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STEROIDAL GLYCOSIDES OF THE 14,15-*SECO*-18-*NOR*-PREGNANE SERIES FROM *CYNANCHUM ASCYRIFOLIUM*

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Key Word Index—*Cynanchum ascyrifolium*; Asclepiadaceae; steroidal glycosides; cynascyrosides A–C; cynajapogenin A.

Abstract—Three new steroidal glycosides named cynascyrosides A–C 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-D-oleandropyranosyl-(1 → 4)-β-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside; cynajapogenin A 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-L-cymaropyranosyl-(1 → 4)-β-L-cymaropyranoside; cynajapogenin A 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-L-cymaropyranoside.

INTRODUCTION

Cynanchum ascyrifolium Matsumura has been used as an antitussive and expectorant in Korea [1]. No phytochemical studies on the roots of this plant have been reported, although species of the genus *Cynanchum* have been studied extensively for their C/D-*cis*-polyoxypregnane glycoside constituents [2]. In this paper, we present the isolation and structural elucidation of three new glycosides named cynascyrosides A–C of which the aglycone was cynajapogenin A, a 14,15-*seco*-18-*nor*-pregnane.

RESULTS AND DISCUSSION

The methanolic extract of the roots of *C. ascyrifolium* was diluted with water, and then re-extracted with *n*-hexane, chloroform and *n*-butanol, respectively. Repeated chromatography of the chloroform-soluble material on silica gel afforded three new glycosides **1**–**3**. These compounds showed positive Liebermann–Burchard and Keller–Kiliani [3] reactions for steroidal glycosides with 2-deoxy sugar residues.

Cynascyroside A (**1**) possessed the molecular formula $C_{40}H_{60}O_{13}$ on the basis of FAB mass spectrometry and ^{13}C NMR data, and its IR spectrum showed the absorption bands for hydroxyl (3451 cm^{-1}), carbonyl (1714 cm^{-1}) and olefinic (1651 cm^{-1}) groups. The ^1H NMR spectrum of **1** revealed the presence of two tertiary methyl groups [δ_H 1.13 (Me-19) and 2.17 (Me-21)], one olefinic proton [δ_H 5.43 (*br d*, $J = 4.8$ Hz, H-6)] and two aromatic protons on a furan ring [δ_H 6.21 and 7.27 (each *d*, $J = 2.0$ Hz, H-16 and H-15)] in its aglycone moiety. The proton signals due to three secondary methyl groups [δ_H 1.24, 1.25 and 1.29 (each *d*, $J = 6.4$ Hz)], two methoxyl groups [δ_H 3.41 and 3.46] and three anomeric protons [δ_H 4.81 (*dd*, $J = 9.8$, 2.0 Hz), 4.87 (*dd*, $J = 9.8$, 1.5 Hz) and 5.03 (*br d*, $J = 3.6$ Hz)] were observed in its sugar moiety. 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 (**4**), and three sugar components, L-cymarose, D-digitoxose and D-oleandrose by comparison with authentic samples. The aglycone was identified as cynajapogenin A (**4**), which is a 14,15-*seco*-18-*nor*-pregnane isolated from *C. atratum*, by comparison of their physical and spectroscopic data [4]. In the ^{13}C NMR spectrum of **1**, glycosidation shifts [5, 6] were observed at C-2 (−2.31 ppm), C-3 (+8.94 ppm) and C-4 (−2.73 ppm) position when compared with ^{13}C chemical shifts of **4**, indicating the attachment of the sugar chain at the C-3 hydroxyl group of the aglycone. The ^1H NMR and ^{13}C NMR spectra of **1** suggested that the three sugar components were β-D-digitoxopyranose, β-L-cymaropyranose and α-D-oleandropyranose, which was supported by the ^1H-^{13}C COSY spectrum. Its negative FAB mass spectrum exhibited the fragment ion peaks at *m/z* 747 $[M–H]^-$, 603 $[747–144]^-$, and 459 [603–

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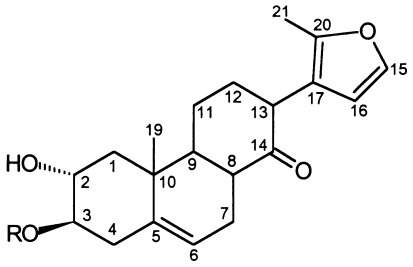
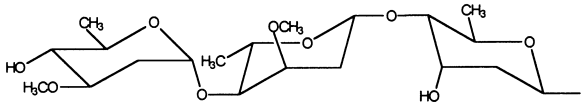
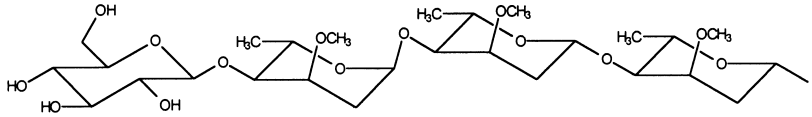
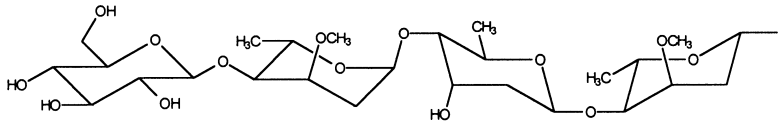


1 R = 

2 R = 

3 R = 

4 R = H

144]$^-$, suggesting the sugar linked to the aglycone in the sugar chain is D-digitoxopyranose. The sequence of these three sugars in compound **1** was confirmed by the HMBC spectrum, which showed distinct cross-peaks of correlation from δ_H 5.03 (H-1‴ of α-D-oleandropyranose) to δ_C 82.17 (C-4″ of β-L-cymaropyranose), from δ_H 4.87 (H-1″ of β-L-cymaropyranose) to δ_C 82.91 (C-4′ of β-D-digitoxopyranose), and from δ_H 4.81 (H-1′ of β-D-digitoxopyranose) to δ_C 85.28 (C-3). Therefore, the structure of compound **1** was elucidated as cynajapogenin A 3-*O*-α-D-oleandropyranosyl-(1 → 4)-β-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Cynascyroside B (**2**) has the molecular formula $C_{47}H_{72}O_{18}$ on the basis of the FAB mass spectrum and ^{13}C NMR data, and its IR spectrum showed the absorption bands for hydroxyl (3472 cm^{-1}), carbonyl (1715 cm^{-1}) and olefinic (1651 cm^{-1}) groups. The ^1H NMR spectrum of **2** gave signals at δ_H 1.13 (3H, *s*, Me-19), 2.17 (3H, *s*, Me-21), 5.43 (1H, *br d*, $J = 4.4$ Hz, H-6), 6.21 (1H, *d*, $J = 2.1$ Hz, H-16) and 7.27 (1H, *d*, $J = 2.1$ Hz, H-15) in its aglycone moiety, which were very similar to those of compound **1**. The proton signals due to three secondary methyl groups at δ_H 1.21, 1.24 and 1.26 (each *d*, $J = 6.4$ Hz), three methoxyl groups at δ_H 3.39, 3.46 and 3.47, and four anomeric protons at δ_H 4.39 (*d*, $J = 7.8$ Hz), 4.74 (*br d*, $J = 10.5$ Hz), 4.80 (*br d*, $J = 10.5$ Hz) and 4.82 (*br d*, $J = 3.6$ Hz) 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 (**4**), and two sugar components, L-cymarose and glaucobiose by comparison with authentic samples. The presence of three moles of L-cymaropyranose and one mole of D-glucopyranose in **2** was inferred from its ^{13}C NMR and ^1H NMR spectra, namely three secondary methyl and three methoxy methyl signals. The signals at δ_C 32.24, 73.27, 78.34, 65.04, 18.47 (C-2‴ to C-6‴ of α-L-cymaropyranose), and 102.18 (C-1⁗ of β-D-glucopyranose) in its ^{13}C NMR spectrum revealed that the terminal β-D-glucopyranose was linked to the C-4‴ hydroxyl group of α-L-cymaropyranose, which was supported by the previous report [7] for the glucosidation shift patterns of α- and β-L-cymaropyranose unit in 4-*O*-β-D-glucopyranosyl-cymaropyranose. In addition, the glycosidation shift of the aglycone carbon signals was observed at C-2 (−2.31 ppm), C-3 (+8.92 ppm) and C-4 (−2.74 ppm) position confirming the attachment of the sugar chain at

the C-3 hydroxyl group of the aglycone. The HMBC spectrum of **2** showed correlation peaks from δ_H 4.39 (H-1‴″ of β-D-glucopyranose) to δ_C 78.34 (C-4‴ of α-L-cymaropyranose), from δ_H 4.82 (H-1‴ of α-L-cymaropyranose) to δ_C 82.12 (C-4″ of β-L-cymaropyranose), from δ_H 4.74 (H-1″ of β-L-cymaropyranose) to δ_C 82.81 (C-4′ of β-L-cymaropyranose), and from δ_H 4.80 (H-1′ of β-L-cymaropyranose) to δ_C 85.26 (C-3). Thus, the structure of **2** was established as cynajapogenin A 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-L-cymaropyranosyl-(1 → 4)-β-L-cymaropyranoside.

Cynascyroside C (**3**) possessed the molecular formula $C_{46}H_{70}O_{18}$ on the basis of the FAB mass spectrum and ^{13}C NMR data, and its IR spectrum was almost the same as that of **2**. The ^1H NMR spectrum of **3** showed signals at δ_H 1.13 (Me-19), 2.17 (Me-21), 5.43 (*br d*, $J = 4.4$ Hz, H-6), 6.21 (*d*, $J = 2.1$ Hz, H-16), and 7.27 (*d*, $J = 2.1$ Hz, H-15), which indicated that the aglycone of **3** is also the same type as **1** and **2**. The presence of four sugar units with one α-linkage and three β-linkages in **3** was inferred from the coupling constants of the anomeric proton signals at δ_H 4.39 (*d*, $J = 7.5$ Hz), 4.80 (*br d*, $J = 10.0$ Hz), 4.85 (*br d*, $J = 10.8$ Hz) and 4.88 (*br s*). This glycoside liberated L-cymarose, D-digitoxose and glaucobiose as sugar components on mild acidic hydrolysis, which were identified by comparison with authentic samples. In its negative FAB mass spectrum, the prominent ion peaks at *m/z* 747 $[M–H–162]^-$, 603 $[747–144]^-$, 473 $[603–130]^-$ and 329 $[473–144]^-$ were attributable to those derived by initial loss of the terminal glucose, followed by cymarose, next followed by digitoxose, and finally the cymarose linked to the aglycone, respectively. Moreover, the glycosidation shifts were observed at C-2 (−2.30 ppm), C-3 (+8.96 ppm) and C-4 (−2.73 ppm) in the ^{13}C NMR data for the aglycone moiety of **3**, suggesting that the sugar chain is linked to the C-3 hydroxyl group of **4**. The sugar sequence of **3** was confirmed by the HMBC spectrum, which showed distinct cross-peaks of correlation from δ_H 4.39 (H-1‴″ of β-D-glucopyranose) to δ_C 78.08 (C-4‴ of α-L-cymaropyranose), from δ_H 4.88 (H-1‴ of α-L-cymaropyranose) to δ_C 80.92 (C-4″ of β-D-digitoxopyranose), from δ_H 4.85 (H-1″ of β-D-digitoxopyranose) to δ_C 82.83 (C-4′ of β-L-cymaropyranose), and from δ_H 4.80 (H-1′ of β-L-cymaropyranose) to δ_C 85.30 (C-3). Consequently, the structure of **3** was assigned as cynajapogenin A 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-L-cymaropyranoside.

EXPERIMENTAL

General

Mps: uncorr. FABMS spectra were measured on Finnigan MAT 90 mass spectrometer. IR spectra were recorded on Perkin–Elmer 1710 spectrometer. NMR spectra were measured with JEOL JNM-LA 300 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C). ^1H NMR were run in $CDCl_3$ soln and ^{13}C NMR in C_5D_5N soln with TMS as int. standard. TLC was performed on precoated silica gel 60 F_{254} (Merck) and 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 identified by Dr Bo Sup Chung, a former professor of 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. Fr. 2 (30 g) was submitted to CC on silica gel with *n*-hexane–EtOAc–MeOH (17:17:1) to give **1** (290 mg). Fr. 3 (12 g) was subjected to the 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** (400 mg) and **3** (750 mg).

Cynascyroside A (**1**). Pale yellow powder, mp 105–107°; $[\alpha]_D^{23}$ −35.7°C (MeOH; *c* 0.1); UV λ_{max}^{MeOH} nm: 221; IR ν_{max}^{KBr} cm^{-1}: 3451, 1714, 1651, 1518, 1000; FABMS (negative) *m/z*: 747 $[M–H]^-$, 603 $[M–H–144]^-$, 459 $[603–144]^-$; ^1H 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), 3.41 (3H, *s*, 3‴-OMe), 3.46 (3H, *s*, 3″-OMe), 4.81 (1H, *dd*, $J = 9.8$, 2.0 Hz, H-1′), 4.87 (1H, *dd*, $J = 9.8$, 1.5 Hz, H-1″), 5.03 (1H, *br d*, $J = 3.6$ Hz, H-1‴), 5.43 (1H, *br d*, $J = 4.8$ Hz, H-6), 6.21 (1H, *d*, $J = 2.0$ Hz, H-16), 7.27 (1H, *d*, $J = 2.0$ Hz, H-15); ^{13}C NMR: see Tables 1 and 2.

Cynascyroside B (**2**). Pale yellow powder, mp 135–137°; $[\alpha]_D^{23}$ −40.0° (MeOH; *c* 0.1); UV λ_{max}^{MeOH} nm: 223; IR ν_{max}^{KBr} cm^{-1}: 3472, 1715, 1651, 1454, 1370, 1061; FABMS (negative) *m/z*: 923 $[M–H]^-$; ^1H NMR: δ 1.13 (3H, *s*, H-19), 1.21, 1.24, 1.26 (each 3H, *d*, $J = 6.4$ Hz, H-6′, H-6″, H-6‴), 2.17 (3H, *s*, H-21), 3.39 (3H, *s*, 3‴-OMe), 3.46, 3.47 (each 3H, *s*, 3′-OMe, 3″-OMe), 4.39 (1H, *d*, $J = 7.8$ Hz, H-1‴″), 4.74 (1H, *br d*, $J = 10.5$ Hz, H-1″), 4.80 (1H, *br d*, $J = 10.5$ Hz, H-1′), 4.82 (1H, *br d*, $J = 3.6$ Hz, H-1‴), 5.43 (1H, *br d*, $J = 4.4$ Hz, 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.

Cynascyroside C (**3**). Pale yellow powder, mp 130–132°; $[\alpha]_D^{23}$ −65.8° (MeOH; *c* 0.1); UV λ_{max}^{EtOH} nm: 212;

Table 1. ^{13}C NMR chemical shifts of the aglycone moieties

C	1	2	3	4
1	45.34	45.33	45.33	45.95
2	70.02	70.02	70.03	72.33
	(−2.31)	(−2.31)	(−2.30)	
3	85.28	85.26	85.30	76.34
	(+8.94)	(+8.92)	(+8.96)	
4	37.60	37.59	37.60	40.33
	(−2.73)	(−2.74)	(−2.73)	
5	138.79	138.80	138.81	139.71
6	121.45	121.46	121.47	120.77
7	25.83	25.83	25.84	25.69
8	51.91	51.92	51.94	52.10
9	45.44	45.44	45.45	45.51
10	38.73	38.73	38.75	39.32
11	25.37	25.38	25.39	25.42
12	33.75	33.76	33.77	33.63
13	47.85	47.80	47.80	47.79
14	209.40	209.39	209.38	209.35
15	140.14	140.15	140.16	140.03
16	111.97	111.97	111.98	111.68
17	117.90	117.89	117.90	117.78
19	19.87	19.89	19.91	20.11
20	148.19	148.19	148.19	148.13
21	11.81	11.83	11.85	11.82

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

Table 2. ^{13}C NMR chemical shifts of the sugar moieties

C	1	2	3
	β-D-Dgt	β-L-Cym	β-L-Cym
1′	97.80	97.77	97.79
2′	39.76	36.90	36.93
3′	68.67	77.60	77.79
4′	82.91	82.81	82.83
5′	67.56	69.15	69.34
6′	18.39	18.12	18.09
3′-OMe	—	58.49	58.92
	β-L-Cym	β-L-Cym	β-D-Dgt
1″	100.48	100.34	100.35
2″	36.98	36.90	38.41
3″	77.81	77.77	68.72
4″	82.17	82.12	80.92
5″	69.35	69.32	67.60
6″	18.14	18.47	18.16
3″-OMe	58.95	58.90	—
	α-D-Ole	α-L-Cym	α-L-Cym
1‴	100.28	98.85	98.30
2‴	35.74	32.24	32.38
3‴	78.74	73.27	73.50
4‴	76.80	78.34	78.08
5‴	69.43	65.04	66.07
6‴	18.54	18.47	18.44
3‴-OMe	57.02	56.85	57.05
		β-D-Glc	β-D-Glc
1⁗		102.18	102.30
2⁗		75.16	75.22
3⁗		78.55	78.30
4⁗		71.70	71.67
5⁗		78.86	78.51
6⁗		62.89	62.84

Cym: cymaropyranosyl; Dgt: digitoxopyranosyl; Glc: glucopyranosyl; Ole: oleandropyranosyl.

IR ν_{max}^{KBr} cm^{-1}: 3472, 1715, 1651, 1454, 1370, 1061; FABMS (negative) *m/z*: 909 $[M–H]^-$, 747 $[M–H–162]^-$, 603 $[747–144]^-$, 473 $[603–130]^-$, 329 [aglycone]$^-$; ^1H NMR: δ 1.13 (3H, *s*, H-19), 1.22, 1.25 (×2) (9H, *d*, $J = 6.4$ Hz, H-6′, H-6″, H-6‴), 2.17 (3H, *s*, H-21), 3.42 (3H, *s*, 3‴-OMe), 3.46 (3H, *s*, 3′-OMe), 4.39 (1H, *d*, $J = 7.5$ Hz, H-1⁗), 4.80 (1H, *br d*, $J = 10.0$ Hz, H-1′), 4.85 (1H, *br d*, $j = 10.8$ Hz, H-1″), 4.88 (1H, *br s*, H-1‴), 5.43 (1H, *br d*, $J = 4.4$ Hz, 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**–**3**)

To a soln 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° for 50 min, and diluted with H_2O (30 ml) and concd to 60 ml. The soln was kept for 60° for further 30 min, then neutralized with aq. satd $Ba(OH)_2$ and the ppt. was filtered off. The filtrate was concd to dryness and chromatographed on a column of silica gel with cyclohexane–isopropylether–MeOH (4:4:1) to afford **4** (16.7, 13.8 and 14.5 mg from **1**, **2** and **3**, respectively) [4], mp 72–74°; $[\alpha]_D^{23}$ −42.0° (MeOH; *c* 0.1). The sugar components in each hydrolysate were identified by TLC comparison with authentic samples. The R_f values of L-cymarose, D-digitoxose and D-oleandrose: 0.47, 0.21 and 0.43 with solvent $CHCl_3$–MeOH (9:1); 0.42, 0.21 and 0.33 with CH_2Cl_2–EtOH (9:1); 0.43, 0.17 and 0.31 with C_6H_6–Me_2CO (5:3), respectively. The R_f value of glaucobiose: 0.31 with $CHCl_3$–MeOH (4:1); 0.20 with $CHCl_3$–MeOH–H_2O (8:2:1, lower phase); 0.21 with C_6H_6–Me_2CO–MeOH (3:3:1).

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REFERENCES

1. Kim, J. G., *Illustrated Natural Drugs Encyclopedia* (Color Edition). Vol. 1, Namsandang, Seoul, 1984, p. 201.
2. Lin, Y.-L., Lin, T.-C. and Kuo, Y.-H., *Journal of Natural Products*, 1995, **58**, 1167.

3. Von Euw, J. and Reichstein, T., *Helvetica Chimica Acta*, 1948, **31**, 883.
4. Zhang, Z.-X., Zhou, J., Hayashi, K. and Kaneko, K., *Phytochemistry*, 1988, **27**, 2935.
5. Kasai, R., Suzuo, M., Asakawa, J. and Tanaka, O., *Tetrahedron Letters*, 1977, 175.
6. Seo, S., Tomita, Y., Tori, K. and Yoshimura, Y., *Journal of the American Chemical Society*, 1978, **100**, 3331.
7. Nakagawa, T., Hayashi, K. and Mitsuhashi, H., *Chemical and Pharmaceutical Bulletin*, 1983, **31**, 2244.