; $[M]^+$, 420.1054.) UV λ_{max}^{MeOH} nm (rel. *A*): 282 (1.00), 338 (0.79); λ_{max}^{NaOMe} nm (rel. *A*): 284 (1.00), 318 (0.49), 388 (0.58); λ_{max}^{NaOAc} nm (rel. *A*): 286 (1.00), 320 sh (0.52), 374 (0.47); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 282 (1.00), 334 (0.74); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 292 (0.97), 317 sh (0.78), 364 (1.00), 430 sh (0.39); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 292 (0.97), 316 sh (0.78), 362 (1.00), 428 sh (0.37). MS *m/z* (rel. int.): 420 $[M]^+$ (75), 405 $[M-Me]^+$ (100), 391 $[M-HCO]^+$ (5), 389 $[M-OMe]^+$ (5), 387 $[M-Me-H_2O]^+$ (7), 375 $[M-CO-OH]^+$ (8), 347 $[M-73]^+$ (5), 197 $[A-Me]^+$ (6), 194 $[B_1-CH_2]^+$ (4), 181 $[B_2]^+$ (6), 169 $[A-COMe]^+$ (4), 165 $[B_1-COMe]^+$ (4).

5,7,4′-*Trihydroxy*-3,6,8-*trimethoxyflavone* (**11**). Isolated as bright yellow needles from MeOH, mp 244–245°; R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.28. (Found $[M]^+$, 360.0842. Calc. for $C_{18}H_{16}O_8$ $[M]^+$, 360.0845.) UV λ_{max}^{MeOH} nm (rel. *A*): 280 (1.00), 340 (0.81); λ_{max}^{NaOMe} nm (rel. *A*): 284 (0.94), 336 (0.65), 408 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 282 (1.00), 310 sh (0.65), 366 (0.54); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 280 (1.00), 340 (0.81); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 288 (0.83), 314 (0.76), 366 (1.00), 425 sh (0.47); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 290 (0.82), 312 (0.76), 364 (1.00), 421 sh (0.43). MS *m/z* (rel. int.): 360 $[M]^+$ (96), 359 (10), 345 $[M-Me]^+$ (100), 331 $[M-HCO]^+$ (5), 330 (6), 327 $[M-Me-H_2O]^+$ (8), 317 $[M-COMe]^+$ (6), 315 $[M-CO-OH]^+$ (5), 302 $[M-Me-COMe]^+$ (5), 197 $[A-Me]^+$ (10), 180 $[M]^{2+}$ (7), 169 $[A-COMe]^+$ (7), 134 $[B_1-CH_2]^+$ (9), 121 $[B_2]^+$ (20), 105 $[B_1-COMe]^+$ (8). The foregoing data are in agreement with values for 'sarothrin', a flavonol described in ref. [23].

5,7,8,4′-*Tetrahydroxy*-3-*methoxyflavone* (**13**). Allowed to crystallize from solns of $CHCl_3$–MeOH–HCO_2H (46:3:1) by slow evaporation and obtained as fine greenish yellow needles, mp 301–302°; R_f (silica gel, $CHCl_3$–MeOH, 9:1), 0.36. (Found $[M]^+$, 316.0573. Calc. for $C_{16}H_{12}O_7$; $[M]^+$ 316.0583.) UV λ_{max}^{MeOH} nm (rel. *A*): 228 (0.90), 284 (1.00), 302 (1.00), 325 sh (0.82), 382 sh (0.30); λ_{max}^{NaOMe} nm (rel. *A*): 238 sh (0.86), 292 (1.00), 330 sh (0.74), 366 (0.97), 430 sh (0.46); λ_{max}^{NaOAc} nm (rel. *A*): 233 sh (0.75), 247 sh (0.48), 296 (1.00), 312 sh (0.88), 400 sh (0.20); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 321 sh (0.92), 296 (1.00), 314 sh (0.85), 406 (0.18); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 238 (0.91), 302 (0.95), 330 (1.00), 362 (0.65), 474 (0.20); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 238 (0.87), 290 (0.95), 318 (0.89), 356 (1.00), 430 (0.32). MS *m/z* (rel. int.): 316 $[M]^+$ (100), 315 (52), 299 $[M-OH]^+$ (7), 298 $[M-H_2O]^+$ (9), 287 $[M-HCO]^+$ (9), 286 $[M-30]^+$ (11), 273 $[M-COMe]^+$ (44), 256 $[M-60]^+$ (5), 242 (8), 227 $[M-COMe-CO-H_2O]^+$ (12), 199 $[M-COMe-2CO-H_2O]^+$ (5), 168 $[A]^+$ (23), 158 $[M]^{2+}$ (7), 131 $[B_1-OH]^+$ (36), 121 $[B_2]^+$ (20), 119 $[B_1-HCO]^+$ (16), 111 $[A-CO-HCO]^+$ (15), 105 $[B_1-COMe]^+$ (15).

5,7,8,3′,4′-*Pentahydroxy*-3-*methoxyflavone* (**15**). Obtained as small greenish yellow plates from MeOH, mp 312–314° (dec.) R_f (silica gel, $CHCl_3$–MeOH, 9:1) 0.24. (Found $[M]^+$, 332.0520. Calc. for $C_{16}H_{12}O_8$, $[M]^+$, 332.0532.) UV λ_{max}^{MeOH} nm (rel. *A*): 262 sh (0.83), 282 (1.00), 342 (0.72), 384 sh (0.49); λ_{max}^{NaOMe} nm (rel. *A*): 290 (1.00), 388 (0.83) 415 sh (0.74); λ_{max}^{NaOAc} nm (rel. *A*): 242 sh (0.77), 265 sh (0.69), 286 (1.00), 330 (0.68), 404 sh (0.35); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 275 sh (0.97), 282 (1.00), 318 sh (0.52), 360 (0.64), 408 sh (0.42); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 292 (1.00), 237 sh (0.46), 402 (0.58), 466 sh (0.39); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 268 sh (0.83), 288 (1.00), 312 (0.67), 366 (0.94), 440 sh (0.39). MS *m/z* (rel. int.): 332 $[M]^+$ (100), 331 (66), 315 $[M-OH]^+$ (11), 314 $[M-H_2O]^+$ (9), 303 $[M-HCO]^+$ (10), 302 (11), 289 $[M-COMe]^+$ (46), 288 (5), 268 (8), 227 $[M-COMe-CO_2-H_2O]^+$ (17), 201 (8), 179 (9), 178 (8), 173 (11), 169 (14), 168 $[A]^+$ (26), 166 $[M]^{2+}$ (8), 163 (11), 159 (11), 152 (12), 147 $[B_1-OH]^+$, (24), 137 $[B_2]^+$ (20), 121 $[B_1-COMe]^+$ (15), 119 $[B_1-CO-OH]^+$ (19), 105 (28).

5,7,8,4′-*Tetrahydroxy*-3,3′-*dimethoxyflavone* (**17**). Bronze needles, mp 253–254.5°, were obtained by recrystallization from MeOH; R_f (silica gel, $CHCl_3$–MeOH, 9:1), 0.36. (Found $[M]^+$, 346.0689. Calc. for $C_{17}H_{14}O_8$, $[M]^+$, 346.0688.) UV λ_{max}^{MeOH} nm (rel. *A*): 238 sh (0.87), 260 (0.74), 282 (1.00), 300 sh (0.80), 332 (0.81), 384 sh (0.42); λ_{max}^{NaOMe} nm (rel. *A*): 240 sh (0.85), 272 (0.80), 292 (0.82), 322 sh (0.46), 388 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 237 sh (0.87), 294 (1.00), 328 sh (0.81), 410 sh (0.26); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 237 sh (1.00), 292 (0.76), 320 (0.66), 400 sh (0.18); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 230 (1.00), 252 sh (0.93), 302 (1.00), 336 (0.89), 368 (0.74), 462 (0.29); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 245 sh (0.73), 265 sh (0.79), 288 (1.00), 316 (0.65), 364 (0.68), 424 (0.44). MS *m/z* (rel. int.): 346 $[M]^+$ (100), 345 (40), 331 $[M-Me]^+$ (26), 317 $[M-HCO]^+$ (5), 316 (6), 315 $[M-OMe]^+$ (6), 304 (9), 303 $[M-COMe]^+$ (57), 288 $[M-Me-COMe]^+$ (6), 268 (5), 260 (6), 169 (10), 168 $[A]^+$ (12), 163 $[B_1-Me]^+$ (5), 161 $[B_1-OH]^+$ (10), 159 (6), 157 (6), 151 $[B_2]^+$ (12), 139 (6), 135 $[B_1-COMe]^+$ (15), 121 (6).

5,7,2′,4′-*Tetrahydroxy*-3,6,8,5′-*tetramethoxyflavone* (**19**). Evaporation of solvent from a MeOH soln gave fine yellow crystals, mp 198–199.5° (dec.); R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.29. (Found $[M]^+$ 406.0899. Calc. for $C_{19}H_{18}O_{10}$, $[M]^+$ 406.0899.) UV λ_{max}^{MeOH} nm (rel. *A*): 270 (1.00), 360 (0.56); λ_{max}^{NaOMe} nm (rel. *A*): 282 (1.00), 344 (0.44), 418 (0.90); λ_{max}^{NaOAc} nm (rel. *A*): 276 (1.00), 330 sh (0.46), 374 (0.58); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 260 (1.00), 360 (0.48); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 282 (1.00), 330 (0.28), 408 (0.47); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 280 (1.00), 328 (0.29), 400 (0.47). MS *m/z* (rel. int.): 406 $[M]^+$ (100), 391 $[M-Me]^+$ (40), 389 $[M-OH]^+$ (6), 375 $[M-HCO]^+$ (15), 373 $[M-Me-H_2O]^+$ (6), 360 $[M-OH-HCO]^+$ (5), 345 $[M-61]^+$ (6), 203 $[M]^{2+}$ (7), 197 $[A-Me]^+$ (43), 194 $[B_1]^+$ (57), 167 $[B_2]^+$ (8), 165 $[B_1-HCO]^+$ (6), 151 $[B_1-COMe]^+$ (7).

5,7,4′-*Trihydroxy*-3,6,8,3′-*tetramethoxyflavone* (**2**) [28]. Obtained as bright yellow needles from MeOH, mp 178–180° after first melting with re-solidification at 105°; R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.39. UV λ_{max}^{MeOH} nm (rel. *A*): 262 (0.83), 280 (0.95), 352 (1.00); λ_{max}^{NaOMe} nm (rel. *A*): 284 (0.78), 346 (0.65), 422 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 282 (1.00), 328 (0.65), 368 (0.70); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 260 (0.87), 280 (1.00), 354 (0.83); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 272 (0.81), 288 (0.86), 310 sh (0.54), 378 (1.00), 424 sh (0.63); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 268 (0.72), 290 (0.83), 374 (1.00), 426 sh (0.50). MS *m/z* (rel. int.): 390 $[M]^+$ (81), 375 $[M-Me]^+$ (100), 360 (12), 357 $[M-Me-H_2O]^+$ (8), 347 $[M-COMe]^+$ (5), 345 (14), 197 $[A-Me]^+$ (9), 195 $[M]^{2+}$ (8), 169 $[A-COMe]^+$ (7), 164 $[B_1-CH_2]^+$ (9), 151 $[B_2]^+$ (11), 135 $[B_1-COMe]^+$ (7). The UV data agree well with those reported by Urbatsch *et al.* [28]; however, their MS data and ours are at some variance.

3,5,7,4′-*Tetrahydroxy*-6,8,3′-*trimethoxyflavone* (**4**) (*limocitrol*)

[30, 31]. Limocitrol (**4**) was obtained from MeOH as bright yellow needles, mp 216° after sintering at 207–210° (lit. [31] mp 210–211°); R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.36. UV λ_{max}^{MeOH} nm (rel. *A*): 262 (0.99), 276 (0.88), 315 sh (0.49), 345 sh (0.80), 380 (1.00); λ_{max}^{NaOMe} nm (rel. *A*): 271 sh (0.95), 282 (1.00), 336 (0.57), 434 (0.95); λ_{max}^{NaOAc} nm (rel. *A*): 265 (0.92), 280 (1.00), 330 (0.70), 388 (0.97); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 260 (1.00), 278 sh (0.81), 343 sh (0.70), 378 (0.88); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 272 (1.00), 320 sh (0.23), 375 sh (0.50), 440 (0.96); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 272 (1.00), 318 sh (0.24), 384 (0.65), 440 (0.97). UV spectra were also run in EtOH and were in good agreement with reported values [31]. MS *m/z* (rel. int.): 376 $[M]^+$ (98), 375 (5), 361 $[M-Me]^+$ (100), 347 $[M-HCO]^+$ (10), 346 (12), 343 $[M-Me-H_2O]^+$ (8), 333 $[M-COMe]^+$ (27), 318 $[M-Me-COMe]^+$ (21), 303 (13), 241 (5), 197 $[A-Me]^+$ (4), 188 $[M]^{2+}$ (8), 172 (9), 157 (8), 151 $[B_2]^+$ (13), 137 $[B_1-CH_2]^+$ (6), 126 (5), 121 $[B_1-COMe]^+$ (5).

5,7,*Dihydroxy*-3,8,3′,4′,5′-*pentamethoxyflavone* (**10**) (*conyzatin* [32]. Obtained as fine yellow needles from MeOH–H_2O, mp 207–208°; (lit. [32] mp 209°); R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.51. UV λ_{max}^{MeOH} nm (rel. *A*): 280 (1.00), 320 sh (0.66), 373 sh (0.49); [lit. [32] λ_{max}^{MeOH} nm (rel. *A*): 276 (1.00), 314 (0.68), 362 (0.30), 402 (0.36)]; λ_{max}^{NaOMe} nm (rel. *A*): 286 (1.00), 315 sh (0.47), 392 (0.31); [lit. [32] λ_{max}^{NaOMe} nm: 288, 305, 416]; λ_{max}^{NaOAc} nm (rel. *A*): 286 (1.00), 322 sh (0.52), 372 (0.34); [lit. [32] λ_{max}^{NaOAc} nm: 287, 305, 414]; $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 280 (1.00), 322 sh (0.61), 370 sh (0.46); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 286 (1.00), 314 (0.70), 352 (0.80), 416 (0.48); [lit. [32] $\lambda_{max}^{AlCl_3}$ nm: 286, 317, 355, 400, 421]; $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 288 (1.00), 314 (0.70), 352 (0.82), 416 (0.45). MS *m/z* (rel. int.): 404 $[M]^+$ (68), 403 (7), 389 $[M-Me]^+$ (100), 375 $[M-HCO]^+$ (4), 374 (6), 373 $[M-OMe]^+$ (5), 361 $[M-COMe]^+$ (7), 359 $[M-CO-OH]^+$ (7), 331 (9), 202 $[M]^{2+}$ (5), 195 $[B_2]^+$ (4), 194 $[B_1-CO]^+$ (9), 179 $[B_1-COMe]^+$ (6), 167 $[A-Me]^+$ (6), 165 $[B_1-CO-HCO]^+$ (5); [lit. [32] MS: 404 $[M]^+$, 403 ('intense'), 389 ('intense')].

5,7,4′-*Trihydroxy*-3,8-*dimethoxyflavone* (**12**) [33]. Isolated as fine yellow needles from MeOH–H_2O, mp 259° after darkening at 248°; (lit. [33] mp 242–244° and 254–256°); R_f (silica gel, $CHCl_3$–MeOH, 9:1) 0.48. UV λ_{max}^{MeOH} nm (rel. *A*): 276 (1.00), 308 (0.68), 330 sh (0.67), 367 sh (0.59); λ_{max}^{NaOMe} nm (rel. *A*): 284 (1.00), 332 (0.53), 406 (0.88); λ_{max}^{NaOAc} nm (rel. *A*): 282 (1.00), 320 sh (0.66), 366 (0.47); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 276 (1.00), 324 (0.65), 356 (0.60); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 284 (1.00), 312 (0.74), 354 (0.89), 412 (0.57); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 284 (1.00), 312 (0.73), 352 (0.92), 414 (0.54). UV spectra obtained in EtOH agreed well with recently reported [33] data for **16**. MS *m/z* (rel. int.): 303 $[M]^+$ (57), 329 (10), 315 $[M-Me]^+$ (100), 301 $[M-HCO]^+$ (5), 297 $[M-Me-H_2O]^+$ (4), 287 $[M-COMe]^+$ (9), 272 $[M-Me-COMe]^+$ (12), 167 $[A-Me]^+$ (5), 139 $[A-COMe]^+$ (12), 134 $[B_1-CH_2]^+$ (10), 131 (5), 121 $[B_2]^+$ (17).

5,7,4′-*Trihydroxy*-3,6,3′-*trimethoxyflavone* (**14**) (*jaceidin*) [34]. Jaceidin, after purification by prep. TLC could not be crystallized and was obtained as a dark yellow gum; (lit. [34] mp 127–133°); R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.43. UV λ_{max}^{MeOH} nm (rel. *A*): 258 (0.85), 272 (0.84), 354 (1.00); λ_{max}^{NaOMe} nm (rel. *A*): 274 (0.66), 338 (0.40), 412 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 274 (1.00), 330 sh (0.74), 362 (0.94); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 258 (0.91), 272 (0.83), 352 (1.00); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 268 (0.92), 285 sh (0.82), 305 sh (0.46), 378 (1.00), 414 sh (0.60); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 268 (0.86), 283 sh (0.77), 305 sh (0.48), 376 (1.00), 408 sh (0.77); the UV data agree well with published spectra of jaceidin [7]. MS *m/z* (rel. int.): 360 $[M]^+$ (100), 359 (22), 345 $[M-Me]^+$ (58), 342 $[M-H_2O]^+$ (14), 331 $[M-HCO]^+$ (6), 327 $[M-Me-H_2O]^+$ (10), 317 $[M-COMe]^+$ (28), 299 $[M-H_2O-COMe]^+$ (14), 274 (9), 227 (6), 167 $[A-Me]^+$ (7), 164 $[B_1CH_2]^+$ (7), 151 $[B_2]^+$ (131), 135 $[B_1-COMe]^+$ (5), the MS data agree well with published [35] data for jaceidin. The ^1H NMR data in Table 1 agree well with the published partial spectrum [36] of jaceidin.

5,7,3′,4′-*Tetrahydroxy*-3,8-*dimethoxyflavone* (**16**) [37]. Obtained from MeOH–H_2O as fine light yellow needles, mp 305–306°; (lit. [37] mp 301–303°); R_f (silica gel, $CHCl_3$–MeOH, 9:1) 0.40. UV λ_{max}^{MeOH} nm (rel. *A*): 260 sh (0.95), 274 (1.00), 335 sh (0.56), 366 (0.74); λ_{max}^{NaOMe} nm (rel. *A*): 280 (1.00), 330 sh (0.35), 416 (0.88); λ_{max}^{NaOAc} nm (rel. *A*): 278 (1.00), 332 (0.52), 380 (0.61); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 268 (1.00), 300 sh (0.30), 386 (0.74); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 282 (1.00), 306 sh (0.33), 344 (0.21), 448 (0.96); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 272 sh (0.98), 282 (1.00), 306 (0.51), 366 (0.76), 416 (0.66). The UV data are in agreement with data (MeOH, $AlCl_3$, $AlCl_3$–HCl only) recently reported for **16** [38]. Some spectra were run in EtOH in order to compare with previously reported [37] data and good agreement was found; MS *m/z* (rel. int.): 346 $[M]^+$ (60), 345 (13), 331 $[M-Me]^+$ (100), 316 (4), 303 $[M-COMe]^+$ (7), 288 $[M-Me-COMe]^+$ (10), 167 $[A-Me]^+$ (5), 150 $[B_1-CH_2]^+$ (9), 139 $[A-COMe]^+$ (10), 137 $[B_2]^+$ (14), 121 $[B_1-COMe]^+$ (9).

5,7,4′-*Trihydroxy*-3,8,3′-*drimethoxyflavone* (**18**) [33]. Obtained as yellow oragne prisms from MeOH, mp 217–218°; (lit. [33] mp 215–217°); R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.38. UV λ_{max}^{MeOH} nm (rel. *A*): 260 (0.90), 276 (1.00), 335 sh (0.73), 360 (0.83); λ_{max}^{NaOMe} nm (rel. *A*): 273 sh (0.88), 284 (0.91), 332 sh (0.34), 420 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 280 (0.68), 286 sh (1.00), 330 (0.64), 366 (0.61); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 260 sh (0.85), 276 (1.00), 334 sh (0.71), 360 (0.83); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 274 sh (0.95), 284 (1.00), 310 sh (0.45), 368 (0.83), 420 (0.69); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 269 sh (0.86), 284 (1.00), 310 sh (0.48), 364 (0.87), 418 (0.66). Spectra obtained in EtOH agreed well with lit. [33] data for **18**. MS *m/z* (rel. int.): 360 $[M]^+$ (50), 359 (7), 345 $[M-Me]^+$ (100), 317 $[M-COMe]^+$ (6), 302 $[M-Me-COMe]^+$ (10), 167 $[A-Me]^+$ (6), 164 $[B_1-CH_2]^+$ (7), 151 $[B_2]^+$ (9), 139 $[A-COMe]^+$ (6), 135 $[B_1-COMe]^+$ (6).

5,4′-*Dihydroxy*-3,6,7,8,3′-*pentamethoxyflavone* (**1**) [39]. Isolated as deep yellow needles from MeOH–H_2O, mp 169–171°; (lit. [39] mp 160–162°; lit. [25] mp 174–176°); R_f (silica gel, $CHCl_3$–MeOH, 19:1) 0.65. UV λ_{max}^{MeOH} nm (rel. *A*): 262 (0.92), 280 (0.92), 354 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 270 (0.65), 424 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 262 (0.97), 280 (0.97), 354 (1.00), 440 sh (0.13); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 262 (1.00), 280 (0.96), 354 (0.99); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 272 sh (0.79), 288 (0.82), 312 sh (0.52), 382 (1.00), 430 sh (0.60); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 274 (0.77), 290 (0.82), 310 sh (0.59), 376 (1.00), 426 sh (0.53); these data are in agreement with those recently reported [25] for **1**. MS *m/z* (rel. int.): 404 $[M]^+$ (70), 403 (6), 389 $[M-Me]^+$ (100), 375 $[M-HCO]^+$ (4), 374 (5), 373 $[M-OMe]^+$ (6), 361 $[M-COMe]^+$ (4), 359 $[M-OH-CO]^+$ (7), 331 (4), 211 $[A-Me]^+$ (6), 202 $[M]^{2+}$ (6), 183 $[A-COMe]^+$ (5), 151 $[B_2]^+$ (9); although the relative intensities are different, similar ions have been recently reported for **1** [40].

5,7,3′,4′-*Tetrahydroxy*-3-*methoxyflavone* (**20**) (3-O-*methylquercetin*) [41]. Recrystallized from MeOH–H_2O as deep yellow fine needles, mp 280–282° (lit. [41] mp 273–275°; lit. [42] mp 282–284°); R_f (silica gel, $CHCl_3$–MeOH, 9:1) 0.40. UV λ_{max}^{MeOH} nm (rel. *A*): 258 (1.00), 271 sh (0.86), 295 sh (0.37), 360 (0.93); λ_{max}^{NaOMe} nm (rel. *A*): 272 (0.95), 330 (0.39), 406 (1.00); λ_{max}^{NaOAc} nm (rel. *A*): 260 (1.00), 268 (0.99), 326 sh (0.61), 364 (0.90); $\lambda_{max}^{NaOAc-H_3BO_3}$ nm (rel. *A*): 262 (1.00), 295 sh (0.30), 378 (0.82); $\lambda_{max}^{AlCl_3}$ nm (rel. *A*): 278 (0.91), 302 sh (0.27), 336 (0.19), 438 (1.00); $\lambda_{max}^{AlCl_3-HCl}$ nm (rel. *A*): 270 (1.00), 302 (0.42), 370 (0.68), 404 (0.82); these data are in close agreement with reported [7, 38, 42] UV data for **20** with the exception of the $AlCl_3$ values reported by Herz *et al.* [42] which, in fact, resemble the $AlCl_3$–HCl spectra. MS *m/z* (rel. int.): 316 $[M]^+$ (100), 315 (71), 301 $[M-Me]^+$ (4), 299 $[M-OH]^+$ (11), 298 $[M-H_2O]^+$ (16), 287 $[M-HCO]^+$

(15), 286 (9), 285 $[M-OMe]^+$ (6), 273 $[M-COMe]^+$ (37), 203 (7), 153 $[A+H]^+$ (23), 144 (10), 137 $[B_2]^+$ (21), 121 $[B_1-COMe]^+$ (11).

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