A New Lupane Glycoside from the Leaves of *Acanthopanax koreanum*

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A new lupane-type saponin, named acankoreoside E (1), was isolated from the methanol extract of the leaves of *Acanthopanax koreanum*, and its structure was established through chemical and spectroscopic analyses as (20*S*) 3α-hydroxy-30-oxolupan-23,28-dioic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Key words *Acanthopanax koreanum*; Araliaceae; lupane-type glycoside; acankoreoside E; (20*S*) lupan-30-al

Acanthopanax genus is a shrub indigenous to Northeast Asia. Its bark, known as Acanthopanis Cortex, is used to treat sinew and bone pains in traditional oriental medicine. Several studies on the triterpenoid constituents of *Acanthopanax koreanum* NAKAI, have been reported.[1—3] As a results of an in-going search for the phytochemical constituents in *Acanthopanax* genus,[4—9] we report on the isolation of a new lupane triterpene glycoside, acankoreoside E (**1**), (20*S*) 3α-hydroxy-30-oxolupan-23,28-dioic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester from the leaves of *Acanthopanax koreanum* and describe the determination of its structure.

The methanolic extract of *A. koreanum* leaves gave acankoreoside E (**1**) by the various chromatographic techniques. Acankoreoside E (**1**) was obtained as a white amorphous powder of molecular formula $C_{48}H_{76}O_{20}$, as determined by HR-FAB-MS (molecular ion peak at *m*/*z* 995.4828 due to $[M+Na]^+$). Its ^{13}C-NMR and DEPT spectra revealed 48 carbons signals, 30 of which were assigned to a triterpenoid sapogenol moiety, and 18 to three monosaccharide moieties. Relevant ^{1}H-NMR data showed the following; four tertiary methyl groups [δ 0.85, 0.96, 1.19 and 1.46 (each 3H, s)], two secondary methyl groups [δ 1.02 (3H, d, J=6.5 Hz) and 1.68 (3H, d, J=6.5 Hz)], and three anomeric protons [δ 4.92 (1H, d, J=7.5 Hz), 5.81 (1H, br s), and 6.29 (1H, d, J=8.5 Hz)]. Moreover, the ^{1}H-NMR spectrum of **1** showed the characteristic signals for H-19 (δ 3.26, 1H, br s), H-13 (δ 2.68, 1H, ddd, J=2.9, 10.2, 10.2 Hz), H-5 (δ 2.49, 1H, d, J=10.2 Hz), and H-3 (δ 4.18, 1H, overlapped), suggesting that the aglycone has an 3α-hydroxylupan-28-oic acid skeleton.[1—3] The structure of **1** was established by comparing its chemical shifts to those of acankoreoside A (**3**),[2] which differed from **1** due to the presence of an aldehyde group (δ_C 204.7) and a secondary methyl group [δ_C 7.0, δ_H 1.02 (3H, d, J=6.5 Hz)] instead of signals from the isopropenyl group of acankoreoside A ($\delta_{C\text{-}20}$ 150.8, $\delta_{C\text{-}29}$ 110.0, $\delta_{C\text{-}30}$ 19.4),[2] indicating that the aldehyde group was located at C-20 of the aglycone.

An analysis of the 1D and 2D-NMR spectra and of the coupling constants in the ^{1}H-NMR spectrum, revealed that the carbon signals at δ 175.0, 181.8 and 204.6 indicated the presence of an esterified carboxyl group at C-28, a free carboxyl group at C-23, and an aldehyde group at C-30 (or C-29) on the triterpene moiety. In the HMBC spectrum, the carbon signal at δ 204.6 was correlated with a proton signal at δ 1.02 (3H, d, J=6.5 Hz, H-29) which coupled to C-20 (δ 50.1) and C-19 (δ 37.3). The carbon signal at δ 181.8 was correlated with the proton signal at δ 1.46 (3H, s, H-24), which correlated with C-3 (δ 72.7), C-4 (δ 51.7) and C-5 (δ 45.6). In addition, the ^{1}H–^{1}H COSY spectrum displayed a proton at δ 2.63 (1H, m, H-20) coupled with the proton signals at δ 1.02 (3H, d, J=6.5 Hz, H-29) and 3.26 (1H, br s, H-19). In the NOESY spectrum, the presence of a cross-peak between H-24 and H-25 indicated that the methyl group at C-24 was axially; which in turn suggested a α-configuration for the carboxyl group at C-4. The absolute configuration at C-20 of **1** was determined to be the *S* form by comparing the chemical shifts of C-20, 29, and 30 [δ_H: 9.70 (1H, s, H-30), δ_C: 204.6 (C-30), 7.0 (C-29), 50.1 (C-20)] with those of (20*S*)-3β-acetoxylupan-29-al [δ_H: 9.60 (1H, s, H-30), δ_C: 205.0 (C-30), 7.4 (C-29), 49.7 (C-20)] and (20*R*)-3β-acetoxylupan-29-al [δ_H: 9.85 (1H, d, J=2 Hz, H-30), δ_C: 207.0 (C-30), 14.5 (C-29), 49.0 (C-20)].[10,11] Sapogenol **2** was obtained as a white amorphous powder by the enzymatic hydrolysis of **1**. The EI-MS of **2** exhibited a molecular ion peak at *m*/*z* 502, suggesting a molecular formula of $C_{30}H_{46}O_6$. Its ^{1}H-NMR spectrum displayed signals due to four tertiary methyl groups, a secondary methyl group, and one oxygen-bearing methine proton. ^{13}C-NMR and DEPT spectra suggested the presence of two carboxyl groups, an oxygen-bearing methine carbon, six methine carbons, ten methylene carbons and five methyl carbons. In the ^{1}H-NMR spectrum, the signal at δ 4.25 (1H, br s) was assigned to H-3, indicating a α-hydroxyl group at C-3.[12,13] Accordingly, the triterpene moiety of compound **1** was determined to be (20*S*)

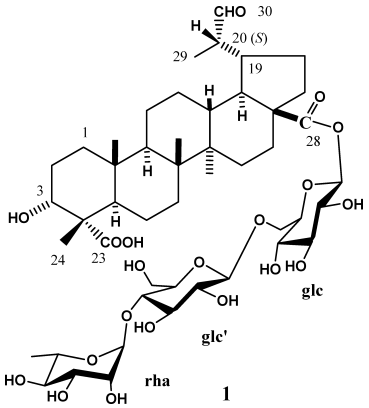

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Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1** and **2** (Pyridine-d_5, 500 MHz, δ in ppm)

Position	**1**		**2**	
	δ_C mult[a)]	δ_H (J/Hz)	δ_C mult[a)]	δ_H (J/Hz)
Aglycone				
1	33.3 t	1.46,[b)] 1.88[b)]	33.1 t	1.46,[b)] 1.83[b)]
2	26.1 t	1.88,[b)] 2.20[b)]	26.2 t	1.88,[b)] 2.02[b)]
3	72.7 d	4.20[b)]	73.0 d	4.25 br s
4	51.7 s	—	52.0 s	—
5	45.6 d	2.49 d (10.2)	45.1 d	2.55 d (10.0)
6	21.7 t	1.68,[b)] 1.79 m	21.8 t	1.84[b)]
7	34.6 t	1.32,[b)] 1.66[b)]	34.8 t	1.38,[b)] 1.78[b)]
8	41.7 s	—	41.7 s	—
9	50.6 d	1.65[b)]	50.7 d	1.66[b)]
10	37.4 s	—	37.4 s	—
11	20.9 t	1.55,[b)] 2.12[b)]	21.0 t	1.58[b)]
12	26.9 t	1.69[b)]	27.0 t	1.74[b)]
13	38.2 d	2.68 ddd (2.9, 10.2, 10.2)	38.5 d	2.75 ddd (3.2, 11.4, 12.8)
14	43.0 s	—	43.1 s	—
15	30.0 t	1.30,[b)] 1.97[b)]	30.0 t	1.27,[b)] 1.78[b)]
16	32.0 t	1.38,[b)] 2.28 dt (11.0)	32.7 t	2.58 m
17	57.0 s	—	56.6 s	—
18	48.5 d	1.50[b)]	49.0 d	1.50[b)]
19	37.3 d	3.26 br s	37.4 d	3.38 br s
20	50.1 d	2.63 dd (2.2, 6.5)	50.2 d	2.69 m
21	24.6 t	1.92,[b)] 1.23[b)]	24.1 t	
22	37.4 t	1.27,[b)] 2.10[b)]	37.5 t	2.12[b)]
23	181.8 s	—	180.1 s	—
24	18.3 q	1.46 s	18.1 q	1.44 s
25	16.8 q	0.96 s	16.7 q	0.95 s
26	16.6 q	1.19 s	16.7 q	1.09 s
27	14.7 q	0.85 s	14.6 q	0.90 s
28	175.0 s	—	178.9 s	—
29	7.0 q	1.02 d (6.5)	7.1 q	1.07 d (5.86)
30	204.6 s	9.70 (s)	204.7 s	9.70 (s)
28-*O*-glc				
1′	95.4 d	6.29 d (8.5)		
2′	73.9 d	4.07[b)]		
3′	78.7 d	4.20[b)]		
4′	70.9 d	4.24[b)]		
5′	78.0 d	4.09[b)]		
6′	69.6 t	4.26,[b)] 4.66[b)]		
6′-*O*-glc				
1″	105.1 d	4.92 d (7.5)		
2″	75.3 d	3.91 dd (8.0, 8.8)		
3″	76.5 d	4.11[b)]		
4″	78.3 d	4.36 dd (9.5, 9.5)		
5″	77.1 d	3.65 dt (9.5)		
6″	61.3 t	4.07,[b)] 4.19[b)]		
4″-*O*-rha				
1	102.7 d	5.81 s		
2	72.5 d	4.66[b)]		
3	72.7 d	4.53 dd (3.3, 9.5)		
4	74.0 d	4.32[b)]		
5	70.3 d	4.92[b)]		
6	18.5 q	1.68 d (6.5)		

All assignments of ^{1}H and ^{13}C signals were conformed by ^{1}H–^{1}H COSY, HMBC, and HMQC spectra. *a*) Multiplicities were obtained from DEPT experiments. *b*) Overlapped signals.

3α-hydroxy-30-oxolupan-23,28-dioic acid. Furthermore, an analysis of its 2D NMR data allowed the complete assignments of the ^{1}H- and ^{13}C-NMR spectra of compounds **1** and **2**. The results obtained are detailed in Table 1.

Acid hydrolysis of **1** provided the monosaccharide components of rhamnose and glucose (identified as TMS derivatives). HMBC correlations of **1** were demonstrated between glc H-1′ (δ 6.29, anomeric proton) and the C-28 of the triterpene moiety (δ 175.0), between glc H-1″ (δ 4.92, anomeric proton) and glc C-6′ (δ 69.6), and between rha H-1 (δ 5.81, anomeric proton) and glc C-4″ (δ 78.3). Large J values (J=7.5, 8.5 Hz) of the anomeric protons of the glucosyl moiety indicated a β glucosidic linkage, and the α-anomeric configuration of rhamnose was suggested based on the ^{13}C-NMR chemical shift of rhamnosyl C-3 (δ 72.7) and C-5 (δ 70.3).[14] The carbon signals due to this sugar moiety were superimposable on those of the characteristic triterpene glycosides isolated from *Acanthopanax* species. Consequently,

supposing that the absolute configurations of the sugars assumed the common forms, L-rhamnose and D-glucose, in naturally occurring glycosides from *Acanthopanax* species,[1—9] the respective sugar components and the sequence of the oligosaccharide chain were determined to be [α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester. Therefore, the structure of acankoreoside E (**1**) was elucidated as (20*S*) 3β-hydroxy-30-oxolupan-23,28-dioic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Experimental

Optical rotation was measured using a JASCO D-1010 spectropolarimeter. FAB-MS were recorded on a JEOL JMS-700 instrument using *m*-nitrobenzyl alcohol as a matrix in the positive ion mode. The NMR spectra were measured in pyridine-d_5 on a Varian UI-500 spectrometer and chemical shifts were relative to tetramethylsilane (TMS). Diaion HP-20 (Mitsubishi Chem. Co), Sephadex LH-20 (Pharmacia Biotech), Silica gel 60 (0.04—0.063 mm, Merck) and LiChroprep RP-18 (40—63 μm, Merck) were used for open column chromatography. TLC was performed using silica gel 60 F_{254} plate (Merck). GLC was performed on a HP5890A gas chromatograph with flame-ionization detector (column: HP-5 fused silica capillary column, 0.32 mm id×30 m).

Plant Material The leaves of *A. koreanum* NAKAI, were collected from the Medical Plant Garden of Kyung Hee University on October 2003. Professor Chang-Soo Yook in the Department of Pharmacognosy at Kyung Hee University identified the plant. A voucher specimen was deposited at the Museum of Oriental Medicine Specimen in Kyung Hee University.

Extraction and Isolation The dried leaves (1.0 kg) were extracted twice with 1.5 l of hot MeOH to give the dried extract (152.1 g), which was chromatographed on a Diaion HP-20P column with H_2O, 30%, 50%, 70%, and 90% MeOH mix in sequence. The fraction eluted by 70% MeOH was subsequently chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2→7 : 3 : 0.5) as eluent to give 8 fractions. A second separation step of fraction No. 4 (6.46 g) was carried out using gel filtration chromatography on a Sephadex LH-20 column with MeOH as eluent. Subsequently, the saponin fraction (5.95 g) from the above separation was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.2) as eluent then by gradients chromatography on a LiChroprep RP-18 column using H_2O/50% to 90% MeOH as eluent to yield compounds **1** (130 mg, yield 0.013%), **3** (acankoreoside A, 1.73 g, yield 0.173%). and **4** (acankoreoside D, 670 mg, yield 0.067%).

Acankoreoside E (**1**): A white powder; mp 223—227 °C; $[\alpha]_D^{26}$ −20.4° (c=0.49 in MeOH); ^{1}H- and ^{13}C-NMR data, see Table 1; Positive FAB-MS *m/z* $[M+Na]^+$ 995.5; HR-FAB-MS *m/z* $[M+Na]^+$ 995.4828 (Calcd for $C_{18}H_{76}O_{20}$·Na 995.4828).

Enzymetic Hydrolysis of 1 A mixture of compound **1** (30 mg) and the esterase, separated and purified from the leaves of *Acanthopanax divaricatus* by the method described in previous paper,[5] was incubated at 37 °C for 5 d. The reaction mixture was extracted with ethyl acetate, concentrated to dryness, and purified by silica gel column chromatography ($CHCl_3$–MeOH 10 : 1) to give compound **2** (8.7 mg). **2**: white amorphous powder, $[\alpha]_D^{26}$ −0.5° (c=0.34 in MeOH); ^{1}H- and ^{13}C-NMR data, see Table 1; EI-MS *m/z* (rel. int.) 502 (M^+, 3), 485 (15), 444 (26), 334 (8), 319 (32), 264 (24), 238 (70), 218 (37), 197 (58), 171 (29), 104 (67), 98 (82), 71 (100).

Acid Hydrolysis of 1 Compound **1** (3 mg) was hydrolyzed with 2 ml of 2 N HCl in H_2O for 4 h at 80 °C, which was followed by neutralization with 2 N NaOH in H_2O and extraction with $CHCl_3$. The aqueous layer was evaporated to dryness under vacuum. The residue was treated with the silylation reagent (Sylon HTP kit, Supelco) for 20 min at room temperature. After removing the excess reagent and organic solvent by condensation, the persilylated products were dissolved in hexane, and this solution was subjected to the GC analysis: column, fused silica capillary column (OV-17, 0.32×30 cm); detector, FID; column temperature, gradient of 100 to 250 °C at 15 °C/min. The retention times of persilylated glucose and rhamnose were founded to be 10.8 and 7.01 min, respectively, when compared with the standard solutions prepared by the same reaction from the standard monosaccharides.

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