Pergamon

0031-9422(95)00236-7

Phytochemistry, Vol. 40, No. 3, pp. 927–929, 1995

0031-9422/95 $9.50 + 0.00

PITURANTHOSIDE FROM *PITURANTHOS TRIRADIATUS*

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(*Received in revised form* 27 *February* 1995)

Key Word Index—*Pituranthos triradiatus*; Apiaceae; shoots; coumarins; pituranthoside; (−)-*S-trans*-marmin-7′-*O*-β-D-glucopyranoside; (−)-*S-trans*-marmin; xanthotoxol; bergapten; umbelliferone; isopimpinellin; spectroscopic analysis.

Abstract—The new monoterpenoid coumarins, pituranthoside [(−)-*S-trans*-marmin-7′-*O*-β-D-glucopyranoside] and (−)-*S-trans*-marmin, as well as four known coumarins, xanthotoxol, umbelliferone, isopimpinellin and bergapten, were isolated from the shoots of *Pituranthos triradiatus* and spectroscopically characterized.

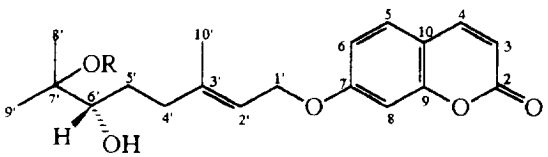
INTRODUCTION

As part of our current work on the chemistry of apiaceous plants growing in Egypt, *Pituranthos triradiatus* was examined for its coumarins. In a previous study, the petrol and diethyl ether extracts of the roots afforded imperatorin, isoimperatorin, isopimpinellin, bergapten, xanthotoxol, marmin, marmesin and umbelliferone [1, 2]. Moreover, cnidilin, imperatorin, isoimperatorin, bergapten and isopimpinellin were reported from the petrol extract of the shoots [3]. In the present study, the coumarins of the ether- and methanol-soluble fractions of the shoots were isolated and fully characterized.

RESULTS AND DISCUSSION

From the shoots of *P. triradiatus*, the new monoterpenoid coumarin glucoside, pituranthoside [(−)-*S-trans*-marmin-7′-*O*-β-D-glucopyranoside, (**2**)] and (−)-*S-trans*-marmin (**1**) as well as four known coumarins, bergapten, isopimpinellin, xanthotoxol and umbelliferone, were isolated and fully characterized [3–9]. The structural elucidation of the new compounds **1** and **2** are described here.

Compound **1**, was obtained as white micro-rosettes from the ether extract of the shoots. The NMR data (Table 1) and FAB–Mass spectrometry (MS) fragmentation pattern (see Experimental) suggested its identity with *trans*-marmin [10, 11]. Natural marmin is known as dextrorotatory with $[\alpha]_D^{30}$ + 25° and the *R* configuration at C-6′ [5, 11]. Since the optical rotation of **1** showed a negative value, $[\alpha]_D^{26}$ − 11°, the *S* configuration at the chiral centre C-6′ is proposed, supporting the structure of **1** as (−)-*S-trans*-marmin.



R = H **1**

R = β-D-Glc **2**

Compound **2**, was obtained as prisms from the methanol-soluble fraction of the shoots. It showed IR bands at 1725, 1710 (δ-lactone), 1620, 1580 and 1505 cm^{-1} (aromatic C=C stretch) and the UV maxima of a 7-oxycoumarin [5, 6]. The ^1H NMR data (Table 1) showed close resemblance to **1** with signals for five aromatic protons at δ6.28 (H-3), 7.98 (H-4), 7.61 (H-5),, 6.95 (H-6), and 7.0 (H-8) of the umbelliferone residue, three methyl resonances a δ1.09, 1.13 and a 1.73, a CH proton at δ3.36 (1H, *d*, *J* = 10.1 Hz, H-6′), CH_2 protons at δ4.65 (2H, *d*, *J* = 6.6 Hz, H_2-1′) and a vinyl CH proton at δ5.46 (1H, *t*, *J* = 6.5 Hz, H-2′) assignable to the monoterpenoid side chain. However, it was significantly different from **1** by the presence of an anomeric 1-proton doublet at δ4.34 (*J* = 7.8 Hz, H-1″) and a diffused absorption integrated for 10 protons resonating at δ3.63–2.92 (6H) and δ5.34–4.40 (4 OH) of a β-linked hexose. Umbelliferone and D-glucose were detected on acid hydrolysis and the FAB-MS spectrum displayed $[M + H]^+$ at *m/z* 495 and a weak $[M]^+$ at *m/z* 494, with 162 mass units higher than that of **1**, consistent with the molecular formula $C_{25}H_{34}O_{10}$ of a marmin glucoside. It also showed significant fragment ions at *m/z* 333

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Table 1. ^1H and ^{13}C NMR spectral data (300, 75 Hz, DMSO-d_6) for compounds **1** and **2**

	δ_H		δ_C^*	
Atom	**1**	**2**	**1**	**2**
Umbelliferone residue				
2		—	161.3 *s*	160.6 *s*
3	6.25 *d* (9.3)	6.28 *d* (9.5)	112.9 *d*	112.6 *d*
4	7.65 *d* (9.3)	7.98 *d* (9.5)	143.5 *d*	144.7 *d*
5	7.37 *d* (8.4)	7.61 *d* (8.6)	128.6 *d*	129.8 *d*
6	6.85 *dd* (8.4, 2.1)	6.95 *dd* (6.8, 2.3)	113.2 *d*	113.2 *d*
7			162.0 *s*	162.0 *s*
8	6.81 *d* (2.1)	7.00 *d* (2.2)	101.5 *d*	101.6 *d*
9		—	155.7 *s*	155.6 *s*
10			112.4 *s*	112.4 *s*
Monoterpene side chain				
1′	4.61 *d* (6.5)	4.65 *d* (6.6)	65.4 *t*	65.6 *t*
2′	5.53 *t* (6.5)	5.46 *t* (6.5)	118.7 *d*	118.7 *d*
3′			142.1 *s*	142.4 *s*
4′	2.39–2.17 *m*	2.28–2.04 *m*	36.4 *t*	36.7 *t*
5′	1.62 1.48 *m*	1.61–1.24 *m*	29.4 *t*	29.4 *t*
6′	3.36 *d* (10.1)	3.36 *d* (10.1)	77.8 *d*	76.9 *d*
7′			73.0 *s*	79.4 *s*
8′-CH_3	1.17 *s*	1.09 *s*	26.4 *q*	22.9 *q*
9′-CH_3	1.22 *s*	1.13 *s*	23.2 *q*	22.0 *q*
10′-CH_3	1.78 *s*	1.73 *s*	16.7 *q*	16.9 *q*
OH	1.86 *s* 2.54 *s*	4.48 *d* (4.2)	—	—
Glucose residue				
1″		4.34 *d* (7.8)	—	96.6 *d*
2″		2.92–3.13 *m*	—	73.9 *d*
3″		2.92–3.13 *m*	—	76.9 *d*
4″		2.92–3.13 *m*	—	70.4 *d*
5″		2.92–3.13 *m*	—	74.6 *d*
6″-*a*		3.63 *dd* (10.8, 5.0)	—	61.3 *t*
6″-*b*		3.39 *dd* (11.7, 6.5)	—	—

*^{13}C multiplicities were determined by the DEPT pulse sequence.

$[M + H\text{-glc}]^+$ and 163 $[M + H - 332]^+$ corresponding to the marmin and umbelliferone moieties of **2**.

Structure **2** was finally established through the analysis of its ^{13}C NMR and DEPT spectral data (Table 1). The presence of 25 carbon signals was revealed and it proved to be a monoside in nature by the anomeric carbon signal at δ96.6. Comparison of the ^{13}C NMR data for **2** with those for **1** (Table 1) revealed, in addition to the close similarity between the carbon resonances of **1** and those of the aglycone moiety of **2**, that the signal of C-7′ in **1** (δ73.0) was shifted downfield by 6.4 ppm in **2** (δ79.4) whereas the two methyl signals at C-8′ and C-9′ positions in **2** appeared upfield by 3.5 and 1.2 ppm, respectively. These observations, together with the significant upfield resonance of the anomeric carbon signal at δ96.6, were indicative of a tertiary-*O*-β-D-glcopyranoside [12, 13]. The geometry about the 2′, 3′-trisubstituted double bond (IR, 840 cm^{-1}) was established, like natural marmin, as *trans* rather than *cis* on the basis of the fine splitting pattern of the 2′-vinyl proton (*t*, J = 6.5 Hz) [10]. The chirality at C-6′ was assigned *S* based on biogenetic analogy with the chiral centre C-6′ of the co-occurring (−)-*S*-*trans*-marmin (**1**). Thus, **2** is (−)-*S*-*trans*-marmin-7′-*O*-β-D-glucopyranoside, named pituranthoside.

It should be pointed out that the present isolation of pituranthoside represents the first report of a 7′-*O*-β-D-glcopyranoside of marmin. It is an isomer of diversoside (marmin-6′-*O*-β-D-glucopyranoside), isolated from the roots of *Ferula diversivittata* growing in the USSR [14]. Although (+)-*R*-*trans*-marmin is an already known coumarin [5], its stereomer (−)-*S*-*trans*-marmin is hitherto unreported.

EXPERIMENTAL

General. Mps, uncorr. UV spectra and optical rotations were measured in MeOH. IR sectra were recorded in KBr. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as an int. standard or in DMSO-d_6. ^{13}C multiplicities were determined by the DEPT pulse sequence [15]. EI-MS were obtained at 70 eV and FAB-MS were recorded at 8 kV in 3-nitrobenzyl alcohol (3-NOBA) as a matrix and xenon as a bombardment gas. Analyt. and prep. TLC were performed on pre-coated silica gel $60F_{254}$ plates (0.25 and 1 mm) and coumarins were detected under UV at 366 nm. CC was performed on silica gel, 230–400 mesh. D-Glucose, D-galactose and L-arabinose (Merck), D-xylose and D-glucuronic acid (Sigma) and L-rhamnose (BDH) were used as reference sugars and detected on cellulose plates (F_{254}) by spraying with aniline hydrogen phthalate followed by heating at 100° for 5 min.

Plant material. The shoots of *P. triradiatus* (Hochst ex Boiss.) Aschers and Schweinf were collected, in early summer, from flowering plants growing wildly in the rocky mountains of Saint Katherine district, Sinai Peninsula. Identification was kindly verified by Dr M. N. El-Hadidi, Department of Botany, Faculty of Science, Cairo University, and a voucher specimen documenting this collection, has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, University of Mansoura.

Extraction and isolation. Air-dried, powdered shoots (0.9 kg) were extracted at room temp. with 70% aq. EtOH. The concd extract was diluted with H_2O, defatted with petrol and then partitioned with Et_2O to afford extract A. The remaining aq. mother liquor was evapd to dryness, dissolved in MeOH and filtered to afford extract B. Evapn of the solvents left the crude extracts A (4.0 g) and B (48.1 g), respectively. CC of the Et_2O extract (A, 4 g) on silica gel (2.5 cm i.d., 100 g), eluted with $CHCl_3$–MeOH gradient followed by prep. TLC on silica in $CHCl_3$ and $CHCl_3$–MeOH (49:1), afforded bergapten (3 mg), isopimpinellin (20 mg), xanthotoxol (11 mg), umbelliferone (30 mg) and **1** (85 mg). CC of the MeOH-soluble fraction (B, 45 g) on silica gel (5 cm i.d., 800 g), eluted with EtOAc, gave **2** (38 mg).

Acid hydrolysis of **2**. Compound **2** (7 mg) was refluxed, for 30 min, with 15 ml 4 N HCl in MeOH. The acid hydrolysate was concd, extracted with $CHCl_3$ and examined by TLC on silica gel in $CHCl_3$ for the liberated aglycone. The acidic mother liquor was neutralized with Ag_2O, filtered, evapd to dryness, and the residue was dissolved in pyridine. The sugar was identified, in the pyridine soln, by TLC on cellulose in EtOAc–pyridine–H_2O–BuOH–HOAc (5:4:4:10:2) against authentic monosaccharides.

(−)-*S-trans-Marmin* (**1**). Condensed microrosettes, mp 117–118° (MeOH–Et_2O, 1:9), $[\alpha]_D^{26}$ − 11° (MeOH; $c = 0.55$), R_f 0.3 ($CHCl_3$–MeOH, 49:1). UV λ_{max} MeOH nm: 216, 253*sh*, 322. ^{1}H and ^{13}C NMR (300, 75 MHz; DMSO-d_6): see Table 1. ^{13}C DEPT (75 MHz, DMSO-d_6, 135°): 3 CH_3, 3 CH_2, 7 CH, 6 C. FAB-MS (+ ve mode, xenon, 3-NOBA): *m/z* (rel. int.) 333 $[M + H]^+$ (15.5), 332 $[M]^+$ (0.15) calculated for $C_{19}H_{24}O_5$, 163 (39).

Pituranthoside [(−)-*S-trans-marmin*-7′-O-β-D-*glucopyranoside*] (**2**). Prisms, mp 185–186° (aq. MeOH), $[\alpha]_D^{26}$ + 12° (MeOH; $c = 0.5$), R_f 0.87 (EtOAc), 0.80 (EtOAc–$CHCl_3$, 1:1). UV λ_{max} MeOH nm: 209, 254*sh*, 320. ^{1}H and ^{13}C NMR (300, 75 MHz; DMSO-d_6): see Table 1. ^{13}C DEPT (75 MHz, DMSO-d_6, 135°): 3 CH_3, 4 CH_2, 12 CH, 6 C. FAB-MS (+ ve mode, xenon, 3-NOBA): *m/z* (rel. int. 495 $[M + H]^+$ (13.3), 494 $[M]^+$ (1.0), calculated for $C_{25}H_{34}O_{10}$, 163 $[M + H - 332]^+$ (51.2), 333 $[M + H - glc]^+$ (35).

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