EXPERIMENTAL

Plant material. The same plant material was used as in ref. [2].

Extraction and purification. Air-dried ground aerial parts of *H. elodes* were defatted with petrol (Soxhlet, 2 hr) and then extracted with MeOH (maceration, 6 hr). The methanolic extract was chromatographed on a cellulose column, and the frs containing **1** were purified by prep. TLC (cellulose) using H_2O.

General. The methods used were those previously reported [2].

Compound **1**. Yellow crystals; R_f values: BAW (0.40), H_2O (0.35), 15% HOAc (0.15), 60% HOAc (0.50); colours at 366 nm: fluorescent yellow, yellow (NH_3), fluorescent yellow ($AlCl_3$), yellow-green (NA); UV λ^{MeOH} nm: 260, 325sh, 365; +NaOMe: 280, 323, 425 (dec); +$AlCl_3$: 267, 350, 432; +$AlCl_3$ + HCl: 270, 310sh, 350, 432; +NaOAc: 275, 315, 395; +NaOAc +H_3BO_3: 267, 325sh, 364; ^{1}H NMR (200 MHz, DMSO-d_6): δ6.14 (*d*, J = 1.8 Hz, H-6), 6.39 (*d*, J = 1.5 Hz, H-8), 6.93 (*d*, J = 7.5 Hz, H-5'), 7.80 (*dd*, J = 2.4 and 7.6 Hz, H-6'), 7.99 (*d*, J = 2.4 Hz, H-2'); ^{13}C NMR (50.3 MHz, DMSO-d_6): δ146.7 (C-2), 136.3 (C-3), 176.2 (C-4), 161.1 (C-5), 98.6 (C-6), 164.5 (C-7), 93.8 (C-8), 156.3 (C-9), 103.4 (C-10), 122.9 (C-1'), 122.6 (C-2'), 141.1 (C-3'), 151.5 (C-4'), 117.6 (C-5'), 125.3 (C-6').

Acknowledgements—We thank Professors Victor Gil and Carlos Geraldes, Faculty of Science and Technology, Coimbra University, for NMR and Instituto Nacional de Investigação Científica for financial support (Fm P_3).

REFERENCES

1. Seabra, R. M. and Alves, A. C. (1990) *Fitoterapia* **61**, 146.
2. Seabra, R. M. and Alves, A. C. (1988) *Phytochemistry* **27**, 3019.
3. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.
4. Markham, K. R. (1982) *Techniques of Flavonoid Identification*. Academic Press, London.
5. Barron, D. and Ibrahim, R. (1987) *Phytochemistry* **26**, 1181.

Phytochemistry, Vol. 30, No 4, pp. 1345–1347, 1991
Printed in Great Britain.

0031 9422/91 $3.00 + 0.00
Pergamon Press plc

8,3'-DIMETHOXY-5,4'-DIHYDROXYFLAVONE 7-GLUCOSIDE FROM *SETARIA ITALICA*

NEERU JAIN, MANSOOR AHMAD, MOHAMMAD KAMIL* and M. ILYAS

Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India

(*Received in revised form* 2 *October* 1990)

Key Word Index—*Setaria italica*; Graminae; foxtail millet; 8,3'-dimethoxy-5,4'-dihydroxyflavone 7-glucoside.

Abstract—A new flavone glycoside has been isolated from the leaves of *Setaria italica* and identified as 8,3'-dimethoxy-5,4'-dihydroxyflavone 7-glucoside by chemical and spectral studies.

INTRODUCTION

In an earlier communication [1] we reported the characterization of a new flavone glycoside, setariciin, from *Setaria italica* Beauv. Recently, K. Gluchoff *et al.* [2] reported the presence of 22 flavonoid glycosides from this plant. We report the isolation and identification of another new flavone glycoside from this source.

RESULTS AND DISCUSSION

The new flavone glycoside (**1**) analysed for $C_{23}H_{24}O_{12}$, responded to the Shinoda test [3], gave a brown-green colour with $FeCl_3$ and a positive Molisch test. The UV spectrum showed absorption maxima at 275 and 355 nm. Its IR spectrum displayed strong bands at 3350 cm^{-1} (OH), 1640 cm^{-1} (C=O), 2950 cm^{-1} (C–H) and a broad band at 1100–1020 cm^{-1} indicating its glycosidic nature. Colour reactions and UV spectral data with diagnostic shift reagents [4, 5] suggested that **1** is a flavone glycoside with free hydroxyl groups at the 5 and 4' positions. Acid hydrolysis yielded glucose (PC and GLC) and an aglycone (**1a**), which gave a bathochromic shift of 15 nm with NaOAc (absent in glycoside) thus showing that the sugar is linked to the 7-position of the aglycone. The negative

*Author to whom correspondence should be addressed.

borate reaction indicated the absence of an *ortho*-dihydroxyl grouping. Negative Gibbs and gossypetone tests [6–8] fixed the position of a methoxyl group in the A-ring at C-8. The aglycone underwent acetylation to a triacetate derivative (**2**), which was characterized as 5,7,4′-tiacetoxy-8,3′-dimethoxyflavone by spectral studies [9]. On methylation the aglycone gave a pentamethylflavone, (**2a**), which was characterized as 5,7,8,3′,4′-pentamethoxyflavone [10] by UV, ^{1}H NMR and mass spectrum. Thus the structure of **1a** was established as 8,3′-dimethoxy-5,7,4′-trihydroxyflavone, which was confirmed by comparison of its spectral data with literature values for natural and synthetic samples [11, 9].

The glycoside formed a crystalline hexaacetate derivative (**1b**). The ^{1}H NMR spectrum exhibited two aromatic acetoxyls integrating for six protons at δ2.31 and δ2.45 assigned for OAc-4′ and OAc-5 and four aliphatic acetoxyls integrating for 12 protons at δ2.05 assigned to sugar acetoxyls. The ^{1}H NMR spectrum showed also a sharp singlet intergrating for one proton at δ6.49, which was assigned to the C-3 proton of the γ-pyrone nucleus. The 5,7,8 trisubstitution was demonstrated by the presence of a remaining singlet at δ6.35 for one proton ascribed to the C-6 proton. The distinction between C-6 and C-3 protons was made by means of signal intensity [5b]. The singlet for C-6 was found to be slightly broadened and hence of lower intensity because of its long range coupling with C-8 methoxyl protons, whereas the C-3 proton showed a sharp singlet. The B-ring protons showed an ABX pattern. A multiplet consisting of a double doublet at δ7.18 (J = 9, 1.8 Hz), a doublet at δ6.97 (J = 1.8 Hz) and another doublet at δ6.64 (J = 9 Hz) were assigned to H-6′, H-2′ and H-5′, respectively. A sharp singlet at δ3.85 integrating for 6 protons was assigned to two methoxyl groups at the C-8 and C-3′ positions.

The mass spectrum of the acetylated glycoside was in agreement with the assigned structure of **1** and showed the presence of an acetylated hexopyranoside at *m/z* 331. The aglycone fragment was observed at *m/z* 330 and a retro-Diels–Alder fragmentation pattern was seen at *m/z* 183, *m/z* 168 and *m/z* 148 leading to fragments $[A_1+H]^+$, $[(A_1+H)\text{-Me}]^+$, $[B_2]^+$. These findings supported the presence of two hydroxyl and one methoxyl groups in the A ring and one hydroxyl and one methoxyl group in the B ring.

Methylation of **1** followed by acid hydrolysis gave a partially methylated compound which showed a bathochromic shift of 12 mm in band II with NaOAc confirming that the C-7, was now free. The methylated sugar was identified as 2,3,4,6-tetra-*O*-methyl-D-glucose by silica gel TLC according to Petek [12] and by alditol acetate formation of 2,3,4,6-tetra-*O*-methyl-D-glucose [13]. Hydrolysis of **1** with almond emulsin showed the β-nature of the glycosidic bond. Quantitative estimation of sugar by the Somogyis Coper Micro method [14] indicated the presence of one mole of sugar per mole of aglycone.

EXPERIMENTAL

Mps: uncorr. A MeOH leaf extract of *S. italica* Beauv. after fractionation on the basis of differential basicity was subjected to CC. The $CHCl_3$–MeOH (7:3, 3:2) eluates were re-chromatographed using $CHCl_3$–MeOH (1:1) as eluting solvent, affording a 1 mp 325° (d), which crystallized as yellow needles. Found C, 56.12; H, 4.89, $C_{00}H_{00}O_0$ requires C, 56.09; H, 4.87. UV λ_{max}^{MeOH} nm: 275, 355; $AlCl_3$ 280, 295sh, 377; $AlCl_3$–Hcl 281, 297sh, 380; NaOAc 275, 330sh, 355; NaOAc–H_3BO_3 276sh, 330sh, 357; NaOMe 272, 405.

Acetylation of glycoside (**1**). The glycoside was acetylated to yield an octaacetate derivative as cream coloured needles (**1b**), mp 142°. ^{1}H NMR (90 MHz, $CDCl_3$, TMS as int. standard) δ: 7.18 (1H, *d*, J = 9, 1.8 Hz, H-6′), 6.97 (1H, *d*, J = 1.8 Hz, H-2′), 6.64 (1H, *d*, J = 9.0 Hz, H-5′), 6.49 (1H, *s*, H-3), 6.35 (1H, *s*, H-6), 2.31, 2.45 (3H each, *s*, OAc-4′, OAc-5), 205 (12H, *s*, 4 × OAc aliphatic acetoxyls), 5.23 (1H, anomeric proton of Glc). MS data: *m/z* 414 $[M-Glc]^+$, *m/z* 372 $[414-Ac]^+$, 330 $[372-Ac]^+$ [aglycone fragment]$^+$, 331 $[Glc(Ac)_4]^+$, 183 $[A_1+H]^+$, 168 $[(A_1+H)-Me]^+$, 302 $[330-CO]^+$, 148 $[B_1]^+$, 151 $[B_2]^+$.

Acid hydrolysis of the glycoside. Compound **1** on acid hydrolysis with 7% HCl gave an aglycone (**1a**) mp 274–276° (Found C, 61.82; H, 4.25 $C_{17}H_{14}O_7$ requires C, 61, 81; H, 4.24%). UV λ_{max}^{MeOH} nm: 253, 276, 343; NaOAc, 268, 291, 362, NaOAC–H_3BO_3 268, 291, 362. IR ν^{KBr} cm^{-1}: 3540, 3470, 3400, 1649, 1613, 1556, 1506. It was characterized as 8,3′-dimethoxy-5,7,4′-trihydroxyflavone by direct comparison of its spectral data with lit. values [11, 9] as well as by comparison of the spectral data of its acetylated and methylated derivatives with known synthetic samples [9, 10]

PP of the aq. hydrolysate on Whatman No. 1 in EtOAc–pyridine–H_2O (12.5:4) and EtOAc–*iso*-PrOH–H_2O (3:1:1) showed glucose as sugar moiety. Glucose was also confirmed by GLC of its alditol acetate.

Methylation of **1** *and hydrolysis of the methylated product.* The glycoside (**1**) was methylated using Me_2SO_4–K_2CO_3 in Me_2CO and hydrolysed with 6% HCl followed by extraction with EtOAc. The partial methyl ether was characterized as 7-hydroxy-5,8,3′,4′-tetramethoxyflavone, mp 162° from spectral studies (Found C, 63, 62; H, 5.10, $C_{19}H_{18}O_7$ requires C, 63.68; H, 5.03%). The concd, filtered permethylated hydrolysate was neutralized with Ag_2CO_3 re-filtered, reduced with $NaBH_4$ and worked-up in the usual manner. The residue was acetylated with Ac_2O–pyridine (1:1) at 100° for 1 hr, purified by silica gel CC and subjected to GLC analysis, using OV-225 on Gas chrom Q at 195°. The peaks corresponding to the alditol acetate of 2,3,4,6-tetra-*O*-methyl-β-D-glucose was identified by comparison of R_f values with lit. values [13].

Enzymic hydrolysis of the glycoside. Almond emulsin soln (10 ml) was added to **1** (20 mg) in EtOH and the reaction mixt. kept at 40° for 45 hr. The hydrolysate gave glucose.

Acknowledgements—Thanks are due to Professor S. A. A. Zaidi, Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh, for provision of facilities. One of us (N.J.) thanks the CSIR, New Delhi for a senior research fellowship.

REFERENCES

1. Jain, N., Alam, M. S., Kamil, M. and Ilyas, M. (1989) *Chem. Ind.* 422.
2. Fiasson, K. G., Jay, M. and Viricel, M. R. (1989) *Phytochemistry* **28**, 2471.
3. Shinoda, J. (1928) *J. Chem. Pharm. Soc. Jpn* **48**, 214.
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
5. Markham, K. R. and Mabry, T. J. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, H. and Mabry, T. J., eds), pp. 48–63, 67. Chapman & Hall, London.
6. Gibbs, H. D. (1972) *J. Biol. Chem.* **72**, 649.
7. King, F. E., King, T. J. and Manning, L. C. (1957) *J. Chem. Soc.*, 563

8. Venkataraman, K. (1962) in *The Chemistry of Flavonoid Compounds* (Geissman, T. A., ed.), p. 75. Pergamon Press, New York.
9. Kenji, F. and Matsuru, N. (1969) *Bull. Chem. Soc. Jpn* **42**, 2327.
10. Morita, N. (1966) *Chem. Pharm. Bull., Tokyo.* **00**, 66.
11. Seaman, F., Rodriguez, E., Charman, N. J. and Mabry, T. J. (1972) *Phytochemistry* **11**, 2626.
12. Petek, F. (1965) *Bull. Soc. Chim. Pr.* **00**, No. 263.
13. Lindberg, B., Longren, J. and Thompson, J. L. (1973) *Carbohydrate Res.* **28**, 351.
14. Somogyis, M. (1952) *J. Biol. Chem.* **19**, 195.

Phytochemistry, Vol. 30, No. 4, pp 1347 1348, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00
Pergamon Press plc

HALENIA ELLIPTICA XANTHONE: A STRUCTURAL REVISION

GRAHAM J. BENNETT and HIOK-HUANG LEE

Department of Chemistry, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511

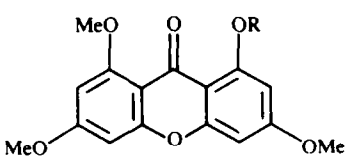
(*Received in revised form* 24 *August* 1990)

Key Word Index—*Halenia elliptica*; Gentianaceae; 1-hydroxy-3,6,8-trimethoxyxanthone; 1-hydroxy-2,3,7-trimethoxyxanthone; structural revision.

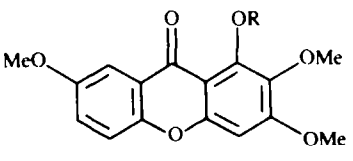
Abstract—The 1,3,6,8-tetraoxygenated xanthone structure assigned to a compound from *Halenia elliptica* is incorrect. Based on published evidence, the xanthone appears to be 1-hydroxy-2,3,7-trimethoxyxanthone.

Recently, Dhasmana and Garg reported the isolation of two 1,3,6,8-tetraoxygenated xanthones (**1** and **2**) from *Halenia elliptica* [1]. This oxygenation pattern is biogenetically unusual and unknown among natural xanthones. ^{1}H NMR and UV spectroscopy were used to support these structures, but the evidence is unsatisfactory in many respects. In particular, the chemical shifts reported for three of the aromatic protons of **1** (Table 1) and **2** are far downfield from those expected for phloroglucinol-ring protons (generally δ6.2–6.5), and the ^{1}H NMR spectrum of the 1-*O*-methyl derivative (**3**) does not reflect the symmetrical nature of the molecule. Furthermore, **1** and **3** have been synthesized previously [2, 3], and their melting points (191° and 219–220°) are quite different from those reported for the *Halenia* xanthone and its derivative (Table 1).

We have repeated the synthesis of **3** and found that its ^{1}H NMR spectrum, as expected, exhibits only two signals due to the four aromatic protons (see Experimental). A more likely structure for the *H. elliptica* aglycone is that of 1-hydroxy-2,3,7-trimethoxyxanthone (4), which has been synthesized by Stout and Balkenhol [4] and is present in *Veratrilla baillonii* [5] and almost certainly in two *Frasera* species (all Gentianaceae) [4, 6]. The physical data reported for **4** and its 1-*O*-methyl derivative (**5**) agree closely with those of the *H. elliptica* xanthone and its derivative (Table 1). In fact, Dhasmana and Garg have themselves recently reported the isolation of **4** from *H. elliptica* [7].



1 R = H
2 R = Primeverosyl
3 R = Me



4 R = H
5 R = Me