Phytochemistry, Vol. 25, No. 9, pp. 2223–2225, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

FLAVANONES FROM *POLYGONUM NEPALENSE**

ANITA RATHORE, S. C. SHARMA and J. S. TANDON

Central Drug Research Institute, Lucknow 226001, India

(*Revised received* 2 *January* 1986)

Key Word Index—*Polygonum nepalense*; Polygonaceae; flavanones.

Abstract—Two new flavonoids from *Polygonum nepalense* have been characterized as 5,4′-dimethoxy-6,7-methylenedioxyflavanone and 5,6,7,2′,3′,4′,5′-heptamethoxyflavanone together with three known compounds, 5,6,7,4′-tetramethoxyflavanone, sitosterol and taraxerone. Their structures were confirmed by ^{13}C and ^{1}H NMR and other spectroscopic methods.

INTRODUCTION

During a screening programme of medicinal plants for various biological activity, *Polygonum nepalense*, a hitherto uninvestigated plant showed spasmolytic activity in its alcohol soluble residue. On further fractionation, the activity was found to be distributed in hexane and chloroform soluble fractions. Three flavanones were isolated.

RESULTS AND DISCUSSION

The major constituent (**3**) $C_{22}H_{26}O_9$ (M^+, 434) gave IR ν_{max}: 1680 (C=O) 1610, 1595, 703 (aromatic) and 1260 cm^{-1} (C–O–C) and absorption in UV at λ_{max} (log ε) 230 (sh), 280 (4.18) and 322 nm (3.65) indicating that it was a highly methoxylated flavanone. The ^{1}H NMR spectrum confirmed the flavanone nucleus by the occurrence of an ABX system centred at δ2.85, 2.92 and 5.65 for H-2 and H-3, together with signals for seven methoxyl functions from δ3.75 to 3.95, the latter being due to a C-5 methoxyl group. Other features of the ^{1}H NMR spectrum of **3** are given in Table 1; RDA fragmentation in MS suggested the presence of three methoxyl groups in ring-A and four in ring-B, with formation of ions at *m/z* 211 and 224. To find out the distribution of methoxyl groups in ring-A, **3** was demethylated by aluminium chloride–chloroform to yield **4**, mp 151–152°; IR ν_{max} cm^{-1}: 3450, 1200 (OH) and 1635 (C=O). The UV spectrum of **4** showed a bathochromic shift of 30 nm with aluminium chloride–HCl suggesting the presence of a C-5 methoxyl with a substituent at C-6 [1]. The substitution at C-6 was further confirmed by the analysis of the ^{1}H NMR in benzene-d_6, where no appreciable chemical shift (0.07 ppm) was observed for the 5-methoxyl [2]. Measuring the Eu(fod)$_3$ shift during the ^{1}H NMR spectrum of **3**, the maximum shift was observed for the 5-methoxyl signal (δ7.46) and the next strongest was the 6-methoxyl (δ5.05). The proton at C-8 was shifted to δ6.80 (0.47 downfield as compared with original values). These observations are in accordance with the structure assigned for **3** in which C-8 is unsubstituted. Furthermore the C-6′ signal at δ6.74 was not affected on addition of excess shift reagent which confirms that C-6′ is free [3].

The ^{13}C NMR spectrum assigned the signals at δ96.36 (*d*) and 137.7 for the C-8 and C-6 nuclei. The ^{13}C NMR values for **3** have been assigned by comparison with various substituted flavanones [4]. It is most likely that the shielding of the C-6′ nucleus which resonates at δ104.63 was due to the *ortho* and *para* methoxyl substituents. Thus the **3** has the structure 5,6,7,2′,3′,4′,5′-heptamethoxyflavanone.

Flavanone 1

The minor constituent **1**, having $C_{18}H_{16}O_6$ (M^+, 328), gave IR ν_{max} cm^{-1}: 1670 (C=O), 1610 (aromatic), 1260 (C–O–C), 935 and 850 and UV λ_{max} (log ε) nm 245 (4.21), 282 (3.97) and 340 (3.51) indicating that **1** was a flavanone. This was further substantiated by the ^{1}H NMR spectrum with an ABX system centred at δ2.83, 3.1 and 5.29 for H-2 and H-3 protons of a flavanone moiety and signals at δ3.76 and 4.03 (each *s*, 3H) in which the later signal is typical of a methoxyl group *peri* to a carbonyl function and *ortho* to an oxygenated substituent [5]. A strong singlet at δ5.89 (2H) was typical of a dioxymethylene group, and a signal at δ6.2 for H-8 and an A_2B_2 system centred at δ7.35 (*d*) and 6.89 (*d*) due to *ortho* coupling. The position of the methylenedioxy group in **1** was assigned by analysis of the ^{1}H NMR in $CDCl_3$, no important shift in the OMe signal at δ4.03 was observed as compared with that measured in C_6D_6 (3.97). Thus the position adjacent to the 5-methoxyl group must be occupied. The MS has clearly shown by the RDA fragmentation pattern that ring-A has one methoxyl group and one dioxymethylene group exhibited by an ion at *m/z* 194 (base peak) and that ring-B has one methoxyl group, *m/z* 134. A literature survey revealed that a very closely related compound **5** [5] has been isolated in which ring-B has a methoxyl group at C-2′ which on comparison was found to be different from **1**. Thus from the above spectral data, compound **1** was assigned as 5,4′-dimethoxy-6,7-methylenedioxyflavanone.

Flavanone 2

Further column chromatography of the hexane fraction yielded compound **2**, $C_{19}H_{20}O_6$, mp 119–120° (ether–hexane); M^+, 344; IR ν_{max} cm^{-1}: 1680 (C=O), 1610, 1580 (aromatic), 1260 (C–O–C). The UV spectrum

*CDRI Communication No. 3761.

was characteristic of a flavanone. The ^{1}H NMR spectrum of **2** displayed an ABX system centred at δ2.85, 3.13 and 5.32 for H-2 and H-3, four methoxyl groups resonated at δ3.84 (6H, *s*) 3.88, 3.92 (each *s*), 6.33 (1H, *s*, H-8) and four aromatic protons as A_2B_2 system centred at δ6.87 and 7.33 (each *d*) due to *ortho* coupling. MS showed the major ion at *m/z* 210 (base peak) and 134, for rings A and B having three and one methoxyl substituents, respectively. The methoxyl group in ring-B is at the C-4′ position, according to the ^{1}H NMR spectrum A_2B_2 system, while the position of the methoxyl groups in ring-A was fixed on conversion of **2** with aluminium chloride–ether into a trimethoxy product **6**; UV λ_{max} 287 and 340 nm (sh), which gave a shift of 25 nm in band II with aluminium chloride–HCl. This suggested a free 5-hydroxyl group with a substituent at C-6. Thus compound **2** is 5,6,7,4′-tetramethoxy flavanone, which was found to be identical with a compound isolated from *Chromolaena arnottiana* (Compositae) [6].

EXPERIMENTAL

All mps are uncorr. MS were recorded at 70 eV; NMR spectra were measured in 400 MHz, EM 360 (60 MHz) and R-32 (90 MHz) using $CDCl_3$ as solvent (unless mentioned), TMS as int. reference. TLC was carried out on silica gel using $CHCl_3$–EtOAc (19:1) as solvent. Flavanones were visualized by spraying with 1% ceric sulphate in 2 N H_2SO_4 and by UV light.

Extraction and separation. Plant material was collected from Mussoorie (U.P.). *Polygonum nepalense* Meissn. syn. *P. alatum* Ham. ex D. Don Var. *nepalense* (Meissn.) Hook. F. was collected and identified by Dr. B. S. Aswal, Botany Division, CDRI, Lucknow and a voucher specimen is deposited in the Medicinal Plant herbarium of the Central Drug Research Institute (Aswal, 11228 CDRI). The plant material was collected in Oct. 1983 during flowering and fruiting. The whole plant was extracted for its chemical investigation. Dry powdered plant (6 kg) was extracted with 95% EtOH (4 × 10 l.) at 40–50° under red. pres. and the concd alcoholic extract (580 g) subsequently extracted with C_6H_{14}, $CHCl_3$ and *n*-BuOH. The concd C_6H_{14}-soluble fraction, was chromatographed over neutral Al_2O_3. Elution with C_6H_{14}–$CHCl_3$ (1:1) gave a viscous mass (850 mg) from which three flavanones (**1**–**3**) were isolated by repeated CC and prep. TLC ($CHCl_3$–EtOAc, 19:1).

5,4′-Dimethoxy-6,7-methylenedioxyflavanone (**1**). Yellow needles (20 mg), mp 135–137° (Et_2O–hexane); IR ν_{max} cm^{-1}: 2910, 1670 (C=O), 1610, 1510, 1465, 1450 (aromatic), 1260 (C–O–C), 1120, 1080, 935, 905, 850; UV λ_{max}^{MeOH} nm (log ε): 220 (4.28), 245 (4.21), 282 (3.97), 340 (3.51); MS *m/z*: 328 $[M]^+$, 285, 221, 194 (base peak), 179, 134; ^{1}H NMR (C_6D_6): δ2.545 (1H, *dd*, *J* = 4, 17 Hz, H-3), 2.715 (1H, *dd*, *J* = 12, 17 Hz, H-3), 3.255, 3.975 (6H, *s*, 4′, 5-OMe), 4.89 (1H, *dd*, *J* = 5, 10 Hz, H-2), 5.021 (2H, *s*–OCH_2O–), 6.26 (1H, *s*, H-8), 6.73 (2H, *d*, *J* = 9 Hz, H-3′,5′), 7.35 (2H, *d*, *J* = 9 Hz, H-2′,6′).

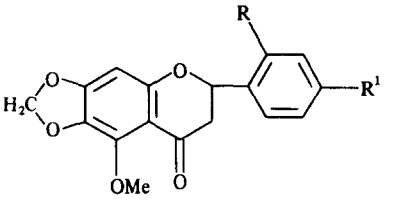
5,6,7,4′-Tetramethoxyflavanone (**2**). Yellow needles (30 mg), mp 119–120° (Et_2O–C_6H_{14}), lit. [0] 129°; IR IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2930, 1680 (C=O), 1610, 1580, 1460 (aromatic), 1418, 1350, 1260 (C–O–C), 1160, 1110, 1020, 830; UV λ_{max}^{MeOH} nm (log ε): 225 (4.29), 275 (3.44), 325 (3.68); MS *m/z* (rel. int.): 344 $[M]^+$ (46.7%), 329, 290 (13.1%), 220 (32.8%), 210 (100%), 195 (83.0%), 167 (38.8%), 134.

5-Hydroxy-6,7,4′-trimethoxyflavanone (**6**). Compound **2** was demethylated with dry $AlCl_3$–K_2CO_3–Et_2O by refluxing for 10 hr under anhydrous conditions, the usual work up and purification by prep. TLC gave **6** as a viscous material which would not crystallize; IR ν_{max}^{neat} cm^{-1}: 3400, 2905, 1630 (C=O),

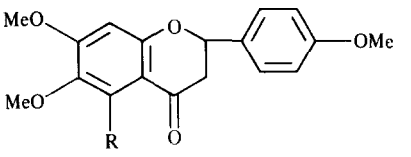
Table 1. ^{1}H NMR (δ) spectra of flavanones **1**–**3***

Flavanone	H-2	H-3	H-8	H-2′,6′	H-3′,5′	OMe
1	5.29 (*dd*, *J* = 5, 10 Hz)	2.83 (1H, *dd*, *J* = 5, 17 Hz), 3.1 (1H, *dd*, *J* = 10, 17 Hz)	6.2 (*s*)	7.35 (2H, *d*, *J* = 9 Hz)	6.89 (2H, *d*, *J* = 9 Hz)	5.89 (2H, *s*) 3.76 (C-4) 4.03 (C-5)
2	5.32 (*dd*, *J* = 5, 10 Hz)	2.85 (1H, *dd*, *J* = 5, 17 Hz), 3.13 (1H, *dd*, *J* = 10, 17 Hz)	6.33 (*s*)	7.33 (2H, *d*, *J* = 9 Hz)	6.87 (2H, *d*, *J* = 9 Hz)	— 3.84 (*s*, 6H), 3.88 (*s*, 3H), 3.92 (*s*, 3H, 5-OMe
3	5.65 (*dd*, *J* = 5, 10 Hz)	2.85 (1H, *dd*, *J* = 5, 17 Hz), 2.92 (1H, *dd*, *J* = 10, 17 Hz)	6.33 (*s*)	6.74 (1H, *s*, 6′H)	—	— 3.80, 3.85 (each *s*, 3H), 3.88 (6H, *s*), 3.90, 3.92, 3.95 (each *s*, 3H)

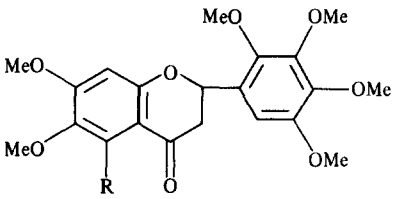
*^{1}H NMR spectra were recorded at R-32 (90 MHz) and 400 MHz. $CDCl_3$ was used as solvent unless indicated otherwise.



1 R = H, R^1 = OMe
5 R = OMe, R^1 = H



2 R = OMe
6 R = OH



3 R = OMe
4 R = OH

1565, 1510, 1445 (aromatic) 1415, 1270 (C–O–C), 1250, 1190, 1155, 1105, 1005, 965, 890, 830; UV λ_{max}^{MeOH} nm: 225, 287, 340; + $AlCl_3$: 312, 395 (sh); + $AlCl_3$–HCl 312, 395 (sh); MS *m/z*: 330 $[M]^+$; ^{1}H NMR: δ2.72 (1H, *dd*, *J* = 4, 12 Hz, H-3), 3.03 (1H, *dd*, *J* = 9, 12 Hz, H-3), 3.77 (6H, *s*, 2 × OMe), 3.8 (3H, *s*, OMe), 5.295 (1H, *dd*, *J* = 5, 12 Hz, H-2), 6.03 (1H, *s*, H-8), 6.89 (2H, *d*, *J* = 9 Hz, H-3′,5′), 7.32 (2H, *d*, *J* = 9 Hz, H-2′,6′), 11.86 (1H, *s*).

5,6,7,2′,3′,4′,5′-Heptamethoxyflavanone (**3**). Yellow needles (80 mg), mp 122–123° (Et_2O–C_6H_{14}); IR ν_{max}^{KBr} cm^{-1}: 2950, 1680 (C=O), 1610, 1595, 1490, 1460, 1420 (aromatic), 1260 (C–O–C), 1210, 1100, 1070, 1020, 860, 760, 703; UV λ_{max}^{MeOH} nm (log ε): 230 (sh) (4.15), 280 (4.18), 322 (3.65); MS *m/z*:434 $[M]^+$, 403 (base peak), 224, 211, 195, 167, 149, 111, 97, 83, 71, 69, 57; ^{1}H NMR (C_6D_6): δ2.63 (1H, *dd*, *J* = 5, 17 Hz, H-3), 2.80 (1H, *dd*, *J* = 10, 17 Hz, H-3), 3.1, 3.37, 3.5 (each s, 3 × OMe), 3.57 (*s*, 2 × OMe), 3.66 (*s*, 3H), 3.88 (*s*, 3H, 5–OMe), 5.65 (1H, *dd*, *J* = 5, 12 Hz, H-2), 6.07 (1H, *s*, H-8), 6.77 (1H, *s*, H-6′); Eu $(fod)_3$: on addition of 2,4,12 and 18 mg of Eu$(fod)_3$ in 40 mg of **3**, the following maximum shifts were observed with 18 mg of Eu$(fod)_3$:5-OMe (δ4.35, 4.75, 6.35, 7.46), 6-OMe (δ3.89, 4.05, 4.63, 5.05), 8-H (δ6.3, 6.35, 6.57, 6.80) respectively. Other signals were at their original positions. ^{13}C NMR: δ74.47 (2, *d*), 45.13 (3, *t*, 189.26 (4), 154.36 (5), 137.7 (6), 159.86 (7), 96.36 (8, *d*), 159.34 (8a), 109,32 (4a), 126.37 (1′), 149.84 (2′), 143.55 (3′)*, 146.96 (4′), 144.84 (5′)*, 104.63 (6′, *d*), 56.39 (5-OMe, *q*), 61.18 (6-OMe, *q*), 56.00 (7-OMe, *q*), 61.51 (2′,-OMe, *q*), 61.00 (3′-OMe, *q*), 61.42 (4′-OMe, *q*), 60.95 (5′-OMe, *q*); (*values are interchangeable).

5-Hydroxy-6,7,2′,3′,4′,5′-hexamethoxyflavanone (**4**). Compound **3** was refluxed with $AlCl_3$–$CHCl_3$ for 8–10 hr and the reaction product worked up and purified by prep. TLC to give **4**, mp 151–152°; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2905, 1635 (C=O), 1560, 1480, 1445, 1405 (aromatic), 1200, 1105, 1060, 955; UV λ_{max}^{MeOH} nm (log ε): 280 (4.28), 330 (sh) (3.86); + $AlCl_3$: 315 (4.27), 380 (sh); + $AlCl_3$–HCl: 310 (4.83), 380 (sh); MS *m/z* (rel. int): 420 (54.7%), 389 (100%), 224 ((81.8%), 208 (40.3%), 195, 180, $C_{21}H_{24}O_9$; ^{1}H NMR: δ2.77 (1H, *dd*, *J* = 4, 16 Hz, H-3), 3.05 (1H, *dd*, *J* = 12, 16 Hz, H-3), 3.88 (2 × OMe), 3.90 (2 × OMe), 3.92, 3.96 (each s, 2 × OMe), 5.72 (2H, *dd*, *J* = 5, 10 Hz, H-2), 6.12 (1H, *s*, H-8) and 6.82 (1H, *s*, H-6′).

Acknowledgement—The authors are grateful to Dr. B. S. Aswal, Scientist, Botany Division for the collection and identification of the plant material.

REFERENCES

1. Mabry, T. J. and Markham, K. R. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds). Chapman & Hall, London.
2. Wilson, R. G., Bowie, J. H. and William, D. H. (1968) *Tetrahedron* **24**, 1407.
3. Okigawa, M., Kawano, N., Rahman, W. and Dhar, M. M. (1972) *Tetrahedron Letters* 4125.
4. Agarwal, P. K. and Rastogi, R. P. (1981) *Heterocycles* **16**, 2181.
5. Geigert, J., Stermitz, F. R., Johnson, G., Maag, D. D. and Johnson, D. K. (1973) *Tetrahedron* **29**, 2703.
6. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1979) *Phytochemistry* **18**, 1177.