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C_{21} steroidal glycosides from the roots of *Cynanchum paniculatum*

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ABSTRACT

As a part of our continuing research for bioactive constituents from *Cynanchum* plants, four new C_{21} steroidal glycosides, cynapanoside D-G (**1**–**4**), together with six known compounds (**5**–**10**) were isolated from the roots of *Cynanchum paniculatum* (Bge.) Kitag. Their structures were elucidated on the basis of 1D- and 2D-NMR spectroscopic data as well as HR-ESI-MS analysis. Compound **8** exhibited potent inhibitory activities against HL-60, HT-29, PC-3 and MCF-7 cell lines with IC_{50} values of 8.3, 7.5, 34.3 and 19.4 μM, respectively and compounds **1**–**4** and **9** displayed moderate cytotoxicity against the four cell lines. The in vitro antioxidant activities of compounds **1**–**4**, **8** and **9** were assayed by DPPH radical scavenging activity. Antibacterial and antifungal activities of compounds **1**–**4**, **8** and **9** were also tested.

1. Introduction

C_{21} steroidal and their glycosides have established themselves as an important class of biologically active compounds. They possess a wide range of pharmacological activities including antitumor, antifungal and cytotoxic activities [1–5]. *Cynanchum paniculatum*, is a perennial herb belonging to the genus *Cynanchum* in the family Asclepiadaceae, which is chiefly distributed in China, Japan and Korea. Its radix and rhizome have been used for rheumatic arthralgia, epigastric pain and distension, toothache, lumbago, traumatic injuries, urticaria and eczema in traditional Chinese medicine [6–8]. It has been reported to be rich in C_{21} steroidal and their glycosides [9], whose chemical structures are classified into normal four-ring C_{21} steroid type and aberrant 13,14:14,15-diseco-pregnane-type [10,11]. As our current interest in the biologically active and structurally unique natural products, the EtOH extract of *C. paniculatum* was investigated, and four new steroidal glycosides, cynapanoside D (**1**), cynapanoside E (**2**), cynapanoside F (**3**) and cynapanoside G (**4**), together with six known ones (**5**–**10**) were isolated.

2. Experimental

2.1. General experiment procedure

Optical rotations were determined using a WZZ-2A (Shanghai base solid Instrument Co., Ltd., Shanghai, China). UV spectra were recorded on a Shimadzu-2201 (Kyoto, Japan). The IR spectrum was obtained from a Bruker IFS-55 spectrophotometer (Karlsruhe, Germany) using KBr pellet. HR-ESI-MS data were measured on a Micro-mass Autospec-Untima TOF mass spectrophotometer (Waters, USA). NMR spectra were run on a Bruker AVANCE-400/-600 spectrometer (Karlsruhe, Germany). Analytical HPLC was carried out on a Shimadzu LC-10AT (Kyoto, Japan) liquid chromatography and preparative HPLC separation was performed on a YMC-Pack ODS-A column(10 × 250 mm, 5 μm; YMC-Pack, Japan), equipped with a Shimadzu LC-8A pump (Kyoto, Japan) and a Shimadzu SPD-10A UV–vis detector (Kyoto, Japan). Sugars analytical HPLC was carried out on a Jasco PU-4180 pump (Kyoto, Japan) and a OR-4090 detector (Kyoto, Japan). HPLC was performed with an Asahipak NH2P-50 4E column (4.6 mm × 250 mm, 5 μm, Japan).

2.2. Plant material

The dried roots (10 kg) of *C. panticulatum* were bought from Anhui Economy People Pharmaceutical Co., Ltd. A voucher specimen was identified by Prof. Jincai Lu of Shenyang Pharmaceutical University and has been deposited in the School of Traditional Chinese Materia Medica of Shenyang Pharmaceutical University (no. 20120913).

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2.3. Extraction and isolation

The dried roots (10 kg) of *C. panticulatum* were extracted three times with 95% EtOH (each 2 h) and the combined solution evaporated to dryness by a vacuum rotary evaporator to afford a syrup (1300 g). The crude extract was successively partitioned with petroleum ether, ethyl acetate and *n*-butanol to yield three layers of extracts. The ethyl acetate extract (170 g) was fractionated by silica gel column chromatography eluting with CH_2Cl_2-MeOH (100:0–0:100, v/v) to obtain twelve fractions (Fr. A-L) based on TLC analyses. Fr. B (18.3 g) was separated into subfractions (Fr. B1-B9) by silica gel column using PE-EtOAc (100:0–0:100, v/v) as the eluent. Fr. B4 (476 mg) was purified via Sephadex LH-20 eluting with CH_2Cl_2-MeOH (1:1, v/v) to afford four fractions (Fr. B4-1-Fr. B4-4). Compound **10** (6.8 mg) was recrystallized from Fr. B4-2. Fr. D (6.8 g) was applied to a silica gel column to give eight fractions (Fr. D1-D8). Fr. D2 (860 mg) was subjected to Sephadex LH-20 eluting with MeOH to afford five fractions (Fr. D2-1-D2-5). Fr. D2-3 (218 mg) was purified by semi-preparative HPLC eluting with 70% MeOH-H_2O (v = 4 mL/min) to yield compound **2** (26 mg, t_R 40.0 min) and compound **8** (45.3 mg, t_R 70.2 min). Fr. D4 (1.4 g) was further separated over a silica gel column using a solvent system of CH_2Cl_2-MeOH (100:0–0:100, v/v) to give five subfractions (Fr. D4-1-D4-5). Fr. D4-2 (863 mg) was subjected to Sephadex LH-20 eluting with MeOH to afford five fractions (Fr. D4-2-1-D4-2-5). Fr. D4-2-2 (215 mg) was purified by preparative reversed-phase HPLC using 68% MeOH-H_2O (v = 4 mL/min) and then purified by preparative TLC using a solvent system of cyclohexane-acetone (3:2, v/v) to obtain compound **9** (26.5 mg). Fr. E (17.2 g) was separated into subfractions (Fr. E1-E7) by silica gel column

Table 1
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data in C_5D_5N for compounds **1–4.**

	1		**2**		**3**		**4**	
No.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	0.91 (m), 1.79[a]	36.2	0.88 (m), 1.80[a]	36.3	0.88 (m), 1.79[a]	36.3	0.99 (m), 1.69 (m)	37.1
2	1.67[a], 2.06[a]	29.8	1.54[a], 2.09[a]	30.3	1.52[a], 2.11[a]	30.1	1.79[a], 2.17 (m)	30.0
3	3.74 (m)	77.3	3.71 (m)	77.2	3.70 (m)	77.2	3.95 (m)	78.5
4	2.27[a], 2.53[a]	38.8	2.28[a], 2.52[a]	38.8	2.26[a], 2.52[a]	38.8	2.64 (m), 2.79[a]	38.8
5	–	140.4	–	140.2	–	140.2	–	139.4
6	5.38 (m)	120.2	5.37 (m)	120.3	5.37 (m)	120.3	5.40 (m)	122.2
7	1.36[a], 1.99[a]	29.7	1.65[a], 2.03[a]	29.8	1.65[a], 2.03[a]	29.9	1.86[a], 2.51 (m)	27.6
8	2.47 (m)	40.4	2.51[a]	40.8	2.50[a]	40.5	1.81[a]	36.8
9	1.20[a]	53.0	1.25[a]	52.7	1.22[a]	52.7	1.10 (m)	46.0
10	—	38.4	—	38.5	—	38.5	—	37.2
11	1.33[a], 2.55[a]	23.7	1.83[a], 2.49[a]	20.6	1.82[a], 2.50[a]	20.7	1.28[a], 1.35[a]	20.8
12	2.11[a], 2.60[a]	28.2	2.14[a], 2.64[a]	28.3	2.13[a], 2.63	28.3	1.18[a], 1.36[a]	38.5
13	–	118.3	–	118.7	–	118.8	–	49.1
14	–	175.2	–	175.3	–	175.4	–	84.7
15	3.92 (dd, 9.3, 9.0) 4.22 (dd, 8.5, 7.3)	67.5	3.97 (t, 9.3) 4.29 (dd, 9.3, 7.5)	66.9	3.97 (t, 9.3) 4.29 (dd, 9.3, 7.5)	67.0	1.79[a], 1.99[a]	34.3
16	5.41 (m)	75.3	5.99 (t, 8.0)	81.8,	5.97 (dd, 8.5, 7.5)	81.8	1.85[a], 2.00[a]	24.2
17	3.53[a]	55.9	–	92.2	–	92.2	2.80[a]	62.8
18	6.45 (s)	143.6	6.63 (s)	144.4	6.65 (s)	144.4	1.05 (s)	15.2
19	0.82 (s)	17.6	0.85 (s)	17.7	0.83 (s)	17.8	0.93 (s)	19.3
20	–	114.1	–	119.5	–	119.5	–	216.5
21	1.51 (s)	24.5	1.73 (s)	20.4	1.73 (s)	20.5	2.17 (s)	32.1
	β-D-Ole		*β*-D-Ole		*β*-D-Ole		*β*-D-Glc	
1′	4.77 (br.d, 9.8)	97.9	4.75 (dd, 9.8, 1.8)	97.9	4.77 (br.d, 9.8)	97.9	5.05 (d, 7.5)	101.1
2′	1.78[a], 2.40[a]	37.7	1.74[a], 2.40[a]	37.7	1.77[a], 2.40[a]	37.8	4.10[a]	84.5
3′	3.55[a]	78.9	3.52[a]	78.9	3.55[a]	78.9	4.21[a]	77.6
4′	3.55[a]	82.9	3.52[a]	82.7	3.51[a]	83.0	4.20[a]	71.2
5′	3.53[a]	71.5	3.53[a]	71.4	3.52[a]	71.5	3.87[a]	78.0
6′	1.44 (s)	18.6	1.42 (s)	18.5	1.41 (s)	18.6	4.30 (m), 4.48 (dd, 11.9, 2.4)	62.3
3′-OMe	3.53 (s)	57.2	3.40 (s)	57.1	3.53 (s)	57.3	–	–
	β-D-Dig		*β*-D-Cym		*β*-D-Dig			
							β-D-Glc	
1″	5.52 (brd, 9.8)	98.5	5.23 (dd, 9.8, 1.8)	98.1	5.51 (brd, 9.8)	98.5	5.26 (d, 7.5)	106.4
2″	1.97[a], 2.41[a]	39.5	1.73[a], 2.06[a]	36.7	1.96[a], 2.39[a]	39.8	4.11[a]	76.8
3″	4.42 (m)	68.5	3.82 (m)	77.6	4.37 (m)	68.8	4.21[a]	77.8
4″	3.57[a]	73.8	3.40[a]	82.0	3.50[a]	82.2	4.28[a]	71.3
5″	4.26 (dd, 8.3, 7.0)	70.3	4.13[a]	69.2	4.57 (br.s)	67.6	3.85[a]	78.8
6″	1.55 (s)	18.8	1.32 (s)	18.3	1.41 (s)	18.4	4.40 (m), 4.54 (dd, 11.9, 2.4)	62.5
3″-OMe	—	—	3.50	58.1	—	—	—	—
			α-L-Cym		*α*-L-Ole			
1‴			4.98 (d, 3.3)	98.7	5.21 (d, 3.0)	100.0		
2‴			1.75[a], 2.39[a]	31.8	1.69[a], 2.44[a]	35.5		
3‴			3.67 (m)	76.1	3.79[a]	78.6		
4‴			3.56[a]	73.1	3.50[a]	76.6		
5‴			4.50 (m)	66.1	4.37[a]	69.3		
6‴			1.50 (s)	18.4	1.48 (s)	18.2		
3‴-OMe			3.34 (s)	56.3	3.30 (s)	56.8		

δ in ppm, *J* values are in parentheses and reported in Hz. The assignments were based on NOESY, HSQC and HMBC experiments. Ole = oleandropyranose, Dig = digitoxopyranose, Cym = cymaropyranose, Glc = glucopyranose.

[a] Overlapped with other signals.