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A BIFLAVONE FROM *OCHNA PUMILA*

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Key Word Index—*Ochna pumila*; Ochnaceae; 7″-*O*-methyl tetrahydroamentoflavone; 7″-*O*-methyl ochnaflavone; ochnaflavone; tetrahydroamentoflavone.

Abstract—7″-*O*-Methyl tetrahydroamentoflavone, together with 7″-*O*-methyl ochnaflavone, ochnaflavone and tetrahydroamentoflavone, has been isolated from the leaves of *Ochna pumila*. The isolation of tetrahydroamentoflavone and 7″-*O*-methyl tetrahydroamentoflavone from *O. pumila* constitutes the first report of their occurrence as a new series of biflavanones.

INTRODUCTION

Ochnaflavone and its mono- and dimethyl ethers have been reported from the leaves of *Ochna squarrosa* L. whereas only ochnaflavone has been reported from *O. atropurpurea* DC. Ochnaflavone thus appeared to be the characteristic biflavone of the genus *Ochna* and a useful taxonomic marker. To examine further the latter proposition, another species of the genus, *O. pumila*, was chemically examined for its flavonoid constituents.

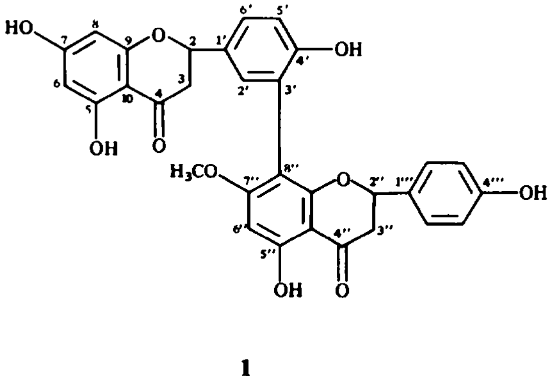
O. pumila, commonly known as 'champa baha', is reported to possess many medicinal properties [1–3]. The root of the plant is used as an antidote to snake bites; its decoction is given for menstrual complaints and for consumption and asthma. It is also reported to be used in epilepsy by Mundas. The leaves are said to be used as a 'poultice' for the treatment of lumbago and ulcers. It is also alleged to have antitubercular activities.

RESULTS AND DISCUSSION

The mixture of biflavonoids obtained on column chromatography of the acetone extract of dried leaves of *O. pumila* was separated by preparative TLC into four fractions: 1, 2, 3 and 4 in order of increasing R_f. The components of fractions 1 and 2 were characterized as ochnaflavone and 7″-*O*-methyl ochnaflavone in our previous work [4].

Fraction 3 gave a positive flavonoid colour test with zinc–hydrochloric acid. Complete methylation with dimethyl sulphate indicated a mixture of the pentamethyl ether of ochnaflavone (minor) and the methyl ether of a biflavonoid (major) which was different from the methyl ethers of known biflavones. Although the shade of the methyl ether of this biflavonid seemed to be identical with that of the pentamethyl ether of ochnaflavone or hinokiflavone under UV light, its R_f value (0.48) was higher than that of ochnaflavone pentamethyl ether (R_f 0.44) and lower than that of hinokiflavone pentamethyl ether (R_f 0.52). Fraction 3 being a mixture was therefore subjected to CCD separation (pH 9.5). After 225 transfers, it yielded only one component, the ^{1}H NMR spectrum of the acetate of which showed the presence of six acetoxy groups and a poorly developed peak at δ4.5 characteristic of biflavanones. The compound present in fraction 3 was therefore expected to be a hexahydroxybiflavanone. It was dehydrogenated by I_2–DMSO/H_2SO_4 reagent [5]. The dehydrogenation product on methylation and TLC examination was found to be the hexamethyl ether of amentoflavone. It was confirmed as 5,7,4′,5″,7″,4‴-hexahydroxy-(3′,8″)biflavone by comparing the physical data of its acetate and methyl ether derivatives with those of authentic samples. The major component of fraction 3 was therefore confirmed as 5,7,4′,5″,7″,4‴-hexahydroxy-2,3,2″,3″-tetrahydroamentoflavone [6].

Fraction 4, on complete methylation and TLC examination, was determined to be a mixture of the methyl ether of the biflavonoid in fraction 3 and ochnaflavone pentamethyl ether. HPLC gave two components, the first of which could not be investigated further due to its low yield. The ^{1}H NMR spectrum of the acetate of the second component (**1**) showed the presence of five acetoxyls and one methoxyl group. A multiplet at δ3.20–3.01 for two H-3 protons and two H-3″ protons together with a double doublet at δ5.45 for H-2 and H-2″ protons indicated a tetrahydrobiflavanone structure for **1**. A multiplet at δ7.96–7.83 for H-2′,6′ protons supported the above observation. The ^{1}H NMR spectrum showed ABX and A_2B_2 systems associated with rings IB and IIB substituted at positions C-3′,C-4′ and C-4‴, respectively. ^{1}H NMR



1

Table 1. ^{1}H NMR data of the pentaacetate of compound **1**, hexahydroxytetrahydroamentoflavone, and 7-*O*-methyl-and 7″-*O*-methyl amentoflavone pentaacetate (100 MHz, $CDCl_3$, TMS as internal standard)

H	1-Acetate	Hexahydroxytetra-hydroamentoflavone	7-*O*-Methyl amentoflavone pentaacetate	7″-*O*-Methyl amentoflavone pentaacetate
8	6.68 (*d*, 1H)	5.92 (*s*, 1H)	6.78 (*d*, 1H)	7.22 (*d*, 1H)
6	6.30 (*d*, 1H)	5.92 (*s*, 1H)	6.98 (*d*, 1H)	6.82 (*d*, 1H)
6″	6.73 (*s*, 1H)	6.08 (*s*, 1H)	7.00 (*s*, 1H)	6.75 (*s*, 1H)
2′ 6′	7.96–7.83 (*m*, 2H)	7.30–7.15 (*m*, 2H)	8.04 (*d*, 1H) 7.91 (*q*, 1H)	7.98 (*q*, 2H)
5′	7.20 (*d*, 1H)	6.85 (*d*, 1H)	7.52 (*d*, 1H)	7.44 (*d*, 1H)
2‴,6‴	7.66 (*d*, 2H)	7.03 (*d*, 2H)	7.48 (*d*, 2H)	7.50 (*d*, 2H)
3‴,5‴	7.03 (*d*, 2H)	6.71 (*d*, 2H)	7.94 (*d*, 2H)	7.04 (*d*, 2H)
3,3″	3.20–3.01 (*m*, 4H)	3.55–2.60 (*m*, 4H)	6.64 (*s*, 2H)	6.64 (*s*, 2H)
2,2″	5.45 (*dd*, 2H)	5.34 (*dd*, 2H)		
7,4′-Ac	2.86, 2.82 (*s*, 6H)	−8.1 to 9.0† (*m*, 4H)	(3.62),* 2.05 (*s*, 3H each)	2.23, 2.05 (*s*, 6H)
7″4‴-Ac	(3.91),* 3.83 (*s*, 3H each)		3.10, 2.15 (*s*, 3H each)	(3.91),* 2.15 (*s*, 3H each)
5,5″-Ac	2.88, 2.90 (*s*, 6H)	−7.82†, −7.73† (*s*, 2H)	2.47, 2.51 (*s*, 6H)	2.25, 2.52 (*s*, 6H)

*The number in parentheses indicates the chemical shifts of methoxy protons.

†These values are the chemical shifts of hydroxy protons when the spectrum was run in DMSO-d_6 using TMS as internal standard at 80 MHz.

Table 2. Chemical shift scale of methoxy and acetoxy protons of 1-acetate and related compounds

	Assigned positions in biflavone nucleus					
Compound	4′	4‴	5	5″	7	7″
1-Acetate	2.82	2.83	2.88	2.90	2.86	(3.91)
Podocarpus flavone-A pentaacetate	2.10	(3.75)	2.44	2.49	2.32	2.06
Biolobetin acetate	(3.72)	2.15	2.47	2.51	2.33	2.10
Sequoiaflavone pentaacetate	2.05	2.15	2.47	2.51	(3.62)	2.10
7″-*O*-Methyl amentoflavone pentaacetate	2.05	2.15	2.25	2.52	2.23	(3.91)

The numbers in parentheses are the chemical shifts of methoxy protons.

values for various protons of the acetate of **1** were also identical with those of hexahydroxytetrahydroamentoflavone (Table 1) supporting the proposed structures. The position of the methoxy groups at C-7″ was confirmed by comparison of the ^{1}H NMR data of 1-acetate with 7-*O*-methyl amentoflavone pentaacetate, 7″-*O*-methyl amentoflavone pentaacetate (Table 1) and other related compounds (Table 2). Proton signals for H-5′,H-3‴,5‴ H-2‴,6‴ and H-6′ at δ7.20, 7.03, 7.56 and 6.73 were comparable to those of 7″-*O*-methyl amentoflavone pentaacetate (δ7.44, 7.04, 7.50 and 6.75) rather than of 7-*O*-methyl amentoflavone pentaacetate. Moreover, the methoxy group at δ3.91 was comparable to 7″-*O*-methyl amentoflavone pentaacetate (δ3.62), 4‴-*O*-methyl amentoflavone pentaacetate (δ3.75) or 4‴-*O*-methyl amentoflavone pentaacetate (δ3.72).

Thus, compound **1** was assigned the structure 5,7,4′,5″,4‴-pentahydroxy-7″-*O*-methyl-2,3,2″,3″-tetrahydroamentoflavone. This constitutes the first report of the isolation and natural occurrence of compound **1**.

EXPERIMENTAL

Mps: uncorr; analytical and prep. TLC: silica gel (BDH), silica gel G (Sthal, Merck) or silica gel NCL (Poona) using C_6H_6–C_5H_5N–HCOOH (36:9:5) as solvent; ^{1}H NMR: 60 MHz and 100 MHz with TMS as internal standard; MS: 70 eV. All reagents used were of Analar grade.

Extraction of plant material. The leaves of *O. pumila* (5 kg) obtained from the Sal forest of Bhikampur (U.P.) and authenticated by FRI.DDN, were dried at room temp., crushed and extracted several times with Me_2CO in a Soxhlet apparatus. The combined Me_2CO extracts were concentrated under red. pres. and the concentrate was extracted successively with petrol (60–80°) and C_6H_6 to remove non-flavonoid and resinous materials. The blackish gummy mass was refluxed with EtOAc for 8 hr and filtered. The filtrate was evaporated to dryness under red. pres. to give a brown residue (30 gm) which responded to the usual colour tests for flavonoids.

CC. The crude flavonoid mixture (30 g) was dissolved in MeOH (30 ml) and adsorbed onto silica gel (25 g). The excess solvent was allowed to evaporate and the dry residue added to a column of silica gel (250 g; 200 × 0.5 cm) prepared in petrol (bp 40–60°). The column was eluted successively with petrol (40–60°), C_6H_6, EtOAc and Me_2CO. TLC indicated the presence of four components.

Physical properties of **1**. Crystallized from MeOH as light yellow needles, mp 154°, R_f 0.57.

Acetylation of **1**. **1** (25 mg) was dissolved in a mixture of Ac_2O (0.5 ml) and dry C_5H_5 (0.5 ml) and kept at room temp. overnight.

Usual work-up afforded 20 mg of a crude acetylated material which was fractionated to give 1-acetate. MS *m/z*: 651 $[M]^+$; ^{1}H NMR: see Table 1. Demethylation of 1 gave 5,7,4′,5″,7″,4‴-hexahydroxy-2,3,2″,3″-tetrahydroamentoflavone. Light tan powder (4 g), mp 234–238°, R_f 0.19; UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 330 (sh), 285, 228 (sh); IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3400, 1605, 1445, 1310, 1235, 1148, 1078, 820; ^{1}H NMR (DMSO-d_6): δ3.46–2.70 (4H, *m*, H_2-3, H_2-3″), 5.45 (2H, *dd*, H-2, H-2″), 5.92 (2H, *s*, H-6, H-6″), 6.08 (1H, *s*, H-8), 6.71 (2H, *d*, H-3‴, H-5‴), 6.85 (1H, *d*, H-5′), 7.23 (2H, *d*, H-2‴, H-6‴), 7.30–7.15 (2H, *m*, H-2′, H-6′), − 8.1 to + 9.0 (4H, *m*, H_2-7,4′, H_2-7′,4‴), − 7.82, − 7.73 (2H, *s* each, OH-5, OH-5″); MS *m/z* (rel. int): 542 $[M]^+$ $C_{30}H_{22}O_{10}$ (10), 524 (14), 404 (11), 389 (13), 378 (9), 312 (12), 311 (10), 270 (11), 253 (11), 252 (24), 230 (10), 226 (37), 213 (17), 186 (13), 179 (22), 160 (21), 153 (54), 152 (23), 147 (43), 132 (23), 126 (100), 120 (66), 119 (31), 94 (38), 91 (58), 85 (35), 78 (31), 77 (34), 69 (73), 65 (39), 58 (76), 57 (92).

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REFERENCES

1. Chopra, R. N., Nayer, S. L. and Chopra, I. C. (1980) *Glossary of Indian Medicinal Plants*, p. 178. CSIR, New Delhi.
2. Kirtikar, K. R. and Basu, B. D. (1980) in *Indian Medicinal Plants*, p. 515. Sudhindra Nath Basu, Allahabad.
3. Chopra, R. N., Chopra, I. C., Handa, K. L. and Kapur, L. D. (1958) in *Chopra's Indigenous Drugs of India*, p. 601. U. N. Dhur, Calcutta.
4. Khan, N. A., Kamil, M., Ilyas, M. and Rahman, W. (1983) *Indian J. Chem.* **228**, 608.
5. Fatima, W., Iqbal, J., Ismail, H., Ishratullah, K., Shaida, W. A. and Rahman, W. (1979) *Chem. Ind.* 315.
6. Ahmad, I., Ishratullah K., Ilyas, M., Rahman, W., Seligmann, O. and Wagner, H. (1981) *Phytochemistry* **20**, 1169.