Two New Lupane-Triterpene Glycosides from Leaves of *Acanthopanax koreanum*

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Two new lupane-triterpene glycosides, acankoreoside A (1) and B (2), were isolated from the leaves of *Acanthopanax koreanum* NAKAI (Araliaceae). Based on spectroscopic data, the chemical structures of 1 and 2 were determined as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and 3α,11α,23-trihydroxy-lup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl- (1→6)-β-D-glucopyranosyl ester, respectively.

Key words *Acanthopanax koreanum*; Araliaceae; leaf; lupane-triterpene glycoside; acankoreoside A and B

The roots and bark of *Acanthopanax* species (Alaliaceae) are used as a tonic and sedative as well as a drug with ginseng-like activity. *Acanthopanax koreanum* NAKAI is indigenous to Korea.[1)]

Although lignan and diterpene derivatives have been reported in the stem and root bark of *A. koreanum*,[2–6)] few constituents have so far been isolated or characterized from the leaves of this plant.

In a previous paper,[7)] we described the isolation and characterization of a lupane-triterpene glycoside (acantrifoside A) from the leaves of *Acanthopanax koreanum* and *A. trifoliatus*. As part of a continuing study on this crude drug, we report here the isolation and structural determination of two new lupane-triterpene glycosides, named acankoreoside A (**1**) and B (**2**), as major components of the leaves of *A. koreanum*.

Acankoreoside A (**1**), obtained as a white powder, mp 225—228 °C (dil. MeOH), $[\alpha]_D$ −41.2° (EtOH), showed absorption bands due to hydroxyl groups at 3417 cm^{-1} and an ester carbonyl group at 1724 cm^{-1} in the IR spectrum. The HR-FAB-MS provided a formula of $C_{48}H_{76}O_{19}$, with a cluster ion peak at 979.4872 $[M+Na]^+$ (Calcd for $C_{48}H_{76}O_{19}Na$: 979.4878). The ^{1}H-NMR spectrum (in pyridine-d_5) showed signals due to five tertiary methyl groups at δ 0.87, 0.95, 1.20, 1.46 and 1.70, one secondary methyl group at δ 1.69 (3H, d, J= 6.1 Hz), three anomeric protons due to two hexosyl residues at δ 4.95 (1H, d, J=7.9 Hz) and 6.34 (1H, d, J= 7.9 Hz) and one methylpentosyl residue at 5.84 (1H, br s) as listed in Table 1.

Therefore, **1** was deduced to be a triterpene glycoside.

The chemical shift of the hexosyl anomeric proton signal appeared at δ 6.34 and the IR absorption at 1724 cm^{-1} of **1** suggested that the sapogenol possessed an ester group, with a hexosyl moiety attached. Therefore, **1** was saponified in 0.5 M aqueous KOH to give an aglycone (**3**), mp 259—262 °C, $[\alpha]_D$ −3.1° (EtOH). It exhibited absorption bands due to a hydroxyl group at 3390 cm^{-1} and a carboxyl group at 1702 cm^{-1} in the IR spectrum. The positive FAB-MS of **3** exhibited a molecular ion peak due to $[M+H]^+$ at m/z 487 ($C_{30}H_{46}O_5+H$). The ^{1}H-NMR spectrum (pyridine-d_5) of **3** displayed signals due to five tertiary methyl groups, two olefinic protons and one oxygen-bearing methine proton (Table 1). The carbon signals observed in the ^{13}C-NMR spectrum (Table 2) suggested the presence of two carboxyl groups, one monosubstituted double bond, one oxygen-bearing methine carbon, five methine carbons, ten methylene carbons and five methyl carbons.

Based on the above data, **3** was identified as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid.[8)] On the other hand, acid hydrolysis of **1** with 2 N HCl gave a mixture of sugars and a sapogenol, which was identical with **3**. The sugar mixture was derivatized to give the trimethylsilyl ethers of the corresponding methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates and analyzed by gas liquid chromatography (GLC) to show that it was composed of glucose and 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 two dimensional (2D) NMR spectra enabled the respective signals to be assigned.

Furthermore, heteronuclear multiple bond correlations (HMBC) from inner glc H-1 at δ 6.34 (1H, d, J=7.9 Hz) to C-28 at δ 174.9 (s) of the aglycone, from outer glc H-1′ at δ 4.95 (1H, d, J=7.9 Hz) to inner glc C-6 at δ 69.4 (t), and from rha H-1 at δ 5.84 (1H, br s) to outer glc C-4′ at δ 78.2 (d) were observed. This evidence suggested the sequence of the sugar linkages of **1**. Moreover, the carbon signals due to this sugar moiety were superimposable on those of chiisanoside isolated from *Acanthopanax chiisanensis* and *A. divaricatus* by Tanaka *et al.*[9–11)]

Consequently, the structure of **1** was determined as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Acankoreoside B (**2**), obtained as a white powder, mp 220—223 °C (dil. MeOH), $[\alpha]_D$ −37.5° (EtOH). The negative FAB-MS exhibited a molecular ion peak due to $[M-H]^-$ at m/z 957 ($C_{48}H_{78}O_{19}-H$). The ^{1}H-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

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Table 1. ^{1}H-NMR Data for Compound **1**—**4** in Pyridine-d_5 (δ in ppm, 500 MHz)[a]

	1	3	2	4
Aglycone				
3	4.28 (1H, brs)	4.29 (1H, brs)	3.92 (1H, brs)	3.93 (1H, brs)
9	1.66 (1H, d, 11.6)	1.70 (1H, d, 11.6)	1.92 (1H, d, 11.0)	1.95 (1H, d, 10.4)
11	1.23[b]	1.59[b]	4.33[b]	4.26 (1H, ddd,
	1.47[b]	1.86[b]		5.5/10.7/10.7)
13	2.67 (1H, m)	2.74 (1H, m)	2.98 (1H, m)	2.94 (1H, m)
19	3.38 (1H, m)	3.54 (1H, m)	3.38[b]	3.52 (1H, m)
23	—	—	3.67 (1H, d,	3.69 (1H, d,
			11.0)	11.0)
			3.88 (1H, d,	3.89 (1H, d,
			11.0)	11.0)
24	1.46 (3H, s)	1.46 (3H, s)	0.83 (3H, s)	1.83 (3H, s)
25	0.95 (3H, s)	0.94 (3H, s)	1.29 (3H, s)	1.28 (3H, s)
26	1.20 (3H, s)	1.12 (3H, s)	1.25 (3H, s)	1.16 (3H, s)
27	0.87 (3H, s)	0.94 (3H, s)	1.00 (3H, s)	1.05 (3H, s)
29	4.72 (1H, brs)	4.77 (1H, brs)	4.61 (1H, brs)	4.65 (1H, brs)
	4.85 (1H, brs)	4.94 (1H, brs)	4.80 (1H, brs)	4.87 (1H, brs)
30	1.70 (3H, s)	1.78 (3H, s)	1.64 (3H, s)	1.70 (3H, s)
C-28 *O*-inner glc				
1	6.34 (1H, d, 7.9)		6.32 (1H, d, 7.9)	
2	4.08[b]		4.08 (1H, t, 9.2)	
3	4.21 (1H, t, 11.0)		4.21 (1H, t, 9.2)	
4	4.30[b]		4.30[b]	
5	4.09[b]		4.11[b]	
6	4.28[b]		4.28[b]	
	4.67 (1H, d, 9.8)		4.67 (1H, d,	
			11.0)	
glc′ (1→6)glc				
1′	4.95 (1H, d, 7.9)		4.94 (1H, d, 7.9)	
2′	3.93 (1H, t, 9.2)		3.94[b]	
3′	4.13[b]		4.15[b]	
4′	4.39 (1H, t, 9.5)		4.39 (1H, t, 9.5)	
5′	3.65 (1H, d, 9.2)		3.67 (1H, d,	
			11.0)	
6′	4.07 (1H, d,		4.11[b]	
	10.4)			
	4.19[b]		4.19[b]	
rha(1→4)glc′				
1	5.84 (1H, brs)		5.83 (1H, brs)	
2	4.66 (1H, brs)		4.66 (1H, brs)	
3	4.54 (1H, d, 9.2)		4.53 (1H, dd,	
			9.2/3.1)	
4	4.34[b]		4.32[b]	
5	4.96[b]		4.93[b]	
6	1.69 (3H, d, 6.1)		1.70 (3H, d, 6.1)	

All assignments were confirmed by ^{1}H–^{1}H chemical shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bonds correlation (HMBC) spectra. *a*) *J* values (in Hz) in parentheses. *b*) Overlapped with other signal(s) and its multiplicity and *J* values were both obscure.

Table 2. ^{13}C-NMR Data for Compound **1**—**4** in Pyridine-d_5 (δ in ppm, 500 MHz)[a]

	1	3	2	4
Aglycone				
1	32.9 t	32.6 t	35.9 t	35.9 t
2	26.2 t	25.9 t	27.1 t	27.1 t
3	73.0 d	72.7 d	75.7 d	75.9 d
4	52.0 s	51.7 s	41.1 s	41.2 s
5	44.9 d	44.8 d	43.8 d	43.9 d
6	21.8 t	21.0 t	18.3 t	18.3 t
7	34.5 t	34.5 t	35.4 t	35.6 t
8	41.8 s	41.5 s	42.7 s	42.8 s
9	51.0 d	50.8 d	55.6 d	56.2 d
10	37.4 s	37.2 s	39.6 s	39.6 s
11	20.9 t	21.5 t	69.8 d	69.9 d
12	26.0 t	25.8 t	38.3 t	38.4 t
13	38.3 d	38.4 d	37.4 d	37.6 d
14	42.9 s	42.7 s	42.9 s	42.8 s
15	30.1 t	30.0 t	30.0 t	30.1 t
16	31.9 t	32.8 t	32.2 t	32.8 t
17	57.0 s	56.4 s	56.9 s	56.5 s
18	49.7 d	49.5 d	49.4 d	49.4 d
19	47.4 d	47.5 d	47.1 d	47.5 d
20	150.8 s	151.1 s	150.4 s	150.8 s
21	30.8 t	30.9 t	30.9 t	31.2 t
22	36.9 t	37.3 t	36.7 t	37.4 t
23	179.0 s	179.6 s	71.9 t	71.9 t
24	18.0 q	17.7 q	18.3 q	18.3 q
25	16.8 q	16.5 q	17.1 q	17.0 q
26	16.7 q	16.5 q	17.7 q	17.7 q
27	14.8 q	14.6 q	14.8 q	14.8 q
28	174.9 s	178.6 s	175.0 s	178.8 s
29	110.0 t	109.7 t	110.0 t	110.0 t
30	19.4 q	19.2 q	19.5 q	19.5 q
C-28 *O*-inner glc				
1	95.2 d		95.3 d	
2	74.0 d		74.0 d	
3	78.7 d		78.6 d	
4	70.8 d		70.8 d	
5	77.9 d		78.0 d	
6	69.4 t		69.4 t	
glc′(1→6)glc				
1′	105.1 d		105.0 d	
2′	75.3 d		75.2 d	
3′	76.4 d		76.4 d	
4′	78.2 d		78.2 d	
5′	77.1 d		77.1 d	
6′	61.3 t		61.3 t	
rha(1→4)glc′				
1	102.7 d		102.7 d	
2	72.5 d		72.5 d	
3	72.7 d		72.7 d	
4	73.9 d		73.9 d	
5	70.3 d		70.3 d	
6	18.5 q		18.5 q	

a) Multiplicities were deduced from a distortionless enhancement by polarization transfer (DEPT) experiment.

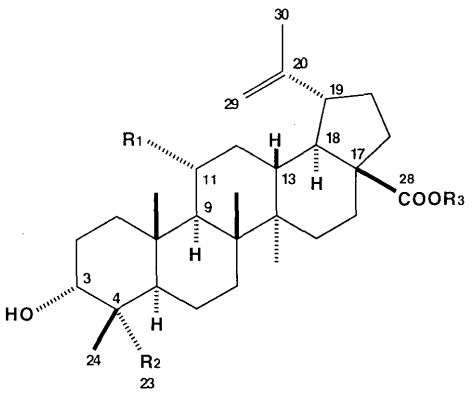
the presence of an ester glycosyl linkage. Therefore, **2** was saponified with aqueous 0.5 M KOH to give an aglycone (**4**), mp 191—196 °C, $[\alpha]_D$ −3.1° (EtOH).

The positive FAB-MS of **4** exhibited a molecula ion peak due to $[M+H]^+$ at *m/z* 489 ($C_{30}H_{48}O_5+H$). The ^{1}H-NMR spectrum (pyridine-d_5) of **4** displayed signals due to five tertiary methyl groups, two olefinic protons, two protons form a hydroxymethyl group and two oxygen-bearing methine protons as listed in Table 2. The carbon signals observed in the ^{13}C-NMR spectrum (Table 2) suggested the presence of one carboxyl group, one monosubstituted double bond, a hydroxymethyl group, two oxygen-bearing methine carbons, five methine carbons, nine methylene carbons and five methyl carbons.

Based on the above data, **4** was identified as the known 3α,11α,23-trihydroxy-lup-20(29)-en-28-oic acid.[12]

^{1}H- and ^{13}C-signals and HMBC correlations of the sugar moiety in **2** were almost identical with those of **1**, suggesting that the sequence of sugar linkages of **2** is the same as that of **1**.

Consequently, the structure of **2** was determined as 3α,11α,23-tri-hydroxy-lup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.



	R1	R2	R3
1	H	COOH	-β-D-glc^p(6→)β-D-glc^p(4→)α-L-rha^p
2	OH	CH_2OH	-β-D-glc^p(6→)β-D-glc^p(4→)α-L-rha^p
3	H	COOH	H
4	OH	CH_2OH	H

Experimental

Melting points (uncorrected) were measured using a 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 JEOL JMS-DX300 and JMS-DX 303HF instruments, and EI-MS on JEOL JMS-01SG and JMS-DX303HF instruments. NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer and chemical shifts were relative to tetramethylsilane (TMS). GLC was performed on a HP5890A gas chromatograph with flame-ionization detector. Column chromatography (CC) was carried out on Silica-gel 60 (0.040—0.063 mm, Merck). TLC was performed on precoated Silica-gel 60F_{254} (Merck) and RP-18 F_{254S} (Merck) plates.

Plant Material The leaves of *A. koreanum* were harvested at Kwang-nung, Kyung-gi Province, in Korea in September 1996.

Isolation The dried leaves of *A. koreanum* (470 g) were extracted with hot MeOH repeatedly to give an extract (105 g), which was partitioned between *n*-hexane and 40% MeOH. The aqueous layer was evaporated to dryness *in vacuo* and chromatographed on Diaion HP-20P (Mitsubishi Chem. Ind. Co., Ltd., Japan) by elution with H_2O, 30%, 50%, 70% and 90% aqueous MeOH successively. A saponin mixture eluted with 70% and 90% MeOH was subsequently chromatographed on Silica-gel using $CHCl_3$–MeOH–H_2O (8:2:0.2→7:3:0.5) to give nine fractions. Fraction Nos. 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, 0.18%). Fraction No. 6 was chromatographed on a reverse phase column, Chromatorex ODS (30—50 μm, Fuji Silysia Chem. Ind. Co., Ltd., Japan), using gradient elution from 50% MeOH to 90% MeOH to give **2** (yields, 0.13%).

Acankoreoside A (1) A white powder. mp 225—228 °C (from dil. MeOH); $[\alpha]_D^{27}$ −41.2° (c=0.39 in EtOH); *Anal.* Calcd for $C_{48}H_{76}O_{19}\cdot 3\,1/2H_2O$: C, 56.51; H, 8.20. Found: C, 56.53; H, 8.27. IR ν_{max}^{KBr} cm^{-1}: 3417 (OH), 2942 (aliphatic CH), 1724 (ester carbonyl), 1641 (C=C), 1066 (s, ether); positive HR FAB-MS *m/z*: 979.4872 $[M+Na]^+$ (Calcd for $C_{48}H_{76}O_{19}Na$: 979.4878); positive FAB-MS *m/z*: 980 $[M+Na+H]^+$; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Alkaline Hydrolysis of 1 Compound **1** (140 mg) was hydrolyzed with 10 ml 0.5 M aqueous KOH for 1 h at 70 °C. The reaction mixture was neutralized with 2 N HCl in H_2O, passed through a 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 (*n*-hexane : acetone = 1 : 1). The obtained aglycone fraction was recrystallized from MeOH to give **3** (40 mg). **3**: Colorless needles. mp 259—262 °C (EtOH); $[\alpha]_D^{26}$ −3.1° (c=0.36 in EtOH); IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2946 (aliphatic CH), 1702 (ester carbonyl), 1643 (C=C); positive FAB-MS *m/z*: 487 $[M+H]^+$; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Acid Hydrolysis of 1 Compound **1** (100 mg) was hydrolyzed with 4 ml 2 N HCl in H_2O for 4 h at 80 °C. The reaction mixture was neutralized with 2 N NaOH in H_2O and extracted with $CHCl_3$. The organic layer was evaporated to give a residue, which was purified using Silica-gel column chromatography (*n*-hexane : acetone = 3 : 1→2 : 1).

The obtained aglycone fraction was recrystallized from MeOH to give **3** (32 mg). On the other hand, the aqueous layer was concentrated to dryness *in vacuo*. The remaining residue was dissolved in dry pyridine and combined with L-cysteine methyl ester hydrochloride. The reaction mixture was then heated for 2 h at 60 °C and concentrated to dryness under a N_2 gas stream. The residue was combined with trimethylsilyl-imidazole and heated for 1 h at 60 °C. The reaction mixture was concentrated to dryness under a N_2 gas stream. 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 °C, detector temp.: 270 °C, injector temp.: 270 °C, carrier gas: He (2.2 kg/cm^2). Two peaks were observed at t_R (min): 4′87″ (L-Rha) and 7′12″ (D-Glc). The standard monosaccharides were subjected to the same reaction and GLC analysis under the same conditions.

Acankoreoside B (2) A white powder. mp 220—223 °C (from dil. MeOH); $[\alpha]_D^{27}$ −37.5° (c=0.37 in EtOH); IR ν_{max}^{KBr} cm^{-1}: 3403 (OH), 2939 (aliphatic CH), 1727 (ester carbonyl), 1641 (C=C); Negative FAB-MS *m/z*: 957 $[M-H]^-$; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Alkaline Hydrolysis of 2 Compound **2** (130 mg) was hydrolyzed with 6 ml 0.5 M KOH in MeOH for 1 h at 70 °C. The obtained aglycone fraction was recrystallized from MeOH to give **4** (46 mg). **4**: Colorless needles. mp 191—196 °C (EtOH); $[\alpha]_D^{29}$ +0.7° (c=0.34 in EtOH); IR ν_{max}^{KBr} cm^{-1}: 3404 (OH), 2939 (aliphatic CH), 1722 (ester carbonyl), 1641 (C=C); Positive FAB-MS *m/z*: 489 $[M+H]^+$, 453 $[M+H-2H_2O]^+$; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Acid Hydrolysis of 2 Compound **2** (80 mg) was treated with acid in the same manner as **1** to give an aglycone **4** (27 mg) and a sugar mixture which was derivatized to the corresponding cysteine derivatives and detected as L-rhamnosyl and D-glucosyl derivatives.

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