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Five new C_{21} steroidal glycosides from *Cynanchum komarovii* Al.Iljinski

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Abstract

Five new C_{21} steroidal glycosides, namely, komarosides D (**1**), E (**2**), F (**3**), G (**4**), and H (**5**), along with two known C_{21} steroidal glycosides cynatratoside E (**6**) and hancoside A (**7**), were isolated from the ethanol extract of the roots of *Cynanchum komarovii* Al.Iljinski (Asclepiadaceae). Their structures were determined by physiochemical and spectroscopic analysis. Among these glycosides, five had an aberrant 13,14:14,15-disecopregnane-type skeleton, and the other two had normal four-ring C_{21} steroid skeletons. The existence of more than one type of C_{21} steroid skeleton in one species is rare in the plants of the family Asclepiadaceae, and this has chemotaxonomic significance for this species.

Keywords: *Cynanchum komarovii* Al.Iljinski; Asclepiadaceae; Steroid; Komarosides D, E, F, G, and H

1. Introduction

Cynanchum komarovii Al.Iljinski is a shrub widely distributed over the northwestern desert of China. The sheep, cattle and camels do not eat this shrub in the pastoral area. In order to determine whether this is due to the chemical constituents of this plant, further chemical investigation was carried out. In a previous paper, we reported the isolation of a phenanthroindlizidine alkaloid [1] from the roots of *Cynanchum komarovii*. In this paper, we describe the isolation and structure determination of five new C_{21} steroidal glycosides named komarosides D (**1**), E (**2**), F (**3**), G (**4**), and H (**5**) along with two known compounds cynatratoside E (**6**) and hancoside A (**7**). Compounds **1**, **2**, **3**, **4** and **6** have an aberrant 13, 14: 14,15-disecopregnane-type skeleton. Compounds **5** and **7** have a normal four-ring C_{21} steroid skeleton.

2. Experimental

2.1. General methods

Optical rotations were measured on a SEPA-300 polarimeter. IR spectral data were determined on a Bio-Rad FTS-135 spectrometer with KBr pellets. The ^{1}D NMR spectrum was obtained on Bruker AM-400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) spectrometers with TMS as the internal standard. The multiplicity of ^{13}C NMR was determined as DEPT. The ^{2}D NMR spectra were recorded on a Bruker AM-500 MHz spectrometer. MS data were obtained on a VG Autospec-3000 mass spectrometer.

2.2. Extraction and isolation

The air-dried roots of *Cynanchum komarovii* Al.Iljinski (12.5 kg) were extracted with 90% EtOH under reflux to give a crude extract. The extract was suspended in water and then partitioned with $CHCl_3$ to afford $CHCl_3$ and aqueous residues (57 and 600 g, respectively). The $CHCl_3$ residue was subjected to CC over Si-gel and eluted with $CHCl_3$/CH_3OH (9:1) to give nine fractions. The sixth

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fraction was repeatedly subjected to CC over Si-gel, Sephadex LH-20 and RP-18 to afford compounds **1**, **2**, **3**, **4** and **6**. The eighth fraction was repeatedly subjected to CC over Si-gel, Sephadex LH-20 and RP-18 to afford compounds **5** and **7**.

Komaroside D (**1**) $C_{47}H_{72}O_{20}$, colorless amorphous (CH_3OH–$CHCl_3$), $[\alpha]_D^{18.3}$ − 7.30 (c = 0.411, CH_3OH), $IR_{\upsilon_{max}}^{KBr}$ (cm^{-1}): 3446, 2933, 1737, 1653, 1648, 1636, 1682, 1448, 1383, 1309, 1162, 1123, 1103, 1079, 1058, 986 cm^{-1}. FAB-MS m/z: 955 $(M - H)^+$, 793 $(M - 162 - H)^+$, 649 $(M - 162 - 144 - H)^+$, 521 $(M - 162 - 144 - 130 - H - H - H)^+$, 375 (aglycone − H)$^+$. HRFAB-MS m/z 955.4572 ([M − H]), calculated: 955.4539. ^{1}H NMR (C_5D_5N, 400 Hz): δ 0.90 (3H, s, H-19), δ 1.54 (3H, s, H-21), δ 6.48 (1H, s, H-18), δ 5.43 (1H, m, H-6), δ 1.37 (3H, s, H-6′ β-D-ole), δ 1. 41 (3H, d, J = 6.3 Hz, H-6″ β-D-digit), δ 1.53 (3H, d, J = 6.4 Hz, H-6‴ α-D-ole), δ 3.53 (3H, s, H-3′-OME β-D-ole), δ 3.32 (3H, s, H-3‴-OME α-D-ole), δ 5.52 (1H, d, 9.4 Hz H-1⁗ β-D-glc), δ 5.17 (1H, s, H-1‴ α-D-ole), δ 5.24 (1H, d, 7.6 Hz H-1″ β-D-digit), δ 4.79 (1H, d, 9.4 Hz H-1′ β-D-ole). For the ^{13}C-NMR spectral data (see Table 1).

*Acid hydrolysis of **1***

A solution of 20 mg of **1** in 5 ml CH_3OH was treated with 5 ml 5% HCl and the mixture kept at room temperature for 15 min. In the hydrolysate of **1**, digitoxose and oleandrose were identified by TLC comparison with authentic samples with solvent A: petrol ether/CH_3COCH_3 (3:2), solvent B: CH_2Cl_2/C_2H_5OH (9:1), and solvent C: $CHCl_3$/CH_3OH (9:1); glucose was identified by TLC comparison with authentic sample with solvent $CHCl_3$/CH_3OH/H_2O (4:3:1).

*Acetylation of **1***

Compound **1** (2 mg) was dissolved in Ac_2O-pyridine (1:1, 0.5 ml). The mixture was heated in a water bath at 60 °C for 30 min, and then incubated at room temperature overnight. The resulting glycoside acetate was subjected to positive FAB-MS analysis, and there were three main peaks at 1208 $[M]^+$, 475 $[glc(OAc)_4 + Ole + H]^+$, 331 $[glc(OAc)_4]^+$.

Komaroside E (**2**) $C_{47}H_{72}O_{20}$, colorless needles (CH_3OH–$CHCl_3$), $[\alpha]_D^{21.7}$ − 20.27 (c = 0.222, CH_3OH), FAB-MS m/z: 955 $(M - H)^+$, 793 $(M - 162 - H)^+$, 649 $(M - 162 - 144 - H)^+$, 521 $(M - 162 - 144 - 130 - H - H - H)^+$, 375 (aglycone − H)$^+$. HRFAB-MS m/z 955.4550 ([M − H]),

Table 1
Chemical shifts of compounds **1**, **2**, **3**, **4**, and **5** (ppm, in C_5D_5N, 100 MHz)

Aglycne moiety	**1**	**2**	**3**	**4**	**5**	Sugar moiety	**1**	**2**	**3**	**4**	**5**
1	44.7	44.6	44.8	44.8	37.4		β-D-ole	β-D-ole	β-D-ole	β-D-3-demeth-	β-D-glc
2	69.9	69.8	69.9	70.0	30.1	1′	98.9	98.9	98.9	99.1	100.7
3	84.9	84.9	84.9	85.0	79.1	2′	37.7	37.5	37.7	39.6	84.9
4	37.3	37.2	37.4	37.4	39.2	3′	78.7[a]	78.9	78.5[a]	70.0	78.1[a]
5	139.7	139.7	139.8	139.8	140.6	4′	82.7	82.4	82.9	88.1	71.1
6	120.8	120.7	120.9	120.9	122.6	5′	71.9	71.8	71.8	71.8	78.2[a]
7	28.4	28.3	28.5	28.5	27.8	6′	18.4[c]	18.0[c]	18.4[c]	18.7[c]	62.5
8	40.2	40.1	40.3	40.3	37.4	OMe	57.5	57.3	57.5	/	/
9	53.0	52.9	53.1	53.1	46.1		β-D-digit	β-D-digit	β-D-3-demeth	β-D-digit	β-D-glc
10	39.9	39.4	39.9	39.9	37.3	1″	98.5	98.3	100.3	99.9	106.8
11	23.8	23.7	23.9	23.9	20.9	2″	39.5	38.4	39.5	38.3	76.5
12	30.0	29.9	30.1	30.0	38.7	3″	68.9	68.9	69.9	69.4	78.0[a]
13	114.3	114.3	114.4	114.4	49.2	4″	82.1[b]	80.8	88.1	81.0	71.2
14	175.4	175.3	175.5	175.5	85.6	5″	67.6	67.7	71.0	67.5	75.8
15	67.8	67.7	67.8	67.9	34.4	6″	18.6[c]	18.4[c]	18.6[c]	18.7[c]	64.8
16	75.6	75.5	75.6	75.6	24.4		α-D-ole	α-L-cym	β-D-ole	α-L-cym	Sinapoyl
17	56.1	56.0	56.2	56.2	63.0	1‴	99.8	98.2	99.7	98.6	1‴ 167.7
18	143.9	143.8	143.9	143.9	15.3	2‴	35.5	32.3	36.3	32.5	2‴ 115.4
19	18.9	18.8	18.9	18.9	19.7	3‴	78.9[a]	73.4	79.2	73.6	3‴ 146.0
20	118.5	118.5	118.6	118.6	216.7	4‴	82.2[b]	78.3[a]	82.6	78.1	4‴ 125.1
21	24.8	24.7	24.8	24.9	32.3	5‴	68.0	65.9	69.9	66.4	5‴ (9‴) 106.8
						6‴	18.4[c]	18.4[c]	18.4[c]	17.8[c]	6‴ (8‴) 149.2
						OMe	56.7	56.9	58.8	57.1	(7‴) 139.6
							β-D-glc	β-D-glc	β-D-glc	β-D-glc	(6‴,8‴-OMe) 56.4
						1⁗	104.9	102.2	106.5	102.5	
						2⁗	75.8	75.2	75.4	75.4	
						3⁗	78.3	78.0	78.3[a]	78.7[a]	
						4⁗	71.9	71.7	71.8	71.8	
						5⁗	78.3	78.5[a]	77.8	78.5[a]	
						6⁗	62.9	62.8	63.0	62.9	

[a–c]: Assignments in each column may be interchangeable; 3-demeth-: 3-demethyl-2-deoxy-thevetopyanosyl; ole: oleandropyranosyl; digit: digitoxoyranosyl; cym: cymaropyranosyl; glc: glucopyranosyl.

calculated: 955.4539. ^{1}H NMR (C_5D_5N, 400 Hz): δ 0.90 (3H, s, H-19), δ 1.54 (3H, s, H-21), δ 6.49 (1H, s, H-18), δ 5.45 (1H, m, H-6), δ 1.41 (3H, d, J = 7.5 Hz H-6′ β-D-ole), δ 1.37 (3H, d, J = 6.3 Hz, H-6″ β-D-digit), δ 1.35 (3H, d, J = 6.6 Hz, H-6‴ α-L-cym), δ 3.53 (3H, s, H-3′-OME β-D-ole), δ 3.46 (3H, s, H-3‴-OMEα-L-cym), δ 5.42 (1H, d, 8.1 Hz H-1⁗ β-D-glc), δ 5.04 (1H, s, H-1‴ α-L-cym), δ 5.02 (1H, d, 7.7 Hz H-1″ β-D-digit), δ 4.79 (1H, d, 9.4 Hz H-1′ β-D-ole). For the ^{13}C NMR spectral data (see Table 1).

Komaroside F (**3**) $C_{47}H_{72}O_{20,}$ colorless amorphous (CH_3OH-$CHCl_3$), $[\alpha]_D^{25.9}$ + 4.45 (c = 0.674, CH_3OH), FAB-MS *m/z*: 955 $(M - H)^+$, 375 (aglycone − H)$^+$. HRFAB-MS *m/z* 955.4509 ([M − H]), calc. 955.4539. ^{1}H-NMR (C_5D_5N, 400 Hz): δ 0.90 (3H, s, H-19), δ 1.52 (3H, s, H-21), δ 6.49 (1H, s, H-18), δ 5.45 (1H, m, H-6), δ 1.56 (3H, d, J = 6.3 Hz H-6′ β-D-ole), δ 1.35 (3H, d, J = 8.8 Hz, H-6″ β-D-3-demethyl-2-deoxy-thevetose), δ 1.38 (3H, d, J = 8.3 Hz, H-6‴ β-D-ole), δ 3.52 (3H, s, H-3′-OME β-D-ole), δ 3.59 (3H, s, H-3‴-OME β-D-ole), δ 3.22 (1H, dd, J = 8.0, 8.0 Hz, H-4″ β-D-3-demethyl-2-deoxy-thevetose), δ 4.90 (1H, d, J = 7.6 Hz H-1⁗ β-D-glc), δ 5.14 (1H, d, 8.3 Hz H-1‴ β-D-ole), δ 4.92 (1H, d, J = 8.1 Hz H-1″ β-D-3-demethyl-2-deoxy-thevetose), δ 4.81 (1H, d, 8.3 Hz H-1′ β-D-ole). For the ^{13}C-NMR spectral data, see Table 1.

Komaroside G (**4**) $C_{46}H_{70}O_{20,}$ colorless amorphous (CH_3OH-$CHCl_3$), $[\alpha]_D^{25.4}$ − 30.66 (c = 0.212, CH_3OH), FAB-MS *m/z*: 941 $(M - H)^+$, 375 (aglycone − H)$^+$. HRFAB-MS *m/z* 941.4348 ([M − H]), calc. 941.4382. ^{1}H-NMR (C_5D_5N, 400 Hz): δ 0.90 (3H, s, H-19), δ 1.53 (3H, s, H-21), δ 6.49 (1H, s, H-18), δ 5.44 (1H, m, H-6), δ 1.36 (3H, H-6′ β-D-demethyl-2-deoxy-thevetose), δ 1.32 (3H, d, 5.8 Hz, H-6″ β-D-digit), δ 1.30 (3H, d, 6.0 Hz, H-6‴ α-L-cym), δ 3.45 (3H, s, H-3‴-OME α-L-cym), δ 3.28 (1H, dd, J = 8.6, 9.0 Hz, H-4′ β-D-3-demethyl-2-deoxy-thevetose), δ 5.03 (1H, d, 7.7 Hz H-1⁗ β-D-glc), δ 5.00 (1H, s, H-1‴ α-L-cym), δ 5.27 (1H, d, J = 9.4 Hz H-1″ β-D-digit), δ 4.88 (1H, d, 9.8 Hz H-1′ β-D-demethyl-2-deoxy-thevetose). For the ^{13}C-NMR spectral data, see Table 1.

*Acid hydrolysis of **2**, **3**, and **4***

Each solution of 2mg of **2**, **3**, and **4** in 2 ml CH_3OH was treated with 2 ml 5% HCl and the mixture kept at room temperature for 15 min. Cymarose, digitoxose, oleandrose, and glucose were identified in the hydrolysate of **2**, oleandrose and glucose were identified in the hydrolysate of **3**, cymarose, digitoxose, and glucose were identified in the hydrolysate of **4** respectively by TLC comparison with authentic samples with the same solvent systems as that of **1**.

Komaroside H (**5**) $C_{44}H_{62}O_{17}$, colorless needles (CH_3OH–$CHCl_3$), $[\alpha]_D^{18.0}$ + 8.73 (c = 0.229, CH_3OH), FAB-MS *m/z*: 861 $(M-1)^+$, 655 (M − sinapoly − H)$^+$, 223 (M − aglycone − 2glc − H)$^+$. HRFAB-MS *m/z* 861.3904 ([M − H]), calculated: 861.3908. $UV_{\lambda_{max}}$ (CH_3OH): 327, 238, 224, 203 nm. $IR_{\upsilon_{max}}^{KBr}$ (cm^{-1}): 3419, 2935, 1693, 1689, 1633, and 1285. ^{1}H NMR (C_5D_5N, 400 Hz): δ 0.96 (3H, s, H-19), δ 1.04 (3H, s, H-18), δ 2.17 (3H, s, H-21), δ 3.84 (6H, s, 6‴, 8‴-OMe), δ 6.68 (1H, d, 15.8 Hz, H-2‴), δ 7.98 (1H, d, 15.8 Hz, H-3), δ 5.08 (1H, d, 7.6 Hz, H-1′ β-D-glc), δ 5.29 (1H, d, 7.6 Hz, H-1″ β-D-glc). For the ^{13}C NMR spectral data (see Table 1).

Cynatratoside E (**6**) $C_{47}H_{72}O_{19,}$ colorless amorphous (CH_3OH–$CHCl_3$), $IR_{\upsilon_{max}}^{KBr}$ (cm^{-1}): 3445, 1735, 1629, 1450, 1382, 1162, 1078, 988, FAB-MS *m/z*: 939 $(M - 1)^+$, 777 (M − glc − H)$^+$, 633 (M − glc − ole − H)$^+$; ^{13}C NMR (C_5D_5N, 100 Hz): 36.5 (C-1), 30.0 (C-2), 77.5 (C-3), 39.0 (C-4), 140.6 (C-5), 120.5 (C-6), 29.9 (C-7), 53.2 (C-8), 40.7 (C-9), 38.7 (C-10), 23.9 (C-11), 28.4 (C-12), 114.3 (C-13), 175.5 (C-14), 67.8 (C-15), 75.6 (C-16), 56.2 (C-17), 143.9 (C-18), 17.9 (C-19), 118.5 (C-20), 24.8 (C-21), 98.1 (C-1′), 37.9 (C-2′), 79.1 (C-3′), 83.2 (C-4′), 71.7 (C-5′), 18.7 (C-6′), 57.5 (C-3′-OMe), 98.6 (C-1″), 39.9 (C-2″), 68.9 (C-3″), 82.2 (C-4″), 67.7 (C-5″), 18.4 (C-6″), 99.8 (C-1‴), 35.4 (C-2‴), 78.5 (C-3‴), 82.1 (C-4‴), 67.9 (C-5‴), 18.6 (C-6‴), 56.8 (C-3‴-OMe), 104.9 (C-1⁗), 75.8 (C-2⁗), 78.3 (C-3⁗), 71.8 (C-4⁗), 78.6 (C-5⁗), 62.9 (C-6⁗), ^{1}H NMR (C_5D_5N, 400 Hz): 0.85 (3H, s, H-19), 1.53 (3H, s, H-21), 5.51 (1H, d, 8.8 Hz, H-1⁗ β-D-glc), 5.45 (1H, s H-1‴, α-D-ole), 5.21 (1H, d, 7.3 Hz, H-1″ β-D-digit), 4.79 (1H, d, 8.6 Hz, H-1′ β-D-ole). The spectral data were similar to those in the literature [2].

Hancoside A (**7**) $C_{44}H_{62}O_{18}$, colorless needles (CH_3OH–$CHCl_3$), FAB-MS *m/z*: 877 $(M - 1)^+$, 671 (M − sinapoyl − H)$^+$, 223 (sinapoyl)$^+$; ^{13}C NMR (C_5D_5N, 100 Hz): 37.5 (C-1), 30.1 (C-2), 78.8 (C-3), 39.1 (C-4), 140.6 (C-5), 122.9 (C-6), 26.1 (C-7), 37.4(C-8), 45.8 (C-9), 37.3 (C-10), 20.7 (C-11), 37.9 (C-12), 48.4 (C-13), 81.9 (C-14), 73.9 (C-15), 35.5 (C-16), 60.4 (C-17), 16.5 (C-18), 19.8 (C-19), 214.5 (C-20), 31.6 (C-21), 100.6 (C-1′), 85.5 (C-2′), 78.2 (C-3′), 71.2 (C-4′), 78.1 (C-5′), 62.5 (C-6′), 106.7 (C-1″), 76.5 (C-2″), 78.0 (C-3″), 71.2 (C-4″), 75.8 (C-5″), 64.8 (C-6″), 167.7 (C-1‴), 115.4 (C-2‴), 145.9 (C-3‴), 125.1 (C-4‴), 106.7 (C-5‴, 9‴), 149.2 (C-6‴, 8‴), 138.9 (C-7‴), 56.4 (C-6‴, 8‴-OMe); ^{1}H NMR (C_5D_5N, 400 Hz): 0.98 (3H, s, H-19), 1.09 (3H, s, H-18), 2.18 (3H, s, H-21), 3.83 (6H, s, 6‴, 8‴-OMe), 6.68 (1H, d, 15.8 Hz, H-2‴), 7.98 (1H, d, 15.8 Hz, H-3‴), 5.06 (1H, d, 7.8 Hz, H-1′ β-D-glc), 5.29 (1H, d, 7.6 Hz, H-1″ β-D-glc). The spectral data were similar to those in the literature [3].

3. Results and discussion

3.1. Komaroside D (1)

Partial hydrolysis of **1** with 5% aqueous HCl yielded glucose, oleandrose, and digitoxose, which were identified by TLC comparisons with authentic samples. The molecular formula of compound **1** was determined to be $C_{47}H_{72}O_{20}$ (955.4572 [M − H], calculated: 955.4539) by the HRFAB-MS data. The ^{1}H NMR spectra showed

signals for the two methyls of the aglycone moiety at δ_H 0.90 (s, 3H, H-19) and 1.54 (s, 3H, H-21), one olefinic proton signal at δ_H 5.43 (m, H-6), one olefinic deshielded proton signal at δ_H 6.48 (s, H-18) connected with the trisubstituted double bond, three oxygen-substituted methine protons at δ_H 5.45 (m, H-16), 3.65 (m, H-3), and 4.22 (m, H-2), and two oxygen-substituted methylene protons at δ_H 3.93 (m, αH-15) and 4.25 (m, βH-15), which were consistent with those of glaucogenin A [4]. The ^{13}C NMR spectral data (Table 1) were very similar to those of glaucogenin A [4] as well. The ^{1}H NMR spectrum of **1** showed three secondary methyl and two methoxyl methyl signals of deoxysugars and four anomeric proton signals at δ 5.52 (1H, d, 9.4 Hz), δ 5.24 (1H, d, 7.6 Hz), δ 5.17 (1H, s), and δ 4.79 (1H, d, 9.4 Hz), indicating the presence of three sugar moieties with three β-linkages and one α-linkage. The comparison of the ^{13}C NMR spectral data (Table 1) with those of cynatratoside E [2] indicated that **1** possessed the same sugar sequence in the oligosaccharide moiety as cynatratoside E. Comparing with glaucogenin A [4], the glycosidation shifts were observed at C-2 (−2.5 ppm), C-3 (+8.2 ppm), and C-4 (−2.8 ppm) in the aglycone moiety, therefore, the sugar moiety was linked to the C-3 hydroxyl group of the aglycone. Thus, this glycoside consisted of glaucogenin A with a sugar linkage at its C-3 hydroxyl group. The NMR assignments for **1** were carried out unambiguously on the basis of HMQC, HMBC, and HMQC-TOCSY experiments. In this way, compound **1** was determined to be glaucogenin A 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-oleandropyranoside (Fig. 1).

3.2. Komaroside E (2)

The molecular formula of **2** was determined to be $C_{47}H_{72}O_{20}$ (955.4550 [M − H], calculated: 955.4539) by its HRFAB-MS data. The acidic hydrolysis of **2** afforded glucose, cymarose, oleandrose, and digitoxose, which were identified by TLC comparison with authentic samples. In the ^{13}C NMR spectra of **1** and **2** (Table 1), the structure of **2** corresponded to **1** except that the α-D-oleandrose was replaced with α-L-cymarose. The NMR data were assigned according to HMQC, HMBC, and HMQC-TOCSY experiments. In addition, the spectral data of the sugar moiety were very close to those of glaucoside J [5]. Thus, the structure of **2** was deduced to be glaucogenin A 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopy-ranosyl-(1 → 4)-β-D-oleandropyranoside (Fig. 1).

3.3. Komaroside F (3)

The molecular formula of **3** was determined to be $C_{47}H_{72}O_{20}$ (955.4509 [M − H], calculated: 955.4539) by its HRFAB-MS data. The negative FAB-MS gave the main fragment ion peaks at m/z 955 $(M - H)^+$, 375 (aglycone − H)$^+$. The ^{1}H NMR spectrum of **3** showed three secondary methyl and two methoxyl methyl signals of deoxysugars

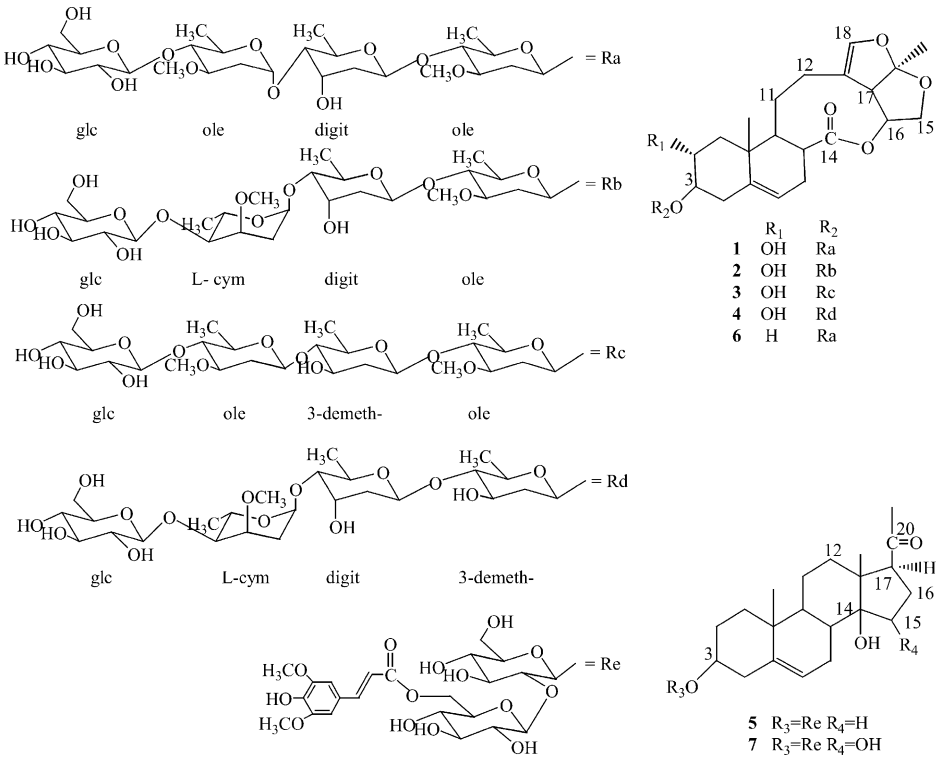

Fig. 1. The structures of compounds **1**, **2**, **3**, **4**, and **5**.

and four anomeric proton signals at δ 5.14 (1H, d, 8.3 Hz), δ 4.92 (1H, d, J = 8.1 Hz), δ 4.90 (1H, d, J = 7.6 Hz), and δ 4.81 (1H, d, 8.3 Hz), indicating the presence of four sugars with β-linkage. Partial hydrolysis of **3** with 5% aqueous HCl yielded glucose and oleandrose, which were identified by TLC comparisons with authentic samples. The authentic sample of another sugar could not be found, but this sugar could be determined by comparison of its NMR spectral data (Table 1) with those in the literature [6]. In the ^{13}C NMR spectrum, there was a characteristic carbon at δ 88.1 (C-4″) of this deoxysugar; the peak at δ 3.22 (dd, 1H, 8 Hz, 8 Hz) in the ^{1}H NMR spectrum was deduced to be linked to the carbon at δ 88.1 from the HMQC experiment. At the same time, the other carbons δ 100.3 (C-1″), δ 39.5 (C-2″), δ 69.9 (C-3″), δ 71.0 (C-5″), and δ 18.6 (C-6″) could be assigned to this sugar, therefore, this deoxysugar was deduced to be β-D-3-demethyl-2-deoxygen-thevetopyranoside by comparison with data in the literature [6]. The NMR data of the other sugars corresponded to **1** except that the β-D-digitoxose and α-D-oleandrose were replaced. Thus, the structure of **3** was determined to be glaucogenin A 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-oleand-ropyranosyl-(1 → 4)-β-D-3-demethyl-2-deoxythevetopyranosyl-(1 → 4)-β-D-oleandropyranoside (Fig. 1).

3.4. Komaroside G (*4*)

The molecular formula of **4** was determined to be $C_{46}H_{70}O_{20}$ (941.4348 [M − H], calculated: 941.4382) by its HRFAB-MS data. The structure of **4** corresponded to one in which the inner β-D-oleandrose of **2** was replaced with β-D-3-demethyl-2-deoxy-thevetose. This was determined by comparison of the spectral data of **4** with those of compound **2**. The NMR data were assigned on the basis of HMQC, HMBC, and HMQC-TOCSY experiments, and the proton at δ 3.28 (dd, 8.6 Hz, 9 Hz) was linked to the carbon at δ 88.1. Compound **4** gave digitoxose, cymarose, and glucose on hydrolysis. Thus, the structure of **4** was concluded to be glaucogenin A 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopy-ranosyl-(1 → 4)-β-D-3-demethyl-2-deoxy-thevetopyranoside (Fig. 1).

3.5. Komaroside H (*5*)

The molecular formula of **5** was determined to be $C_{44}H_{62}O_{17}$ (861.3904 [M − H], calculated: 861.3908) by its HRFAB-MS data. The ^{1}H NMR spectrum of **5** in C_5D_5N showed three secondary methyl and two methoxyl methyl peaks and two anomeric proton signals at δ 5.29 (1H, d, 7.6 Hz) and δ 5.08 (1H, d, 7.6 Hz). This indicated the presence of two sugars with β-linkage. In the ^{13}C NMR spectrum of **5** in C_5D_5N (Table 1), the data of the aglycone moiety were similar to those of 3β, 14β-dihydroxy-pregn-5-en-20-one [7] except that the glycosidation shifts were observed at C-2 (−2.3 ppm), C-3 (+7.9 ppm), and C-4 (−4.1 ppm) in the aglycone moiety, so the sugar was linked to the C-3 hydroxyl group of the aglycone. And the data of the sugar moiety were the same as those of hancoside A [3]. Therefore, the structure of **5** was proposed to be 3β, 14β-dihydroxy-pregn-5-en-20-one 3-*O*-β-D-(6-sinapoyl)-glucopyranosyl-(1 → 2)-β-D-gluco-pyranoside (Fig. 1). This conclusion was further confirmed by the ^{2}D spectra.

From the plants of the family Asclepiadaceae, it is rare to isolate phenanthroindolizidine alkaloids and C_{21} steroidal glycosides with normal and aberrant pregnane skeletons at the same time. The chemotaxonomical study on the genus *Cynanchum* and its allied genera has already provided some important information. For instance, the phenanthroindolizidine alkaloids were present in *Tylophora* [8–10] and *Vincetoxicum* [11,12], the C_{21} steroidal glycosides with a normal pregnane skeleton were present in the genus *Cynanchum* [13–17], and the C_{21} steroidal glycosides with an aberrant pregnane skeleton were found in both *Tylophora* [18] and *Vincetoxicum* [2,19–23]. Therefore, the presence of phenanthroindolizidine alkaloids and C_{21} steroidal glycosides with normal and aberrant pregnane skeletons simultaneously in *C. komarovii* was of taxonomic interest and indicated that this species could potentially be of intermediate taxa in *Asclepiadaceous* plants.This is consistent with the taxonomic treatment proposed by Qiu et al. [24] and Li et al. [25], which separated *Vincetoxicum* from *Cynanchum* on the basis of evidence from the study of steroidal aglycones of glycosides, and also is consistent with the analysis by Liede [26]. Further investigation will be required to explore this theory in depth.

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