New Pregnane Glycosides from *Cynanchum ascyrifolium*

Hosup YEO, Young Hae CHOI, and Jinwoong KIM*

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University; Seoul 151–742, Korea. Received December 17, 2001; accepted February 1, 2002

Two new pregnane glycosides, cynascyrosides D and E, were isolated from the roots of *Cynanchum ascyrifolium*. The structures of these compounds were determined on the basis of spectroscopic and chemical evidence as cynajapogenin A 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-L-cymaropyranoside and cynajapogenin A 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranoside.

Key words pregnane glycoside; *Cynanchum ascyrifolium*; cynascyroside D; cynascyroside E

Cynanchum ascyrifolium MATSUMURA (Asclepiadaceae) is a widely distributed species in eastern Asia, the roots of which have been used as an antitussive and expectorant in Korea.[1)] Previously, we isolated three steroidal glycosides with a 14,15-*seco*-18-*nor*-pregnane skeleton, cynascyrosides A—C, from the roots of this plant.[2)] As a continuation of the phytochemical investigation of this plant, we now describe the isolation and structural elucidation of two additional pregnane glycosides, cynascyrosides D and E.

Experimental

General Experimental Procedures Melting points: uncorr. FAB-MS was measured on a Finnigan MAT 90 mass spectrometer. IR spectra were recorded on a Perkin-Elmer 1710 spectrometer. NMR spectra were measured with a JEOL JNM-LA 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). ^{1}H-NMR was run in $CDCl_3$ solution and ^{13}C-NMR in C_5D_5N solution with tetramethylsilane (TMS) as an internal standard. Optical rotations were determined with a JASCO DIP-1000. TLC was performed on precoated silica gel 60 F_{254} (Merck), and column chromatography (CC) was carried out on silica gel (230—400 mesh, Merck).

Plant Material The roots of *Cynanchum ascyrifolium* MATSUMURA were collected at Bakbong (Korea) in June 1987, and taxonomically identified by Dr. Bo Sup Chung, a former professor of the College of Pharmacy, Seoul National University. A voucher specimen has been deposited in the herbarium of our institute.

Extraction and Isolation The air-dried roots of *C. ascyrifolium* (2 kg) were cut into pieces and extracted with MeOH. The MeOH extract was evaporated *in vacuo* to give a crude extract (430 g), which was successively extracted with *n*-hexane, $CHCl_3$ and *n*-BuOH. The $CHCl_3$ extract (120 g) was fractionated by CC over silica gel using a $CHCl_3$–MeOH gradient to give three fractions (fr. 1: 1.5 g, fr. 2: 37 g, fr. 3: 19 g), of which fr. 2 and fr. 3 showed positive Liebermann–Burchard and Keller–Kiliani reactions. Fraction 2 (30 g) was submitted to CC on silica gel with *n*-hexane–EtOAc–MeOH (17 : 17 : 1) to give **1** (730 mg). Fraction 3 (12 g) was subjected to repeated CC over silica gel with $CHCl_3$–MeOH–7% HCO_2H (10 : 3 : 2) and EtOAc–MeOH–H_2O (25 : 2 : 1) to afford **2** (300 mg).

Cynascyroside D (**1**): Pale yellow powder, mp 102—104 °C; $[\alpha]_D^{23}$ −54.3° (c=0.1, MeOH); UV λ_{max}^{EtOH} nm: 212; IR ν_{max}^{KBr} cm^{-1}: 3451, 1714, 1651, 1518, 1100; FAB-MS (negative) *m/z*: 747 $[M-H]^-$, 603 $[M-H-144]^-$, 473 $[603-130]^-$; ^{1}H-NMR: δ 1.13 (3H, s, H-19), 1.23, 1.26 (×2) (9H, d, J=6.4 Hz, H-6′, H-6″, H-6‴), 2.18 (3H, s, H-21), 3.42 (3H, s, 3‴-OMe), 3.46 (3H, s, 3′-OMe), 4.81 (1H, dd, J=9.6, 2.0 Hz, H-1′), 4.85 (1H, dd, J=9.6, 1.6 Hz, H-1″), 4.91 (1H, br d, J=3.9 Hz, H-1‴), 5.43 (1H, br d, J=4.4 Hz, H-6), 6.22 (1H, d, J=2.1 Hz, H-16), 7.27 (1H, d, J=2.1 Hz, H-15); ^{13}C-NMR: see Tables 1 and 2.

Cynascyroside E (**2**): Pale yellow powder, mp 135—137 °C; $[\alpha]_D^{23}$ −65.0° (c=0.1, MeOH); UV λ_{max}^{EtOH} nm: 219; IR ν_{max}^{KBr} cm^{-1}: 3472, 1715, 1651, 1454, 1370, 1061; FAB-MS (negative) *m/z*: 909 $[M-H]^-$, 747 $[M-H-162]^-$, 603 $[747-144]^-$, 459 $[603-144]^-$, 329 [aglycone]$^-$; ^{1}H-NMR: δ 1.13 (3H, s, H-19), 1.24, 1.25, 1.29 (each 3H, d, J=6.4 Hz, H-6′, H-6″, H-6‴), 2.17 (3H, s, H-21), 3.43, 3.46 (each 3H, s, 3″-OMe, 3‴-OMe), 4.45 (1H, d, J=7.2 Hz, H-1⁗), 4.81 (1H, br d, J=9.9 Hz, H-1′), 4.85 (1H, br d, J=9.3 Hz, H-1‴), 5.09 (1H, br s, H-1″), 5.44 (1H, m, H-6), 6.21 (1H, d, J=2.1 Hz, H-16), 7.27 (1H, d, J=2.1 Hz, H-15); ^{13}C-NMR: see Tables 1 and 2.

Acidic Hydrolysis of Each Glycoside (1, 2) To a solution of each compound (100 mg) in 15 ml MeOH was added 30 ml of 0.1 N H_2SO_4, respectively. Each reaction mixture was kept at 50 °C for 50 min, then diluted with H_2O (30 ml) and concentrated to 60 ml. The solution was maintained at 60 °C for another 30 min, then neutralized with aq. saturated $Ba(OH)_2$, and the precipitate were filtered off. The filtrate was concentrated to dryness and chromatographed on a column of silica gel with cyclohexane–isopropyl ether–MeOH (4 : 4 : 1) to afford **3** (17.1 and 14.2 mg from **1** and **2**, respectively) (4), mp 72—74 °C; $[\alpha]_D^{23}$ −42.0° (c=0.1, MeOH). The sugar components in each hydrolysate were identified by TLC comparison with authentic samples. The Rf values of L-cymarose, D-digitoxose and L-diginose: 0.42, 0.04 and 0.35 with solvent *n*-hexane–Me_2CO (1 : 1); 0.59, 0.42 and 0.56 with $CHCl_3$–MeOH–H_2O (12 : 3 : 1, lower layer), respectively.

Results and Discussion

The $CHCl_3$-soluble extract of the roots of *C. ascyrifolium* was pre-fractionated using CC on silica gel to obtain a crude mixture of steroidal glycosides with 2-deoxy sugar residues which exhibited positive Liebermann–Burchard and Keller–Kiliani reactions. The crude glycoside mixture was subjected to repeated silica gel CC with various solvent systems to afford pure pregnane glycosides **1** and **2**.

Cynascyroside D (**1**) possessed the molecular formula $C_{40}H_{60}O_{13}$ on the basis of FAB mass spectrum and ^{13}C-NMR data, and its IR spectrum showed absorption bands for hydroxyl (3451 cm^{-1}), carbonyl (1714 cm^{-1}) and olefinic (1651 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** revealed the presence of two tertiary methyl groups [δ 1.13 (Me-19), 2.18 (Me-21)], one olefinic proton [δ 5.43 (br d, J=4.4 Hz, H-6)] and two aromatic protons on a furan ring [δ 6.22, 7.27 (each d, J=2.1 Hz, H-16, H-15)] in its aglycone moiety. Proton signals due to three secondary methyl groups [δ 1.23, 1.26 (9H, each d, J=6.4 Hz)], two methoxyl groups [δ 3.42, 3.46] and three anomeric protons [δ 4.81 (dd, J=9.6, 2.0 Hz), 4.85 (dd, J=9.6, 1.6 Hz), 4.91 (br d, J=3.9 Hz)] in its sugar moiety were observed. The splitting patterns of anomeric proton signals indicated that **1** has three sugar units, with one α-linkage and two β-linkages. Mild acidic hydrolysis of **1** afforded an aglycone (**3**), and two sugar components, L-cymarose and D-digitoxose, by comparison with authentic samples. The aglycone was identified as cynajapogenin A (**3**), which is a 14,15-*seco*-18-*nor*-pregnane isolated from *Cynanchum atratum*, by comparison of their physical and spectroscopic data.[3)] In the ^{13}C-NMR spectrum of **1**, glycosidation shifts[4,5)] were observed at the C-2 (−2.28 ppm), C-3 (+9.03 ppm) and C-4 (−2.68 ppm) positions when compared with ^{13}C chemical shifts of **3**, indicat-

* To whom correspondence should be addressed. e-mail: jwkim@snu.ac.kr

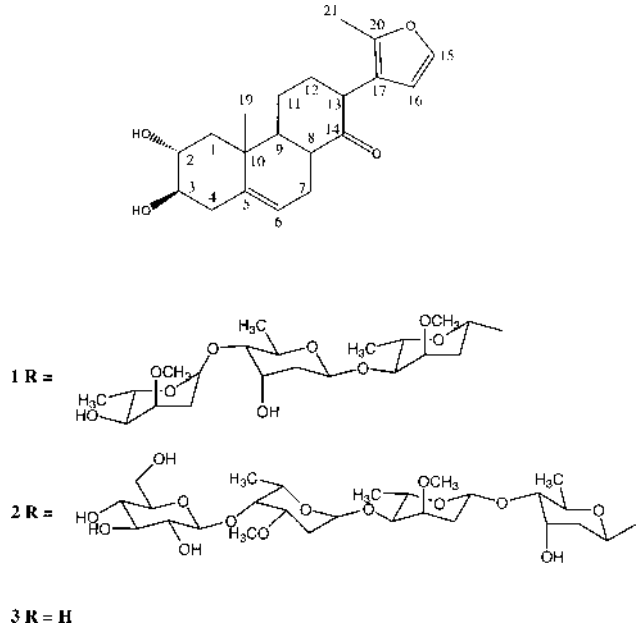

Fig. 1. Chemical Structures of Compounds **1**—**3**

Table 1. ^{13}C-NMR Chemical Shifts of the Aglycone Moieties

C	1	2	3
1	45.36	45.31	45.95
2	70.05	70.01	72.33
	(−2.28)	(−2.32)	
3	85.37	85.25	76.34
	(+9.03)	(+8.91)	
4	37.65	37.58	40.33
	(−2.68)	(−2.75)	
5	138.84	138.77	139.71
6	121.49	121.45	120.77
7	25.86	25.81	25.69
8	51.97	51.90	52.10
9	45.49	45.43	45.51
10	38.77	38.72	39.32
11	25.43	25.36	25.42
12	33.80	33.73	33.63
13	47.82	47.82	47.79
14	209.35	209.40	209.35
15	140.16	140.14	140.03
16	111.99	111.96	111.68
17	117.91	117.88	117.78
19	19.94	19.88	20.11
20	148.22	148.17	148.13
21	11.87	11.82	11.82

δ values (ppm) from internal TMS in C_5D_5N. Glycosidation shifts are given in parentheses.

ing the attachment of the sugar chain at the C-3 hydroxyl group of the aglycone. The ^{1}H- and ^{13}C-NMR spectra of **1** suggested that the three sugar components were β-L-cymaropyranose, β-D-digitoxopyranose and α-L-cymaropyranose, which were supported by the ^{1}H–^{13}C correlation spectroscopy (COSY) spectrum. Its negative FAB mass spectrum exhibited fragment ion peaks at *m/z* 747 $[M-H]^-$, 603 $[747-144]^-$, and 473 $[603-130]^-$, suggesting that the sugar at the middle position in the sugar chain is D-digitoxopyranose. The ^{1}H- and ^{13}C-NMR chemical shifts of the sugar moiety were identical with those reported for the sugar portion of glycoside-C, a known compound isolated from *Cynanchum glaucescens*.[6] Based on the above data, the structure of compound **1** was determined to be cynajapogenin A 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-L-cymaropyranoside.

Table 2. ^{13}C-NMR Chemical Shifts of the Sugar Moieties

C	1	2
	β-L-Cym	β-D-Dgt
1′	97.84	97.78
2′	36.98	39.58
3′	77.82	69.34
4′	82.86	82.89
5′	69.36	67.63
6′	18.18	18.47
3′-OMe	58.96	—
	β-D-Dgt	β-L-Cym
1″	100.38	100.42
2″	38.38	36.97
3″	68.80	77.81
4″	80.77	82.12
5″	67.77	68.63
6″	18.38	18.12
3″-OMe	—	58.96
	α-L-Cym	α-L-Dgn
1‴	98.41	100.71
2‴	32.22	32.13
3‴	76.52	74.47
4‴	72.65	74.86
5‴	67.17	67.98
6‴	18.49	17.65
3‴-OMe	56.81	55.48
		β-D-Glc
1⁗		105.33
2⁗		75.15
3⁗		78.43
4⁗		71.62
5⁗		78.08
6⁗		62.89

Cym: cymaropyranosyl, Dgt: digitoxopyranosyl, Dgn: diginopyranosyl, Glc: glucopyranosyl.

Cynascyroside E (**2**) has the molecular formula $C_{46}H_{70}O_{18}$ on the basis of FAB mass spectrum and ^{13}C-NMR data. Its IR and ^{1}H-NMR spectrum indicated that the aglycone of **2** is the same type as **1**. Proton signals due to three secondary methyl groups at δ 1.24, 1.25 and 1.29 (each d, J=6.4 Hz), two methoxyl groups at δ 3.43 and 3.46, and four anomeric protons at δ 4.45 (d, J=7.2 Hz), 4.81 (br d, J=9.9 Hz), 4.85 (br d, J=9.3 Hz) and 5.09 (br s) were observed in its sugar moiety. These findings indicated that there were four sugar units with one α-linkage and three β-linkages in **2**. Mild acidic hydrolysis of compound **2** yielded an aglycone (**3**), and four sugar components, D-digitoxose, L-cymarose, L-diginose and D-glucose, by comparison with authentic samples. Its negative FAB mass spectrum showed fragments at *m/z* 909 $[M-H]^-$, 747 $[909-162]^-$, 603 $[747-144]^-$, 459 $[603-144]^-$ and 329 $[459-130]^-$, which indicated the presence of glucose at the terminal position and digitoxose linked to the aglycone. In the ^{13}C-NMR spectrum of **2**, a glycosidation shift of the aglycone carbon signals was observed at the C-2 (−2.32 ppm), C-3 (+8.91 ppm) and C-4 (−2.75 ppm) positions, confirming the attachment of the sugar chain at the C-3 hydroxyl group of the aglycone. The sequence of these four sugars in compound **2** was confirmed by the heteronuclear multiple bond correlation (HMBC) spectrum, which

showed distinct cross-peaks of correlation from δ_H 4.45 (H-1‴″ of β-D-glucopyranose) to δ_C 74.86 (C-4‴ of α-L-diginopyranose), from δ_H 4.85 (H-1‴ of α-L-diginopyranose) to δ_C 82.12 (C-4″ of β-L-cymaropyranose), from δ_H 5.09 (H-1″ of β-L-cymaropyranose) to δ_C 82.89 (C-4′ of β-D-digitoxopyranose), and from δ_H 4.81 (H-1′ of β-D-digitoxopyranose) to δ_C 85.25 (C-3). Thus, the structure of compound **2** was elucidated as cynajapogenin A 3-*O*-β-D-glucopyranosyl-(1(4)-α-L-diginopyranosyl-(1→4)-β-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranoside.

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References

1) Kim J. G., "Illustrated Natural Drugs Encyclopedia," Vol. 1, Namsandang, Seoul, 1984, p. 201.
2) Yeo H., Kim K. W., Kim J., Choi Y. H., *Phytochemistry*, **49**, 1129—1133 (1998).
3) Zhang Z. X., Zhou J., Hayashi K., Kaneko K., *Phytochemistry*, **27**, 2935—2941 (1988).
4) Kasai R., Suzuo M., Asakawa J., Tanaka O., *Tetrahedron Lett.*, **1977**, 175—178.
5) Seo S., Tomita Y., Tori K., Yoshimura Y., *J. Am. Chem. Soc.*, **100**, 3331—3339 (1978).
6) Nakagawa T., Hayashi K., Wada K., Mitsuhashi H., *Tetrahedron*, **39**, 607—612 (1983).