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TORTUOSIDE, A NEW NATURAL COUMARIN GLUCOSIDE FROM *SESELI TORTUOSUM*

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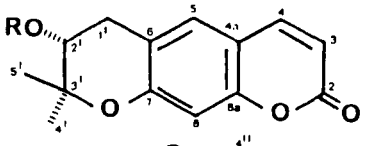
ABSTRACT.—A new coumarin glucoside, tortuoside [**3**], has been isolated from the aerial parts of *Seseli tortuosum*. Its structure was established on the basis of spectroscopic data and chemical evidence.

During the course of our investigations on metabolites from *Seseli tortuosum* L.B.S. Eur. (Umbelliferae), we have isolated and characterized, in addition to previously reported compounds (1,2), three coumarin derivatives. Two of these, (−)-2′-senecioyloxy-1′,2′-dihydroxanthyletin [**1**] and (−)-2′-isovaleryloxy-1′-2′-dihydroxanthyletin [**2**], have been reported from various species of Seseli (3,4) while the third, named tortuoside [**3**], is described here.

Chromatographic procedures applied to the Me_2CO extract of the air-dried aerial parts of *S. tortuosum* provided tortuoside [**3**], $C_{20}H_{26}O_{10}$, mp 212–216°, $[\alpha]_D$ +18.9°. An analysis of its ^{1}H-nmr spectrum clearly indicated that **3** contains one 7,8-disubstituted coumarin nucleus and a β-glycoside unit, as evidenced from the large ^{1}H coupling constant (7.6 Hz) of the anomeric proton (δ 4.56). The carbon resonances of **3** were in agreement with a β-D-glucosyl residue (5). However, the chemical shift of the anomeric carbon (δ 98.2) seemed anomalous. This upfield shift from the expected values was indicative of a *tert-O*-β-D-glucopyranoside (6,7).

The aglycone moiety, isolated from the hydrolysis of **3**, was identified as 7-hydroxy-8-(2′,3′-dihydroxy-3′-methylbutyl)-coumarin [**4**], on analytical and spectroscopical bases (1). The ^{13}C,^{1}H-COSY of **4** led to the full clarification of the carbon and proton signals (Table 1 and Experimental) and corroborated the assigned structure. The sugar isolated as the methylglycoside was identical in all respects with an authentic sample of methyl-α-D-glucoside. Placement of this residue at the tertiary carbon on the aglycone was confirmed by comparison of the chemical shifts of the C-3′, C-4′, and C-5′ in **3** with those of the corresponding signals of **4** (6). ^{13}C-nmr data of acetates **5** and **6** were consistent structure for these compounds. Further elution



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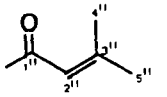

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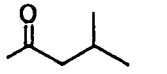

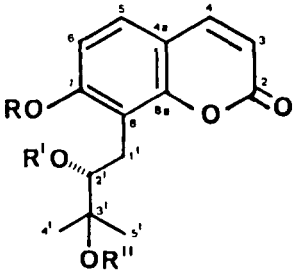

3 R=H, R′=H, R″=β-D-glucosyl
4 R=H, R′=H, R″=H
5 R=Ac, R′=Ac, R″=β-D-glucosyl acetate
6 R=Ac, R′=Ac, R″=H
7 R=Me, R′=H, R″=H

TABLE 1. ^{13}C nmr Data.[a]

Carbon	Compound						
	1	**2**	**3**	**4**	**5**[b]	**6**[c]	**7**
C-2	160.9	160.9	163.9	163.8	159.9	160.2	163.8
C-3	113.0	113.1	111.9	111.9	115.5	115.4	112.7
C-4	142.9	142.9	146.5	146.5	143.2	143.6	143.9
C-4a	112.6	112.6	113.5	113.4	116.3	116.4	113.0
C-5	128.5	128.1	128.3	128.2	126.3	126.4	126.8
C-6	115.9	115.8	114.3	114.2	118.8	118.9	107.4
C-7	156.3	156.2	155.1	155.1	152.1[d]	152.0[d]	160.5
C-8	140.4	104.3	115.5	115.5	119.3	119.5	115.8
C-8a	154.1	154.1	161.1	161.3	153.5[d]	153.3[d]	153.3
C-1′	27.7	27.7	26.3	26.4	23.6	23.9	25.9
C-2′	69.0	69.1	79.3	79.4	79.3	78.2	78.0
C-3′	76.6	76.4	81.4	74.0	77.2	71.8	72.8
C-4′	24.9	24.9	22.9[d]	25.3[d]	23.6[e]	25.1[e]	24.0[d]
C-5′	27.2	27.2	22.9[d]	25.5[d]	24.3[e]	25.3[e]	25.4[d]
C-1″	165.5	171.8	98.2		95.4		
C-2″	115.4	43.2	75.3		72.9		
C-3″	158.0	25.5	78.2[e]		71.7[f]		
C-4″	20.1	22.0	71.7		68.9		
C-5″	22.9	22.1	78.1[e]		71.4[f]		
C-6″			62.8		62.4		
7-OMe							56.1

[a]Compounds **1**, **2**, **5**–**7** in $CDCl_3$; compounds **3** and **4** in CD_3OD.
[b]*MeCO*: 168.6, 170.3, 170.1, 169.8, 169.3, 168.9; *CH*$_3$CO: 20.3, 20.4, 20.5, 20.7, 21.7.
[c]*MeCO*: 168.6, 170.5; *CH*$_3$CO: 20.7, 20.5.
[d–f]Signals in any vertical column with the same superscript may be reversed.

with $CHCl_3$-MeOH (9:1) gave 0.9 g of tortuoside [**3**]: $[\alpha]_D$ +18.9° (c = 0.01, MeOH), mp 212–216°; ^1H nmr (CD_3OD) δ 1.38 (3H, s, 3′-Me), 1.42 (3H, s, 3′-Me), 2.58–3.89 (10H, m, H-1′, H-2′, and glucose-H), 4.56 (1H, d, anomeric-H), 6.18 (1H, d, J = 9.4 Hz, H-3), 6.82 (1H, d, J = 8.5 Hz, H-5), 7.33 (1H, d, J = 8.5 Hz, H-6), 7.88 (1H, d, J = 9.4 Hz, H-4); ^{13}C nmr see Table 1. *Anal.* calcd for $C_{20}H_{26}O_{10}$, C 56.33, H 6.15; found C 56.12, H 6.25.

HYDROLYSIS OF TORTUOSIDE [**3**].—A solution of 0.25 g of tortuoside [**3**] in 10 ml of 10% methanolic HCl was refluxed under magnetic stirring for 4 h. After being cooled, the reaction mixture was neutralized with a $NaHCO_3$ solution, diluted with H_2O, and extracted with $CHCl_3$. The combined organic layers were washed with H_2O, dried, and evaporated. Chromatography of the residue and elution with $CHCl_3$-MeOH (95:5) gave 0.15 g of aglycone **4**: $[\alpha]_D$ +60.6° (c = 0.009, MeOH); mp 138–140°; ^{13}C nmr see Table 1. *Anal.* calcd for $C_{14}H_{16}O_5$, C 63.63, H 6.10; found C 63.48, H 6.17.

The aqueous phase was lyophilized to give a methyl-α-D-glycoside identical in all respects with an authentic sample of methyl-α-D-glucoside.

ACETYLATION OF TORTUOSIDE [**3**].—A solution of 0.1 g of the glucoside **1** and 2 ml of Ac_2O in 6 ml of C_5H_5N was kept at room temperature for 12 h. After usual workup, the crude product was chromatographed. Elution with $CHCl_3$ gave 0.12 g of acetate **5**: ^1H nmr ($CDCl_3$) δ 1.30 (3H, s, 3′-Me), 1.40 (3H, s, 3′-Me), 1.81 (3H, s, 2′-OAc), 2.01, 2.03, 2.04, 2.07 (12H, each s, OAc-glucose), 2.45 (3H, s, 7-OAc), 3.04 (1H, dd, J = 7.8, 14.6 Hz, H-1′), 3.16 (1H, dd, J = 2.4, 14.6 Hz, H-1′), 3.60–5.35 (8H, m, H-2′ and glucose-

H), 6.38 (1H, d, $J=9.5$ Hz, H-3), 7.04 (1H, d, $J=8.4$ Hz, H-5), with the proposed structures. The absolute stereochemistry of the new natural glycoside was determined by comparing the optical rotation value of the methyl derivative **7** with that of natural meranzin hydrate (8). Consequently, tortuoside [**3**] is expressed as 7-hydroxy-8-[(2′*R*)-hydroxy-3′-*O*-β-D-glucopyranosyl-3′-methylbutyl]-coumarin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Reichert micro hotstage and are uncorrected. Elemental analyses were carried out on a Carlo Erba Model 1106 Elemental Analyzer. All nmr experiments were performed on a Bruker AC 200 spectrometer with 5-mm ^1H and ^{13}C probes operating at 200 and 50 MHz respectively. All ^1H-nmr and ^{13}C-nmr chemical shifts were referred to internal TMS. Cc was carried out on 0.063–0.200 mesh Merck Si gel. All extracts were dried over Na_2SO_4.

PLANT MATERIAL.—Plant material was collected in September 1987, near Perugia, Umbria, Italy, and voucher specimens (Number 2315) were deposited in the Herbarium of the Dipartimento di Biologia Vegetale of the University of Perugia.

EXTRACTION AND ISOLATION OF THE COMPONENTS.—Dried and finely powdered *S. tortuosum* aerial parts (2 kg) were extracted exhaustively with Me_2CO. The resulting extracts were concentrated under vacuum. The crude gum was chromatographed over Si gel, and elution with $CHCl_3$ gave, in addition to previously reported compounds (1), 1.5 g of **1** and 0.3 g of **2** (2,3), whose ^{13}C-nmr data are presented for the first time (Table 1), and fully supported the 7.38 (1H, d, $J=8.4$ Hz, H-6), 7.69 (1H, d, $J=9.5$ Hz, H-4); ^{13}C nmr see Table 1. *Anal.* calcd for $C_{32}H_{44}O_{16}$, C 56.14, H 6.48; found C 54.24, H 6.36.

ACETYLATION OF **4**.—A solution of 50 mg of aglycone **4** and 1 ml of Ac_2O in 3 ml of C_5H_5N was kept at room temperature for 12 h. After usual workup, the crude product was chromatographed. Elution with $CHCl_3$ afforded 54 mg of acetate **6**: ^1H nmr ($CDCl_3$) δ 1.29 (3H, s, 3′-Me), 1.31 (3H, s, 3′-Me), 1.83 (3H, s, 2′-OAc), 2.38 (3H, s, 7-OAc), 3.09 (1H, dd, $J=10.3$, 14 Hz, H-1′), 3.20 (1H, dd, $J=2.4$, 14 Hz, H-1′), 5.17 (1H, dd, $J=2.4$, 10.3 Hz, H-2′), 6.40 (1H, d, $J=9.4$ Hz, H-3), 7.04 (1H, d, $J=8.5$ Hz, H-5), 7.38 (1H, d, $J=8.5$ Hz, H-6), 7.72 (1H, d, $J=9.4$ Hz, H-4); ^{13}C nmr see Table 1. *Anal.* calcd for $C_{18}H_{20}O_7$, C 62.06, H 5.79; found C 62.22, H 5.62.

METHYLATION OF **4**.—To a solution of **4** (75 mg) in Me_2CO (5 ml) were added 67 mg of K_2CO_3 and 0.13 ml of MeI under N_2 and magnetic stirring. The solution was refluxed for 2 h. After cooling, the mixture was diluted with H_2O and extracted with $CHCl_3$. The combined organic layers were washed with H_2O, dried, and evaporated. Chromatography of the residue and elution with $CHCl_3$-MeOH (95:5) gave 61 mg of (+)-meranzin hydrate [**7**]: $[\alpha]_D$ +51.2° ($c=0.01$, EtOH) [lit. (8) (−)-meranzin hydrate: $[\alpha]_D$ −57°].

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