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Original Article

Paniculatumoside G, a new C_{21} steroidal glycoside from *Cynanchum paniculatum*

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ABSTRACT

A new C_{21} steroidal glycoside, paniculatumoside G, together with neocynapanogenin C isolated for the first time from the natural source and two known compounds were isolated and characterized from the roots and rhizomes of *Cynanchum paniculatum* (Bunge) Kitag. ex H.Hara, Apocynaceae, a commonly used Traditional Chinese Medicine. On the basis of spectroscopic analysis, including HR-ESI-MS, 1D and 2D NMR spectral data, the structure of the new C_{21} steroidal glycoside was elucidated as neocynapanogenin H 3-*O*-*β*-D-oleandropyranoside.

Introduction

Cynanchum paniculatum (Bunge) Kitag. ex H.Hara, Apocynaceae, a perennial herb native to east Asia, is commonly called 'Xu Chang Qing' in Chinese, and has been used as a Traditional Chinese Medicine for the treatment of peratodynia, gastroenteritis, venomous snake bite, and ascites (Jiang and Li, 1977). Previous phytochemical investigations on *C. paniculatum* have revealed the presence of phenolic derivatives, alkaloids, flavonoids, polysaccharides, triterpenoids, and C_{21} steroidal glycosides (Niu et al., 2015; Fu et al., 2015). The reported bioactivities of the plant extracts and isolated constituents include anti-adipogenic (Jang et al., 2014), neuroprotective (Weon et al., 2013), anti-tumor (Kim et al., 2012), anti-inflammatory, anti-nociceptive, sedative (Choi et al., 2006), araricidal (Kim et al., 2013a), and herpes simplex encephalitis inducing impairment preventive activities (Li et al., 2012). Our previous phytochemical investigation on ethanol extract of this source resulted in the isolation of nine C_{21} steroidal aglycones and glycosides (Chu et al., 2015). In our continuing study on this source, one new steroidal glycoside (**1**) together with three known compounds (**2**–**4**) were isolated and identified. It should be noted that compound **2** was isolated for the first time from the natural source. Their structures were elucidated by detailed interpretation of NMR and MS data.

Materials and methods

General experimental procedures

Optical rotations were measured by using a JASCO P-1020 automatic digital polarimeter (JASCO Corporation, Tokyo, Japan). The NMR spectral data were recorded on a Bruker AV-500 FT-NMR (500 MHz for ^{1}H and 125 MHz for ^{13}C) in C_5D_5N, using visual C_5D_5N resonances (^{1}H δ 7.21, 7.58, and 8.73, ^{13}C δ 123.5, 135.5, and 149.0) for internal reference. All chemical shifts (δ) are given in ppm. HR-ESI-MS and ESI-MS were obtained with a Bruker microTOFQ mass spectrometer (Bruker Daltonics, Bremen, Germany). Column chromatography was performed with macroporous resin HPD100 (Cangzhou Bon Adsorber Technology Co., Ltd, Cangzhou, China) and RP-18 reversed-phase silica gel (S-50 mm, YMC, Kyoto, Japan). TLC analysis was carried out on pre-coated TLC plates with silica gel RP-18 60 F_{254} (Merck, Darmstadt, Germany, 0.25 mm). Detection was achieved by spraying with 10% H_2SO_4 in MeOH followed by heating. Preparative HPLC was performed on a NP7005C pump connected with a SHODEX RI-102 detector (Shoko Scientific Co., Ltd, Tokohama, Japan), using Megres ODS column (250 mm × 10 mm, i.d., 5 μm, Hanbang Sci. & Tech., Haian, China). HPLC-grade MeOH was purchased from Merck. HPLC-grade water was purified using

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a Milli-Q system (millipore, Boston, MA, USA). All solvents used for the chromatographic separations were distilled before use.

Plant material

The roots and rhizomes of *Cynanchum paniculatum* (Bunge) Kitag. ex H.Hara, Apocynaceae, were obtained in Jingde Pharmaceutical Company, Bozhou, Anhui Province of China, and identified by Prof. Baomin Feng, Dalian University, China. A voucher specimen (CPXCQ-2014-03) was deposited at the College of Pharmacy, Qingdao University, China.

Extraction and isolation

The roots and rhizomes of *C. paniculatum* (10 kg) were reflux extracted twice with 90% ethanol for 1.5 h and the solvent was evaporated under reduced pressure to give an EtOH extract (1.5 kg). The EtOH extract (1.2 kg) was dissolved with water and subjected to column chromatography on HPD-100 macroporous resin and eluted with EtOH-H_2O (0:100, 30:70, 70:30, and 95:5), successively. The fraction eluted with 70% ethanol (100 g) was chromatographed over a D941 macroporous resin column, eluting with 95% ethanol and a total of 15 g residue was collected. The residue was chromatographed further on a RP-C_{18} silica gel and eluted with a gradient increasing MeOH (30–80%) in water to give sixteen subfractions (Fr.C1–C16) on the basis of TLC analyses. Fr.C14 was purified by preparative HPLC using MeOH/H_2O (60:40) at a flow rate of 2 ml/min (Megres C_{18} column, 250 mm × 10.0 mm, 5 μm) to yield compound **1** (4.91 mg, t_R = 41.0 min). Compound **2** (5.60 mg, t_R = 16.0 min) and compound **3** (8.25 mg, t_R = 60.0 min) were obtained from Fr.C13 and Fr.C12 by preparative HPLC (Megres C_{18} column, 250 mm × 10.0 mm, 5 μm; flow rate, 2.0 ml/min) employing MeOH/H_2O (55:45) and MeOH/H_2O (52:48) as the mobile phase, respectively. The fraction eluted with 95% ethanol (10 g) was separated chromatographically on a RP-C_{18} silica gel to get five subfractions (Fr.C1′–C5′) on the basis of TLC analysis. Fr.C4′ was isolated by preparative HPLC using MeOH/H_2O (60:40) at a flow rate of 1.6 ml/min (Megres C_{18} column, 250 mm × 10.0 mm, 5 μm) to yield compound **4** (62.29 mg, t_R = 140 min).

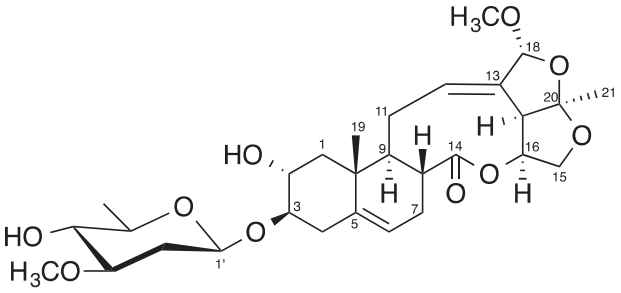
Spectral data

Neocynapanogenin H 3-O-β-D-oleandropyranoside (**1**): An amorphous powder; $[\alpha]_D^{25}$ +45.7 (*c* 0.01, MeOH); ^{1}H-(C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz) see Table 1; HR-ESI-MS *m/z* 573.2667 $[M+Na]^+$ (calcd for $C_{29}H_{42}NaO_{10}$, 573.2676).

Neocynapanogenin C (**2**): An amorphous powder; $[\alpha]_D^{25}$ −65.4 (c 0.01, MeOH); ^{1}H- (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz) see Table 2; HR-ESI-MS *m/z* 399.1783 $[M+Na]^+$ (calcd for $C_{21}H_{28}NaO_6$, 399.1784).

Results and discussion

Compound **1** was obtained as white amorphous powder, and showed positive Liebermann–Burchard and Keller–Kiliani reactions, suggesting it to be a steroidal glycoside with a 2-deoxysugar moiety (Zhu et al., 1999). Its molecular formula was determined as $C_{29}H_{42}O_{10}$ on the basis of positive HR-ESI-MS adduction $[M+Na]^+$ at *m/z* 573.2667 (calcd for $C_{29}H_{42}NaO_{10}$: 573.2676), which was further supported by the ^{1}H- and ^{13}C-NMR spectral data (Table 1). The ^{13}C-NMR and DEPT spectra revealed 29 carbon signals due to five methyl carbons, six methylene carbons, thirteen methine carbons, and five nonprotonated carbons, of which 22 carbons were assigned to the aglycone part including two tertiary methyl carbons (δ_C 20.6 and 24.3), one methoxyl carbon (δ_C 55.0), one oxygenated methylene carbon (δ_C 70.4), four oxygenated methine carbons (δ_C 70.0, 78.1, 84.8, and 104.3), four olefinic carbons (δ_C 120.4, 131.0, 139.4 and 142.3), one acetalic carbon (δ_C 114.6), and one carbonyl carbon (δ_C 179.3), which exhibited the characteristics of 13,14:14,15-disecopregnane-type steroidal glycoside. The ^{1}H-NMR spectrum of the aglycone moiety showed two angular methyl protons at δ_H 1.09 (3H, s) and 1.73 (3H, s), two geminal coupled oxygenated-methylene protons at δ_H 4.14 (1H, dd, *J* = 10.0, 4.8 Hz) and 4.42 (1H, dd, *J* = 9.9, 7.4 Hz), four oxygen-substituted methine protons at δ_H 3.69 (1H, m), 4.02 (1H, ddd, *J* = 12.6, 9.0, 4.6 Hz), 5.62 (1H, s), and 5.74 (1H, ddd, *J* = 8.1, 7.4, 4.8 Hz), together with two olefinic protons at δ_H 5.43 (1H, m) and 5.47 (1H, m). In addition, one methoxy group resonated at δ_H 3.50 (3H, s) was observed in the ^{1}H-NMR spectrum of the aglycone moiety. Comparison of the



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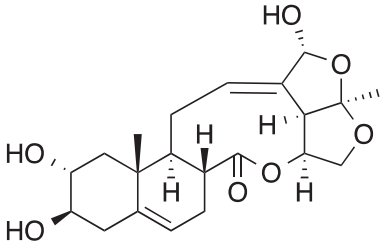

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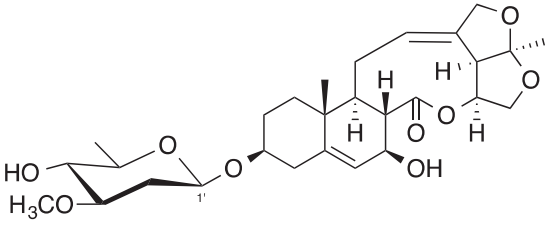

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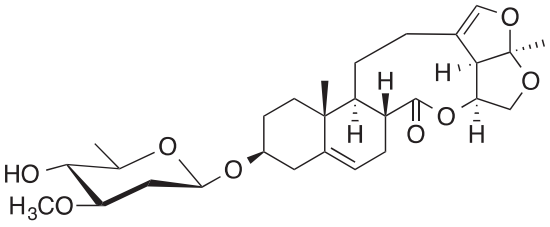

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Table 1
^{1}H-NMR and ^{13}C-NMR spectral data of compound **1** (500 and 125 MHz, C_5D_5N, δ ppm, J in Hz).

Position	**1**		Paniculatumoside A[a]
	δ_H	δ_C	δ_C
Aglycone			
1α	1.40 (t, J = 12.2 Hz)	45.5 (t)	37.2 (t)
1β	2.42 (dd, J = 13.0, 4.6 Hz)		
2	4.02 (ddd, J = 12.6, 9.0, 4.6 Hz)	70.0 (d)	29.7 (t)
3	3.69 (m)	84.8 (d)	77.0 (d)
4α	2.65 (m)	37.5 (t)	39.1 (t)
4β	2.59 (m)		
5	–	139.4 (s)	140.3 (s)
6	5.43 (m)	120.4 (d)	120.1 (d)
7α	2.62 (m)	29.2 (t)	30.4 (t)
7β	2.50 (m)		
8	2.47 (m)	40.9 (d)	41.4 (d)
9	2.13 (td, J = 11.4, 5.2 Hz)	51.9 (d)	52.1 (d)
10	–	38.6 (s)	37.8 (s)
11α	2.55 (m)	30.4 (t)	30.3 (t)
11β	2.29 (ddd, J = 11.9, 7.4, 4.4 Hz)		
12	5.47 (m)	131.0 (d)	133.2 (d)
13	–	142.3 (s)	139.4 (s)
14	–	179.3 (s)	179.4 (s)
15α	4.42 (dd, J = 9.9, 7.4 Hz)	70.4 (t)	70.5 (t)
15β	4.14 (dd, J = 10.0, 4.8 Hz)		
16	5.74 (ddd, J = 8.1, 7.4, 4.8 Hz)	78.1 (d)	78.0 (d)
17	3.29 (d, J = 8.1 Hz)	56.1 (d)	56.0 (d)
18	5.62 (s)	104.3 (d)	107.3 (d)
19	1.09 (s)	20.6 (q)	19.6 (q)
20	–	114.6 (s)	115.1 (s)
21	1.73 (s)	24.3 (q)	24.3 (q)
18-OCH_3	3.50 (s)	55.0 (q)	
Sugar			
1′(Ole)	4.84 (dd, J = 9.8, 1.8 Hz)	99.3 (d)	98.3 (d)
2′α	2.59 (m)	37.3 (t)	37.5 (t)
2′β	1.78 (ddd, J = 12.0, 9.8, 4.5 Hz)		
3′	3.51 (m)	81.5 (d)	81.7 (d)
4′	3.46 (m)	76.1 (d)	76.5 (d)
5′	3.65 (m)	73.1 (d)	72.9 (d)
6′	1.56 (d, J = 6.1 Hz)	18.4 (q)	18.8 (q)
3′-OCH_3	3.49 (s)	57.1 (q)	57.1 (q)

[a] Data from Li et al. (2004).

aglycone spectral data of **1** with those of neocynapanogenin C, the aglycone of paniculatumoside B (Li et al., 2004), the main differences were the presence of signal for an additional methoxyl ($\delta_{H/C}$ 3.50/55.0) and the changes of the chemical shifts in C-1 (+8.2 ppm), C-2 (+39.7 ppm), and C-3 (+7.7 ppm), as well as in C-18 (+5.6 ppm) and C-13 (−3.4 ppm) in the NMR spectra of **1**. The aglycone moiety of compound **1** was therefore proposed to be a 2-hydroxyl-18-methoxyl derivative of neocynapanogenin C, which were proved by the HMBC correlations from δ_H 1.40 and 2.42 (H-1) to δ_C 70.0 (C-2), 84.8 (C-3), 139.4 (C-5), 38.6 (C-10), 20.6 (C-19), from δ_H 2.59 and 2.65 (H-4) to δ_C 70.0 (C-2), 84.8 (C-3), 139.4 (C-5), 120.4 (C-6), 38.6 (C-10), and from δ_H 3.50 (18-OCH_3) to δ_C 104.3 (C-18) (Fig. 1). The relative configuration of the aglycone was elucidated by the NOESY spectrum and the vicinal proton-proton coupling constant. The coupling constant between H-2 and H-3 (9.0 Hz) was typical for *trans*-diaxial protons, indicating that both oxygenated substituents were equatorial. Observed 1,3-diaxial NOE correlations for H-2/H-4β, H-2/H-19, H-4β/H-19 and H-1α/H-3 (Fig. 2) further supported the β-orientation of H-2 and α-orientation of H-3 and revealed the chair conformation of the A ring. The *trans*-diaxial relationship of H-8 and H-9, namely, the β-orientation of H-8 and α-orientation of H-9, was suggested by the splitting pattern of H-9 (td, J = 11.4, 5.2 Hz) and the NOESY correlations for H-8/H-19 and H-1α/H-9 (Bai et al., 2005). In addition, the NOE correlation from the methoxyl group at C-18 to H_3-21 confirmed the methoxyl group at C-18 as α-orientation. Thus the structure for the aglycone of compound **1**

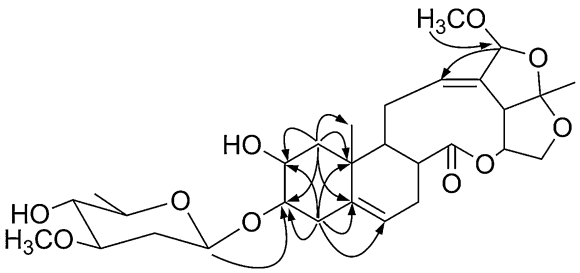

Fig. 1. Key HMBC correlations of compound **1**.

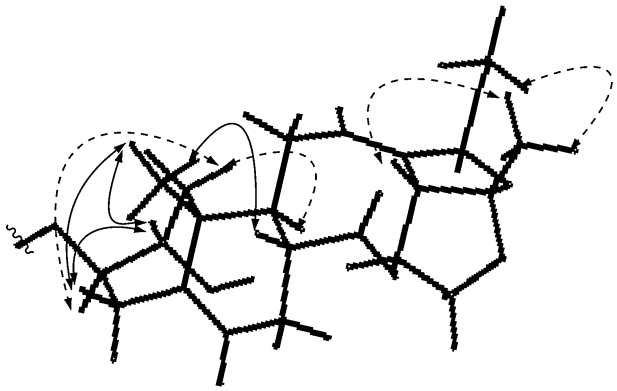

Fig. 2. Key NOESY correlations of compound **1**.

was deduced and a trivial name neocynapanogenin H was assigned. Proton signals were also assigned to one secondary methyl group at δ_H 1.56 (d, J = 6.1 Hz), one methoxyl group at δ_H 3.49 (s), and one anomeric proton at δ_H 4.84 (dd, J = 9.8, 1.8 Hz), whose multiplicities suggested the presence of one 2,6-dideoxy-sugar in a saccharide chain and β-configuration of the hexose unit. The ^{13}C NMR and DEPT data indicated the existence of one oleandropyranosyl unit. It was confirmed by the observed DQFCOSY and HMBC correlations. For the deoxysugars, since only D-form authentic samples could be obtained, their absolute configurations could not be assigned by GC analysis, but determined to be D-forms by comparison of their ^{13}C-NMR spectroscopic data with those reported data. The most significant differences in the ^{13}C-NMR data between D- and L-configuration oleandropyranosyl involve the resonances of C-2. The chemical shift of C-2 in the L-oleandropyranosyl is less than 35 ppm, but that of C-2 in the D-oleandropyranosyl appears above 36 ppm. Therefore, the oleandropyranosyl unit of **1** was determined to be D-configuration based on its ^{13}C-NMR chemical shift of C-2 at 37.3 ppm (Table 1) (Li et al., 2004; Ma et al., 2007; Yang et al., 2011; Kim et al., 2013b), and its location was determined to be C-3 by the H-1′/C-3 HMBC correlation (Fig. 1). Thus, the structure of **1** was finally established as neocynapanogenin H 3-*O*-*β*-D-oleandropyranoside.

Compound **2** was obtained as white amorphous powder, and showed positive Liebermann–Burchard reaction. Its molecular formula was determined as $C_{21}H_{28}O_6$ on the basis of positive HR-ESI-MS adduction $[M+Na]^+$ at m/z 399.1783 (calcd for $C_{21}H_{28}NaO_6$: 399.1784), which was further supported by the ^{1}H-NMR and ^{13}C-NMR data (Table 2). The ^{1}H-NMR data showed two olefinic protons at δ_H 5.34 (1H, br d, J = 4.6 Hz) and 5.55 (1H, d, J = 11.0 Hz), three oxygen-substituted methine protons at δ_H 3.82 (1H, m), 5.77 (1H, ddd, J = 8.1, 7.7, 5.2 Hz), and 6.33 (1H, br d, J = 6.0 Hz), two geminal coupled oxygenated-methylene protons at δ_H 4.16 (1H, dd, J = 9.8, 5.0 Hz) and 4.39 (1H, dd, J = 9.8, 7.2 Hz), two methyl signals at δ_H 1.04 (3H, s) and 1.84 (3H, s). The ^{13}C-NMR spectrum showed 21 carbon signals, including two tertiary methyl carbons (δ_C 19.8 and 25.0), an oxygenated methylene carbon (δ_C 70.0), three oxygenated methine carbons (δ_C 70.7, 78.3, and 98.7), four olefinic carbons (δ_C 119.4, 130.2, 141.1 and 145.5), an acetalic carbon (δ_C 113.6), and a carbonyl carbon (δ_C 179.6), which exhibited the characteristics of 13,14:14,15-disecopregnane-type steroidal glycoside. Comparison of the spectral data of **2** with those of paniculatumoside B, a new C_{21} steroidal glycoside isolated from the dried root of *C. paniculatum* (Li et al., 2004), the changes of the chemical shifts in C-2 (+2.5 ppm), C-3 (−6.4 ppm), C-4 (+4.0 ppm) showed that it has no linkage of the sugar moiety at the C-3 hydroxyl group of the aglycone. Thus, the structure of **2** was established as neocynapanogenin C, the aglycone of paniculatumoside B. It should be noted that compound **2** was isolated for the first time from the natural source.

Compounds **3** and **4** were identified by comparing the ^{1}H- and ^{13}C-NMR, as well as MS spectra with those reported in the literatures. They were determined to be cynapanoside A (**3**) (Sugama et al., 1986) and cynatratoside A (**4**) (Zhang et al., 1985).

Table 2
^{1}H-NMR and ^{13}C-NMR spectral data of compound **2** (500 and 125 MHz, C_5D_5N, δ ppm, J in Hz).

Position	δ_H	δ_C
1	1.17 (m) 1.83 (m)	37.6 (t)
2	1.74 (m) 2.08 (m)	32.5 (t)
3	3.82 (m)	70.7 (d)
4	2.54 (m) 2.62 (m)	43.1 (t)
5	–	141.1 (s)
6	5.34 (br d, J = 4.6 Hz)	119.4 (d)
7	2.58 (m) 2.90 (q, J = 12.2 Hz)	29.1 (t)
8	2.52 (m)	41.6 (d)
9	2.08 (m)	52.2 (d)
10	–	37.7 (s)
11	2.27 (m) 2.51 (m)	30.4 (t)
12	5.55 (d, J = 11.0 Hz)	130.2 (d)
13	–	145.5 (s)
14	–	179.6 (s)
15	4.16 (dd, J = 9.8, 5.0 Hz) 4.39 (dd, J = 9.8, 7.2 Hz)	70.0 (t)
16	5.77 (ddd, J = 8.1, 7.7, 5.2 Hz)	78.3 (d)
17	3.38 (d, J = 8.1 Hz)	56.8 (d)
18	6.33 (br d, J = 6.0 Hz)	98.7 (d)
19	1.04 (s)	19.8 (q)
20	–	113.6 (s)
21	1.84 (s)	25.0 (q)

Authors' contributions

HG, WXC, HLY, and QG performed the extraction, isolation, and elucidation of the constituents. KL, YL, and XHL contributed to checking and confirming all of the procedures of the isolation and identification. WW designed the study, supervised the laboratory work, and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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