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ARBUTIN DERIVATIVES IN *GENTIANA PYRENAICA*

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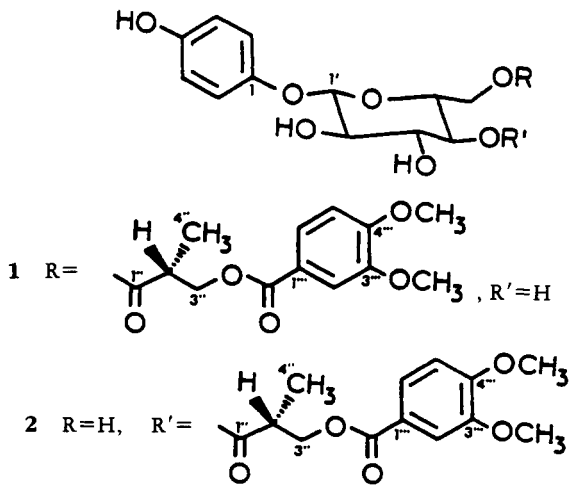
ABSTRACT.—6′-*O*-[(2*R*)-methyl-3-veratroyloxy-propanoyl] arbutin [**1**] and 4′-*O*-[(2*R*)-methyl-3-veratroyloxy-propanoyl] arbutin [**2**], two new arbutin derivatives, have been isolated from the leaves of *Gentiana pyrenaica*. Their structures were determined by spectral and chemical means.

Previous investigations on the leaf constituents of *Gentiana pyrenaica* L. (Gentianaceae) reported the presence of *C*-glycosylflavones (1). In this paper we describe the isolation and structure elucidation of two phenolic glucoside esters which are new arbutin derivatives. These compounds were identified as 6′-*O*-[(2*R*)-methyl-3-veratroyloxy-propanoyl] arbutin [**1**] and 4′-*O*-[(2*R*)-methyl-3-veratroyloxy-propanoyl] arbutin [**2**] on the basis of spectroscopic studies and chemical methods. This is the first report of arbutin derivatives in the Gentianaceae.

Compounds **1** and **2** were obtained from the Me_2CO extract of dried and powdered leaves of *G. pyrenaica* after chromatographic purification.

Fragments at *m/z* 523 $[M+H]^+$ and *m/z* 521 $[M-H]^-$ in the negative fabms spectra indicated for **1** a mol wt of 522. Its ^{1}H-nmr spectrum revealed the presence of a *p*-disubstituted benzene unit (A_2B_2 system), a glucosyl moiety, an alkyl chain, two phenolic methoxyl groups, and a 1,3,4-trisubstituted aromatic ring. The latter was identified as veratric acid by comparison of its nmr features with the literature (2). This result was supported by the uv absorptions at 220, 260, 287 nm and by fabms data which showed peaks at *m/z* 181 (fab^-) and *m/z* 165 (fab^+) (2). The ^{1}H-nmr spectrum of **1**, completed by spin decoupling experiments, exhibited further signals assigned to a β-hydroxyisobutyric acid (3). Resonances of the H-3″ at 4.43 and 4.39 ppm as well as that of the corresponding carbon at 67.0 ppm indicated this acid to be acylated on C-3″. The fact that this acylation was effected by veratric acid was suggested by fab^+ms data which displayed fragments at *m/z* 268 and 251 attributed to the β-hydroxyisobutyric acid esterified with veratric acid.



Alkaline hydrolysis of **1** afforded arbutin, which was identified by comparison with an authentic sample (tlc, uv, ^{1}H nmr), veratric acid, and β-hydroxyisobutyric acid with an *R* configuration [because of its negative optical rotation value $[\alpha]^{25}D$ −29° (*c* = 0.082, MeOH)] (4). The chemical shift values of the H-6′ at 4.54 and 4.19 ppm indicated that the glucose moiety was acylated on C-6′. This fact was confirmed by the deshielding of C-6′ at 65.3 ppm, C-5′ being shifted upfield at 75.4 ppm when compared to arbutin (5). This result was in agreement with fab$^+$ms data which showed a fragment at *m/z* 413 arising from the glucose part esterified with the 2-methyl-3-veratroyloxy-propanoic acid.

From the above data, compound **1** was concluded to be 6′-*O*-[(2*R*)-methyl-3-veratroyloxy-propanoyl] arbutin, a new natural product.

Compound **2** presented uv and fabms spectra identical to those of **1**. ^{1}H- and ^{13}C-nmr data of both compounds were also similar, the difference lying in the signals for the glucose moiety. In the ^{1}H-nmr spectrum of **2**, H-4′ appeared at 4.89 ppm indicating location of an ester linkage at C-4′. This result was corroborated by the resonance of C-4′ at 72.6 ppm and by the upfield shift of the carbon in the β position (C-5′ and C-3′) when compared to arbutin (5).

Thus, the structure of **2** was established as 4′-*O*-[(2*R*)-methyl-3-veratroyloxy-propanoyl] arbutin, a new natural compound.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The general experimental procedures were similar to those described by Garcia and Chulia (6). ^{1}H- and ^{13}C-nmr spectra were obtained with a Bruker AM 300 spectrometer with TMS as internal standard. Fabms spectra were recorded on a Nermag R 10-10C spectrometer. Optical rotation was determined on a Perkin-Elmer 241 polarimeter.

ISOLATION.—*G. pyrenaica* was collected during flowering at Puymorens, Pyrénées Orientales, France. An herbarium specimen is deposited in the Herbarium of the Department of Botany (Grenoble University) where the material was identified by Professor J.F. Dobremez. The dried and powdered leaves (185 g) were successively extracted at room temperature with *n*-hexane, C_6H_6, $CHCl_3$, Me_2CO, and MeOH. The Me_2CO extract (7 g) was chromatographed on Sephadex LH 20 eluting with $CHCl_3$-MeOH (20:80).

TABLE 1. ^{1}H-nmr data (300 MHz, CD_3OD) of Compounds **1** and **2**.[a]

Proton	Compound	
	1	**2**
H-2, -6	6.91 d (8.5)	6.95 d (8.5)
H-3, -5	6.65 d (8.5)	6.68 d (8.5)
H-1′	4.66 d (7)	4.75 d (7.5)
H-2′		
H-3′	3.30–3.43 m	3.30–3.66 m
H-4′		4.89 m[b]
H-5′	3.58 ddd (9.5, 7, 2)	
H-6′a	4.54 dd (12, 2)	3.30–3.66 m
H-6′b	4.19 dd (12, 7)	
H-2″	2.99 quint d (7, 5.5)	3.03 quint d (7, 5.5)
H-3″a	4.43 dd (10.5, 7)	4.47 dd (11, 7)
H-3″b	4.39 dd (10.5, 5.5)	4.44 dd (11, 5.5)
H-4″	1.27 d (7)	1.30 d (7)
H-2‴	7.47 d (2)	7.53 d (2)
H-5‴	6.93 d (8.5)	7.08 d (8.5)
H-6‴	7.59 dd (8.5, 2)	7.65 dd (8.5, 2)
OMe	3.86–3.82 2s	3.89–3.87 2s

[a]Values in parentheses are coupling constants in Hz.
[b]Partially covered by HDO signal.

Early fractions were subjected to centrifugal tlc and eluted by $CHCl_3$/MeOH with increasing MeOH content. Fractions eluted with $CHCl_3$-MeOH (85:15) were fractionated on polyamide column with C_6H_6-MeOH as eluent. Elution with C_6H_6-MeOH (92:8) afforded a mixture of **1** and **2**; these compounds were separated by hplc on a Si gel column [hexane-iPrOH-MeOH (70:15:15)].

The final purification was performed on hplc using RP-18 and eluting with MeOH-H_2O (50:50) for **1** (5 mg) and MeOH-H_2O (55:45) for **2** (5 mg).

TABLE 2. ^{13}C-nmr data (75.46 MHz, CD_3OD) of Compounds **1** and **2**.

Carbon	Compound	
	1	**2**
C-1	153.9	153.9
C-2, -6	119.5	119.4
C-3, -5	116.6	116.6
C-4	152.3	152.3
C-1′	103.7	103.6
C-2′	74.9	75.1
C-3′	77.8	75.6
C-4′	71.8	72.6
C-5′	75.4	75.8
C-6′	65.3	62.2
C-1″	175.3	175.0
C-2″	40.8	40.6
C-3″	67.0	67.1
C-4″	14.0	14.0
Ar-CO	167.5	167.6
C-1‴	123.4	123.3
C-2‴	113.2	113.3
C-3‴	150.1	150.2
C-4‴	154.8	154.9
C-5‴	111.9	111.9
C-6‴	125.0	125.0
OMe	56.4	56.4

COMPOUND **1**.—Uv λ max (MeOH) 220, 260, 287; fab$^+$ms $[M+Na]^+$ 545, $[M+H]^+$ 523, 413, 268, 251, 165; fab$^-$ms $[M-H]^-$ 521, 181; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

COMPOUND **2**.—Uv and fabms identical to those of **1**; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

ALKALINE HYDROLYSIS.—Each compound (4.5 mg) was dissolved in 1 ml MeOH and hydrolyzed with 1 N NaOH for 2 h at room temperature. The mixture was neutralized with HCl and extracted with $CHCl_3$ and EtOAc. The $CHCl_3$ layer afforded veratric acid, and the EtOAc layer gave β-hydroxyisobutyric acid, both identified by their ^{1}H-nmr data. The aqueous layer yielded arbutin, identified by comparison with an authentic sample (^{1}H nmr, uv, tlc).

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LITERATURE CITED

1. A. Marston, K. Hostettmann, and A. Jacot-Guillarmod, *Helv. Chim. Acta*, **59**, 2596 (1976).
2. K.S. Verma, G.R. Sood, S.R. Gupta, and V.K. Gujral, *J. Chem. Soc., Perkin Trans. 1*, 2473 (1979).
3. A.G. Gonzalez, J.M. Arteaga, and J.L. Breton, *Phytochemistry*, **14**, 2039 (1975).
4. W. Choy, P. Ma, and S. Masamune, *Tetrahedron Lett.*, **22**, 3555 (1981).
5. R.A. Dommisse, L. Van Hoof, and A.J. Vlietinck, *Phytochemistry*, **25**, 1201 (1986).
6. J. Garcia and A.J. Chulia, *Planta Med.*, **52**, 327 (1986).

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