Journal of Natural Products
Vol. 52, No. 5, pp. 996-1002, Sep-Oct 1989

SECOIRIDOIDS AND A PHENOLIC GLUCOSIDE FROM *GENTIANA PYRENAICA*

JULIAN GARCIA,* EMMANUEL MPONDO MPONDO, GILBERT CARTIER,

Laboratoire de Pharmacognosie, UFR de Pharmacie, Université Joseph Fourier-Grenoble I, Domaine de la Merci, F-38706 La Tronche Cedex, France

and CLAUDE GEY

CERMAV, BP 53X, F-38041 Grenoble Cedex, France

ABSTRACT.—Morroniside [**1**], 4′-*p*-coumaroylmorroniside [**2**] and 6′-*O*-[(2*R*)-methyl-3-veratroyloxypropanoyl]morroniside [**3**], three secoiridoid glucosides, have been isolated from the leaves of *Gentiana pyrenaica* along with pyrenoside [**4**], a new phenolic glucoside. Their structures were determined by spectral and chemical means.

In a previous paper (1) the presence of several *C*-glycosylflavones was reported in the leaves of *Gentiana pyrenaica* L. (Gentianaceae). Our investigation of *G. pyrenaica* resulted in the isolation of morroniside [**1**], 4′-*p*-coumaroylmorroniside [**3**], and 6′-*O*-[(2*R*)-methyl-3-veratroyloxypropanoyl]morroniside [**4**], three secoiridoid glucosides. Besides these compounds, *G. pyrenaica* was found to contain an original phenolic glucoside which we have named pyrenoside [**6**].

Dried and powdered leaves of *G. pyrenaica* were extracted as described in the Experimental section. Repeated chromatography of the Me_2CO extract led to the isolation of compounds **1**, **3**, **4**, and **6**.

Morroniside [**1**] was identified by spectral evidence. Nmr data of **1** and its pentaacetate derivative **2** were in good agreement with those reported in the literature (2–4). The ^{1}H-nmr spectrum showed that **1** was a mixture of 7α-OH and 7β-OH isomers (2:1, respectively, from the relative signal intensities), because of a hemiacetal structure at C-7 (3,4,11).

Compound **3** presented a positive vanillin reaction identical to that of **1** and an intense uv absorption at 230 nm typical of a conjugated enol-ether functionality, these all suggesting that **3** was a secoiridoid. A comparison of ^{1}H-nmr spectra of **1** and **3** confirmed the close relationship in the aglycone protons of these compounds and indicated that **3** consisted also of a mixture of 7α/β-OH isomers. The ^{1}H-nmr spectrum of **3** displayed further signals arising from the AA′BB′ system of a *p*-coumaroyl moiety which presented *trans*-olefinic protons at 7.67 and 6.37 ppm (J = 16 Hz) as well as *cis*-olefinic protons at 6.91 and 5.81 ppm (J = 13 Hz). The presence of this moiety in the molecule was supported by fabms data which exhibited fragments at m/z = 161 (fab^-) and 147 (fab^+) attributed to the *p*-coumaroyl unit. The resonance of H-4′ at 4.85 ppm established the position of the acylation as C-4′. This fact was confirmed by the downfield shift of C-4′ at 72.4 ppm while C-5′ and C-3′ were shifted upfield by 1.8 and 2.3 ppm, respectively, when compared to **1** (4). This result was in agreeement with fab^+ ms data which displayed a peak at m/z = 309 corresponding to the glucose part esterified with *p*-coumaric acid.

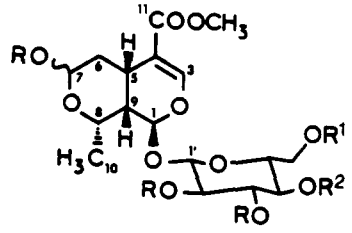
From the above data the structure of **3** was determined to be 4′-*p*-coumaroylmorroniside, a new natural product.

Compound **4** was isolated as a major constituent of the Me_2CO extract. The proton-proton connectivities were determined by a homonuclear 2D nmr experiment (COSY DQF), and ^{13}C-nmr assignments were established by heteronuclear correlation spectroscopy (XHDEPT). The ^{1}H-nmr spectrum contained resonances attributed to a mor-

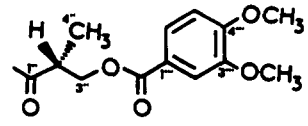
TABLE 1. ^{1}H-nmr Data (400 MHz, CD_3OD) of Compounds **3** and **4**.[a]

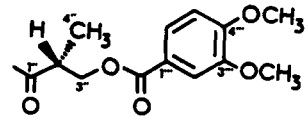
Moiety	Proton	Compound			
		3 (7α-OH)	**3** (7β-OH)	**4** (7α-OH)	**4** (7β-OH)
	H-1	5.84 d (9)	5.89 d (9)	5.77 d (9)	5.78 d (9)
	H-3	7.52 s	7.51 s	7.48 s	7.47 s
	H-5	2.93 dt (13, 4.5)	3.14 dt (13, 4.5)	2.79 dt (13, 4.5)	3.10 dt (13, 4.5)
	H-6A	1.18 td (13, 10)	1.50 td (13, 3.5)	1.17 td (13, 10)	1.43 td (13, 3.5)
	H-6β	2.03 m	1.90 dd (13, 4.5)	1.99 ddd (13, 4.5, 2.5)	1.83 dd (13, 4.5)
	H-7	4.86 m[b]	5.24 d (3.5)	4.78 dd (10, 2.5)	5.21 d (3.5)
	H-8	3.96 m	4.56 m	3.94 m	4.50 m
	H-9	1.78 ddd (9, 5, 2)	1.83 m	1.74 ddd (9, 4.5, 2.5)	1.77 m
	H-10	1.41 d (7)	1.35 d (7)	1.36 d (7)	1.27 d (7)
	11-OMe	3.70 s	3.69 s	3.71 s	3.70 s
	H-1′	4.86 m[b]	4.86 m[b]	4.79 d (8)	4.76 d (8)
	H-4′	4.85 m[b]	4.85 m[b]		
	H-6′A			4.57 dd (12, 2)	4.60 dd (12, 2)
	H-6′B			4.24 dd (12, 4.5)	4.26 dd (12, 4.5)
trans-p-coumaric acid	H-α	6.37 d (16)			
	H-β	7.67 d (16)			
	H-2″–6″	7.48 d (8.5)			
	H-3″–5″	6.81 d (8.5)			
cis-p-coumaric acid	H-α	5.81 d (13)			
	H-β	6.91 d (13)			
	H-2″–6″	7.69 d (8.5)			
	H-3″–5″	6.75 d (8.5)			
β-hydroxyisobutyric acid . .	H-2″			3.03 m	
	H-3″A			4.50 dd (11, 7)	
	H-3″B			4.42 dd (11, 5.5)	
	H-4″			1.31 d (7)	
veratric acid	H-2‴			7.52 d (2)	
	H-5‴			7.03 d (8.5)	
	H-6‴			7.65 dd (8.5, 2)	
	Ar-OMe			3.88–3.92 2s	

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



1 R=R^1=R^2=H
2 R=R^1=R^2=Ac
3 R=R^1=H, R^2=*p*-coumaroyl

4 R=R^2=H, R^1= 

5 R=R^2=Ac, R^1= 

roniside-type structure possessing the characteristic 7α/β-OH isomerism. Three aromatic protons at 7.03, 7.52, and 7.65 ppm corresponding to a 1,3,4-trisubstituted benzene ring as well as two methoxy groups at 3.88 and 3.92 ppm were assigned to a veratroyl moiety. This result, in accordance with the uv absorptions at 255 and 290 nm, also agreed with fabms data which displayed peaks at *m/z* = 181 (fab$^-$) and *m/z* = 165 (fab$^+$) (5). The ^{1}H nmr spectrum of **4** exhibited further signals arising from a β-hydroxyisobutyroyl unit (6). Acylation of the latter by veratric acid was indicated by resonance of both the H-3″ protons at 4.42 and 4.50 ppm along with that of the corresponding carbon at 67.0 ppm. This result was supported by fragments at *m/z* = 251 and 268 in the fab$^+$ mass spectrum. The β-hydroxyisobutyroyl group was linked to the C-6′ position of the glucose moiety because both the H-6′ protons were deshielded at 4.24 and 4.57 ppm as compared with **1** (4). The ^{13}C-nmr spectrum of **4** corroborated the proposed structure. Thus, the C-6′ signal was shifted downfield by 1.9 ppm when compared to **1** (4) while the signal for the β-carbon C-5′ was shifted upfield by 2.9 ppm. This finding was in agreement with the occurrence of a fragment at *m/z* = 413 (fab$^+$ms) arising from the glucose part esterified with the 2-methyl-3-veratroyloxypropanoic acid. The ^{1}H-nmr spectrum of compound **5**, the acetate derivative of **4**, dis-

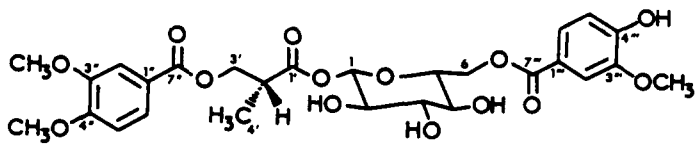


TABLE 2. ^{13}C-nmr Data (75.46 MHz) of Compounds **3**, **4**, and **5**.

Moiety	Carbon	Compound					
		3[a] (7α-OH)	**3**[a] (7β-OH)	**4**[a] (7α-OH)	**4**[a] (7β-OH)	**5**[b] (7α-OH)	**5**[b] (7β-OH)
	C-1	97.1	95.7	96.4	96.1	94.8	94.5
	C-3	154.4	154.4	154.4	154.4	152.4	152.4
	C-4	110.0	111.9	111.3	111.3	110.2	110.0
	C-5	32.0	27.4	31.8	27.2	30.0	25.9
	C-6	37.2	34.6	37.1	34.4	32.9	31.2
	C-7	96.1	92.4	96.9	92.1	93.8	91.2
	C-8	74.1	65.9	73.9	65.6	73.5	67.2
	C-9	40.0	40.6	40.1	40.7	38.8	39.1
	C-10	19.8	19.8	19.8	19.8	18.7	18.7
	C-11	168.7	168.7	168.6	168.6	166.4	166.4
	11-OMe	51.7	51.7	51.7	51.7	51.3	51.3
	C-1′	100.1	100.1	100.3	100.3	96.8	96.8
	C-2′	75.2	75.2	74.8	74.8	71.0	71.0
	C-3′	75.7	75.7	77.8	77.8	72.5	72.5
	C-4′	72.4	72.4	71.7	71.8	68.5	68.5
	C-5′	76.7	76.7	75.6	75.6	72.0	72.0
	C-6′	62.5	62.5	64.8	64.8	62.1	62.1
trans-p-coumaric acid	Ar-CO	168.5					
	C-α	114.8					
	C-β	147.2					
	C-1″	127.2					
	C-2″–6″	131.2					
	C-3″–5″	116.9					
	C-4″	161.4					
cis-p-coumaric acid	Ar-CO	167.3					
	C-α	116.1					
	C-β	146.0					
	C-1″	127.6					
	C-2″–6″	133.8					
	C-3″–5″	115.8					
	C-4″	160.4					
β-hydroxyisobutyric acid	C-1″			175.2		173.2	
	C-2″			40.7		32.3	
	C-3″			67.0		65.5	
	C-4″			14.1		13.8	
veratric acid	Ar-CO			167.5		165.8	
	C-1‴			123.4		122.4	
	C-2‴			113.4		112.2	
	C-3‴			150.2		148.7	
	C-4‴			155.0		153.1	
	C-5‴			112.0		110.3	
	C-6‴			124.9		123.6	
	OMe			56.5		56.0	

[a]In CD_3OD.
[b]In $CDCl_3$.

played four acetyl groups between 1.99 and 2.11 ppm. Of these, three were assigned to the glucose moiety and one to the C-7 hydroxyl group. This compound was obtained as a mixture of 7α/β-OH isomers but in a 5:1 ratio. Alkaline hydrolysis afforded morroniside, veratric acid, and β-hydroxyisobutyric acid. The latter was found to have the *R* configuration from its negative optical rotation value $[\alpha]^{25}D -28°$ ($c = 0.634$, MeOH) (7).

Thus, it was concluded that **4** is 6′-*O*-[(2*R*)-methyl-3-veratroyloxypropanoyl]morroniside. This compound and 4′-*p*-coumaroylmorroniside are, to our knowledge, new morroniside esters. In the Gentianaceae, morroniside has so far been isolated only from *Gentiana thunbergii* Griseb. (8) which, like *G. pyrenaica*, belongs to the Chondrophylla

section. Thus, occurrence of such compounds in the latter species may have chemotaxonomic significance.

The ^{1}H-nmr spectrum of **6** displayed signals of two 1,3,4-trisubstituted aromatic rings, three phenolic methoxy groups, an alkyl chain, and a sugar unit. The location of the three methoxy groups at C-3″, C-4″, and C-3‴ was suggested by the observation of long-range coupling correlations in a 2D nmr spectrum (COSY DQF) and confirmed by nOe experiments. Thus, the benzene rings were deduced to be veratric acid and vanillic acid. These results were in agreement with fabms data which showed fragments at $m/z = 151$ (fab^+) and 167 (fab^-) arising from the vanilloyl moiety and at $m/z = 165$ (fab^+) and 181 (fab^-) due to the veratroyl unit. As for **4**, the ^{1}H-nmr spectrum of **6** exhibited signals attributed to a β-hydroxyisobutyric acid esterified at C-3′ by veratric acid. The sugar moiety was identified by its nmr features as D-glucopyranose with a β configuration ($J_{1',2'} = 7.5$ Hz). Resonances of the anomeric proton and carbon at 5.55 and 96.0 ppm, respectively, indicated esterification of this glucose unit on the C-1 position (9). Further acylation on the C-6 hydroxy group was deduced from the downfield shift of both the H-6 protons at 4.48 and 4.19 ppm. This fact was confirmed by the deshielding of C-6 at 65.0 ppm, C-5 being shifted upfield to 76.3 ppm. The linkage of the vanillic acid to the C-6 hydroxy group was suggested by fab^+ ms, which showed a peak at $m/z = 313$ corresponding to the 6-*O*-vanilloylglucose oxonium ion. Thus, it was deduced that the β-hydroxyisobutyric acid acylated with the veratric acid was attached to

TABLE 3. ^{1}H- and ^{13}C-nmr Data (300/75 MHz, CD_3OD) of Compound **6**.[a]

Atom	^{1}H	^{13}C
1	5.55 d (7.5)	96.0
2		73.9
3	3.31–3.50 m	78.1
4		71.6
5	3.69 ddd (9.5, 6.5, 2)	76.3
6A	4.48 dd (12, 2)	65.0
6B	4.19 dd (12, 6.5)	
1′	—	174.6
2′	3.07 quint.d (7,5)	40.4
3′A	4.43 dd (11, 5)	67.2
3′B	4.36 dd (11, 7)	
4′	1.29 d (7)	13.7
1″	—	123.2
2″	7.33 d (2)	113.6
3″	—	150.0
4″	—	154.8
5″	6.70 d (8.5)	111.7
6″	7.49 dd (8.5, 2)	124.9
7″	—	167.6[b]
1‴	—	122.4
2‴	7.36 d (2)	115.8
3‴	—	148.6
4‴	—	152.8
5‴	6.73 d (8.5)	113.9
6‴	7.39 dd (8.5, 2)	125.1
7‴	—	167.9[b]
3″-OMe	3.73 s	56.4–56.5
4″-OMe	3.80 s	
3‴-OMe	3.84 s	

[a]Values in parentheses are coupling constants in Hz.
[b]Interchangeable.

the C-1 position of the glucose moiety. These results were corroborated by acid hydrolysis under mild conditions which yielded 6-*O*-vanilloyl-D-glucopyranose and 2-methyl-3-veratroyloxy-propanoic acid. It was noteworthy that **6** was rather unstable when left at room temperature for a few days. It spontaneously afforded two products which were further purified and appeared to be identical to those obtained by mild acid hydrolysis. Alkaline hydrolysis gave veratric acid, vanillic acid, D-glucose, and β-hydroxyisobutyric acid with the *R* configuration.

From the above findings, it was concluded that **6** is 1-*O*-[(2*R*)-methyl-3-veratroyloxy propanoyl]-6-*O*-vanilloyl-β-D-glucopyranoside, a new natural product for which we propose the name of pyrenoside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General isolation procedures and instrumentation were similar to those given in Garcia and Chulia (10). ^{1}H- and ^{13}C-nmr spectra were measured on Bruker AM 400 or AM 300 spectrometers with TMS as internal standard. Fabms spectra were registered on a Nermag R 10-10C spectrometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter.

ISOLATION.—*G. pyrenaica* was collected when in flower at Puymorens in the Pyrenees (Pyrénées Orientales, France). A voucher specimen is preserved in the laboratory. The dried and powdered leaves and stems (185 g) were successively extracted with *n*-hexane, C_6H_6, $CHCl_3$, Me_2CO, and MeOH at room temperature. The Me_2CO extract (7 g) was fractionated on Sephadex LH 20 with $CHCl_3$-MeOH (20:80) as eluent. Head fractions were chromatographed on centrifugal tlc, eluting by $CHCl_3$/MeOH with increasing MeOH content. Elution with $CHCl_3$-MeOH (95:5) afforded compound **4** (60 mg) which was purified by hplc on a Si gel column [C_6H_{14}-iPrOH-MeOH (70:15:15)]. Fractions eluted with $CHCl_3$-MeOH (85:15) were further chromatographed on a polyamide column to give 9 mg of **6** [C_6H_6-MeOH (94:6)] and 9 mg of **3** [C_6H_6-MeOH (85:15)]. Final purification of the latter compound was performed on hplc using a Si gel column as above. Compound **6** was purified by hplc on RP-18 [MeOH-H_2O (50:50)]. Fractions eluted with $CHCl_3$-MeOH (60:40) were subjected to hplc on RP-18 [MeOH-H_2O (35:65)] to afford **1** (20 mg), which was purified by hplc on a Si gel column as above.

MORRONISIDE [**1**].—Identified by comparison of its nmr data and that of its acetate derivative with literature values (2–4).

4′-*p*-COUMAROYL MORRONISIDE [**3**].—Oil: uv λ max (MeOH) 230, 310; fab$^+$ms $[M+K]^+$ 591, $[M+Na]^+$ 575, 309, 227, 147; fab$^-$ms $[M-H]^-$ 551, [aglycone − H]$^-$ 243, 163.

COMPOUND **4**.—Oil: uv λ max (MeOH) 225, 240, 255, 290; fab$^+$ms $[M+K]^+$ 695, $[M+Na]^+$ 679, 413, 268, 251, 165; fab$^-$ms $[M-H]^-$ 655, [aglycone − H]$^-$ 243, 181.

PYRENOSIDE [**6**].—Colorless amorphous powder: uv λ max (MeOH) 218, 260, 292; fab$^+$ms $[M+Na]^+$ 603, $[M+H]^+$ 581, 313, 268, 251, 165, 151; fab$^-$ms $[M-H]^-$ 579, 181, 167.

ACETYLATION OF **4**.—Compound **4** (10 mg) was treated with pyridine/Ac_2O at room temperature for 12 h. After usual workup the tetraacetate derivative **5** (12 mg) was purified by hplc on a Si gel column [C_6H_{14}-iPrOH-MeOH (70:15:15)]. Cims (%) $[M+NH_4]^+$ 842 (5.8), 782 (100), [M − aglycone]$^+$ 539 (23.4), 269 (14.7), 200 (79.7), 182 (21.3), 165 (55.4).

ALKALINE HYDROLYSIS OF **4**.—Compound **4** (45 mg) was dissolved in 1 ml MeOH and hydolyzed with 1 ml in NaOH for 1 h at room temperature. After neutralization with HCl the mixture was extracted with $CHCl_3$ and EtOAc. The organic layers yielded veratric acid and β-hydroxyisobutyric acid, both identified by their nmr data and the β-hydroxyisobutyric acid by optical rotation. The aqueous layer gave morroniside, identified by tlc, hplc, and nmr.

ALKALINE HYDROLYSIS OF **6**.—Compound **6** (5 mg) was hydrolyzed as above. The $CHCl_3$ layer afforded veratric acid and vanillic acid, and the EtOAc layer gave β-hydroxyisobutyric acid, identified by their nmr data. The aqueous layer yielded D-glucose, identified by tlc.

MILD ACID HYDROLYSIS OF **6**.—Compound **6** (2 mg) was warmed for 1 h at 60° with 2 ml 0.5 N HCl. After cooling the solution was extracted with $CHCl_3$. The organic layer yielded 2-methyl-3-veratroyloxy-propanoic acid which was purified by hplc on RP-18 [MeOH-H_2O (50:50)]. ^{1}H nmr (300 MHz, CD_3OD) 1.26 (3H, d, J = 7 Hz, H-4′), 2.97 (1H, quint.d, J = 7, 6 Hz, H-2′), 3.86 and 3.89 (2 × 3H, s, OMe), 4.39 (1H, d, J = 6 Hz, H-3′A), 4.40 (1H, d, J = 7 Hz, H-3′B), 7.02 (1H, d, J = 8.5 Hz, H-5″), 7.51 (1H, d, J = 2 Hz, H-2″), 7.62 (1H, dd, J = 8.5, 2 Hz, H-6″).

The aqueous layer afforded 6-*O*-vanilloyl-D-glucopyranose which was purified as above. ^{1}H-nmr (300 MHz, CD_3OD) 3.16–4.60 (sugar protons), 3.89 (3H, s, OMe), 4.51 (1H, d, J = 8 Hz, H-1β), 5.11 (1H, d, J = 3.5 Hz, H-1α), 6.83 (1H, d, J = 8.5 Hz, H-5‴), 7.56 (1H, d, J = 2 Hz, H-2‴), 7.57 (1H, dd, J = 8.5, 2 Hz, H-6‴).

ACKNOWLEDGMENTS

We are grateful to Mr. C. Bosso (CERMAV, Grenoble) for the mass spectra and Mrs. M.-C. Micciche for secretarial help.

LITERATURE CITED

1. A. Marston, K. Hostettmann, and A. Jacot-Guillarmod, *Helv. Chim. Acta*, **59**, 2596 (1976).
2. H. Inouye, S. Tobita, Y. Akiyama, K. Ito, and T. Shingu, *Chem. Pharm. Bull.*, **21**, 846 (1973).
3. K. Bock, S.R. Jensen, and B.J. Nielsen, *Acta Chem. Scand., Ser. B*, **30**, 743 (1976).
4. G.A. Gross and O. Sticher, *Helv. Chim. Acta*, **69**, 1113 (1986).
5. K.S. Verma, G.R. Sood, S.R. Gupta, and V.K. Gujral, *J. Chem. Soc., Perkin Trans. 1*, 2473 (1979).
6. A.G. Gonzalez, J.M. Arteaga, and J.L. Breton, *Phytochemistry*, **14**, 2039 (1975).
7. W. Choy, P. Ma, and S. Masamune, *Tetrahedron Lett.*, **22**, 3555 (1981).
8. H. Inouye and Y. Nakamura, *Yakugaku Zasshi*, **91**, 755 (1971).
9. K. Yoshimoto, Y. Itatani, and Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 2065 (1980).
10. J. Garcia and A.J. Chulia, *Planta Med.*, **52**, 327 (1986).
11. A. Rumbero-Sanchez and P. Vazquez, *Heterocycles*, **27**, 2863 (1988).

Received 6 *March* 1989