Lupane-Triterpene Glycosides from the Leaves of *Acanthopanax gracilistylus*

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A novel lupane-triterpene glycoside, called wujiapioside B (1), was isolated from the leaves of *Acanthopanax gracilistylus* (Araliaceae) together with three known lupane-triterpene glycosides, acankoreoside C (2), acantrifoside A (3) and 3-epibetulinic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (4). Based on spectroscopic data, the chemical structure of 1 was determined as 3α,23-dihydroxy-lup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester. Compounds 2—3 were obtained for the first time from this plant and compound 4 has not been isolated from *Acanthopanax* genus yet.

Key words *Acanthopanax gracilistylus*; lupane-triterpene glycoside; wujiapioside B; acankoreoside C; acantrifoside A; 3-epibetulinic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester

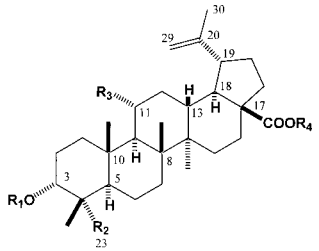
Acanthopanax gracilistylus W. W. SMITH (Araliaceae) is widely distributed in China, and the root bark of which has been listed in the Chinese pharmacopoeia as Cortex Acanthopanacis (Wujiapi) and has been used to relieve rheumatic conditions, tonify liver and kidney, and strengthen tendons and bones.[1)] Lignan and diterpene derivatives have been isolated from the root and stem barks of this plant.[2,3)] In our previous papers,[4,5)] we also reported the isolation and structures of volatile components, lignans, diterpenes and phytosterols from the same source. But there has yet been no report on the constituents of the leaves of this plant.

In a continuing study on *Acanthopanax* genus, we describe here the isolation and structure determination of a novel lupane-triterpene glycoside, called wujiapioside B (**1**), from the leaves of *A. gracilistylus*. Together with compound **1**, three known lupane-triterpene glycosides were also isolated and determined as acankoreoside C (**2**),[6)] acantrifoside A (**3**),[7,8)] and 3-epibetulinic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**4**)[9)] by comparing their spectroscopic data with the previously reported ones. Compounds **2** and **3** were obtained for the first time from this plant, and especially compound **4** has not been isolated from *Acanthopanax* genus so far.

A MeOH extract of the leaves of *A. gracilistylus* was fractioned on Diaion HP20P column. Further separation of each fraction with a combination of silica gel, Chromatorex ODS and Sephadex LH-20 columns led to the isolation of compounds **1**—**4**.

Compound **1** was obtained as a white powder, mp 218—220 °C (from dil. MeOH), $[\alpha]_D$ −47.3° (c=0.55 in MeOH) and gave positive responses in Liebermann–Burchard and Molish tests. Its IR spectrum showed absorption bands due to a hydroxyl group at 3419 cm^{-1}, an ester carbonyl group at 1729 cm^{-1} and a C=C group at 1638 and 885 cm^{-1}. The negative FAB-MS spectrum provided a formula of $C_{48}H_{78}O_{18}$ from a molecular ion peak due to $[M-H]^-$ at m/z 941 as well as a fragment ion peak due to [M−sugar residues]$^-$ at m/z 471. The formula was also confirmed by ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) experiment (Table 1). The ^{1}H-NMR spectrum (in pyridine-d_5) showed signals due to five tertiary methyl groups at δ 0.79, 0.86, 0.95, 1.19 and 1.65, one secondary methyl group at δ 1.70 (3H, d, J=6.1 Hz), three anomeric protons due to two hexosyl residues at δ 6.32 (1H, d, J=8.1 Hz) and 4.94 (1H, d, J=8.0 Hz) and one methylpentosyl residue at δ 5.83 (1H, br s), and two olefinic protons at δ 4.73 (1H, br s) and 4.85 (1H, br s) as illustrated in Table 1. The chemical shift at δ 6.32 assignable to a hexosyl anomeric proton and the IR absorption at 1729 cm^{-1} suggested the presence of an ester glycosyl linkage. Total forty eight carbon signals observed in the ^{13}C-NMR spectrum (Table 1) suggested the presence of one carbonyl group at δ 175.0, one 1,1-disubstituted double bond at δ 110.0 and 150.9, one oxygen-bearing methine carbon at δ 75.8, one hydroxymethylene carbon δ 71.4, and three anomeric carbons at δ 95.3, 102.7 and 105.1. From the above facts, **1** was deduced to be a lupane-triterpene glycoside.

Furthermore, the heteronuclear multiple bond correlations (HMBC) from inner glucose H-1 at δ 6.32 (1H, d, J=8.1 Hz) to C-28 at δ 175.0 (s) of the aglycone, from outer glucose H-



	R_1	R_2	R_3	R_4
1	H	CH_2OH	H	**S**
1a	H	CH_2OH	H	H
1b	H	CH_2OH	OH	H
2	-β-D- glc	CH_3	OH	**S**
3	H	CH_3	OH	**S**
4	H	CH_3	H	**S**

S =-β-D- glc^6-β-D- glc^4-α-L- rha

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Table 1. ^{1}H- and ^{13}C-NMR Data and Selected Heteronuclear Multiple Bond Connectivity (HMBC) Correlations in **1** (in Pyridine-d_5, ^{1}H; 500 MHz, ^{13}C; 125 MHz)

Position	δ_C [a]	δ_H [b]	Cross peaks (δ_C) in HMBC spectrum
Aglycone			
1	33.8 t	1.28 (*), 1.49 (*)	16.7 (C-25)
2	26.7 t	1.71 (*)	
3	75.8 d	3.92 (1H, br s)	33.8 (C-1), 71.4 (C-23)
4	41.4 s		
5	43.7 d	2.08 (1H, d, 11.2)	41.4 (C-4), 18.4 (C-6), 37.5 (C-10), 18.0 (C-24)
6	18.4 t	1.40 (*), 1.51 (*)	
7	34.3 t	1.27 (*), 1.48 (*)	18.4 (C-6)
8	40.8 s		
9	50.9 d	1.81 (1H, d, 12.1)	37.5 (C-10)
10	37.5 s		
11	21.1 t	1.48 (*)	26.1 (C-11)
12	26.1 t	1.19 (*), 1.95 (*)	
13	38.4 d	2.68 (1H, m)	
14	42.9 s		
15	30.1 t	1.18 (*), 1.95 (*)	
16	32.3 t	1.51 (*), 2.61 (*)	175.0 (C-28)
17	57.0 s		
18	49.8 d	1.72 (*)	38.4 (C-13), 57.0 (C-17), 47.5 (C-19), 175.0 (C-28)
19	47.5 d	3.39 (1H, m)	
20	150.9 s		
21	30.7 t	1.48 (*), 2.18 (*)	49.8 (C-18), 47.5 (C-19)
22	36.9 t	1.50 (*), 2.20 (*)	49.8 (C-22)
23	71.4 t	3.66 (1H, d, 11.2), 3.86 (1H, d, 11.2)	75.8 (C-3)
24	18.0 q	0.79 (3H, s)	75.8 (C-3), 41.4 (C-4), 43.7 (C-5), 71.4 (C-23)
25	16.7 q	1.19 (3H, s)	33.8 (C-1), 43.7 (C-5), 50.9 (C-9), 37.5 (C-5)
26	16.5 q	0.86 (3H, s)	34.3 (C-7), 40.8 (C-8), 50.9 (C-9), 42.9 (C-14)
27	14.9 q	0.95 (3H, s)	42.9 (C-14), 30.1 (C-15)
28	175.0 s		
29	110.0 t	4.73 (1H, br s), 4.85 (1H, br s)	47.5 (C-19), 19.4 (C-30)
30	19.4 q	1.65 (3H, s)	150.9 (C-20), 110.0 (C-29)
C-28 *O*-inner glc			
1	95.3 d	6.32 (1H, d, 8.1)	175.0 (C-28), 74.0 (glc C-3)
2	74.0 d	4.0 (*)	95.3 (glc C-1), 78.3 (glc C-3)
3	78.3 d	4.20 (*)	74.0 (glc C-2), 70.9 (glc C-4)
4	70.9 d	4.31 (*)	78.3 (glc C-3)
5	77.2 d	4.10 (*)	69.5 (glc C-6)
6	69.5 t	4.64 (1H, d, 11.1)	105.1 (glc C-1′)
glc′(1→6)glc			
1′	105.1 d	4.94 (1H, d, 8.0)	69.5 (glc C-6), 75.3 (glc C-3′)
2′	75.3 d	3.94 (*)	105.1 (glc C-1′), 76.5 (glc C-3′)
3′	76.5 d	4.12 (*)	78.7 (glc C-4′)
4′	78.7 d	4.38 (1H, t-like)	61.3 (glc C-6′), 102.7 (rha C-1)
5′	78.0 d	3.63 (*)	61.3 (glc C-6′)
6′	61.3 t	4.10 (*), 4.20 (*)	78.0 (glc C-5′)
rha(1→4)glc			
1	102.7 d	5.83 (1H, br s)	78.7 (glc C-4′), 72.6 (rha C-2), 70.3 (rha C-5)
2	72.6 d	4.64 (1H, br s)	102.7 (rha C-1)
3	72.8 d	4.53 (1H, dd, 9.4, 4.0)	74.0 (rha C-4)
4	74.0 d	4.30 (*)	72.8 (rha C-3)
5	70.3 d	4.96 (*)	18.5 (rha C-6)
6	18.5 q	1.70 (3H, d, 6.1)	74.0 (rha C-4), 70.3 (rha C-5)

All assignments of ^{1}H- and ^{13}C-NMR signals were confirmed by ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and HMBC spectra. Glc: β-D-glucopyranosyl; rha: α-L-rhamnopyranosyl. s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet; br s: broad singlet; dd: double doublet. *a*) Multiplicities were deduced from a DEPT experiment. *b*) *J* values (in Hz) in parentheses. * Overlapped with other signal(s) and its multiplicity and *J* values were both obscure.

1′ at δ 4.94 (1H, d, J=8.0 Hz) to inner glucose C-6 at δ 69.5 (t), and from rhamnose H-1 at δ 5.83 (1H, br s) to outer glucose C-4′ at δ 78.7 (d) were observed. These findings suggested the sequence of the sugar linkage of **1**.

Alkaline hydrolysis of **1** with 5% KOH in MeOH gave a sapogenol (**1a**), a white powder, mp 245—248 °C, together with a mixture of sugars. The sugar mixture was identified to be composed of D-glucose and L-rhamnose by TLC.

Based on above fact and the coupling constants of anomeric protons, the sugar moiety of **1** was found to be composed of β-D-glucopyranose and α-L-rhamnopyranose. Measurements of two dimensional (2D)-NMR spectra of ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectroscopy enabled the respective signals to be assigned (Table 1). Moreover, the carbon

signals due to this sugar moiety were identical with those of lupane-triterpene glycosides isolated from *A. koreanum*.[6,7]

The electron impact (EI)-MS of the sapogenol (**1a**) exhibited a molecular ion peak at *m/z* 472. The ^{1}H-NMR spectrum (in pyridine-d_5) of **1a** displayed signals due to five tertiary methyl groups, two olefinic protons, two oxygen-bearing methylene protons and one oxygen-bearing methine proton. The carbon signals observed in the ^{13}C-NMR and a DEPT experiments suggested the presence of one carboxyl group, one 1,1-disubstituted double bond, one oxygen-bearing methylene carbon, one oxygen-bearing methine carbon, five methine carbons, ten methylene carbons, five methyl carbons. All the ^{13}C-NMR signals of **1a** were very similar to those of 3α,11α,23-trihydroxy-lup-20(29)-en-28-oic acid (**1b**),[6] except for the absence of one oxygen-bearing methine carbon signal of C-11 at δ 69.8 (d) observed in **1b** and the presence of one methylene carbon signal at δ 21.1 (t) in **1a**. This fact was supported by significant upfield shifts of the C-9 signal (4.7 ppm) and the C-12 signal (12.1 ppm) by an α-effect of dehydroxylation at C-11 in **1a**. Measurements of 2D-NMR spectra of **1a** enabled the respective signals to be assigned. Based on the above data, **1a** was identified as 3α,23-dihydroxy-lup-20(29)-en-28-oic acid. To our best knowledge, this sapogenol has not been reported yet.

Based on the above spectroscopic data, the structure of **1** was determined as 3α,23-dihydroxy-lup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1$\rightarrow$4)-β-D-glucopyranosyl-(1$\rightarrow$6)-β-D-glucopyranosyl ester. This compound appears to be found for the first time in the plant kingdom and we called it wujiapioside B.

Experimental

Melting points (uncorrected) were measured using a Boetius micromelting 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 was obtained in a glycerol matrix in the negative ion mode using a JEOL JMS-DX300 and JMS-DX303HF 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. Column chromatography was carried out on Diaion HP20P (Mitsubishi Chem. Ind. Co.), silica gel 60 (0.040—0.063 mm, Merck), Sephadex LH-20 (Pharmacia Biotech Co.) and Chromatorex ODS (30—50 μm, Fuji Silysia Chem. Ind. Co.). TLC was performed on a precoated silica gel 60F_{254} (Merck) and RP-18F_{254S} (Merck) plates.

Plant Material The leaves of *A. gracilistylus* were collected at Changsha, Hunan province of China, in August 2000 and were botanically identified by one of the authors, Prof. Chang-Soo Yook; the specimen has been deposited in the Herbarium of the College of Pharmacy, Kyung-Hee University.

Extraction and Isolation The dried leaves of the plant (350 g) were extracted repeatedly with hot MeOH to give an extract (50 g), which was chromatographed on Diaion HP20P column by using gradient elution with H_2O, 30% MeOH, 50% MeOH, 80% MeOH and MeOH. A saponin mixture eluted with 80% MeOH was evaporated to dryness *in vacuo*, and was subsequently chromatographed on a silica gel 60 by using gradient elution with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1$\rightarrow$7 : 3 : 0.5) to give seven fractions. Each of fractions 3, 4 and 6 were chromatographed again on a silica gel 60 column by using gradient elution with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.2$\rightarrow$7 : 3 : 0.5) to give **4** (20 mg), **1** (40 mg) and **2** (12 mg), respectively. On the other hand, Fraction 5 was chromatographed on a silica gel column by using gradient elution with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.2$\rightarrow$7 : 3 : 0.5), a Sephadex LH-20 column by using elution with MeOH and a Chromatorex ODS column by using gradient elution with MeOH–H_2O (6 : 4$\rightarrow$10 : 0) to give **3** (15 mg).

Alkaline Hydrolysis of 1 Compound **1** (25 mg) was hydrolyzed with 8 ml of 5% KOH in MeOH for 2 h at 80 °C. The reaction mixture was neutralized with 2 N HCl in H_2O and extracted with EtOAc. The aqueous layer was filtered, concentrated and chromatographed on TLC plate in which D-glucose and L-rhamnose were detected. The EtOAc layer was evaporated *in vacuo* and the residue was chromatographed on a silica gel column by using elution with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1). The obtained sapogenol fraction was recrystallized from dil. MeOH to give **1a** (6 mg).

Compound **1a**: Colorless needles, mp 245—248 °C. EI-MS *m/z* (rel. int. %): 472 (M^+, 25), 454 (M^+-H_2O, 22), 424 (28), 259 (20), 205 (64), 189 (99), 187 (100), 119 (69), 95 (78), 69 (67), 55 (72); ^{1}H-NMR (500 MHz, in pyridine-d_5) δ: 0.76, 0.87, 0.97, 1.09, 1.76 (each 3H, s, H-24, 25, 26, 27 and 30), 1.74 (1H, m, H-18), 1.84 (1H, d, J=10.4 Hz, H-9), 2.09 (1H, d, J=11.2 Hz, H-5), 2.22 (1H, m, H-13), 3.53 (1H, m, H-19), 3.92 (1H, br s, H-3β), 3.66 (1H, d, J=11.0 Hz, H-23A), 3.85 (1H, d, J=11.0, H-23B), 4.75 (1H, br s, H-29A), 4.92 (1H, br s, H-29B); ^{13}C-NMR (125 MHz, in pyridine-d_5) δ: 33.8 (t, C-1), 26.8 (t, C-2), 75.8 (d, C-3), 41.3 (s, C-4), 43.8 (d, C-5), 18.4 (t, C-6), 34.5 (t, C-7), 40.9 (s, C-8), 50.9 (d, C-9), 37.6 (s, C-10), 21.1 (t, C-11), 26.2 (t, C-12), 38.6 (d, C-13), 42.9 (s, C-14), 30.3 (t, C-15), 32.9 (t, C-16), 56.6 (s, C-17), 49.8 (d, C-18), 47.8 (d, C-19), 151.4 (s, C-20), 31.2 (t, C-21), 37.5 (t, C-22), 71.4 (t, C-23), 18.0 (q, C-24), 16.7 (q, C-25), 16.5 (q, C-26), 14.9 (q, C-27), 178.9 (s, C-28), 109.9 (t, C-29), 19.5 (q, C-30).

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