*Phytochemistry*, Vol. 26, No. 7, pp. 2079–2083, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00


# FOUR 6-HYDROXYFLAVONOLS FROM *BLUMEA MALCOMII**

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(*Revised received* 29 *November* 1986)

**Key Word Index**—*Blumea malcolmii*; Compositae; 6-hydroxyflavonols; 5-methoxyflavonols; 2'-methoxyflavonols.

**Abstract**—Four new 6-hydroxyflavonols have been isolated from *Blumea malcolmii* and identified as 6-hydroxy-3,5,7,4'-tetramethoxyflavone, 6,2',5'-trihydroxy-3,5,7-trimethoxyflavone, 6,5'-dihydroxy-3,5,7,2'-tetramethoxyflavone and 6-hydroxy-3,5,7,2',5'-pentamethoxyflavone, by spectral data coupled with some chemical correlations.

## INTRODUCTION

*Blumea malcolmii* Hook. f. grows as a weed in the hilly areas of Western ghats of Maharastra, India. No chemical work has been reported on this plant although other *Blumea* species are reported to contain monoterpenes [1, 2], sesquiterpenes [2, 7], acetylenic compounds [3] and flavonoids [4–6].

## RESULTS AND DISCUSSION

As part of our work on bioactive principles in members of the Compositae we now report the isolation and characterization of four new flavonols (**1a**, **2a**–**2c**) from aerial parts of *B. malcolmii*.

*6-Hydroxy-3,5,7,4'-tetramethoxyflavone.*

Compound **1a**, $C_{19}H_{18}O_7$, $[M]^+$ 358, exhibited signals in its $^1H$ NMR spectrum (Table 1) assignable to four methoxy groups ($\delta$3.56–3.97), a singlet (1H) at $\delta$6.5 and an $A_2B_2$ quartet centered at $\delta$7.06 and $\delta$8.09. These data coupled with the UV spectral data (Table 2) and the relative intensities of the ion peaks in the mass spectrum (Table 3) particularly the base peak assignable to $B_2$ fragment ion indicated that **1a** is a flavonol [8–10]. The position of the hydroxyl and methoxyl groups were established as follows. The $A_2B_2$ pattern in the $^1H$ NMR suggested that C-4' is substituted and similarly the absence of a signal around $\delta$8.0 other than the two aromatic protons of the above $A_2B_2$ system revealed that C-5 is also substituted [8, 9]. The characteristic shifts with sodium methoxide, aluminium trichloride and sodium acetate in the UV spectrum (Table 2) ruled out the presence of hydroxyl groups at C-3, C-4', C-5 and C-7 [8, 9] and therefore C-3, C-4' and C-5 must be substituted with methoxyl groups. The position of the hydroxyl group at C-6 was deduced by shift reagent studies in the $^1H$ NMR of **1a** when a shift of $\delta$1.08 (in S-value) of the signal at $\delta$6.50 was observed indicating the presence of a proton at C-8 [9].

Table 1. $^1H$ NMR data for flavonol methyl ethers **1a** and **1b**

| Proton | 1a | 1b |
|---|---|---|
| H-8 | 6.50 *s* | 6.764 *s* |
| H-2',6' | 8.095 *d* (9) | 8.077 *d* (9) |
| H-3',5' | 7.066 *d* (9) | 7.0212 *d* (9) |
| OMe | 3.56, 3.91 3.93, 3.97 | 3.871, 3.890, 3.922, 3.974, 4.023 |

90 MHz, $\delta$-scale in ppm, TMS as int. standard; Figures in parentheses denote coupling constants

The mass spectral data (Table 3) with ion peaks assignable to $[M-1]^+$, $[M-1-18]^+$, $[B_2]^+$, $[B_2-28]^+$, $[A_1-31]^+$, $[A_1-43]^+$, etc. were in full agreement with the structure proposed for **1a** [10]. $[M]^+$ or $[M-15]^+$ ion peaks are generally observed as the most intense peaks in flavonols [9]. However, in **1a** the base peak was assigned to the $B_2$ fragment ion and its intensity may be attributed to the presence of a 6-hydroxyl group which stabilizes it in preference to $A_1$. This was evident by the change in the ratio of intensities in the ion peaks of $A_1$ and $B_2$ and related fragments in the mass spectrum of the 6-methoxylated compound **1b** obtained by methylation of **1a**. Compound **1b** was found to be identical with the methylated product of penduletin [11, 12] and **1a** has been synthesized previously [12].

With a view to study the origin of the $[M-1]^+$ ion in **1a**, the hydroxyl group at C-6 was deuterated by $D_2O$ exchange to obtain a mixture of deuterated (55%)† and undeuterated (45%) **1a**. The ratio of $[M_{Deut}-1]^+$:$[M_{Deut}-2]^+$ when compared with the ratio of $[M]^+$:$[M-1]$ revealed that $[M-1]^+$ ion originates from $[M]^+$ ion and not from $[M_{Deut}]^+$ ion as $[M_{Deut}-2]^+$. This observation indicates that the proton loss is not from the hydroxyl group at C-6 but could be an aromatic proton, as postulated by Schmid *et al.* [10].

*NCL Communication No. 4136.

†This was calculated from the ratio of ion peaks $(M_{Deut}-15)^+$:$[M-15]^+$, $[M_{Deut}-43]^+$:$[M-43]^+$, $(A_{1\,Deut}-31)^+$:$A_1-31)^+$ and $(A_{1\,Deut}-43)^+$:$(A_1-43)^+$.

The mass spectral studies of deuterated **1a** provided additional support for the presence of a hydroxyl group in ring A by the observation of ion peaks corresponding to $(A_{1\,Deut}-15)^+$, $(A_{1\,Deut}-31)^+$, $(A_{1\,Deut}-43)^+$, etc. (Table 4).

Table 2. UV spectral data for 6-hydroxyflavonols **1a**, **2a–2c** $\lambda_{max}$ (nm)

| | Flavonols | MeOH Band II/ Band I | NaOMe | $AlCl_3$ | $AlCl_3$ + HCl | NaOAc |
|---|---|---|---|---|---|---|
| **1a** | 6-OH- | 255 272 | 295 | 270 | 278 | 260 270 |
| | 3,5,7,4'- | (3.61)* (3.64) | (3.71) | | | |
| | OMe | 345 | 385 | 355 | 360 | 345 |
| | | (3.57) | (3.25) | | | |
| **2a** | 6,2',5'-OH- | 260 | 280 | 280 | 263 | 262 |
| | 3,5,7-OMe | (3.65) | (3.57) | | | |
| | | 355 | 400 | 420 | 370 | 365 |
| | | (3.45) | (3.32) | | | |
| **2b** | 6,5'-OH | 255 275 | 255 280 | 270 285 | 270 285 | 260 270 |
| | 3,5,7,2'- | (3.38) (3.35) | (3.34) (3.44) | | | |
| | OMe | 350 | 390 | 370 | 370 | 355 |
| | | (3.2) | (3.16) | | | |
| **2c** | 6-OH- | 257 267 | 300 340 | 270 290 | 270 290 | 260 280 |
| | 3,5,7,2', | (3.55) (3.49) | (3.63) (3.43) | | | |
| | 5'-OMe | 355 | 400 | 375 | 375 | 356 |
| | | (3.60) | (3.04) | | | |

*Figures in parentheses denote log $\varepsilon$.

Table 3. MS data for flavonol methyl ethers **1a**, **1b**, **2a–2c**, **2e** and deuterated **1a** and **2c**

| Flavonol methyl ethers | $[M]^+$ | $[M-1]^+$ | $[M-1-18]^+$ | $[M-15]^+$ | $[M-43]^+$ |
|---|---|---|---|---|---|
| 6-OH 3,5,7,4'- OMe **1a** | 358 (67.42) | 357 (46.34) | 339 (28.48) | 343 (47.13) | 315 (34.89) |
| 3,5,6,7, 4'-OMe **1b** | 372 (7.89) | 371 (4.37) | | 357 (16.16) | |
| 6-OD + 6-OH | 359 (85) | 357 (44.91) | 340 (32.10) | 344 (64.95) | 316 (35.93) |
| 3,5,7,4'- OMe | 358 (100) | | 343 (57.60) | 339 (54.33) | 315 (33.92) |
| 6,2',5'- OH 3,5,7-OMe **2a** | 360 (47.89) | 359 (25.87) | 341 (18.67) | 345 (27.66) | 317 (10.30) |
| 6,5'-OH 3,5,7,2'- OMe **2b** | 374 (68.74) | 373 (37.15) | 353 (19.86) | 359 (40.3) | 331 (12.21) |
| 6-OH 3,5,7,2', 5'-OMe **2c** | 388 (76.87) | 387 (39.75) | 369 (21.88) | 373 (66) | 345 (15.69) |
| 3,5,6,7,2' 5'-OMe **2e** | 402 (5.59) | 401 (3.26) | | 387 (11.32) | |
| 6-OD + 6-OH | 389 (79.3) | 387 (21.25) | 370 (16.56) | 374 (72.15) | 346 (16.54) |
| 3,5,7,2', 5'-OMe | 388 (80.75) | | 369 (33.80) | 373 (40.38) | 345 (13.27) |

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

Table 3. cont.

| Flavonol methyl ethers | $[A_1-15]^+$ | $[A_1-31]^+$ | $[A_1-43]^+$ | $[A_1-18-29]$ | $[B_2]^+$ | $[B_2-28]^+$ |
|---|---|---|---|---|---|---|
| 6-OH 3,5,7,4'-OMe **1a** | 181 (28.14) | 165 (86.15) | 153 (45.03) | 149 (30.6) | 135 (100) | 107 (17.81) |
| 3,5,6,7, 4'-OMe **1b** | 195 (7.88) | 179 (3.86) | 167 (41.44) | | 135 (49.82) | 107 (14.78) |
| 6-OD + 6-OH | 182 (22.80) | 166 (49.12) | 154 (31.53) | 150 (35.18) | 135 | 107 |
| 3,5,7,4'-OMe | 181 (21.61) | 165 (43.34) | 153 (25.19) | 149 (49.75) | (68) | (9.42) |
| 6.2',5'-OH 3,5,7-OMe **2a** | 181 (27.84) | 165 (6.26) | 153 (58.35) | 149 (27.80) | 137 (93.76) | 109 (61.74) |
| 6,5'-OH 3,5,7,2'-OMe **2b** | 181 (29.53) | 165 (26.71) | 153 (73.21) | 149 (79.37) | 151 (100) | 123 (33.82) |
| 6-OH 3,5,7,2', 5'-OMe **2c** | 181 (23.44) | 165 (100) | 153 (56.5) | 149 (70) | 165 (100) | 137 (23.75) |
| 3,5,6,7,2' 5'-OMe **2e** | 195 (13.86) | 179 (7.69) | 167 (43.81) | | 165 (50.47) | 137 (17) |
| 6-OD + 6-OH | 182 (17.84) | 166 (15.69) | 154 (27.27) | 150 (21.59) | 165 (100) | 137 (12.94) |
| 3,5,7,2', 5'-OMe | 181 (36.41) | 165 (100) | 153 (23.38) | 149 (59.48) | | |

*6,2',5'-Trihydroxy-3,5,7-trimethoxyflavone* **2a**

Compound **2a**, $C_{18}H_{16}O_8$ $[M]^+$ 360 showed the presence of three methoxyl groups ($\delta$4.04–4.12), a singlet at $\delta$6.62 (1 H), a doublet at $\delta$7.49 and a quartet at $\delta$7.95 in its $^1$H NMR spectrum. This data coupled with its UV (Table 1) and mass spectral data (Table 3) suggested that **2a** is closely related to **1a**. Furthermore, the UV and mass spectral fragmentation pattern of **2a** showed that the substitution in ring A and C was identical with that of **1a**. However, the peak assignable to fragment $B_2$ showed two hydroxyl groups in ring B. That **2a** has three hydroxyl groups was confirmed by acetylation to the triacetate **2d**. The sodium methoxide and aluminium trichloride induced shifts in the UV spectrum of **2a** ruled out the presence of an hydroxyl at C-4' and the *ortho* relationship of both the hydroxyl groups respectively [8, 9]. The $^1$H NMR pattern of B-ring protons (Table 4), a doublet ($J$ = 9 Hz, *ortho* coupling) and a quarter ($J$ = 9, 2 Hz,

Table 4. $^1$H NMR data for flavonol methyl ethers **2a–2f**

| Proton | **2a*** | **2b** | **2c** | **2d** | **2e** | **2f** |
|---|---|---|---|---|---|---|
| H-8 | 6.62 *s* | 6.52 *s* | 6.512 *s* | 6.86 *s* | 6.757 *s* | 6.865 *s* |
| H-3' | 7.49 *d* (9) | 7.05 *d* (9) | 6.999 *d* (9) | 7.34 *d* (9) | 7.108 *d* (9) | 7.075 *d* (9) |
| H-4',6' | 7.95 *q* (9, 2) | 7.80 *q* (9, 2) | 7.738 *q* (9, 2) | 8.04 *q* (9, 2) | 7.731 *q* (9, 2) | 8.052 *q* (9, 2) |
| OMe | 4.04 (6H) 4.12 | 3.88 3.94, 3.98, 4.01 (9H) | 3.875, 3.932, 3.978 | 3.829, 3.850, 4.0 | 3.873, 3.927, 3.976 (9H) 4.025 | 3.809, 3.837, 3.922, 3.991 |
| OAc | | | | 2.33, 2.34, 2.51 | | 2.360, 2.517 |

90 MHz, $\delta$-scale in ppm, TMS as int. standard. Figures in parentheses denote coupling constants.

*$C_5D_5N$ 80 MHz.

*ortho–meta* coupling) requires the hydroxyl groups to be placed at the C-2′ and C-5′ positions. From the above data **2a** is identified as 6,2′,5′-trihydroxy-3,5,7-trimethoxyflavone. **2a** on methylation gave a pentamethoxy compound identical in all respects (IR, $^1$H NMR, mp and mmp) to **2c**.

### 6,5′-*Dihydroxy*-3,5,7,2′-*tetramethoxyflavone* **2b**

Compound **2b**, isolated as a crystalline compound, $C_{19}H_{18}O_8$ $[M]^+$ 374 gave UV (Table 2) and $^1$H NMR spectra (Table 4 similar to those of **2a** except that four methoxyl groups were revealed in the $^1$H NMR spectrum. The molecular formula indicated that one of the hydroxyl groups in **2a** is methylated in **2b** and further, that the methylated hydroxyl group was attached to ring B was evident from the extra 14 mass units in the $B_2$ fragment ion in the mass spectrum of **2b**. But for this difference in the substitution in ring B the mass spectral fragmentation showed a similar substitution pattern for rings A and C to **2a**. Further, the correlation between **2b** and **2a** fixed the positions of hydroxyl and methoxyl groups in ring B at C-2′/C-5′ or C-5′/C-2′. However, the exact positions of these groups were deduced by the chemical shifts of ring B protons in the acetate **2f** prepared from **2b**.

In the $^1$H NMR spectrum of **2f** a down-field shift of 22 Hz in the quartet (*ortho–meta* coupling) due to H-4′ and H-6′ protons as compared with a downfield shift of 2.5 Hz due to the H-3′ proton favoured the placement of hydroxyl group at C-5′ and the methoxyl group at C-2′ in **2b**. Thus **2b** can be represented as 6,5′-dihydroxy-3,5,7,2′-tetramethoxyflavone.

### 6-*Hydroxy*-3,5,7,2′,5′-*pentamethoxyflavone* **2c**

Compound **2c** was obtained as a crystalline compound, $C_{20}H_{20}O_8$ $[M]^+$ 388 which had 14 and 28 mass units more than **2b** and **2a**, respectively suggesting it was a monomethyl ether of **2b** and a dimethyl ether of **2a**. This was confirmed by the presence of five methoxyl groups in the $^1$H NMR spectrum; otherwise the UV (Table 2). $^1$H NMR (Table 4) and mass spectral data (Table 3) were very similar to those of **2a** and **2b**. The $B_2$ fragment ion in the mass spectrum of **2c** revealed the presence of two methoxyl groups in ring B. The correlation between **2a** and **2c** established earlier (*vide infra*) fixes the position of these methoxyls at C-2′ and C-5′.

The hydroxyl group at C-6 in **2c** when subjected to $D_2O$ exchange as in the case **1a** afforded a mixture containing deuterated and undeuterated compounds (55:45). The mass spectral fragmentation of this mixture supported our earlier findings that $[M-1]^+$ originated by the loss of aromatic proton [C-6′] and not from the hydroxy group at C-6.

## EXPERIMENTAL

The plant material collected near Lonavala, Maharastra, India, during November 1980. A voucher specimen has been deposited in the N.C.L. Herbarium. Mps are uncorr., UV–visible spectra were recorded in MeOH and the reagent solutions for UV–visible spectra such as NaOAc, NaOMe, $AlCl_3$ and HCl were prepared by standard methods. $^1$H NMR spectra were recorded in $CDCl_3$ unless otherwise stated, with TMS as the int. standard. Mass spectra were recorded on a Finnigan MAT 1020 C double focussing direct inlet system at 70 eV.

*Isolation of compounds* **1a**, **2a–2c**. The powdered plant material of *B. malcolmii* was extracted with $Me_2CO$ to yield an extract (4%), which was chromatographed over silica gel using $Me_2CO$–petrol (60–80°) as the elution gradient. Four broad fractions, A, B, C and D were collected of which fraction A consisted of straight chain compounds and a mixture of sterols.

*Compound* **1a**. Fraction B on repeated chromatography afforded 60 mg of **1a** (0.12% based on the extract); mp 178–180°, Lit. mp 199–200° [12]. Spot appearance: (UV) brown; (UV/$NH_3$) brown (TLC, solvent system; $Me_2CO$–petrol 3:7); UV, $^1$H NMR and MS (Tables 2, 1 and 3 respectively).

*Methylation of* **1a**. Twenty mg of **1a** was refluxed with dimethyl sulphate and dry $K_2CO_3$ in dry $Me_2CO$ for 5 hr to give, after the usual work up, **1b**, mp 151°, Lit. 152° [11], 153–154° [12]; Spot appearance: (UV) light blue, (UV/$NH_3$) light blue; $^1$H NMR (Table 1); for MS see Table 3.

*Compound* **2a**. This was obtained from the most polar fraction D, yield 500 mg (1% of the extract), mp 252° ($Me_2CO$). Spot appearance (UV) brown, (UV/$NH_3$) brown. UV, $^1$H NMR and MS (Tables 2, 4 and 3, respectively).

*Acetylation of* **2a**. **2a** (50 mg) was acetylated using $Ac_2O$ and pyridine at room temp. and the usual work up gave **2d** (30 mg), mp 148–149°, $^1$H NMR (Table 4; MS: *m/z* (rel. int.) 486 $[M]^+$ (5), 442 $[M-42]$ (100), 427 $[M-59]$ (7), 402 (11), 401 (10), 387 (30), 359 (48), 345 (27), 341 (24), 231 (14), 181 (16), 167 (18), 153 (18), 137 (21), 69 (26).

*Methylation of* **2a**. Twenty mg of **2a** was methylated as described earlier to yield **2c** identical in all respects (IR, $^1$H NMR, mp mmp.) with the natural product.

*Compound* **2b**. Fraction C on repeated chromatography gave **2b**, 40 mg (0.08% based on the extract), mp 189–191°; Spot appearance (UV): brown, (UV/$NH_3$) brown. UV, $^1$H NMR and MS (Tables 2, 4 and 3, respectively).

*Acetylation of* **2b**. **2b** (10 mg) was acetylated as described earlier to give after the usual work up **2f**. $^1$H NMR (Table 4).

*Methylation of* **2b**. Twenty mg of **2b** was methylated as described above to give **2e**, mp 138–139°. Spot appearance (UV): light blue; (UV/$NH_3$) light blue.

*Compound* **2c**. This was obtained from fraction C by repeated chromatography, yield 200 mg (0.4% of the extract), mp 166–168°; Spot appearance (UV): brown; (UV/$NH_3$) brown. UV, $^1$H NMR and MS (Tables 2, 4 and 3, respectively).

*Methylation of* **2c**. Twenty mg of **2c** was methylated as described earlier to yield 8 mg of **2e** identical with the methylation product of **2b** in all respects (IR, $^1$H NMR, mp and mmp.). $D_2O$ exchange of 6-OH in **1a** and **2c**: 10 mg of each compound was taken in $CDCl_3$ and shaken with 0.1 ml of $D_2O$ for 5 min. and the $H_2O$ layer separated. To the $CDCl_3$ layer fresh $D_2O$ was added and the process repeated twice, $CDCl_3$ was removed under vacuum and used for MS studies.

*Acknowledgements*—We thank the authorities of the Botanical Survey of India, Pune, for identification of the plant material and Dr P. S. Kulkarni for helpful discussions regarding the interpretation of mass spectral data.

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