Pergamon

PII: S0031-9422(97)00801-7

Phytochemistry, Vol. 48, No. 7, pp. 1259–1261, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

6-HYDROXY-3,5,7,4′-TETRAMETHOXYFLAVONE 6-RHAMNOSIDE FROM ROOTS OF *PTEROCARPUS MARSUPIUM*

R. N. YADAV* and RAJESH KR. SINGH

Department of Chemistry, Dr Hari Singh Gour University, Sagar 470 003 (M.P.), India

(*Received in revised form* 1 *July* 1997)

Key Word Index—*Pterocarpus marsupium*; Leguminosae; roots; flavonol glycoside; 6-hydroxy-3,5,7,4′-tetramethoxyflavone 6-*O*-rhamnopyranoside.

Abstract—A new flavonol glycoside was isolated from roots of *Pterocarpus marsupium*. Its structure was determined as 6-hydroxy-3,5,7,4′-tetramethoxyflavone 6-*O*-rhamnopyranoside by spectral data and chemical degradation.

INTRODUCTION

Earlier workers have reported the presence of isoflavonoids [1], an isoflavonoid glycol [2], aurone glycosides [3], benzofuranone [4], phytosterols, pterostilbene, liquiritigenin, isoliquiritigenin [5, 6] from the heartwood, and aurone glycosides [7] from the flowers of *Pterocarpus marsupium* Roxb. A sesquiterpene alcohol, terpenes and a number of phenolic components have been characterized from the roots of this plant [8, 9]. The present paper deals with the isolation and structural elucidation of a novel flavonol glycoside (**1**) from the roots of *P. marsupium*.

RESULTS AND DISCUSSION

Compound **1**, $C_{25}H_{27}O_{11}$, $[M]^+$ *m/z* 503 gave a positive response to the Shinoda [10] test and Molisch test, confirming it to be a flavonoid glycoside. Acid hydrolysis of **1** yielded 6-hydroxy-3,5,7,4′-tetramethoxyflavone (**2**) identified by EIMS and ^{1}H NMR (see Experimental) and rhamnose. The UV spectrum of **2** with $AlCl_3$–HCl gave a bathochromic shift of 27 nm in band 1 relative to methanol indicating a free 6-hydroxyl group and a 3-*O*-substituent [11]. A free 6-hydroxyl in **2** [12] was further supported by a strong peak at *m/z* 357 (aglycone-H) in the mass spectrum which was indicative of a sugar attached at this position in **1**. The structure of the aglycone (**2**) was confirmed by alkaline degradation, which yielded two products, 3,6-hydroxy-2,4-dimethoxy acetophenone, (**2a**), identified by comparison of its mp, IR and ^{1}H NMR data with a known sample [13], and *p*-methoxybenzoic acid (**2b**) (Co–PC, Co–TLC, mmp).

The ^{1}H NMR spectrum (270 MHz, $CDCl_3$) of **1** showed two *ortho* coupled doublets at δ 7.80 (2H, $J = 8.5$ Hz) and 7.20 (2H, $J = 8.5$ Hz) assigned to H-2′,6′ and H-3′,5′ protons, respectively, confirming ring B to be symmetrically substituted. Resonance signals indicating four methoxyl groups were assigned as δ 3.92 (3H, *s*, OMe-3), δ 4.00 (3H, *s*, OMe-5), δ 3.88 (3H, *s*, OMe-7) and δ 3.80 (3H, *s*, OMe-4′). The higher δ value of a singlet at 6.65 on ring B was assigned to H-8 proton and was correlated with compounds having similar oxygenation pattern [14]. A signal for an anomeric proton was observed at δ 4.63 (1H, *br s*, H-1″) and a complex signal at δ 1.28 was due to the rhamnosyl methyl. The mass spectral analysis of **1** was in full agreement with the proposed structure. The molecular ion peak, as expected, was not observed. The base peak at *m/z* 358 (100%) corresponded to the aglycone fragment. The structure of **1** was further confirmed by retro-Diels Alder fragments in the mass spectrum of the aglycone. Thus, a fragment at *m/z* 196 $[A_1^+]^+$ indicated the presence of two methoxyl and one hydroxyl group in the A-ring of the aglycone, while a fragment at *m/z* 135 $[B_2^+]^+$ showed the presence of one methoxyl group in the B-ring.

Permethylation of **1** followed by acid hydrolysis yielded the aglycone **2** and 2,3,4-tri-*O*-methyl rhamnose identified according to Petek [16], which showed C_1-OH attachment of rhamnose to the C_6-OH of the aglycone. Enzymic hydrolysis of **1** by Takadiastase liberated one mole of L-rhamnose confirming α-linage between aglycone and L-rhamnose.

On the basis of these findings, the structure of **1** was elucidated as 6-hydroxy-3,5,7,4′-tetramethoxyflavone 6-*O*-rhamnopyranoside.

* Author to whom correspondence should be addressed.

EXPERIMENTAL

Plant material

The roots of *P. marsupium* Roxb. were collected in the Sagar region and identified by staff of the Botany Department. A voucher specimen (No. X-XV) has been deposited in the Natural Product Laboratory, Department of Chemistry, Dr H. S. Gour University, Sagar (M.P.), India.

General

UV spectra were run in MeOH and IR spectra measured in KBr. ^{1}H NMR spectra were run at 270 MHz using TMS as int. standard and $CDCl_3$ as solvent. ^{13}C NMR spectra (400 MHz) were measured using DMSO-d_6 as solvent. Mps are uncorr.

Extraction and identification

Air-dried and powdered roots of *P. Marsupium* were extracted with 95% MeOH. The concentrated MeOH extract was successively partitioned with *n*-hexane, $CHCl_3$ and EtOAc. The concentrated EtOAc soluble part was chromatographed over a silica gel column using solvents with increasing polarity. The fraction collected from $CHCl_3$–MeOH (9:1) gave **1**, which crystallized from Et_2O as light yellow needles, $C_{25}H_{27}O_{11}$, mp 244° and gave a single spot on silica gel TLC in C_6H_6–HOAc–H_2O (40:20:1), [M$^+$] 503, (Found: C, 59.62; H, 5.41; calcd C, 59.63; H, 5.40). IR ν_{max}^{KBr} cm^{-1}, 1658 (α,β-unsat. C=O), 2865 (OMe), 1535, 1600, 1130, 1205, 870. UV λ_{max}^{MeOH} 352, 295, 260; (+NaOMe) 350, 295, 260: (+NaOAc) 352, 292, 265; (+$AlCl_3$) 357, 294, 262; (+$AlCl_3$/HCl) 350, 294, 260. ^{1}H NMR (270 MHz, $CDCl_3$); δ 3.92 (3H, *s*, OMe-3), 4.00 (3H, *s*, OMe-5), 3.88 (3H, *s*, OMe-7), 3.80 (3H, *s*, OMe-4′), 6.65 (1H, *s*, H-8), 7.80 (2H, *d*, *J* = 8.5 Hz, H-2′,6′), 7.20 (2H, *d*, *J* = 8.5 Hz, H-3′,5′), 4.63 (1H, *br s*, H-1″), 1.28 (complex signal rhamnosyl methyl). ^{13}C NMR of **1** (400 MHz, DMSO-d_6); 157.2 (C-2), 134.6 (C-3), 177.7 (C-4), 155 (C-5), 132.4 (C-6), 148.2 (C-7), 94.5 (C-8), 152 (C-9), 105.5 (C-10), 120.7 (C-1′), 127.7 (C-2′), 116.6 (C-3′), 160.4 (C-4′), 115.6 (C-5′), 127.8 (C-6′), 59.65 (OMe), 62.0 (OMe), 58.9 (OMe), 60.85 (OMe). EIMS*m/z* 503 [M$^+$] absent, 358 [M$^+$–sugar]$^+$ (100%), 357 [M$^+$–sugar–H]$^+$, 315 [M$^+$–COMe]$^+$ (10%), 196 [A_1^+]$^+$ (20%), 168 [A_1^+–CO]$^+$ (18%), 135 [B_2^+]$^+$ (45%), 132 [B_1^+]$^+$ (15%), 107 [B_2^+–CO]$^+$ (16%).

Acid hydrolysis of **1**. Compound **1** was hydrolysed by heating with 0.2 N HCl on a steam bath for 1 h. The aglycone (**2**) which precipitated out on cooling was recrystallized from Et_2O as a yellow amorphous powder and was identified as 6-hydroxy-3,5,7,4′-tetramethyoxyflavone, $C_{19}H_{18}O_7$, [M$^+$] 358, mp 173°, (found: C, 63.60; H, 5.02; calcd.: C, 63.68; H, 5.06). IR ν_{max}^{KBr} cm^{-1} 1655, 2860, 1533, 1606, 1132, 1210, 875. UV λ_{max}^{MeOH} 346, 288, 252; (+NaOMe) 345, 288; (+NaOAc) 346, 287, 250; (+$AlCl_3$/HCl) 373, 286. ^{1}H NMR (270 MHz, $CDCl_3$), δ 3.80 (3H, *s*, OMe-4′), 3.86 (3H, *s*, OMe-7), 3.98 (3H, *s*, OMe-5), 3.92 (3H, *s*, OMe-3), 6.42 (1H, *s*, H-8), 7.80 (2H, *d*, *J* = 8.5 Hz, H-2′, 6′), 7.20 (2H, *d*, *J* = 8.5 Hz, H-3′, 5′), EIMS *m/z* 358 [M$^+$] (100%), 357 [M$^+$–Ḣ]$^+$ (85%), 315 [M$^+$–COMe]$^+$ (10%), 196 [A_1^+]$^+$ (20%), 168 [A_1^+–CO]$^+$ (18%), 135 [B_2^+]$^+$ (45%), 132 [B_1^+]$^+$ (15%), 107 [B_2^+–CO]$^+$ (16%).

The concd filtrate from the acid hydrolysis of **1** gave rhamnose identified on PC in BAW (4:1:5, top layer) at R_f 0.36 in comparison with an authentic marker using aniline hydrogen phthalate as detecting agent.

Alkaline degradation of **2**. Alkaline degradation with KOH in MeOH afforded **2a**, which was identified as 3,6-dihydroxy-2,4,-dimethoxyacetophenone, $C_{10}H_{12}O_5$, mp 160–161° (lit. 162° [15], [M$^+$] 213, (Found: C, 56.59; H, 5.67. Calcd C, 56.60; H, 5.69) and *p*-methoxybenzoic acid (**2b**), $C_8H_8O_3$, mp 216–217° [M$^+$] 152, (by Co-PC, Co-TLC).

Permethylation of **1** *followed by acid hydrolysis.* Compound **1** was permethylated with MeI/Ag_2O/DMF and the product acid hydrolysed to yield the aglycone **2** and 2,3,4-tri-*O*-methyl-L-rhamnose, identified according to Petek [16].

Enzymic hydrolysis of **1**. Compound **1** was treated with 3 ml of takadiastase at 38° for 36 h. The product was identified as L-rhamnose (R_f = 0.36) by PC (BAW, 4:1:5, top layer) using aniline hydrogen phthalate as detecting reagent.

Acknowledgements—Thanks are due to the Director of the Central Drug Research Institute, Lucknow, for spectral analysis and to Prof. V. K. Saxena, Department of Chemistry, and Dr H. S. Gour University, Sagar, India, for critical suggestions.

REFERENCES

1. Mitra, J. and Joshi, T., *Phytochemistry*, 1983, **22**, 2326.
2. Rao, A. V. S. and Mathew, J., *Phytochemistry*, 1982, **21**, 1837.
3. Mohan, P. and Joshi, T., *Phytochemistry*, 1989, **28**, 2529.
4. Maurya, R., Ray, A. B., Duah, F. K., Slatkin, D. J. and Schiff, P. L., *Heterocycles*, 1982, **19**, 2103.
5. Sawhney, P. L. and Seshadri, T. R., *J. Sci. Ind. Res. Sect. C*, 1956, **15**, 154.
6. Bhargava, P. N., *Proc. Indian Acad. Sci. Sect. A*, 1946, **24**, 497, 501.
7. Mohan, P. and Joshi, T., *Phytochemistry*, 1989, **28**, 1287.
8. Adinarayan, D. and Syamasundar, K. V., *Phytochemistry*, 1982, **21**, 1083.
9. Adinarayan, D., Sayamsundar, K. V., Seligmann, D. and Wanger, H., *Z. Naturforsch, C.; Biosci.*, 1982, **37C**, 145.

10. Shinoda, J., *J. Chem. Pharm. Soc. Jpn*, 1928, **48**, 214.
11. Sakakibara, M. and Mabry, T. J., *Rev. Latinoam. Quim.*, 1978, **9**, 92.
12. Rodriguez, E., Carman, N. J., Vander Velde, G., McReynolds, J. H. and Mabry, T. J., *Phytochemistry*, 1972, **11**, 3509.
13. Quijano, J. S., Calderon, F., Gomer and Rios, T., *Phytochemistry*, 1982, **21**, 2575.
14. Sachdev, K. and Kulshreshtha, D. K., *Phytochemistry*, 1983, **22**, 1253.
15. Isoma, C., *Chem. Abst.*, 1969, **07**, 37385a.
16. Petek, F., *Bull. Soc. Chim. Fr.*, 1965, **263**.