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A LUPANE-TRITERPENE GLYCOSIDE FROM LEAVES OF TWO *ACANTHOPANAX*

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Key Word Index— *Acanthopanax trifoliatus*; *A. koreanum*; Araliaceae; leaves; lupane-triterpene glycoside; acantrifoside A.

Abstract—A new lupane triterpene glycoside, acantrifoside A, was isolated from the leaves of *Acanthopanax trifoliatus* and *A. koreanum*. Based on spectroscopic data, the compound was identified as 3α,11α-dihydroxy-lup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Acanthopanax trifoliatus (L.) MERR. is distributed widely in India, China and Formosa [1] whereas *Acanthopanax koreanum* NAKAI grows in Korea [2]. The barks of *Acanthopanax* species are used as a tonic and sedative as well as a drug with ginseng-like activities. As for the constituents in the leaves of *A. trifoliatus*, Adam *et al.* [3–6] reported the presence of the lupane-triterpene sapogenols, while Kim *et al.* [7–11] reported lignan and diterpene derivatives from the stem bark and root bark of *A. koreanum*. We have now characterised some of the constituents of the leaves of *A. koreanum*.

The leaves of *A. trifoliatus* were collected at Mt. Yangming in Formosa and the leaves of *A. koreanum* were harvested at Kwang-nung, Kyung-gi province of Korea. As described in the Experimental, a new lupane glycoside designated as acantrifoside A was obtained in a yield 0.014% from the former plant and 0.47% from the latter plant. Here we describe the structure characterization of this compound.

RESULTS AND DISCUSSION

Acantrifoside A (**1**), obtained as a white powder, mp 265–267°C (dil. MeOH), $[\alpha]_D$ −42.6° (MeOH), showed absorptions due to hydroxyl groups at 3415 cm^{-1} and ester carbonyl group at 1745 cm^{-1} in the IR spectrum. The HR FAB-mass spectrum with an ion at *m/z* 965.5073 $[M+Na]^+$ provided the formula $C_{48}H_{78}O_{18}$ (Calcd for $C_{48}H_{78}O_{18}Na$: 965.5027). The positive FAB-mass spectrum also exhibited a peak due to $[M+Na+H]^+$ at *m/z* 966, [M-methylpentose $+Na+H]^+$ at *m/z* 820 [M-methylpentose-hexose $+Na+H]^+$ at *m/z* 658 and [M-methylpentose-2× hexose $+Na+H]^+$ at *m/z* 496. The ^1H NMR spectrum (in pyridine-d_5) showed signals due to six tertiary methyl groups at δ 0.96, 0.98, 1.23 (×2), 1.26 and 1.65, one secondary methyl group at δ 1.68 (3H, *d*, *J* = 6.1 Hz), three anomeric protons due to two hexosyl residues at δ 4.93 (1H, *d*, *J* = 7.9 Hz) and 6.30 (1H, *d*, *J* = 7.9 Hz) and one methylpentosyl residue at 5.80 (1H, *br s*) as illustrated in Table 1. Therefore, taking into consideration of the molecular formula, compound **1** was deduced to be a triterpene glycoside.

The chemical shift of the hexosyl anomeric proton signal appeared at δ 6.30 and the IR absorption at 1745 cm^{-1}, suggesting that the sapogenol possessed an ester carbonyl group, to which a hexosyl moiety was attached. Therefore, compound **1** was saponified with 0.5 M KOH in methanol to give an aglycone (**2**), which had mp 230–232°, $[\alpha]_D$ 0.57° (EtOH), and absorptions due to hydroxyl groups at 3421 cm^{-1} and carboxyl group at 1700 cm^{-1} in the IR spectrum. The molecular weight was estimated as 472 from the EI-mass spectrum. The ^1H NMR spectrum (pyridine-d_5) of **2** displayed signals due to six tertiary methyl groups at δ 0.96, 1.01, 1.13, 1.24 (×2) and 1.71, two olefinic protons at δ 4.66 (1H, *br s*) and 4.87 (1H, *br s*), two oxygen bearing protons at δ 4.23 (1H, *ddd*, *J* = 5.5, 10.7, 10.7 Hz) and 3.63 (1H, *br s*, $W_{1/2}$ = 6.7 Hz) as illustrated in Table 2. The carbon signals observed in

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Table 1. ^1H and ^{13}C NMR (500 MHz) Spectral data of Compound **1** in pyridine-d_5 (δ values in ppm)[1]

C	δ_C	δ_H*	Cross peaks (δ_C) in HMBC spectrum
1	36.2 CH_2	2.22 (*m*†)3.08 (*br d*, 12.8)	26.9 (2), 49.6 (5), 75.3 (3)
2	26.9 CH_2	1.78 (*m*†), 2.15 (*m*†)	36.2 (1)
3	75.3 CH	3.61 (*br s*)	22.9 (24), 36.2 (1), 49.6 (5)
4	38.5 C		
5	49.6 CH	1.75 (*m*)	18.6 (6), 22.9 (24), 38.5 (4)
6	18.6 CH_2	1.38 (*m*†), 1.50 (*m*†)	49.6 (5), 35.7 (7)
7	35.7 CH_2	1.34 (*m*†), 1.47 (*m*†)	56.2 (9)
8	42.8 C		
9	56.2 CH	1.83 (*d*, 10.4)	16.9 (25), 35.7 (7), 39.9 (10), 42.8 (8), 69.8 (11)
10	39.9 C		
11	69.8 CH	4.27 (*m*†)	
12	38.3 CH_2	1.58 (*m*†), 2.36 (*m*)	37.4 (13), 69.8 (11)
13	37.4 CH	2.85 (*m*)	14.8 (27), 43.0 (14), 49.5 (18)
14	43.0 C		
15	30.0 CH_2	1.19 (*m*†), 1.94 (*m*)	43.0 (14)
16	32.3 CH_2	1.51 (*m*†)2.63 (*dt*, 12.8)	43.0 (14), 49.5 (18), 56.9 (17)
17	56.9 C		
18	49.5 CH	1.70 (*m*†)	37.4 (13), 47.2 (19), 56.9 (17), 150.4 (20), 175.0 (28)
19	47.2 CH	3.37 (*m*)	
20	150.4 C		
21	30.9 CH_2	1.41 (*m*†), 2.14 (*m*†)	36.8 (22), 47.2 (19), 49.5 (18)
22	36.8 CH_2	1.47 (*m*†), 2.18 (*m*†)	30.9 (21), 56.9 (17), 49.5 (18)
23	29.8 CH_3	1.23 (*s*†)	22.9 (24), 38.5 (4), 75.3 (3)
24	22.9 CH_3	0.96 (*s*)	29.8 (23), 38.5 (4), 49.6 (5), 75.3 (3)
25	16.9 CH_3	1.26 (*s*)	36.2 (1), 39.9 (10), 49.6 (5), 56.2 (9)
26	17.7 CH_3	1.23 (*s*†)	35.7 (7), 43.0 (14), 56.2 (9)
27	14.8 CH_3	0.98 (*s*)	30.0 (15), 37.4 (13), 42.8 (8), 43.0 (14)
28	175.0 C		
29	110.2 CH_2	4.61 (*br s*)4.80 (*br s*)	19.5 (30), 47.2 (19)
30	19.5 CH_3	1.65 (*s*)	47.2 (19), 110.2 (29), 150.4 (20)
C-28 *O*-inner glc			
1	95.3 CH	6.30 (*d*, 7.9)	175.0 (28)
2	73.9 CH	4.07 (*m*†)	78.7 (g-3)
3	78.7 CH	4.19 (*m*†)	70.9 (g-4), 73.9 (g-2), 78.0 (g-5)
4	70.9 CH	4.29 (*m*†)	78.7 (g-3)
5	78.0 CH	4.09 (*m*†)	95.3 (g-1)
6	69.5 CH_2	4.27 (*m*†)4.66 (*d*, 11.6)	105.1 (g-1′)
glc′(1→6)glc			
1′	105.1 CH	4.93 (*d*, 7.9)	69.5 (g-6)
2′	75.2 CH	3.92 (*t*, 8.5)	76.4 (g-3′), 105.1 (g-1′)
3′	76.4 CH	4.11 (*m*†)	75.2 (g-2′), 78.3 (g-4′)
4′	78.3 CH	4.36 (*t*, 9.2)	75.2 (g-2′), 77.1 (g-5′), 102.7 (r-1)
5′	77.1 CH	3.64 (*dt*, 9.2)	78.3 (g-4′)
6′	61.3 CH_2	4.08 (*m*†), 4.19 (*m*†)	
rha(1→4)glc′			
1	102.7 CH	5.80 (*br s*)	70.3 (r-5), 72.7 (r-3), 78.3 (g-4′)
2	72.5 CH	4.64 (*br s*)	70.3 (r-5), 72.7 (r-3)
3	72.7 CH	4.51 (*dd*, 9.2, 3.1)	74.0 (r-4)
4	74.0 CH	4.33 (*m*†)	18.5 (r-6), 70.3 (r-5), 72.5 (r-2)
5	70.3 CH	4.93 (*m*†)	
6	18.5 CH_3	1.68 (*d*, 6.1)	70.3 (r-5), 74.0 (r-4)

glc, β-*d*-glucopyranosyl; rha, α-*l*-rhamnopyranosyl. All assignments of ^1H and ^{13}C signals were conformed by ^1H-^1H COSY, HMQC and HMBC spectra. * *J* values (in Hz) in parentheses. † Overlapped signals.

the ^{13}C NMR spectrum Table 2 suggested the presence of a carboxyl group at δ 179.3, monosubstituted double bond at δ 151.0 and 110.0, and two oxygen bearing methine carbons at δ 75.2 and 69.9, five methine carbons at δ 37.7, 47.6, 49.5, 49.6 and 56.2, nine methylene carbons at δ 18.6, 27.0, 30.2, 31.3, 33.0, 36.0, 36.3, 37.5 and 38.4 and six methyl carbons at δ 14.8, 16.8, 17.8, 19.6, 22.9, and 29.9. Based on the above

Table 2. ^1H and ^{13}C NMR (500 MHz) Spectral data of Compound **2** in pyridine-d_5 (δ values in ppm)[2]

C	δ_C	δ_H*	Cross peaks (δ_C) in HMBC spectrum
1	36.3 CH_2	2.25 (*m*†)3.13 (*br d*, 13.4)	27.0 (2), 49.6 (5), 75.2 (3)
2	27.0 CH_2	1.81 (*m*†), 2.14 (*m*†)	36.3 (1)
3	75.2 CH	3.63 (*br s*)	22.9 (24), 36.3 (1), 38.5 (4), 49.6 (5)
4	38.5 C		
5	49.6 CH	1.72 (*m*†)	16.8 (25), 18.6 (6), 22.9 (24), 36.0 (7), 38.5 (4)
6	18.6 CH_2	1.42 (*m*†), 1.55 (*m*†)	36.0 (7), 39.9 (10), 49.6 (5)
7	36.0 CH_2	1.37 (*m*†), 1.53 (*m*†)	56.2 (9)
8	42.8 C		
9	56.2 CH	1.86 (*d*, 10.4)	16.8 (25), 36.0 (7), 39.9 (10), 42.8 (8), 69.9 (11)
10	39.9 C		
11	69.9 CH	4.23 (*ddd*, 5.5, 10.7, 10.7)	
12	38.4 CH_2	1.62 (*m*†), 2.44 (*m*)	37.7 (13), 43.0 (14), 69.9 (11)
13	37.7 CH	2.93 (*m*)	49.5 (18)
14	43.0 C		
15	30.2 CH_2	1.21 (*m*†), 1.78 (*m*)	56.4 (17)
16	33.0 CH_2	1.51 (*m*†) 2.62 (*dt*, 12.8)	43.0 (14), 49.5 (18)
17	56.4 C		
18	49.5 CH	1.78 (*m*†)	37.7 (13), 47.6 (19), 56.4 (17), 151.0 (20), 179.3 (28)
19	47.6 CH	3.52 (*m*)	
20	151.0 C		
21	31.3 CH_2	1.49 (*m*†), 2.24 (*m*†)	49.5 (18)
22	37.5 CH_2	1.54 (*m*†), 2.25 (*m*†)	31.3 (21), 49.5 (18)
23	29.9 CH_3	1.24 (*s*†)	38.5 (4), 49.6 (5), 75.2 (3)
24	22.9 CH_3	0.96 (*s*)	29.9 (23), 38.5 (4), 49.6 (5), 75.2 (3)
25	16.8 CH_3	1.24 (*s*†)	36.3 (1), 39.9 (10), 49.6 (5), 56.2 (9)
26	17.8 CH_3	1.13 (*s*)	36.0 (7), 43.0 (14), 56.2 (9)
27	14.8 CH_3	1.01 (*s*)	30.2 (15), 37.7 (13), 42.8 (8), 43.0 (14)
28	179.3 C		
29	110.0 CH_2	4.66 (*br s*), 4.87 (*br s*)	19.6 (30), 47.6 (19)
30	19.6 CH_3	1.71 (*s*)	47.6 (19), 110.0 (29), 151.0 (20)

All assignments of ^1H and ^{13}C signals were conformed by ^1H-^1H COSY, HMQC and HMBC spectra. * *J* values (in Hz) in parentheses. † Overlapped signals.

data, compound **2** was identified as 3α,11α-dihydroxy-lup-20(29)-ene-28-oic acid [3].

Acid hydrolysis of **1** with 2 N HCl gave the sapogenol, which was identical with **2**, together with a mixture of sugars. The sugar mixture was derivatised to give the trimethylsilyl ethers of the corresponding methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates followed by GLC analysis to identify D-glucose and L-rhamnose. Therefore, based upon the coupling constants of the anomeric protons, it was determined that the D-glucosyl bond had a β-linkage and the L-rhamnose had an α-linkage. Measurements of ^1H-^1H and ^1H-^{13}C 2D NMR spectra enabled the respective signals to be assigned as indicated in Table 1.

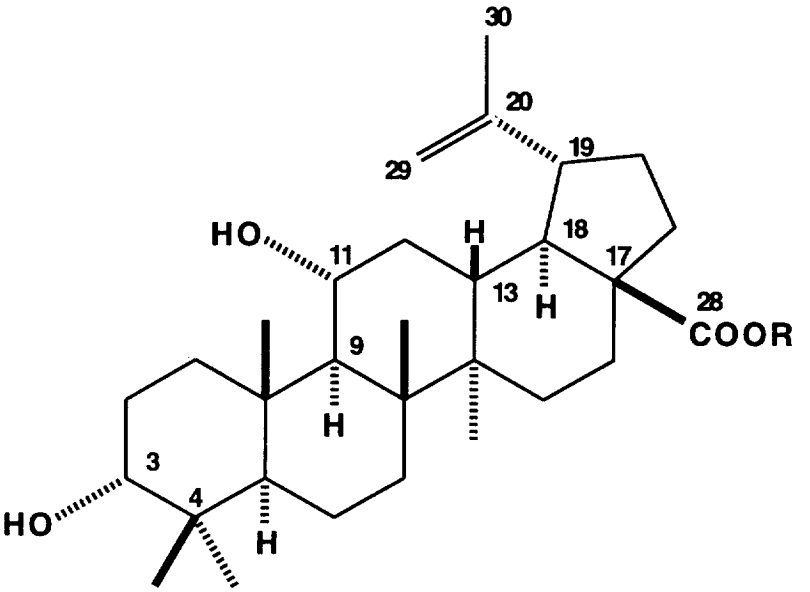
The heteronuclear multiple bonds correlation (HMBC) from inner glc H-1 at δ 6.30 (1H, *d*, $J=7.9$ Hz) to C-28 at δ 175.0 (*s*) of the aglycone, from outer glc H-1′ at δ 4.93 (1H, *d*, $J=7.9$ Hz) to inner glc C-6 at δ 69.5 (*t*), and from rha H-1 at δ 5.80 (1H, *br s*) to outer glc C-4′ at δ 78.3 (*d*) were observed as shown in Table 1. This evidence suggested the sequence of sugar linkages of **1**. Moreover, the sugar moiety was identical with that of chiisanoside isolated from *Acanthopanax chiisanensis* and *A. divaricatus* by Tanaka *et al.* [12–14].

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

EXPERIMENTAL

General

Mps (uncorr.) 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 spectrometer. 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 MHz spectrometer and chemical shifts were referenced to TMS. GLC was performed on a HP5890A gas



1 R= - β-D-glc · pyr^{6}— β-D-glc · pyr^{4}— α-L-rha · pyr

2 R=H

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).

Plant material

The leaves of *A. trofoliatus* were collected at Mt. Yangming in Formosa in February, 1983, and the leaves of *A. koreanum* were harvested at Kwang-nung, Kyung-gi province of Korea on September, 1996.

Isolation of compounds

The dried leaves (500 g) of *A. trifoliatus* were extracted with MeOH to give an extract (90 g), which was partitioned between Et_2O and H_2O. The H_2O layer was evaporated to dryness *in vacuo*, and chromatographed on silica gel with $CHCl_3$-MeOH-H_2O (8:2:0.2) followed by recrystallization from MeOH to yield **1** (yield, 0.014%). The dried leaves of *A. koreanum* (470 g) were extracted repeatedly with hot MeOH 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. Ltd., 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. Fr-6 was chromatographed on a reverse phase column, Chromatorex ODS (30–50 μm, Fuji Silysia Chem. Ind. Co. Ltd., Japan), with gradient elution from 50% MeOH to 90% MeOH, and Fr-6E and Fr-6F were recrystallized from MeOH-H_2O to yield **1** (yield, 0.47%).

Compound 1

A white powder. mp 265–267°C (from MeOH-H_2O); $[\alpha]_D^{20}$ −42.6° (*c* 0.39 in MeOH). IR $\upsilon_{max}^{KBr}cm^{-1}$: 3415 (*br* OH), 1745 (ester carbonyl), 1641 (C=C): positive HR FAB-MS *m/z*: 965.5073 $[M+Na]^+$ (Calcd for $C_{48}H_{78}O_{18}Na$: 965.5027); positive FAB-MS *m/z*: 966 $[M+Na+H]^+$, 820 [M-methylpentose $+Na+H]^+$, 658 [M-methylpentose-hexose $+Na+H]^+$, 496 [M-methylpentose-2× hexose $+Na+H]^+$; ^1H, ^{13}C NMR and 2D NMR correlations: see Table 1.

Alkaline hydrolysis of 1

Compound **1** (130 mg) was hydrolyzed with 6 ml of 0.5 M KOH in MeOH for 1 hr at 70°. The reaction mixture was neutralized with 2 N HCl in MeOH, 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 (*n*-hexane-acetone = 2:1). The obtained aglycone fraction was recrystallized from MeOH to give **2** (32 mg). Compound **2**: Colorless needles, mp 230–232° (MeOH); $[\alpha]_D^{24}$ +0.57 (*c* 0.07 in EtOH); IR $\upsilon_{max}^{KBr}cm^{-1}$: 3421 (*br* OH), 1700 (carbonyl), 1641 (C=C); EI-MS *m/z*: 472 $[M]^+$, 454 $[M\text{-}H_2O]^+$, 436 $[M\text{-}2H_2O]^+$; ^1H, ^{13}C NMR and 2D NMR correlations: see Table 2.

Acid hydrolysis of 1

Compound **1** (100 mg) was hydrolyzed with 4 ml of 2 N HCl in H_2O for 4 hr at 80°. 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 CC (*n*-hexane-acetone = 3:1 → 2:1).

The obtained aglycone fraction was recrystallized from MeOH to give **2** (18 mg). Colorless needles, mp 235–236° (MeOH), $[\alpha]_D^{25}$0.98 (*c* 0.11 in EtOH), identical with **2**. The aq. layer was concentrated to dryness *in vacuo*. The remaining residue was dissolved in dry pyridine and L-cysteine methyl ester hydrochloride added. The reaction mixture was heated for 2 hr at 60°C and concentrated to dryness under N_2. Trimethylsilylimidazole was added and the mixture heated for 1 hr at 60°. The reaction mixture was then taken to dryness under N_2. 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). Two peaks were observed at R_t (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.

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