A New Secoiridoid Glucoside, Amaronitidin, from the Peruvian Folk Medicine "Hercampuri" (*Gentianella nitida*)

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A new secoiridoid glucoside designated amaronitidin (1) was isolated from the Peruvian folk medicine "Hercampuri" (*Gentianella nitida*) along with three known secoiridoid glucosides. Their structures were determined by extensive spectroscopic investigation.

Key words amaronitidin; *Gentianella nitida*; Hercampuri; Gentianaceae; secoiridoid glucoside

In previous papers,[1,2] we reported the isolation of two novel sesterterpenoids with a new skeleton designated as nitidasin and nitiol from the dichloromethane extract of the whole plant of *Gentianella* (*G.*) *nitida* (Gentianaceae), a biennial medicinal plant growing in the Andes region and used in traditional Peruvian folk medicine. Commonly known as "Hercampuri" or "Hircampure", it is used as a remedy for hepatitis, as a cholagogue, and in treatment of obesity.[3] Further investigation of the EtOAc extract of the above medicinal plant led us to isolate a new secoiridoid glycoside designated amaronitidin (**1**), along with three known secoiridoid glucosides, amarogentin (**2**), amaroswerin (**3**) and decentapicrin A (**4**). The structural elucidation of the above compound **1** is reported in this paper.

The methanolic extract (518 g) of the whole plant (1.35 kg) was partitioned between EtOAc and H_2O. The EtOAc extract (18.8 g) was fractionated by silica gel column chromatography using a $CHCl_3$–MeOH gradient. The $CHCl_3$–MeOH (5 : 1) elute was subjected to a Sephadex LH-20 with a $CHCl_3$–MeOH (3 : 1) solvent system followed by HPLC with a $CHCl_3$–MeOH (10 : 1) solvent system to afford compounds **1**—**4**. Compounds **2**, **3** and **4** were identified as amarogentin, amaroswerin and decentapicrin A, respectively, on the basis of the ^{1}H- and ^{13}C-NMR spectra and finally comparison with the literature data.[4,5]

Compound **1**, in the form of colorless amorphous, $[\alpha]_D$ −76.1° (*c* 1.40, MeOH), gave a quasi-molecular ion at *m*/*z* 585 $(M+H)^+$ in positive ion FAB mass spectrometry, and high-resolution FAB-MS determined the molecular formula $C_{29}H_{29}O_{13}$ ($[M+H]^+$; *m*/*z* 585.1625). The IR and UV spectra suggested the presence of carbonyl groups conjugated with double bonds and hydroxyl groups. The ^{13}C-NMR spectrum of **1** (Table 1) displayed twenty sp^2 carbons, including two carbonyl carbons (δ 165.7, 171.0), and a sugar moiety (δ 62.5, 71.7, 74.4, 75.0, 78.4, 96.8). The ^{1}H-NMR spectrum of **1** (Table 1) exhibited twenty-two nonexchangeable protons, including five olefinic proton signals at δ 7.30 (br s), 5.55 (br dd, *J*=1.8, 3.7 Hz), 5.66 (ddd, *J*=6.7, 10.4, 17.1 Hz), 5.12 (ddd, *J*=1.2, 1.5, 10.4 Hz) and 5.15 (ddd, *J*=1.2, 1.5, 17.1 Hz), which were assignable to H-3, H-6, H-8 and H_2-10 of the secoiridoid skeleton, respectively. Furthermore, the appearance of two *meta*-coupled aromatic proton signals at δ 6.16 and 6.30 (each 1H, d, *J*=2.4 Hz) and disubstituted aromatic proton signals at δ 6.69 (dd, *J*=1.5, 2.4 Hz), 6.72 (ddd, *J*=0.9, 1.5, 7.9 Hz), 6.77 (ddd, *J*=0.9, 2.4, 7.9 Hz) and 7.16 (t, *J*=7.9 Hz) indicated the presence of a 3,3′,5-trihydroxy-2-biphenylcarboxyl group related to **2** and **3**. The other ^{1}H-NMR spectral data were quite similar to those of gentiopicroside (**5**)[6] except for the signal of H-2′. A downfield shift (1.53 ppm) of the signal due to H-2′ suggested that a 3,3′,5-trihydroxy-2-biphenyl-carboxyl group was attached to C-2′ of **5**. The 3,3′,5-trihydroxy-2-biphenylcarboxyl group was determined to be attached at the C-2′ position of the β-glucopyranosyl moiety due to the correlation between the H-2′ (δ 4.67) and carbonyl signal at δ 171.0 ppm observed in the heteronuclear multiple-bond correlation (HMBC) spectrum.

To confirm the structure of **1** as 3,3′,5-trihydroxy-2-biphenylcarboxylate, **1** was deacylated with methanolic 25% ammonia. TLC and HPLC identified the products as gentiopicroside and 3,3′,5-trihydroxy-2-biphenylcarboxylic acid methyl ester. Therefore, the structure of amaronitidin was de-

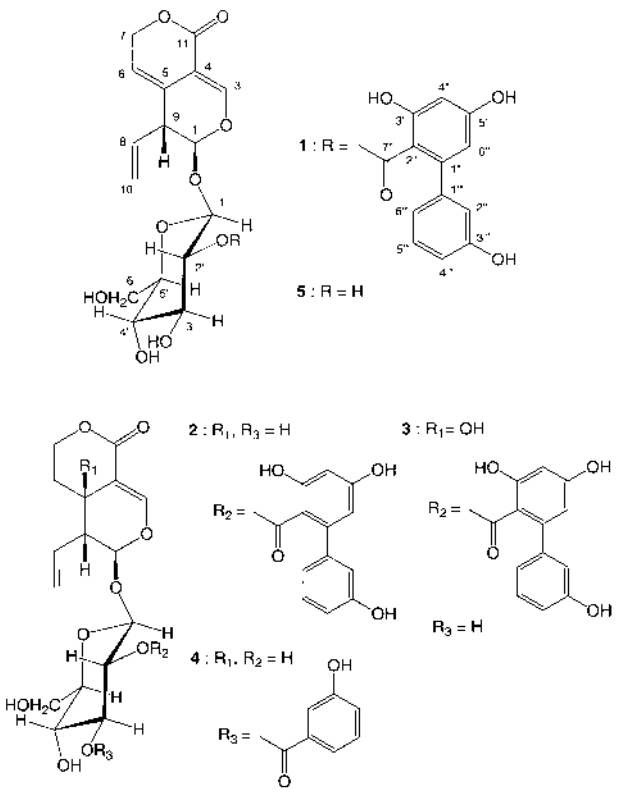

Chart 1

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Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of **1** in CD_3OD

Position	$^{1}H^{a)}$	$^{13}C^{b)}$
1	5.57 (d, 1.8)	97.1 d
3	7.30 (br s)	149.7 d
4		106.0 s
5		126.6 s
6	5.55 (br dd, 1.8, 3.7)	117.7 d
7	4.83 (br dd, 3.7, 18.0)	70.5 t
	4.91 (br dd, 1.8, 18.0)	
8	5.66 (ddd, 6.7, 10.4 17.1)	134.7 d
9	3.20 (ddt, 1.5, 1.8, 6.7)	46.2 d
10	5.12 (ddd, 1.2, 1.5, 10.4)	118.2 t
	5.15 (ddd, 1.2, 1.5, 17.1)	
11		165.7 s
1′	4.17 (d, 7.9)	96.8 d
2′	4.67 (dd, 7.9, 9.5)	74.4 d
3′	2.84 (t, 9.5)	75.0 d
4′	3.21 (t, 9.5)	71.7 d
5′	3.06 (ddd, 2.1, 6.4, 9.5)	78.4 d
6′	3.59 (dd, 6.4, 11.9)	62.5 t
	3.83 (dd, 2.1, 11.9)	
1″		148.6 s
2″		104.0 s
3″		166.0 s
4″	6.30 (d, 2.4)	103.0 d
5″		163.7 s
6″	6.16 (d, 2.4)	112.8 d
7″		171.0 s
1‴		146.5 s
2‴	6.69 (dd, 1.5, 2.4)	116.6 d
3‴		157.4 s
4‴	6.77 (ddd, 0.9, 2.4, 7.9)	114.5 d
5‴	7.16 (t, 7.9)	129.3 d
6‴	6.72 (ddd, 0.9, 1.5, 7.9)	121.2 d

a) *J* values (in Hz) in parentheses. *b*) Multiplicities and assignments made by the HMBC and DEPT techniques.

termined as shown in Chart 1.

A large number of acylated secoiridoid glucosides, including **2** and **3**, have been isolated from the Gentianaceae family, while **1** is the first example of a 3,3′,5-trihydroxy-2-biphenylcarboxylate of **5**.

Experimental

General Procedures Optical rotations were measured with a JASCO DIP-370 spectrometer. FAB-MS and HR-FAB-MS were obtained on a JEOL JMS-SX102 spectrometer. Ultraviolet (UV) and IR spectra were recorded on a Hitachi U-2000 spectrophotometer and a JASCO IR-5300 spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL α-500 spectrometer at 500 MHz and at 125 MHz, respectively, using tetramethylsilane as an internal standard. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck) and Sephadex LH-20 (Pharmacia). HPLC was performed on a column of LiChrospher Si 60 (250×10 mm i.d., Merck). Thin layer chromatography (TLC) was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected under UV light.

Plant Material The whole plants of *Gentianella nitida* were collected in 1996 in Houaroz, Peru. A voucher specimen has been deposited at the National Institute of Health Sciences, Japan.

Extraction and Isolation The whole plant of *G. nitida* (1.35 kg) was crushed and extracted with MeOH (3 l×5) to give an extract (518 g), which was partitioned between CH_2Cl_2 and H_2O, and then EtOAc and H_2O to give CH_2Cl_2 extract (50.3 g) and EtOAc extract (23.2 g). Eighteen and eight-tenth grams of EtOAc extract was fractionated by silica gel column chromatography using a $CHCl_3$–MeOH gradient. The $CHCl_3$–MeOH (5 : 1) elute was subjected to a Sephadex LH-20 with a $CHCl_3$–MeOH (3 : 1) solvent system followed by HPLC with a $CHCl_3$–MeOH (10 : 1) solvent system to afford amaronitidin (**1**, 25 mg), amarogentin (**2**, 250 mg), amaroswerin (**3**, 18 mg) and decentapicrin A (**4**, 47 mg).

Compound **1**: Colorless amorphous powder. $[\alpha]_D^{23}$ −76.1° (*c* 1.40, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1701 (CO), 1657 (CO). UV λ (log ε): 224 sh (4.66), 266 (4.28), 296 sh (3.90). Positive FAB-MS *m/z*: 585 $(M+H)^+$, HR-FAB-MS *m/z*: Calcd for $C_{29}H_{29}O_{13}$: 585.1608. Found: 585.1625. ^{1}H- and ^{13}C-NMR: Table 1.

Alkaline Hydrolysis of 1 Compound **1** (2 mg) was dissolved in MeOH (0.5 ml) containing 25% ammonia (0.5 ml) and the solution was kept at room temperature for 12 h. The reaction mixture was poured into ice water, neutralized with 10% H_2SO_4 and extracted with $CHCl_3$ and EtOAc. The $CHCl_3$ layer was subjected to TLC ($CHCl_3$–MeOH, 10 : 1) and HPLC (t_R, 4.0 min) analysis to identify the 3,3′,5-trihydroxy-2-biphenylcarboxylic acid methyl ester. The EtOAc layer was also subjected to TLC ($CHCl_3$–MeOH, 3 : 1) and HPLC (t_R, 4.5 min) analysis to identify gentiopicroside. HPLC conditions: column, TSK-GEL ODS-80TS (TOSOH Co. Ltd.), 150×4.6 mm (i.d.); solvent, CH_3CN–H_2O (9 : 91, V/V); flow rate, 0.5 ml/min.

3,3′,5-Trihydroxy-2-biphenylcarboxylic Acid Methyl Ester Compound **2** (6 mg) was dissolved in MeOH (0.5 ml) containing 1 N KOH (0.5 ml) and the solution was kept at room temperature for 1 h. The reaction mixture was poured into ice water, neutralized with 10% H_2SO_4 and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and the residue was purified by HPLC with $CHCl_3$–MeOH (30 : 1) to give 3,3′,5-trihydroxy-2-biphenylcarboxylic acid methyl ester (1.5 mg) as an amorphous powder. ^{1}H-NMR ($CDCl_3$): δ 3.49 (3H, s, OMe), 6.27 (1H, d, *J*=2.7 Hz, H-6), 6.43 (1H, d, *J*=2.7 Hz, H-4), 6.70 (1H, br dd, *J*=1.8 and 2.1 Hz, H-2′), 6.77 (1H, br d, *J*=7.9 Hz, H-4′), 6.99 (1H, br dd, *J*=2.1, 7.9 Hz, H-6′), 7.20 (1H, t, *J*=7.9 Hz, H-5′), 11.17 (1H, s, OH). ^{13}C-NMR ($CDCl_3$): δ 51.6 (COOMe), 102.5 (C-4), 105.3 (C-2), 111.1 (C-6), 113.8 (C-4′), 114.9 (C-2′), 120.7 (C-6′), 128.7 (C-5′), 144.2 (C-1′), 146.7 (C-1), 154.9 (C-3′), 159.8 (C-5), 164.1 (C-3), 171.1 (COOMe).

References and Notes

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6) The reference standard was purchased from Toray Techno Co., Ltd.