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EMODIN GLYCOSIDES FROM *VENTILAGO CALYCULATA*

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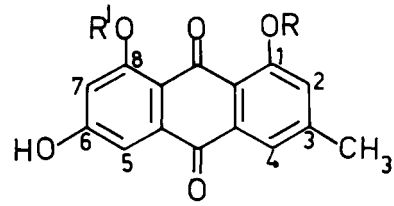
In our previous communications, we reported the presence of a number of anthraquinones (1,2), naphthoquinones (3,4), naphthalenes, xanthone (4) and naphthoquinone lactones, and extended quinones (5) from the root bark of *Ventilago calyculata* Tulasne (Rhamnaceae). The present paper documents the isolation and structure elucidation of three anthraquinone glycosides: emodin-1-*O*-α-L-rhamnoside, emodin-8-*O*-α-L-rhamnoside, and emodin-8-*O*-β-D-glucoside from the methanolic extract of the root bark of the title plant.

The first compound was obtained as yellow needles from MeOH. It analyzed for $C_{21}H_{20}O_9$. In acid hydrolysis, it gave emodin (identified by mp, mmp, and co-tlc) and L-rhamnose in a 1:1 ratio. The characteristic C-CH_3 signal at δ1.2 integrating for three protons in the ^{1}H-nmr spectrum of its acetate further established the sugar moiety to be L-rhamnose (6,7). The glycoside was methylated using Me_2SO_4/anhydrous K_2CO_3 and dry Me_2CO until negative to ferric ion reaction. The aglycone obtained by acid hydrolysis of the methylated product was identified as physcion-8-methyl ether by mp, mmp (8), and spectral data (9). This established the attachment of sugar moiety at position-1. The glycoside was completely hydrolyzed with taka-diastase, indicating an α-linkage between the aglycone and the sugar moiety. The coupling constant (J=2 Hz) of the anomeric proton gave further support to the α-linkage (10). Therefore, the structure of the first glycoside is emodin-1-*O*-α-L-rhamnoside (**1**). To the best of our knowledge, this is the first report from a natural source.

The second glycoside crystallized from MeOH as yellow microcrystals. It also analyzed for $C_{21}H_{20}O_9$. Acid hydrolysis yielded emodin and L-rhamnose. Therefore, the first two compounds are isomers differing in the position of attachment of the sugar moiety to the aglycone. Methylation followed by acid hydrolysis gave physcion-1-methyl ether (8) (mp, mmp, ir). The glycoside also was completely hydrolyzed by the enzyme taka-diastase. Thus, the second glycoside is emodin-8-*O*-α-L-rhamnoside (**2**), previously isolated from *Cassia javanica* (11). However, this report is the first record of the presence of emodin-8-*O*-α-L-rhamnoside in the Rhamnaceae.

The attachment of the sugar moiety to position-8 was established by ^{13}C studies (not reported earlier). In a ^{13}C-nmr spectral comparison of emodin-8-*O*-α-L-rhamnoside with emodin (^{13}C signals were assigned from References 12,13), it is apparent that the 8-hydroxyl in emodin is substituted with the sugar because the C-8 in the glycoside was shifted upfield (1.67) and the related *ortho*-carbons were shifted downfield (14,15). The other benzenoid ring carbons are less affected (see Experimental section).

The third glycoside gave emodin and glucose on acid hydrolysis. It was completely hydrolyzed with emulsin, indicating a β-linkage between the aglycone and sugar moiety. The mp, uv, and ir



1 R=L-rhamnose; R^1=H
2 R=H; R^1=L-rhamnose
3 R=H; R^1=D-glucose

data suggested emodin-8-*O*-β-D-glucoside (**3**), and comparison with an authenic sample established its identity (16).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Silica gel G was used for tlc and Whatman No. 1 for paper chromatography (ascending). System I, $CHCl_3$-MeOH (4:1) was used for tlc; System II, *n*-BuOH-HOAc-H_2O (4:1:5) was used for pc. Melting points were uncorrected. The plant material was collected from the Kondapalli forest, A.P., India. The voucher specimen was deposited in the Nagarjuna University Herbarium, No. NUH-238.

EXTRACTION AND ISOLATION.—The powdered root bark (2.1 kg) was extracted first with Me_2CO and then with MeOH. The MeOH extract (50 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$-MeOH (9:1), (4:1), and (1:1), respectively. The $CHCl_3$-MeOH (4:1) eluted glycosides **1** and **2**. These were separated into pure compounds by tlc (C_6H_6-MeOH, 4:1), yield: glycoside **1**, 95 mg; glycoside **2**, 200 mg. Glycoside **3** was eluted with $CHCl_3$-MeOH (1:1) and purified by repeated crystallization from MeOH, yield: 400 mg.

HYDROLYSIS.—Acid hydrolysis was performed by refluxing each glycoside (20 mg) with 6% aqueous HCl for 2 h. Aglycones were extracted with EtOAc. The sugar moiety in the hydrolysate was examined on pc in system II. Each glycoside (5 mg) was separately hydrolyzed with enzyme at 37 ° for 48 h. The sugar was identified by pc with an authenic sample.

EMODIN-1-*O*-α-L-RHAMNOSIDE (**1**).—Mp 184°; tlc system I, Rf 0.54; *Anal.* Calcd for $C_{21}H_{20}O_9$: C, 60.58; H, 4.84. Found: C, 60.40; H, 4.65%; uv (MeOH) log ε 225(4.32), 290(4.38), 420(3.96); ir (KBr) 3400, 2900, 1630, 825 cm^{-1}. Acetate (Ac_2O/pyridine) light yellow needles, ^1H nmr (100 MHz, $CDCl_3$) δ 1.2 (3H, d, J=6 Hz, CH_3-rhamnose), 2.0 (3H, s, $-COCH_3$), 2.04 (3H, s, $-COCH_3$), 2.19 (3H, s, $-COCH_3$), 2.40 (6H, s, 2 X-$COCH_3$), 2,48 (3H, s, Ar-CH_3), 3.72-5.52 (4H, m, rhamnose protons), 5.64 (1H, d, J=2 Hz, rhamnose-H-1′), 7.04 (1H, d, J=2 Hz, H-7), 7.16 (1H, broad signal, H-2), 7.84 (1H, d, J=2 Hz, H-5), 7.96 (1H, broad signal, H-4). Acid hydrolysis yielded emodin and L-rhamnose. Enzymic hydrolysis with taka-diastase gave emodin and L-rhamnose. The gylcoside was methylated using Me_2SO_4/K_2CO_3 in dry Me_2CO for 24 h. The product obtained was subjected to acid hydrolysis without purification. The product was identified as physicion-8-methyl ether, mp 213°.

EMODIN-8-*O*-α-L-RHAMNOSIDE (**2**).—Mp 200°; tlc system I, Rf 0.5; ^{13}C nmr[1] (67.89 MHz, DMSO-d_6) δ 17.81 (q, rhamnose-CH_3), 21.47 (q, Ar-CH_3, 69.77 (d, C-5′), 70.25 (d, C-2′), 70.33 (d, C-3′), 71.65 (d, C-4′), 98.64 (d, C-1′), 108.54 (d, C-7), 109.07 (d, C-5), 110.61 (s, C-12), 113.28 (s, C-13), 120.52 (d, C-4), 124.08 (d, C-2), 132.66 (s, C-14), 134.81 (s, C-11), 148.56 (s, C-3), 161.51 (s, C-1), 162.78 (s, C-8), 163.84 (s, C-6), 180.77 (s, C-10), 189.94 (s, C-9). For emodin 21.42 (Ar-CH_3), 107.77 (C-7), 108.78 (C-5, C-12), 113.08 (C-13), 120.25 (C-4), 123.84 (C-2), 132.53 (C-14), 134.81 (C-11), 147.99 (C-3), 161.39 (C-1), 164.45 (C-8), 165.57 (C-6), 180.86 (C-10), 189.43 (C-9). Acid hydrolysis gave emodin and L-rhamnose. Hydrolysis with taka-diastase gave emodin and L-rhamnose. Methylation (Me_2SO_4/K_2CO_3 in dry Me_2CO for 24 h) followed by acid hydrolysis physcion-1-methyl ether was obtained; mp 204°.

EMODIN-8-*O*-β-D-GLUCOSIDE (**3**).—Mp 193°, tlc system I, Rf 0.45. Acid hydrolysis as well as enzymic gave emodin and glucose.

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LITERATURE CITED

1. B. Kesava Rao, T. Hanumaiah, C.P. Rao, G.S.R. Rao, K.V.J. Rao, and R.H. Thomson, *Phytochemistry*, **22**, 2583 (1983).
2. B. Kesava Rao, T. Hanumaiah, J.U.M. Rao, K.V.J. Rao, and R.H. Thomson, *Phytochemistry*, **23**, 2104 (1984).
3. T. Hanumaiah, D.S. Marshall, B.K. Rao, C.P. Rao, G.S.R. Rao, J.U.M. Rao, K.V.J. Rao, and R.H. Thomson, *Phytochemistry*, **24**, 2373 (1985).
4. T. Hanumaiah, B.K. Rao, C.P. Rao, G.S.R. Rao, J.U.M. Rao, K.V.J. Rao, D.S. Marshall, and R.H. Thomson, *Phytochemistry*, **24**, 1811 (1985).
5. T. Hanumaiah, D.S. Marshall, B.K. Rao, J.U.M. Rao, K.V.J. Rao, and R.H. Thomson, *Phytochemistry*, **24**, 2669 (1985).
6. M. Viscontini, D. Hoch, and P. Karrer, *Helv. Chim. Acta.*, **38**, 642 (1955).

[1] Numbering followed as in Höfle (13).

7. T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, 1970, p. 269.
8. D.W. Cameron and M.J. Crossley, *Aust. J. Chem.*, **30**, 1161 (1977).
9. O.R. Gottleib, H. Martins, and M.J. Magalhaes, *Phytochemistry*, **10**, 1379 (1971).
10. T.J. Mabry, J. Kagan, and H. Rösler, *Phytochemistry*, **4**, 487 (1965).
11. R.D. Tiwari and R. Singh, *Phytochemistry*, **18**, 906 (1979).
12. Y. Berger and A. Castonguay, *Org. Magn. Reson.*, **11**, 375 (1978).
13. G. Höfle, *Tetrahedron*, **33**, 1963 (1977).
14. K.R. Markham, B. Ternai, R. Stanley, H. Geiger, and T.J. Mabry, *Tetrahedron*, **34**, 1389 (1978).
15. H. Itokawa, K. Mihara, and K. Takeya, *Chem. Pharm. Bull.*, 31, 2353 (1983).
16. T. Hanumaiah, B. Kesava Rao, and K.V. Jagannadha Rao, *Acta. Ciencia Indica*, **9C**, 209 (1983).

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