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C_{21} Steroidal Glycosides from the Root of *Cynanchum paniculatum*

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Cynanchum paniculatum (Bunge) Kitag. (Asclepiadaceae) grows in areas of Asia, such as China and Korea, and has been used in Chinese traditional medicine for treating snake bites and chronic bronchitis.[1] The root of this plant has been used as a diuretic in Korean folk medicine.[2] Steroidal glycosides[3] and phenolic compounds were reported in *C. paniculatum* and showed neuroprotective activity and analgesic effect.[4-7] The EtOH extract of *C. paniculatum* shows protective activity for treating herpes simplex encephalitis.[8] In our continuing efforts to study the secondary metabolites of natural plant sources, the MeOH extract of *C. paniculatum* was investigated, and two new steroidal glycosides (**1** and **2**) and five known ones (**3-7**) (Figure 1) were isolated. The structures of the new isolated compounds were determined based on spectroscopic analyses (^{1}H- and ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, and ROESY).

Compound **1** was obtained as an amorphous gum. The molecular formula was established as $C_{41}H_{60}O_{15}$ as evidenced from the $[M+Na]^+$ ion peak at *m/z* 815.3830 (calcd. for $C_{41}H_{60}NaO_{15}$: 815.3830) in the HR-FABMS. The ^{1}H-NMR spectrum of **1** showed the presence of five methyl groups [δ_H 1.09 (3H, s), 1.47 (6H, d, J = 6.0 Hz), 1.59 (3H, d, J = 6.0 Hz) and 1.66 (3H, s)], two oxygenated methines [δ_H 3.78 (1H, m) and 5.73 (1H, t, J = 12.0 Hz)], one oxygenated methylene [δ_H 4.05 (1H, dd, J = 5.0, 10.5 Hz) and 4.42 (1H, dd, J = 5.0, 10.0 Hz)], two olefinic protons [δ_H 5.27 (1H, t, J = 2.5 Hz) and 6.12 (1H, dd, J = 2.0, 8.5 Hz)], and three anomeric protons [δ_H 4.75 (1H, dd, J = 2.0, 9.5 Hz), 5.19 (1H, d, J = 3.5 Hz), and 5.52 (1H, dd, J = 2.0, 10.0 Hz)] signals. The ^{13}C-NMR spectrum showed a total of 41 carbon signals, of which 21 carbons were assigned to the aglycone and the remaining 20 carbons were assigned to the sugar moieties. The ^{13}C-NMR and DEPT spectra of the aglycone showed two carbonyl carbons (δ_C 167.5 and 179.3), four olefinic carbons (δ_C 119.8, 129.9, 140.1 and 146.8), two oxygenated methine carbons (δ_C 76.9 and 77.3), one oxygenated methylene carbon (δ_C 71.3), and one acetalic carbon (δ_C 113.3) signals. From these data, **1** was presumed to be of 15,20:18,20-diepoxy-13,14:14,15-disecopregnane-type steroid skeleton.[9,10] Comparison of the ^{1}H- and ^{13}C-NMR spectra of the aglycone of **1** with those of the aglycone of atratoglaucosides B indicated that the aglycone of **1** was the same as that of atratoglaucosides B.[11] Besides, ^{1}H- and ^{13}C-NMR spectra of **1** displayed the presence of three sugars; two oleandropyranose {[δ_C 18.8, 37.9, 57.4, 71.7, 79.2, 83.2 and 98.1; δ_H 1.47, 2.44, 3.50-3.61 (3H), 3.54 and 4.74] and [δ_C 18.6, 35.8, 57.0, 69.0, 76.9, 78.8 and 100.3; δ_H 1.47, 2.44, 3.50-3.61 (3H), 3.53 and 5.19]}[12,13] and a digitoxopyranose [δ_C 18.6, 38.9, 69.5, 78.9, 83.4 and 98.5; δ_H 1.59, 1.76, 3.50-3.61 (3H) and 5.52].[13] The sugar configurations were determined through the J values of the anomeric protons to be α-oleandropyranose (δ_H 4.74, dd, J = 2.0, 10.0 Hz),[12-14] β-digitoxopyranose (δ_H 5.52, dd, J = 2.0, 10.0 Hz),[13,14] and β-oleandropyranose (δ_H 5.19, dd, J = 3.0, 5.0 Hz).[14,15] The sugar sequence of **1** was determined by HMBC correlations of H-1"/C-4' and H-1'"/C-4" (Figure 2). Comparing these data with those of amplexicoside B isolated from *Cynanchum amplexicaule*,[12] the sugar parts of **1** were confirmed to be α-oleandropyranosyl-(1→4)-β-digitoxopyranosyl-(1→4)-β-oleandropyranoside and its location was determined to be C-3 by the H-1'/C-3 HMBC correlation. The relative stereochemistry of **1** was presumed to be similar with that of aglycone of atratoglaucosides B[11] based on the NMR data, and reconfirmed by ROESY correlations of H-19/H-8, H-12/H-9 and H-17, and H-16/H-17 and H-21 (Figure 2). Thus, the structure of **1** was established as stauntogenin 3-*O*-α-oleandropyranosyl-(1→4)-β-digitoxopyranosyl-(1→4)-β-oleandropyranoside.

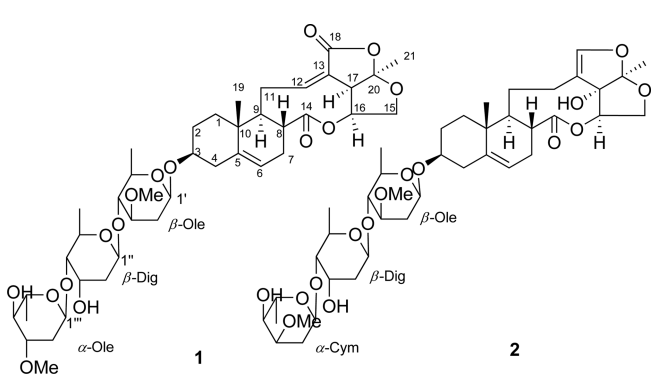

Figure 1. Chemical structures of new compounds **1** and **2**.

Compound **2** was obtained as an amorphous gum. The molecular formula was $C_{41}H_{62}O_{15}$ as evidenced from the $[M+Na]^+$ ion peak at *m/z* 817.3984 (calcd. for $C_{41}H_{62}NaO_{15}$: 817.3986) in the HR-FABMS. The ^{1}H-NMR spectrum of **2** showed five methyl groups [δ_H 0.82 (3H, s), 1.38 (3H, d, J = 6.5 Hz), 1.47 (6H, d, J = 8.5) and 1.71 (3H, s)], two oxygenated methines [δ_H 3.71 (1H, m), 5.97 (1H, t, J = 9.0 Hz)], one oxygenated methylene [δ_H 4.06 (1H, m), and 4.35 (1H,

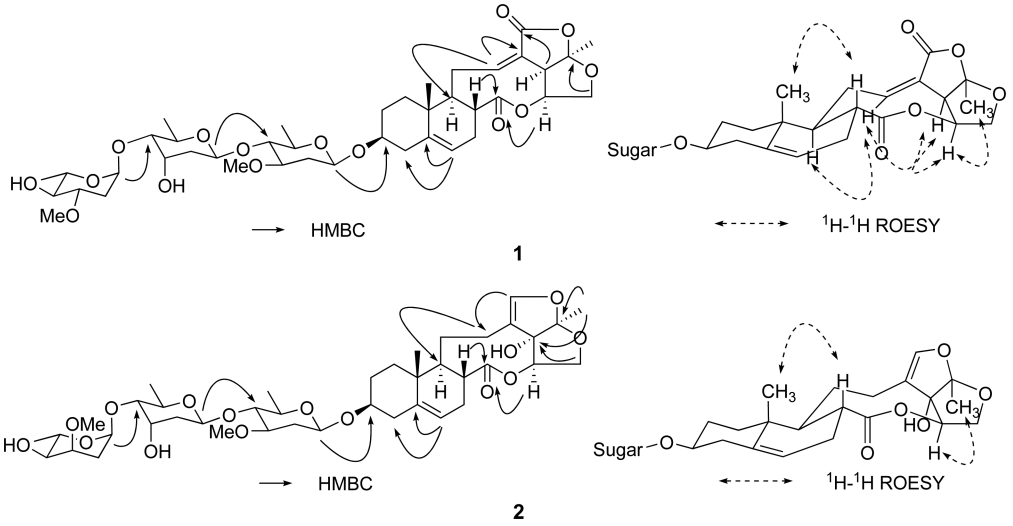

Figure 2. Key HMBC and ROESY correlations of compounds **1** and **2**.

br s)], two olefinic protons [δ_H 5.38 (1H, dd, J = 2.5, 9.5 Hz) and 6.61 (1H, s)], and three anomeric protons [δ_H 4.75 (1H, dd, J = 2.0, 10.0 Hz), 5.07 (1H, d, J = 3.5 Hz), and 5.43 (1H, dd, J = 2.0, 10.0 Hz)] signals. The ^{13}C-NMR spectrum showed 41 carbon signals, of which 21 carbons were assigned to the aglycone and the remaining 20 carbons were assigned to the sugar moieties. The ^{13}C-NMR and DEPT spectra of the aglycone showed one carbonyl carbon (δ_C 175.6), four olefinic carbons (δ_C 118.9, 120.5, 140.4, and 144.6), three oxygenated methine carbons (δ_C 76.4, 82.0, and 92.3), one oxygenated methylene carbon (δ_C 67.0), and one acetalic carbon (δ_C 119.7) signals. From these data, **2** was also indicated to have a 15,20:18,20-diepoxy-13,14:14,15-disecopregnane skeleton.[9,10] Comparison of the ^{1}H- and ^{13}C-NMR spectra of the aglycone of **2** with those of the aglycone of (3β,8β,9α.16α,17α)-14,16β:15,20α:18.20β-triepoxy-16β:17α-dihydroxy-14-oxo-13,14:14,15-disecopregna-5,13(18)-dian-3-yl α-cymaropyranosyl-(1→4)-α-digitoxopyransyl-(1→4)-α-oleandropyranoside showed that the aglycone of **2** was the same as that of (3β,8β,9α.16α,17α)-14,16β:15,20α:18.20β-triepoxy-16β:17α-dihydroxy-14-oxo13,14:14,15-disecopregna-5,13(18)-dian-3-yl α-cymaropyranosyl-(1→4)-α-digitoxopyransyl-(1→4)-α-oleandropyranoside.[12-14] The ^{1}H- and ^{13}C-NMR spectra of **2** displayed three sugars; a cymaropyranose [δ_C 18.3, 38.4, 56.7, 67.1, 72.7, 82.0 and 98.3; δ_H 1.47, 2.39, 3.50, 3.53, 3.72, 4.47 and 5.07],[12] a digitoxopyranose [δ_C 18.5, 38.7, 67.8, 69.1, 80.7 and 98.4; δ_H 1.38, 1.90, 3.43, 4.09, 4.43 and 5.43],[14] and a oleandropyranose [δ_C 18.7, 37.9, 57.3, 71.6, 79.2, 83.0 and 98.1; δ_H 1.47, 2.39, 3.52, 3.52-3.55 (2H), 3.54 and 4.79].[15] The configurations of the sugars were determined through the J values of the anomeric protons to be α-cymaropyranose (δ_H 5.07, dd, J = 3.0, 5.0 Hz),[12-14] α-digitoxopyranose (δ_H 5.43, dd, J = 2.0, 10.0 Hz),[10,11] and β-oleandropyranose (δ_H 4.75, dd, J = 2.0, 10.0 Hz).[14-16] The sugar sequence of **2** was identified by the H-1"/C-4' and H-1'"/C-4" HMBC correlations (Figure 2). Comparing these data with those of cynatratoside B isolated from *Cynanchum atratum*,[11] the sugar parts of **2** were confirmed to be α-cymaropyranosyl-(1→4)-β-digitoxopyranosyl-(1→4)-β-oleandropyranoside and its location was determined at C-3 by the H-1'/C-3 HMBC correlation. The relative stereochemistry of **2** was presumed to be similar with that of the aglycone of (3β,8β,9α.16α,17α)-14,16β:15,20α:18.20β-triepoxy-16β:17α-dihydroxy-14-oxo-13,14:14,15-disecopregna-5,13(18)-dian-3-ylα-cymaropyranosyl-(1→4)-α-digitoxopyransyl-(1→4)-α-oleandropyranoside based on NMR data, and reconfirmed by H-19/H-8 and H-16/H-21 ROESY correlations (Figure 2). Acid hydrolysis of **1** and **2** was attempted at several conditions (0.05 N, 1 N and 2 N HCl, and 1 N and 2 N H_2SO_4), but all trials failed. Steroidal glycosides containing oleandropyranose, cymaropyranose and digitoxopyranose could not be hydrolyzed, and many reports[16-18] have been published without hydrolysis. Thus, the structure of **2** was established as (3β,8β,9α.16α,17α)-14,16β:15,20α:18.20β-triepoxy-16β:17α-dihydroxy-14-oxo-13,14:14,15-disecopregna-5,13(18)-dian-3-yl α-cymaropyranosyl-(1→4)-β-digitoxopyransyl-(1→4)-β-oleandropyranoside.

Compounds **3-7** were identified by comparing the ^{1}H- and ^{13}C-NMR, and MS spectra with the literature to be (3β,8β,9α.16α,17α)-14,16β:15,20α:18.20β-triepoxy-16β:17α-dihydroxy-14-oxo-13,14:14,15-disecopregna-5,13(18)-dian-3-yl α-D-oleandropyranosyl-(1→4)-α-D-digitoxopyranosyl-(1→4)-α-L-cymaropyranoside (**3**),[18] (3β,8β,9α.16α,17α)-14,16β:15,20α:18.20β-triepoxy-16β:17α-dihydroxy-14-oxo-13,14:14,15-disecopregna-5,13(18)-dian-3-yl α-D-oleandropyranosyl-(1→4)-α-D-digitoxopyranosyl-(1→4)-α-D-oleandropyranoside (**4**),[20] cynapanoside A (**5**),[20] cynatratoside B (**6**),[19] and cynatratoside C (**7**).[19]

Experimental Section

Plant Materials. The roots of *C. paniculatum* were collected in Taebaek City, Korea during June 2011, and the plant was identified by one of the authors (K. R. Lee). A voucher specimen (SKKU-NPL 1103) of the plant was

deposited at the herbarium of the School of Pharmacy at Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The roots of *C. paniculatum* (3.6 kg) were extracted with 80% MeOH at room temperature and filtered. The filtrate was evaporated under reduced pressure to give a MeOH extract (750 g), which was suspended in water (800 mL) and solvent-partitioned to give *n*-hexane (75 g), $CHCl_3$ (50 g), EtOAc (9 g), and BuOH (60 g) fractions. The $CHCl_3$ (20 g) fraction was separated over a silica gel column (230-400 mesh, 500 g) with *n*-hexane: EtOAc:MeOH (3:1:0.5). Seven crude fractions (fr. A-G) were collected based on a thin-layer chromatography analysis. Fr. C (4 g) was chromatographed further on a RP-C_{18} silica gel (230-400 mesh, 150 g) and eluted with a gradient solvent system of MeOH/H_2O (3:2, 4:1, 9:1, and 1:0) to give seven subfractions (fr. C1-C7). Fr. C3 (700 mg) was separated on a silica gel column with $CHCl_3$:MeOH (60:1) to give three subfractions (fr. C31-C33). Fr. C31 (60 mg) was purified by silica gel column preparative high performance liquid chromatography (HPLC) with $CHCl_3$:MeOH (60:1) at a flow rate of 2.0 mL/min (Alltech Econosil® Silica 5 μm column; 250 × 10 mm; 10 μm particle size, Shodex RI-101 refractive index detector) to yield **1** (5 mg, t_R = 20.0 min). Fr. C33 (80 mg) was purified by preparative reversed-phase HPLC with 80% MeOH at a flow rate of 2.0 mL/min (Econosil RP-18 10 μm column; 250 × 10 mm; 10 μm particle size; Shodex refractive index detector) to yield **2** (6 mg, t_R= 16.5 min). Fr. B1 (1 g) was separated on a silica gel column (230-400 mesh, 40 g) with $CHCl_3$:MeOH (60:1) and further separated by preparative reversed-phase HPLC using a solvent of 80% MeOH at a flow rate of 2.0 mL/min (Econosil RP-18 10 μm column; 250 × 10 mm; 10 μm particle size; Shodex refractive index detector) to yield **3** (6 mg, t_R = 18.3 min) and **4** (65 mg, t_R = 23.0 min). Fr. C1 (1 g) was separated on a silica gel column (230-400 mesh, 40 g) with $CHCl_3$:MeOH (50:1) and further separated by preparative reversed-phase HPLC using 70% MeOH to yield **5** (5 mg, t_R = 18.3 min), **6** (9 mg, t_R = 23.0 min), and **7** (30 mg, t_R = 28.0 min).

Stauntogenin 3-*O*-α-oleandropyranosyl-(1→4)-β-digitoxopyranosyl-(1→4)-β-oleandropyranoside (1): Amorphous gum, $[\alpha]_D^{25}$ −16.5 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε): 238 (10.5) nm; IR (KBr) ν_{max}: 3418, 3079, 3030, 1641, 1583, 1216, 1148, 1068, 1031 cm^{-1}; ^{1}H- (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz) see Table 1; HR-FABMS *m/z* 815.3830 $[M+Na]^+$ (calcd for $C_{41}H_{60}NaO_{15}$, 815.3830).

(3β,8β,9α.16α,17α)-14,16β:15,20α:18.20β-Triepoxy-16β:17α-dihydroxy-14-oxo-13,14:14,15-disecopregna-5,13(18)-dian-3-yl α-cymaropyranosyl-(1→4)-β-digitoxopyransyl-(1→4)-β-oleandropyranoside (2): Amorphous gum, $[\alpha]_D^{25}$ –25.6 (*c* 0.40, MeOH); IR (KBr) ν_{max}: 3415, 3060, 3034, 1643, 1587, 1216, 1148, 1068, 1032 cm^{-1}; ^{1}H- (C_5D_5N, 500

Table 1. ^{1}H- and ^{13}C-NMR spectral data of **1**[a]

Position	Aglycone		Position	Sugar	
	δ_H	δ_C		δ_H	δ_C
1a	1.07 dd (3.0, 13.0)	37.3 t	1'(Ole)	4.74 dd (2.0, 10.0)	98.1 d
1b	1.76 ddd (4.0, 4.5, 13.0)		2'	2.44 m	37.9 t
2a	2.05 m	30.0 t	3'	3.50-3.61 m[b]	79.2 d
2b	1.65 m		4'	3.50-3.61 m[b]	83.2 d
3	3.78 m	77.3 t	5'	3.50-3.61 m[b]	71.7 d
4a	2.52 m	38.4 t	6'	1.47 d (6.0)	18.8 d
4b	2.49 m		-OCH_3	3.54 s	57.4 t
5		140.1 s	1"(Dig)	5.52 dd (2.0, 10.0)	98.5 d
6	5.27 t (2.5)	119.8 d	2"	1.76 d (10.0)	38.9 t
7a	2.36 m	30.1 t	3"	3.50-3.61 m[b]	78.9 d
7b	2.07 m		4"	3.50-3.61 m[b]	83.4 d
8	2.52 m	40.9 d	5"	3.50-3.61 m[b]	69.5 d
9	2.05 m	52.2 d	6"	1.59 d (6.0)	18.6 q
10		37.9 s	1'"(Ole)	5.19 d (3.5)	100.3 d
11a	2.37 m	27.1 t	2'"	2.44 m	35.8 t
11b	4.10 d (12.0)		3'"	3.50-3.61 m[b]	78.8 d
12	6.12 dd (2.0, 8.5)	146.8 d	4'"	3.50-3.61 m[b]	76.9 d
13		129.9 s	5'"	3.50-3.61 m[b]	69.0 d
14		179.3 s	6'"	1.47 d (6.0)	18.6 q
15a	4.41 dd (5.0, 10.0)	71.3 t	-OCH_3	3.53 s	57.0 q
15b	4.05 dd (5.0, 10.5)				
16	5.73 ddd (5.0, 7.0, 8.0)	76.9 d			
17	3.54 m	54.5 d			
18		167.5 s			
19	1.09 s	20.0 q			
20		113.3 s			
21	1.66 s	23.4 q			

[a] ^{1}H- and ^{13}C-NMR run at 500 MHz (C_5D_5N), proton coupling constants (*J*) in Hz are given in parentheses. [b]Overlapped signals.

Table 2. ^{1}H- and ^{13}C-NMR spectral data of **2**[a]

Position	Aglycone		Position	Sugar	
	δ_H	δ_C		δ_H	δ_C
1a	0.88 d (10.0)	36.3 t	1'(Ole)	4.75 dd (2.0, 10.0)	98.1 d
1b	1.82 m		2'	2.39 m	37.9 t
2a	2.06 m	29.8 t	3'	3.54 m	79.2 d
2b	1.07 m		4'	3.52-3.55 m[b]	83.0 d
3	3.71 m	76.4 d	5'	3.52-3.55 m[b]	71.6 d
4a	2.50 m	38.8 t	6'	1.47 d (8.5)	18.7 q
4b	2.32 t (13.5)		-OCH_3	3.50 s	57.3 q
5		140.4 s	1"(Dig)	5.43 dd (2.0, 10.0)	98.4 d
6	5.38 dd (2.5, 9.5)	120.5 d	2"	1.90 (*t*-like)	38.7 t
7a	2.65 t (14.5)	28.3 t	3"	4.43 m	69.1 d
7b	2.12 t (19.0)		4"	3.43 dd (2.0, 9.0)	80.7 d
8	2.49 t (12.0)	38.4 d	5"	4.09 m	67.8 d
9	2.39 t (7.5)	38.8 d	6"	1.38 d (6.5)	18.5 q
10		40.5 s	1'"(Cym)	5.07 dd (3.0, 5.0)	98.3 d
11a	2.52 m	20.0 s	2'"	2.39 m	38.4 t
11b	1.86 t (10.0)		3'"	3.72 m	72.7 d
12 a	2.12 br s	30.7 t	4'"	3.53 m	82.0 d
12 b	1.52 m		5'"	4.47 m	67.1 d
13		118.9 s	6'"	1.47 d (8.5)	18.3 q
14		175.6 s	-OCH_3	3.50 s	56.7 q
15 a	4.35 br s	67.0 t			
15 b	4.06 m				
16	5.97 t (9.0)	82.0 d			
17		92.3 s			
18	6.61 s	144.6 d			
19	0.82 s	17.7 q			
20		119.7 s			
21	1.71 s	20.4 q			

[a] ^{1}H- and ^{13}C-NMR run at 500 MHz (C_5D_5N), proton coupling constants (*J*) in Hz are given in parentheses. [b]Overlapped signals.

MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz) see Table 2; HR-FABMS *m/z* 817.3984 $[M+Na]^+$ (calcd for $C_{41}H_{62}NaO_{15}$, 817.3986).

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Supporting Information. The general experimental procedures and the spectral data of **1** and **2** are available on request from the corresponding author.

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