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Lupane-triterpene glycosides from leaves of *Acanthopanax koreanum*

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Abstract

Two new lupane-triterpene glycosides named acankoreosides C and D, were isolated from the leaves of *Acanthopanax koreanum*. Based on spectroscopic data, the chemical structures were determined as 3-*O*-*β*-D-glucopyranosyl 3α,11α-dihydroxylup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester and 3α,11α-dihydroxylup-23-al-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester, respectively.

Keywords: *Acanthopanax koreanum*; Araliaceae; Leaf; Lupane-triterpene glycoside; Acankoreoside C and D

1. Introduction

In previous papers (Yook, Kim, Hahn, Nohara, & Chang, 1998; Chang, Yook & Nohara, 1998), we reported the isolation and structures of three lupane-triterpene glycosides, acantrifoside A, acankoreosides A and B, from the leaves of *Acanthopanax koreanum* NAKAI, which is indigenous to Korea and is a plant with tonic and sedative actions as well as ginseng-like activity (Yook, 1993). In a continuing study on this crude drug, we describe the isolation and structure determination of an additional two new lupane-triterpene glycosides, named acankoreosides C (**1**) and D (**2**), as minor components of the leaves of *A. koreanum*.

2. Results and discussion

Acankoreoside C (**1**), obtained as colorless needles, m.p. 247–249° (dil. MeOH), $[\alpha]_D$ −44.6° (EtOH), showed absorptions due to hydroxyl groups at 3427 cm^{-1} and an ester carbonyl group at 1748 cm^{-1} in the IR spectrum. The negative FAB-mass spectrum provided the molecular formula $C_{54}H_{88}O_{23}$ from a cluster of ion peaks at *m/z* 1103 $[M-H]^-$. The ^1H NMR spectrum (in pyridine-d_5) showed signals due to six tertiary methyl groups at *δ* 0.77, 0.89, 1.16, 1.20, 1.22 and 1.66, one secondary methyl group at *δ* 1.66 (3H, *d*, *J* = 6.1 Hz), four anomeric protons due to three hexosyl residues at *δ* 4.82 (1H, *d*, *J* = 7.9 Hz), *δ* 4.88 (1H, *d*, *J* = 7.9 Hz) and 6.24 (1H, *d*, *J* = 7.9 Hz) and one methylpentosyl residue at *δ* 5.75 (1H, *br s*) as listed in Table 1. Therefore, **1** was deduced to be a triterpene tetraglycoside.

The chemical shift of the hexosyl anomeric proton signal appeared at *δ* 6.24 and the IR absorption at 1748 cm^{-1} of **1** suggested that the sapogenol possessed an ester group, wherein a hexosyl moiety was attached. Therefore, **1** was saponified with 0.5 M aqueous KOH to give a prosapogenin (**3**), colorless needles, m.p. 264–266°, $[\alpha]_D$ −18.7°(EtOH). It exhibited absorptions due to hydroxyl groups at 3390 cm^{-1} and a carboxyl group at 1683 cm^{-1} in the IR spectrum. The negative FAB mass spectrum of **3** exhibited a molecular ion peak at *m/z* 633 ($C_{36}H_{57}O_9$) due to $[M-H]^-$. The ^1H NMR spectrum (pyridine-d_5) of **3** displayed signals due to six tertiary methyl groups, two olefinic protons,

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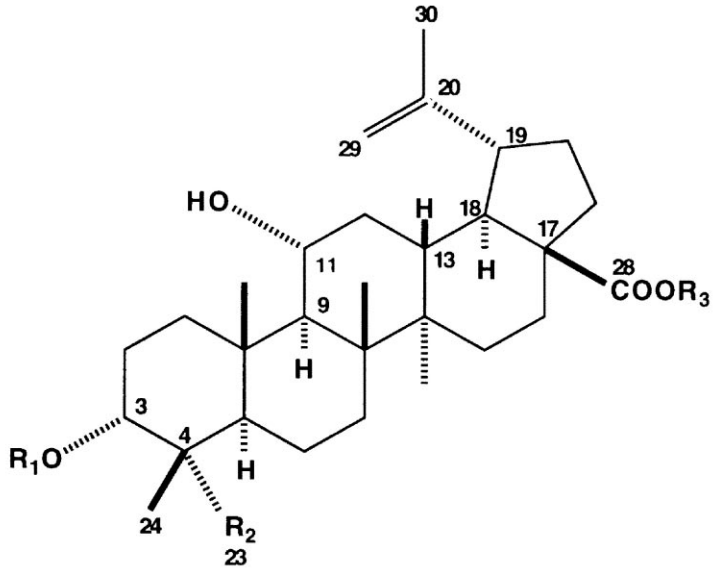


	R_1	R_2	R_3
1	-β-D-glc^p	CH_3	-β-D-glc^p(6→) β-D-glc^p(4→) α-L-rha^p
2	H	CHO	-β-D-glc^p(6→) β-D-glc^p(4→) α-L-rha^p
3	-β-D-glc^p	CH_3	H
4	H	CH_3	H
5	H	CHO	H

two oxygen bearing methine protons and one anomeric proton due to a hexosyl residue at δ 4.89 (1H, *d*, $J = 7.9$ Hz) (Table 1). The carbon signals of **3** observed on the ^{13}C-NMR spectrum (Table 2) suggested the presence of one ester carboxyl group, one di-substituted double bond, two oxygen-bearing methine carbons, five methine carbons, nine methylene carbons and six methyl carbons. Acid hydrolysis of **3** with 2 N HCl gave a sugar and a sapogenol (**4**), colorless needles, m.p. 228–230°, $[\alpha]_D$ +0.6° (EtOH), the latter of which was identical with 3α,11α-dihydroxylup-20(29)-en-28-oic acid (Yook *et al.*, 1998; Ty *et al.*, 1984). The sugar fraction was converted into the trimethylsilyl ether of the corresponding methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylate and analyzed by GLC to reveal that it was composed of D-glucose (Hara, Okabe & Mihashi, 1987). From the above facts, and the coupling constant of the anomeric proton, **3** was found to be composed of a β-D-glucopyranosyl moiety. Measurements of ^{1}H–^{1}H and ^{1}H–^{13}C 2D NMR spectra enabled the respective signals to be assigned. Furthermore, heteronuclear multiple bond correlation (HMBC) from the glucose H-1 at δ 4.89 (1H, *d*, $J = 7.9$ Hz) to C-3 at δ 81.5 (*d*) of the aglycone was observed. This evidence suggested the location of the sugar linkage of **3**. Consequently, the structure of **3** was determined as 3-*O*-β-D-glucopyranosyl 3α,11α-dihydroxylup-20(29)-en-28-oic acid.

On the other hand, acid hydrolysis of **1** with 2 N HCl gave a mixture of sugars and a sapogenol, which was identical with **4**. The sugar mixture was also analyzed by GLC which revealed that it was composed of D-glucose and L-rhamnose. From the above facts and the coupling constants of anomeric protons, **1** was found to be composed of β-D-glucopyranosyl and α-L-rhamnopyranosyl moieties. Measurements of ^{1}H–^{1}H and ^{1}H–^{13}C 2D-NMR spectra enabled the respective signals to be assigned. Furthermore, the HMBC from the glucose H-1 at δ 4.82 (1H, *d*, $J = 7.9$ Hz) to C-3 at δ 81.3 (*d*) of the aglycone, from the glucose inner H-1 at δ 6.24 (1H, *d*, $J = 7.9$ Hz) to C-28 at δ 174.8 (*s*) of the aglycone, from the outer glucose H-1′ at δ 4.88 (1H, *d*, $J = 7.9$ Hz) to the inner glucose C-6 at δ 69.4 (t), and from rhamnose H-1 at δ 5.75 (1H, *br s*) to the

Table 1
^{1}H NMR data for compounds **1**, **2**, **3** and **5** in pyridine-d_5 (δ in ppm, 500 MHz)*

	1	**3**	**2**	**5**
Aglycone				
3	3.67 (1H, *br s*)	3.72 (1H, *br s*)	4.01 (1H, *br s*)	4.02 (1H, *br s*)
9	1.71†	1.75†	1.97 (1H, *d*, 10.4)	2.00 (1H, *d*, 10.4)
11	4.17†	4.20†	4.30†	4.26 (1H, *ddd*, 5.5/10.7/10.7)
13	2.79 (1H, *m*)	2.89 (1H, *m*)	2.86 (1H, *m*)	2.94 (1H, *m*)
19	3.33 (1H, *m*)	3.53 (1H, *m*)	3.38 (1H, *m*)	3.52 (1H, *m*)
23	1.22 (3H, *s*)	1.27 (3H, *s*)	10.05 (1H, *s*)	10.07 (1H, *s*)
24	0.89 (3H, *s*)	0.89 (3H, *s*)	1.18 (3H, *s*)	1.18 (3H, *s*)
25	1.20 (3H, *s*)	1.21 (3H, *s*)	1.26 (3H, *s*)	1.26 (3H, *s*)
26	1.16 (3H, *s*)	1.11 (3H, *s*)	1.22 (3H, *s*)	1.14 (3H, *s*)
27	0.77 (3H, *s*)	0.83 (3H, *s*)	1.01 (3H, *s*)	1.06 (3H, *s*)
29	4.66 (1H, *br s*)	4.72 (1H, *br s*)	4.62 (1H, *br s*)	4.66 (1H, *br s*)
	4.80 (1H, *br s*)	4.90 (1H, *br s*)	4.80 (1H, *br s*)	4.88 (1H, *br s*)
30	1.66 (3H, *s*)	1.74 (3H, *s*)	1.65 (3H, *s*)	1.71 (3H, *s*)
C-28 *O*-inner glc				
1	6.24 (1H, *d*, 7.9)		6.32 (1H, *d*, 8.5)	
2	4.02†		4.10†	
3	4.15†		4.22†	
4	4.24†		4.31†	
5	4.06†		4.11†	
6	4.27†		4.27†	
	4.64†		4.68 (1H, *d*, 11.0)	
glc′(1 → 6)glc				
1′	4.88 (1H, *d*, 7.9)		4.94 (1H, *d*, 7.9)	
2′	3.98†		3.93†	
3′	4.09†		4.15†	
4′	4.35†		4.39 (1H, *t*, 9.5)	
5′	3.61†		3.65 (1H, *d*, 9.8)	
6′	4.04†		4.10†	
	4.18†		4.22†	
rha(1 → 4)glc′				
1	5.75 (1H, *br s*)		5.83 (1H, *br s*)	
2	4.62 (1H, *br s*)		4.67 (1H, *br s*)	
3	4.43†		4.53 (1H, *d*, 9.7)	
4	4.27†		4.34†	
5	4.90†		4.95†	
6	1.66†		1.70 (3H, *d*, 6.1)	
C-3 *O*-glc				
1	4.82 (1H, *d*, 7.9)	4.89 (1H, *d*, 7.9)		
2	3.87†	4.08 (1H, *t*, 9.2)		
3	4.19†	4.23†		
4	4.17†	4.21†		
5	3.88†	3.95†		
6	4.33†	4.40 (1H, *dd*, 11.6/5.5)		
	4.51†	4.57 (1H, *dd*, 11.6/3.1)		

All assignments were conformed by ^{1}H–^{1}H COSY, HMQC and HMBC spectra.*J values (in Hz) in parentheses.†Owing to overlapping with other signal(s), their multiplicities and J values were obscure.

outer glucose C-4′ at δ 78.2 (*d*) were observed. This evidence suggested the sequence of sugar linkage of **1**. Moreover, the carbon signals due to this sugar moiety at C-28 were superimposable on those of acantrifoside A, acankoreoside A and B (Yook *et al.*, 1998; Chang *et al.*, 1998). Consequently, **1** was a bidesmoside and the structure of it was determined as 3-*O*-β-D-glucopyranosyl 3α,11α-dihydroxylup-20(29)-en-28-oic acid

Table 2
^{13}C NMR data for compounds **1**, **2**, **3** and **5** in pyridine-d_5 (δ in ppm, 500 MHz)*

	1	3	2	5
Aglycone				
1	36.1 *t*	36.2 *t*	34.9 *t*	35.4 *t*
2	21.8 *t*	21.9 *t*	26.8 *t*	27.2 *t*
3	81.3 *d*	81.5 *d*	72.7 *d*	73.1 *d*
4	37.8 *s*	37.9 *s*	52.6 *s*	53.0 *s*
5	50.5 *d*	50.6 *d*	43.9 *d*	44.3 *d*
6	18.4 *t*	18.4 *t*	21.0 *t*	21.4 *t*
7	35.4 *t*	35.7 *t*	35.2 *t*	35.6 *t*
8	42.6 *s*	42.7 *s*	42.4 *s*	42.9 *s*
9	55.8 *d*	55.9 *d*	55.6 *d*	56.0 *d*
10	39.6 *s*	39.7 *s*	38.7 *s*	39.0 *s*
11	69.7 *d*	69.9 *d*	69.4 *d*	69.8 *d*
12	38.1 *t*	38.3 *t*	37.8 *t*	38.3 *t*
13	37.3 *d*	37.7 *d*	37.0 *d*	37.6 *d*
14	42.9 *s*	42.9 *s*	43.0 *s*	43.3 *s*
15	30.0 *t*	30.2 *t*	29.6 *t*	30.1 *t*
16	32.2 *t*	32.9 *t*	31.9 *t*	32.9 *t*
17	56.8 *s*	56.6 *s*	56.6 *s*	56.6 *s*
18	49.4 *d*	49.4 *d*	49.1 *d*	49.4 *d*
19	47.1 *d*	47.5 *d*	46.8 *d*	47.5 *d*
20	150.4 *s*	150.9 *s*	150.1 *s*	150.8 *s*
21	30.8 *t*	31.3 *t*	30.6 *t*	31.3 *t*
22	36.7 *t*	37.4 *t*	36.4 *t*	37.4 *t*
23	29.8 *q*	29.9 *q*	209.7 *d*	210.0 *d*
24	23.0 *q*	23.0 *q*	14.6 *q*	15.0 *q*
25	16.8 *q*	16.8 *q*	16.5 *q*	16.8 *q*
26	17.6 *q*	17.6 *q*	17.4 *q*	17.8 *q*
27	14.7 *q*	14.8 *q*	14.4 *q*	14.8 *q*
28	174.8 *s*	178.9 *s*	174.6 *s*	178.8 *s*
29	110.1 *t*	110.1 *t*	109.9 *t*	110.1 *t*
30	19.6 *q*	19.6 *q*	19.2 *q*	19.5 *q*
C-28 *O*-inner glc				
1	95.2 *d*		94.9 *d*	
2	73.9 *d*		73.7 *d*	
3	78.6 *d*		78.3 *d*	
4	70.8 *d*		70.5 *d*	
5	77.9 *d*		77.7 *d*	
6	69.4 *t*		69.1 *t*	
glc′(1 → 6)glc				
1′	105.0 *d*		104.7 *d*	
2′	75.0 *d*		74.9 *d*	
3′	76.3 *d*		76.1 *d*	
4′	78.2 *d*		77.9 *d*	
5′	77.0 *d*		76.8 *d*	
6′	61.3 *t*		61.0 *t*	
rha(1 → 4)glc′				
1	102.6 *d*		102.4 *d*	
2	72.4 *d*		72.2 *d*	
3	72.6 *d*		72.4 *d*	
4	73.9 *d*		73.6 *d*	
5	70.2 *d*		70.0 *d*	
6	18.4 *q*		18.2 *q*	
C-3 *O*-glc				
1	101.8 *d*	101.9 *d*		
2	75.1 *d*	75.1 *d*		
3	78.8 *d*	78.9 *d*		
4	72.1 *d*	72.2 *d*		
5	78.1 *d*	78.2 *d*		
6	63.1 *t*	63.2 *t*		

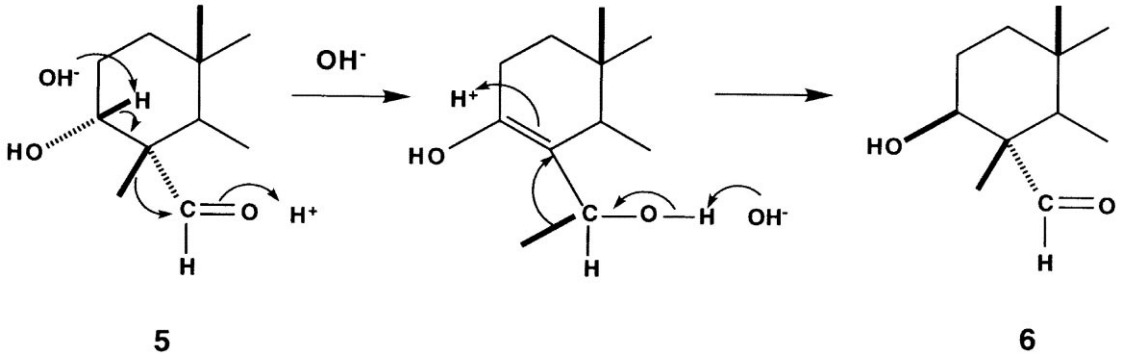
* Multiplicities were deduced from a DEPT experiment.

28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester.

Acankoreoside D (**2**) was obtained as a white powder, m.p. 222–225° (dil. MeOH), $[\alpha]_D$ −40.8° (EtOH). The negative FAB-MS exhibited a molecular ion peak due to $[M-H]^-$ at m/z 955 ($C_{48}H_{75}O_{19}$). The ^1H NMR spectrum (in pyridine-d_5) showed signals due to five tertiary methyl groups, one secondary methyl group, three anomeric protons due to two hexosyl residues and one methylpentosyl residue (see Table 1). Therefore, taking into consideration the molecular formula, **2** was also deduced to be a triterpene glycoside. The chemical shift at δ 6.32 assignable to a hexosyl anomeric proton suggested the occurrence of an ester glycosyl linkage. Therefore, **2** was hydrolyzed with a mixture of hesperidinase and cellulase to give an aglycone (**5**), m.p. 207–209°. The positive FAB-mass spectrum of **5** exhibited a molecular ion peak due to $[M + H]^+$ at m/z 469 ($C_{30}H_{46}O_5$ + H). The ^1H NMR spectrum (pyridine-d_5) of **5** displayed signals due to five tertiary methyl groups, two olefinic protons, one aldehyde proton and two oxygen bearing methine protons as listed in Table 1. The carbon signals observed on the ^{13}C NMR spectrum (Table 2) suggested the presence of one ester carboxyl group, one di-substituted double bond, an aldehyde carbonyl group, two oxygen bearing methine carbons, five methine carbons, nine methylene carbons and five methyl carbons. Based on the above data, **5** was identified as the known 3α,11α-dihydroxy-lup-20(29)-en-23-al-28-oic acid (Ty, Lischewski, Phiet, Preiss, Nguyen & Adam, 1985).

On the other hand, alkaline hydrolysis of **2** gave a sapogenol (**6**) different from **4**, which showed a double doublet signal (J = 4.9 11.0 Hz) at δ 4.15 assigned to H-3α. Therefore, the structure of **6** was deduced to be 3β,11α-dihydroxylup-20(29)-en-23-al-28-oic acid. Production of **6** was assumed to occur by action of alkali (Scheme 1).

The ^1H NMR and ^{13}C NMR signals supported structure **2**. The signals due to the sugar moiety in **2** were superimposable on those of **1**, suggesting that the sugar linkage of **2** is the same with that of **1**. Consequently, the structure of **2** was determined to be 3α,11α-dihydroxylup-23-al-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester.



Scheme 1.

3. Experimental

3.1. General

M.p.'s (uncorr.) were measured using Boetius micro-melting point apparatus. Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter ($l = 0.5$). IR spectra were obtained with a Hitachi 270-30 type spectrophotometer. FAB-MS were obtained in a glycerol matrix in the positive ion mode using a JEOL JMS-DX300 and JMS-DX 303HF, and EI-MS on a JEOL JMS-01SG and JMS-DX303HF. NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer and chemical shifts were referenced to TMS. GLC was performed on a HP5890A gas chromatograph with flame ionization detector. CC was carried out with silica gel 60 (0.040–0.063 mm, Merck). TLC was performed on a precoated silica gel $60F_{254}$ (Merck) and RP-18 F_{254S} (Merck).

3.2. Plant material

The leaves of *A. koreanum* were harvested at Kwang-nung, Kyung-gi province of Korea on September 1996, and identified by Prof Chang-Soo Yook.

3.3. Isolation

The dried leaves (470 g) of *A. koreanum* were extracted with hot MeOH repeatedly to give an extract (105 g), which was partitioned between *n*-hexane and 40% MeOH. The aq. layer was evaporated to dryness *in vacuo* and chromatographed on Diaion HP-20P (Mitsubishi Chem. Ind. Co., Japan) by eluting with H_2O, 30, 50, 70 and 90% aq. MeOH, successively. A saponin mixture eluted with 70 and 90% MeOH was subsequently chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (8:2:0.2 → 7:3:0.5) to give 9 fractions. Fractions No. 7, 8 and 9 were evaporated to dryness *in vacuo*, dissolved in H_2O and passed through Amberlite IR-120. The eluents were evaporated to dryness *in vacuo* and recrystallized from MeOH–H_2O to yield **1** (yield, 0.045%). On the other hand fraction No. 6 was chromatographed on a reverse phase column, Chromatorex ODS (30–50 μm, Fuji Silysia Chem. Ind. Co., Japan), by gradiently eluting with from 50% MeOH to 90% MeOH to give **2** (yield, 0.028%).

3.4. Acankoreoside C (**1**)

A white powder, m.p. 247–249°C (from dil. MeOH); $[\alpha]_D^{26}$ −44.6° (*c* 0.36 in EtOH). IR ν_{max}^{KBr} cm^{-1}: 3427 (OH), 2928 (aliphatic CH), 1748 (carbonyl), 1640 (C=C), 1066 (ether). negative FAB-MS *m/z*: 1104 [M]− , 957 [M–methylpentose]$^-$, 634 [M–methylpentose-2×hexose]$^-$. ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2.

3.5. Alkaline hydrolysis of **1**

Compound **1** (113 mg) was hydrolyzed with 4 ml of 0.5 M aq. KOH for 1 h at 70°C. The reaction mixture was neutralized with 2 N HCl in H_2O, passed through MCI-gel CHP20P column, washed with H_2O and then eluted with MeOH. The eluate was evaporated *in vacuo* and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH–H_2O = 9:1:0.1). The obtained prosapogenol fraction was recrystallized from MeOH–H_2O to give **3** (52 mg). Compound was **3** a white powder, m.p. 264–266° (dil. MeOH), $[\alpha]_D^{26}$ −18.7° (*c* 0.38 in EtOH). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2958 (aliphatic CH), 1683 (carbonyl), 1641 (C=C). negative FAB-MS *m/z*: 633 [M–H]$^-$, 472 [M–hexose]$^-$. ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2.

3.6. Acid hydrolysis of **3**

Compound **3** (41 mg) was hydrolyzed with 5 ml of 2 N HCl in H_2O for 4 h at 80°. The reaction mixture was neutralized with 2 M NaOH in H_2O and extracted with $CHCl_3$. The organic layer was evaporated to give a residue, which was purified using silica gel CC (*n*-hexane–Me_2CO, 2:1). The obtained aglycone fraction was recrystallized from MeOH to give **4** (28 mg). Compound **4**: Colorless needles, m.p. 228–230°C, $[\alpha]_D$ +0.6° (*c* 0.02 in EtOH). The identification of **4** was established by direct comparison (TLC, EI-MS, ^{1}H NMR and ^{13}C NMR) with an authentic sample (Yook, 1998). On the other hand, the aq. layer was concentrated to dryness *in vacuo*. The remaining residue was dissolved in dry pyridine and mixed with L-cysteine methyl ester hydrochloride. The reaction mixture was heated for 2 h at 60° and concentrated to dryness under N_2 gas. The residue was added with trimethylsilylimidazole and heated for 1 h at 60°. The reaction mixture was concentrated to dryness under N_2 gas. The residue was extracted with *n*-hexane and H_2O and the organic layer was analyzed by GLC; column: OV-17 (0.32 mm×30 m), detector: FID, column temp.: 230°, detector temp.: 270°, injector temp.: 270°, carrier gas: He (2.2 kg/cm^2). One peak was observed at R_t (min); 7′12″ (D-Glc). The standard monosaccharides were subjected to the same reaction and GLC analysis under the same condition.

In the same manner as described as above, **1** was acid-hydrolyzed to provide a sapogenol identical with **4** and sugar mixture, which was converted to the corresponding trimethylsilyl ethers of methyl 2-polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates to be checked by GLC. D-glc: 7′25″, L-rha: 5′35″ under the same conditions as above.

3.7. Acankoreoside D (**2**)

A white powder, m.p. 222–225° (from dil. MeOH), $[\alpha]_D^{26}$ −40.8° (*c* 0.37 in EtOH). IR ν_{max}^{KBr} cm^{-1}: 3403 (OH), 2937 (aliphatic CH), 1724 (ester carbonyl), 1643 (C=C). Negative FAB-MS *m/z*: 956 $[M]^-$, 955 $[M-H]^-$, 810 $[M + H–methylpentose]^-$, 648 $[M + H–methylpentose–hexose]^-$. ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2.

3.8. Enzymatic hydrolysis of **2**

Compound **2** (20 mg) was hydrolyzed by incubation with a mixture of hesperidinase (Sigma Co., H-8137) and cellulase (from *Asperigillus niger*, Sigma Co., 0.45 units/mg) (2:1) for 7 days at 37°C. The obtained aglycone fraction was recrystallized from MeOH to give **5** (16 mg). Compound **5**: Colorless needles, m.p. 207–209°C (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 2931 (aliphatic CH), 1720 (carbonyl), 1643 (C=C). Positive FAB-MS *m/z*: 469 $[M + H]^+$, 451 $[M + H–H_2O]^+$. ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2.

3.9. Alkaline hydrolysis of **2**

Compound 2 (32 mg) was hydrolyzed with 2 ml of 0.5 M aq. KOH for 1 h at 70°. The reaction mixture was neutralized with 2 N HCl in H_2O, passed through MCI-gel CHP20P column, washed with H_2O and then eluted with MeOH. The eluate was evaporated *in vacuo* and the residue was purified by silica gel CC (solv. $CHCl_3$–MeOH–H_2O = 9:1:0.1). The obtained prosapogenol fraction was recrystallized from dil. MeOH to give **6** (4 mg). Compound **6**: A white powder. ^{1}H NMR (pyridine-d_5) δ: 1.09, 1.14, 1.23, 1.42, 1.73 (each 3H, *s*, H_3-26, -27, -25, -24 and -30), 4.15 (1H, *dd*, *J* = 4.9, 11.0 Hz, H-3α), 4.26 (1H, *m*, H-11β), 4.66, 4.88 (each 1H, *br s*, H_2-29). ^{13}C NMR (pyridine-d_5) δ: 41.6, 27.8, 71.3, 57.2, 48.4, 21.6, 35.2, 42.8, 56.2, 38.4, 69.6, 38.3, 37.5, 43.0, 30.2, 32.9, 56.6, 49.4, 47.5, 150.9, 31.3, 37.4, 207.3, 9.6, 16.9, 17.6, 14.8, 178.8, 110.1, 19.6 (C-1-30).

3.10. Acid hydrolysis of **2**

Compound **2** (11 mg) was treated with acid in the same manner as **1** to give sugar mixture which was derived into the cysteine derivatives and detected as L-rhamnosyl and D-glucosyl derivatives.

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