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NEW SECOIRIDOID GLUCOSIDES FROM *GENTIANA VERNA*

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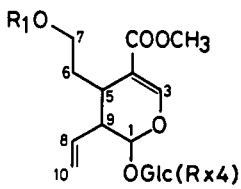
ABSTRACT.—Secologanol [**1**], 7-acetylsecologanol [**2**], and 7-(2,5-dihydroxybenzoyl)secologanol [**3**], three new secoiridoid glucosides, have been isolated from the aerial parts of *Gentiana verna*. Their structures were determined by spectral methods.

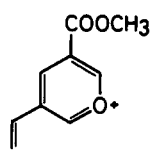
Previous studies reported the presence of flavonoids and xanthones (1) in *Gentiana verna* L. (Gentianaceae). Our investigation led to the isolation of three new secologanin-type of secoiridoids. These include secologanol [**1**], 7-acetylsecologanol [**2**], and 7-(2,5-dihydroxybenzoyl)secologanol [**3**]. Their structural elucidation by spectral means is discussed herein.

Dried and powdered aerial parts of *G. verna* were extracted with solvent of increasing polarities. The Me_2CO extract was fractionated to give the three secoiridoids **1–3** after chromatographic workup (see Experimental).

When submitted to H_2SO_4-vanillin spray, the three compounds gave a red color characteristic of the secologanin type of secoiridoids. ^1H and ^{13}C nmr of these compounds (Tables 1 and 2) indicated that they consist of a glucoside and a secoiridoid unit. Their fab$^+$ms displayed a peak **4** at $m/z = 165$ characteristic of these secoiridoids (2,3).



1 R=R_1=H
2 R=H, R_1=Ac
3 R=H, R_1=gentisoyl



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The ^1H- and ^{13}C-nmr spectra of compound **1** indicated the presence of protons at 1.69 and 1.88 ppm (H-6) and 3.55 ppm (2H, H-7) and their corresponding carbons at 33.6 ppm (C-6) and 61.3 ppm (C-7). The multiplicities of these carbons were found by DEPT experiments to be CH_2 groups. Furthermore, the ^1H-nmr spectrum of the acetylated derivative of **1** displayed five alcoholic acetyls, among which four belong to the glucoside moiety and one to the terpene. These facts suggested that H-7 is included in a primary alcohol function. This structure was in good agreement with fabms data, which showed pseudomolecular ion peaks at $m/z = 389$ $[M-H]^-$ and $m/z = 391$ $[M+H]^+$. The stereochemistries at C-5 and C-8 were determined by comparing the ^{13}C-nmr values of **1** with those of similar compounds reported by Jensen *et al.* (4). From the above findings, compound **1** was determined to be secologanol, an alcoholic derivative of secologanin.

Compound **2** exhibited a uv spectrum identical to that of **1**. Its fab$^+$ms, m/z 433 $[M+H]^+$, and fab$^-$ms, $m/z = 431$ $[M-H]^-$, were indicative of an increase of 42 amu in comparison with **1**. The ^1H-nmr spectrum of **2** was similar to that of **1** except for the occurrence of a natural alcoholic acetyl in **2** which reso-

TABLE 1. ^{1}H-nmr Data of the Secoiridoids.[a]

Proton	1	2	3	1 acetylated	3 acetylated
H-1	5.52 d (6.5)	5.52 d (6.5)	5.59 d (6.5)	5.35 d (5)	5.35 d (5)
H-3	7.43 s	7.44 s	7.51 s	7.35 s	7.36 s
H-4	—	—	—	—	—
H-5	2.85 br dd (13,6)	2.85 br dd (12.5,6)	2.97 br dd (13,6)	2.78 m	2.83 m
H-6A	1.88 dt (16,6)	1.93 dt (16,6)	2.11 dt (15,6)	2.23 m	1.90–2.35
H-6B	1.69 ddd (16,13,6)	1.81 ddd (16,12.5,6)	1.99 ddd (15,13,6)	1.90–2.10	1.90–2.35
H-7	3.55 m	4.08 m	4.35 t (6)	4.06 m	4.35 m
H-8	5.77 ddd (17,10,8.5)	5.77 ddd (17,10,8.5)	5.82 ddd (17,10.5,8.5)	5.60 ddd (17,10,8)	5.60 ddd (17,10,8)
H-9	2.63 ddd (8.5,6.5,6)	2.66 ddd (8.5,6.5,6)	2.69 ddd (8.5,6.5,6)	2.67 m	2.70 m
H-10A	5.28 dd (17,1.5)	5.30 dd (17,1.5)	5.33 dd (17,1.5)	5.26–5.34	5.25–5.35
H-10B	5.23 dd (10,1.5)	5.25 dd (10,1.5)	5.25 dd (10.5,1.5)	5.26–5.34	5.25–5.35
OAc	3.68 s	3.68 s	3.68 s	3.71 s	3.7 s
Ac	—	2.01 s	—	1.93–2.10 5s	1.92–2.34 6s
H-1′	4.62 d (8)	4.69 d (8)	4.71 d (8)	4.87 d (8)	4.87 d (8)
H-2′–6′	3.20–3.89	3.19–3.90	3.20–3.90	3.73–5.01	3.70–5.01
H-3″	—	—	6.78 d (9)	—	7.09 d (8.5)
H-4″			6.97 dd (9,3)		7.32 dd (8.5,2.5)
H-6″			7.23 d (3)		7.75 d (2.5)

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

TABLE 2. ^{13}C-nmr Data of the Secoiridoids.

Carbon	1	2	3
C-1	97.8	97.8	97.8
C-3	153.5	153.6	153.8
C-4	111.8	111.5	111.5
C-5	30.9	30.0	30.2
C-6	33.6	31.4	31.7
C-7	61.3	64.2	65.0
C-8	135.8	135.6	135.7
C-9	45.4	45.4	45.4
C-10	119.5	119.5	119.6
$COOCH_3$	169.5 and 51.7	169.2 and 51.7	169.2 and 51.7
$COCH_3$	—	172.9 and 20.8	—
C-1′	100.3	100.2	100.3
C-2′	74.7	74.7	74.7
C-3′	78.0	78.1	78.1
C-4′	71.6	71.6	71.6
C-5′	78.4	78.4	78.4
C-6′	62.8	62.8	62.9
C-1″			113.5
C-2″			156.1
C-3″			115.6
C-4″			118.9
C-5″			150.7
C-6″			125.0
C-7″ (C=O)			171.8

nated at 2.01 ppm. The location of this acetyl was determined to be on position 7, according to the deshielding of H-7 at 4.08 ppm, as compared with **1**. This fact was confirmed by the ^{13}C-nmr spectrum, which showed a downfield shift for the C-7 signal ($\Delta\delta = +2.9$ ppm) while the carbon at the β position (C-6) was shifted upfield by a similar amount when compared with **1**. Moreover, the ^{1}H-nmr of the acetylated derivatives of **2** and **1** were identical. Compound **2** was therefore identified as 7-acetylsecologanol.

Comparison of the ^{1}H-nmr spectra of **3** and **1** revealed a secologanol-like structure for **3**. Further resonances above 6.78 ppm were attributed to a 1,2,5-trisubstituted benzene ring which was identified, by comparison with the literature values (5), as 2,5-dihydroxybenzoic acid or gentisic acid. This conclusion agreed with the presence of fragments at $m/z = 137$ (fab^+) and at $m/z = 153$ (fab^-) in the mass spectrum. The acetylated derivative of **3** displayed four alcoholic acetyls at 1.92, 2.00, 2.05, and 2.09 ppm corresponding to the glucose moiety and two phenolic acetyls at 2.31 and 2.34 ppm attributed to the benzoic acid. The deshielding of both the H-7 at 4.35 ppm in the ^{1}H-nmr spectrum of **3** indicated that the gentisoyl unit was located at C-7. This result was corroborated by the ^{13}C-nmr data which showed a downfield shift of the C-7 signal ($\Delta\delta = +3.7$ ppm) while C-6 was shifted upfield by 1.9 ppm, as compared with **1**. The proposed structure was in agreement with the acid hydrolysis of **3** which yielded D-glucose and gentisic acid, both identified by comparison with authentic samples. From the evidence set forth, **3** was identified as 7-(2,5-dihydroxybenzoyl)secologanol or 7-gentisoylsecologanol, a new natural compound.

Secologanol and 7-acetylsecologanol have been reported as a part of bisglycosidic iridoids and secoiridoids isolated from *Dipsacus sylvestris* (4). This is

the first report of their occurrence alone as natural compounds. 7-Gentisoylsecologanol is a new natural product. To our knowledge, this is the first isolation of these secoiridoids from a member of the Gentianaceae.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on Bruker AM 300 and AC 200 spectrometers. Ms were obtained using a Nermag R 10-10C spectrometer with a glycerol matrix. The uv spectra were performed on a Beckman model 25, and hplc was realized using Waters Associates model 510 and 440. H_2SO_4/vanillin was sprayed on Si gel plates of the three compounds, which developed a red color after heating. Acetylation of compounds was carried out using standard procedures with pyridine/Ac_2O, and the final purification was obtained by hplc on a Si gel column with *n*-hexane–iPrOH–MeOH (70:15:15).

ISOLATION PROCEDURE.—*G. verna* was collected when in flower at the Col du Galibier (Isère, France). A voucher specimen was deposited at the Pharmacognosy Laboratory Herbarium. Dried and powdered aerial parts (180 g) were successively extracted with *n*-hexane, C_6H_6, Me_2CO, and MeOH. The Me_2CO extract (6 g) was fractionated on a Si gel column ($CHCl_3$ with increasing MeOH content) and over Si gel centrifugal tlc with the same solvent. The middle fractions afforded **2** and **3** after purification by hplc on RP-18 [H_2O-MeOH (60:40)]. The last fractions yielded **1** after purification by hplc on RP-18 [H_2O-MeOH (65:35)].

Compound **1** *(10 mg).*—Uv λ max (MeOH) 235; ^{1}H nmr (300 MHz, CD_3OD/TMS) see Table 1; ^{13}C nmr (75.46 MHz, CD_3OD) see Table 2; fab$^+$ms $[2M+H]^+$ 781, $[M+H]^+$ 391, $[M+H-Glc]^+$ 229, 211, 179, 165, 133, 105; fab$^-$ms $[2M-H]^-$ 779, $[M-H]^-$ 389, $[M-Glc]^-$ 227, 195, 179, 157, 119, 101. ^{1}H nmr of **1** acetylated (2 mg, 200 MHz, $CDCl_3$/TMS) see Table 1.

Compound **2** *(9 mg).*—Uv λ max (MeOH) 235; ^{1}H nmr (300 MHz, CD_3OD/TMS) see Table 1; ^{13}C nmr (50 MHz, CD_3OD) see Table 2; fab$^+$ms $[M+H]^+$ 433, $[M+H-Ac]^+$ 391, $[M+H-Glc]^+$ 271, 211, 179, 165, 139, 133, 105; fab$^-$ms $[2M-H]^-$ 863, $[M-H]^-$ 431, $[M-Glc]^-$ 269, 199, 179, 155, 129, 119, 101.

Compound **3** *(12 mg).*—Uv λ max (MeOH) 235, 335; ^{1}H nmr (300 MHz, CD_3OD/TMS) see Table 1; ^{13}C nmr (50 MHz, CD_3OD) see Table 2; fab$^+$ms $[M+H]^+$ 527, $[M+H-benzoyl]^+$ 391, 373, $[M+H-Glc]^+$ 365, 211, 179, 165, 139, 137, 109, 105; fab$^-$ms $[M-H]^-$ 525, 153, 135, 127, 109, 101. ^{1}H nmr of acetylated **3** (3 mg, 200 MHz, $CDCl_3$/TMS) see Table 1.

ACID HYDROLYSIS OF **3**.—Compound **3** (3 mg) was refluxed with 5 ml 2 N HCl for 1 h. After evaporation the residue was examined by tlc in comparison with authentic samples of D-glucose and gentisic acid.

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