Arch Pharm Res Vol 25, No 6, 831-836, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

A New Lupane-Triterpene Glycoside from the Leaves of *Acanthopanax gracilistylus*

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(Received August 27, 2002)

A new and two known lupane-triterpene glycosides were isolated from the hot MeOH fraction of the leaves of *Acanthopanax gracilistylus* W. W. Smith. Based on the physical properties and spectroscopic data, their chemical structures were determined as acankoreoside A (**1**), acankoreoside D (**2**), and 3α-hydroxy-lup-23-al-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**3**), respectively. To our best knowledge, compand **3** appears to be novel, which was named as wujiapioside A.

Key words: *Acanthopanax gracilistylus*, Lupane-triterpene glycoside, Wujiapioside A, 3α-Hydroxy-lup-23-al-20(29)-en-28-oic acid 28-O-α-L-Rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1 →6)-β-D-glucopyranosyl ester, Acankoreoside A and acankoreoside D

INTRODUCTION

There are more than 26 species of *Acanthopanax* (Araliaceae) widely distributed in China, and the dried roots and stem barks of *Acanthopanax gracilistylus* W.W. Smith are listed officially in the Chinese pharmacopoeia as Cortex Acanthopanacis (Wujiapi), which has been used medicines for the treatment of paralysis, arthritis, rheumatism, lameness, and liver disease (Pharmacopoeia of China, 2000). Some of lignan and diterpene derivatives from the stem and root barks of *Acanthopanax gracilistylus* have been isolated and identified (Xiang *et al*, 1983; Tang *et al*, 1995; Song *et al*, 1983). In our previous papers (Liu *et al*, 2001; Liu *et al*, 2002a; Liu *et al*, 2002b; Yook *et al*, 2002), we have reported the isolation and structures of volatile components, lignans, diterpenes, phytosterols and lupane-triterpene glycosides from the same plant source.

In this manuscript as a continuing study on *Acanthopanax* genus, we describe the isolation and structure determination of a novel lupane-triterpene glycoside, called wujiapioside A (**3**) from the leaves of *A. gracilistylus*, together with two known lupane-triterpene glycosides, which were determined as acankoreoside A (**1**), and acankoreside D (**2**) by comparing their spectroscopic data with the previously reported ones. A hot MeOH extract of the leaves of *A. gracilistylus* was fractioned on Diaion HP-20P 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 **1~3**.

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MATERIALS AND METHODS

General procedure

Melting points (uncorrected) were measured using a Boetius micromelting point apparatus. Optical rotations were determined on a JASCO DIP-1000KUY 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-DX303HF instruments, and NMR spectra were measured in pyridine-d_5 on a JEOL-α-500 spectrometer and chemical shifts were relative to tetramethylsilane (TMS). Column chromatography (CC) was carried out on silica-gel 230~400 mesh (Merck). TLC was performed on precoated silica gel 60GF_{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 dried in the shade, which was identified by 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 *A. gracilistylus* (350 g) were extracted with hot MeOH repeatedly to give an extract (50 g), which was chromatographed on Diaion HP-20P (Mitsubishi Chem. Ind. Co., Ltd., Japan) by elution with H_2O, 30%, 50%, 80% and 100% MeOH successively. The elution of 80%MeOH was evaporated to dryness *in vacuo*, and was subsequently chromatographed on silica-gel using $CHCl_3$-MeOH-H_2O (9:1:0.1→7:3:0.5) to give seven fractions. Fraction No.7 was chromatographed on silica-gel column using $CHCl_3$-MeOH-H_2O (7:3:0.5) to give **1** (1.2 g). Fraction No.6 was chromatographed on Sephadex LH-20 column using MeOH to obtain **2** (16 mg). Fraction No.3 was chromatographed on silica-gel column using $CHCl_3$-MeOH-H_2O (7:3:0.2→7:3:0.5), and a reverse phase column, Chromatorex ODS (30-50 μm, Fuji Silysia Chem. Ind. Co. Ltd., Japan), using gradient elution 60% MeOH to 100% MeOH to give **3** (70 mg).

Acankoreoside A (**1**). White powder, mp 226~228°C (from dil. MeOH); $[\alpha]_D$-41.2°(c=0.5 in EtOH); ^{1}H-NMR (500 MHz, pyrindine-d_5, δ in ppm, *J* in Hz): 4.28 (1H, brs, H-3), 1.66 (1H, d, *J* = 11.6, H-9), 2.67 (1H, m, H-13), 3.38 (1H, m, H-19), 1.46 (3H, s, H-24), 0.95 (3H, s, H-25), 1.20 (3H, s, H-26), 0.87 (3H, s, H-27), 4.72 (1H, brs, H-29a), 4.85 (1H, brs, H-29b), 1.70 (3H, s, H-30); C-28-*O*-inner glc: 6.34 (1H, d, *J* = 7.9, H-1), 4.08 (m, H-2), 4.21 (m, H-3), 4.30 (m, H-4), 4.09 (m, H-5), 4.28 (H-6) and 4.67 (1H, d, *J* = 9.8, H-6); glc: 4.95 (1H, d, *J* = 7.9, H-1), 3.93 (1H, t, *J* = 9.2, H-2), 4.13 (H-3), 4.39 (1H, t, *J* = 9.5, H-4), 3.65 (1H, d, *J* = 9.2, H-5), 4.07 (1H, d, *J* = 10.4, H-6) and 4.19 (H-6); rha: 5.84 (1H, brs, H-1), 4.66 (1H, brs, H-2), 4.54 (1H, d, *J* = 9.2, H-3), 4.34 (H-4), 4.96 (H-5) and 1.69 (3H, d, *J* = 6.1, H-6). ^{13}C-NMR data (125 MHz, pyrindine-d_5): see Table I.

Acankoreoside D (**2**). White powder, mp 222~224°C (from dil. MeOH); $[\alpha]_D$-40.8°(c=0.5 in EtOH); ^{1}H-NMR (500 MHz, pyrindine-d_5, δ in ppm, *J* in Hz): 4.02 (1H, brs, H-3), 1.90 (1H, d, *J* = 13.0, H-9), 4.30 (m, H-11), 2.88 (1H, dt, *J* = 11.0 and 9.2, H-13), 3.39 (1H, m, H-19), 1.18 (3H, s, H-24), 1.26 (3H, s, H-25), 1.22 (3H, s, H-26), 1.01 (3H, s, H-27), 4.63 (1H, brs, H-29a), 4.81 (1H, brs, H-29b), 1.64 (3H, s, H-30); C-28-*O*-inner glc: 6.35 (1H, d, *J* = 7.9, H-1), 4.10 (m, H-2), 4.22 (m, H-3), 4.31 (m, H-4), 4.11 (H-5), 4.27 (H-6) and 4.68 (1H, d, *J* = 11.2, H-6); glc: 4.95 (1H, d, *J* = 8.0, H-1), 3.94 (1H, t, *J* = 9.7, H-2), 4.15 (H-3), 4.39 (1H, t, *J* = 9.6, H-4), 3.65 (1H, brd, *J* = 10.0, H-5), 4.11 (H-6) and 4.22 (H-6); rha: 5.82 (1H, brs, H-1), 4.70 (1H, brs, H-2), 4.54 (1H, d, *J* = 9.6, H-3), 4.34 (H-4), 4.95 (H-5) and 1.71 (3H, d, *J* = 6.0, H-6). ^{13}C-NMR data (125 MHz, pyrindine-d_5): see in Table I.

Wujiapioside A (**3**). A white powder, mp 230~234°C (from dil. MeOH); $[\alpha]_D$-34.4°(c=1.0 in MeOH); Negative HRFAB-MS m/z 939.4999[M-H]$^-$(cald. For $C_{48}H_{76}O_{18}$-H: 939.4955); IR ν_{max}^{KBr} cm^{-1}: 3416 (OH), 2940 (alphatic CH), 1721 (ester carbonyl), 1639 (C=C), 1067 (ether). ^{1}H-NMR (500 MHz, pyrindine-d_5) and ^{13}C-NMR (125 MHz, pyrindine-d_5) data see Table I; Its HMBC correlations were in Table II.

Alkaline hydrolysis of 3

Compound **3** (30 mg) was hydrolyzed with 8 ml of 5%KOH in MeOH for 2 hrs at 80°C. The reaction mixture was neutralized with 2 N HCl in H_2O, extracted with EtOAC three times. The aqueous layer was filtered and concentrated, D-glucose and L-rhamnase were detected by TLC. The organic layer was evaporated *in vacuo* and residue was purified by silica gel CC (solv. $CHCl_3$-MeOH-H_2O = 9:1:0.1). The obtained aglycone fraction was recrystallized from dil. MeOH to give **4** (6 mg). Compound **4**: A white powder, ^{1}H-NMR (500 MHz in pyridine-d_5) δ ppm: 1.07, 1.08, 1.21, 1.33 (each 3H, s, H-24, 25, 26, 27), 1.80 (3H, s, H-30), 2.23 (m, H-18), 2.62 (m, H-13), 3.50 (m, H-19), 4.16 (1H, dd, *J* = 4.9 and 11.2 Hz, H-3α), 4.76 and 4.93 (each 1H, brs, H-29). ^{13}C-NMR data (125 MHz, pyrindine-d_5): see Table I.

Acid hydrolysis of 3

Compound **3** (25 mg) was hydrolyzed with 5 ml of 2 N HCl in MeOH for 3 hrs at 80°C. The reaction mixture was neutralized with 2 N NaOH and extracted with EtOAC three times. The organic layer 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 aglycone fraction was recrystallized from dil MeOH to give **5** (5 mg). Compound **5**: A white powder, ^{1}H-NMR (500 MHz, pyridine-d_5) δ ppm: 0.86, 0.96, 1.14, 1.16 (each 3H, s, H-24, 25, 26, 27), 1.78 (3H, s, H-30), 2.25 (m, H-18), 2.60 (1H, d, *J* = 10.1 Hz, H-5), 2.70 (1H, m, H-13), 3.52 (1H, brs, H-19), 3.68 (1H, H-3β), 4.53 and 4.78 (each 1H, brs, H-29). ^{13}C-NMR data (125 MHz, pyrindine-d_5): see Table I.

RESULTS AND DISCUSSION

A hot MeOH extract of the leaves of *A. gracilistylus* was fractioned on Diaion HP-20P 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 **1~3**.

Compound **1** was a white powder, mp 226~228°C (dil. MeOH), $[\alpha]_D$-41.2° (c=0.5 in EtOH), gave a positive reaction in the Liebermann-Burchard and the Molish tests. ^{1}H-NMR

Table 1. ^{13}C-NMR data for compounds **1~5** (125 MHz, pyridine-d_5, δ in ppm)

Position	1	2	3	4	5
Aglycone					
1	32.9 t	35.2 t	33.1 t	38.9 t	34.2 t
2	26.2 t	27.2 t	26.1 t	27.3 t	27.1 t
3	73.0 d	73.1 d	73.0 d	71.7 d	72.9 d
4	52.0 s	53.0 s	52.5 s	56.6 s	53.0 s
5	44.9 d	44.2 d	44.1 d	47.9 d	44.6 d
6	21.8 t	21.4 t	21.0 t	21.6 t	21.1 t
7	34.5 t	35.6 t	34.6 t	34.1 t	34.7 t
8	41.8 s	42.8 s	41.8 s	41.5 s	42.0 s
9	51.0 d	56.0 d	50.6 d	50.8 d	50.7 d
10	37.4 s	39.0 s	37.7 s	36.3 s	38.1 s
11	20.9 t	69.4 d	21.1 t	21.1 t	21.3 t
12	26.0 t	38.2 t	26.0 t	26.0 t	26.7 t
13	38.3 d	37.4 d	38.4 d	38.6 d	38.4 d
14	42.9 s	43.4 s	42.9 s	42.9 s	42.8 s
15	30.1 t	30.0 t	30.1 t	30.2 t	30.3 t
16	31.9 t	32.3 t	32.3 t	32.8 t	32.6 t
17	57.0 s	56.9 s	57.0 s	56.5 s	57.2 s
18	49.7 d	49.5 d	49.8 d	49.7 d	49.8 d
19	47.4 d	47.2 d	47.5 d	47.9 d	47.6 d
20	150.8 s	150.4 s	150.9 s	151.3 s	150.8 s
21	30.8 t	30.9 t	30.9 t	31.2 t	30.8 t
22	36.9 t	36.8 t	36.9 t	37.4 t	36.9 t
23	179.0 s	210.1 s	209.8 s	207.4 s	209.7 s
24	18.0 q	15.0 q	14.8 q	9.4 q	14.7 q
25	16.8 q	16.9 q	16.4 q	16.3 q	16.6 q
26	16.7 q	17.8 q	16.5 q	16.5 q	16.7 q
27	14.8 q	15.0 q	14.6 q	14.9 q	14.8 q
28	174.9 s	175.0 s	175.0 s	178.8 s	178.3 s
29	110.0 t	110.2 t	110.1 t	110.0 t	110.4 t
30	19.4 q	19.5 q	19.4 q	19.5 q	19.6 q
C 28-O-inner glc					
1	95.2 d	95.3 d	95.3 d		
2	74.0 d	74.0 d	74.0 d		
3	78.7 d	78.3 d	78.7 d		
4	70.8 d	70.9 d	70.8 d		
5	77.9 d	77.1 d	77.1 d		
6	69.4 t	69.7 t	69.4 t		
glc'(' →6)glc					
1	105.1 d	105.1 d	105.1 d		
2	75.3 d	75.3 d	75.3 d		
3	76.4 d	76.5 d	76.5 d		
4	78.2 d	78.7 d	78.3 d		
5	77.1 d	78.0 d	78.0 d		
6	61.3 t	61.3 t	61.4 t		
rha(' →4)glc'					
1	102.7 d	102.7 d	102.7 d		
2	72.5 d	72.6 d	72.6 d		
3	72.7 d	72.8 d	72.8 d		
4	73.9 d	74.1 d	74.1 d		
5	70.3 d	70.3 d	70.3 d		
6	18.5 q	18.5 q	18.6 q		

All assignments of ^{13}C-NMR signals were confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC spectra. glc: β-D-glucopyranosyl; rha: α-L-rhamnopyranosyl. s: singlet; d: doublet; t: triplet; q: quartet; Multiplicities were deduced from a DEPT experiment.

Table II. ^{1}H-NMR and HMBC correlations in compound **3** (in pyridine-d_5, δ ppm, 500 MHz)[a)]

H-Position	δH	Cross peaks (C) in HMBC spectrum
Aglycone		
1	1.26 (m*)	26.1 (C-2), 73.0 (C-3), 16.4 (C-23)
2	1.84 (m)	33.1 (C-1)
3	4.01 (1H, brs)	33.1 (C-1), 14.8 (C-24)
4		
5	2.41 (1H,brd, 9.6)	14.8 (C-24)
6	1.46 (m*)	
7	1.26 (m*)	50.6 (C-9)
8		
9	1.68 (1H, d, 11.8)	37.7 (C-10)
10		
11	1.47 (m*), 1.15 (m*)	26.0 (C-12)
12	1.72 (m*)	
13	2.65 (m*)	
14		
15	1.98 (m*), 1.19 (m*)	38.4 (C-13), 42.9 (C-14)
16	2.65 (m*)	175.0 (C-28)
17		
18	1.70 (m*)	47.5 (C-19), 150.9 (C-20), 175.0 (C-28)
19	3.39 (1H, m)	
20		
21	2.01 (m*), 1.43 (m*)	
22	2.20 (m*)	49.8 (C-18), 175.0 (C-28)
23		
24	1.12 (3H, s)	73.0 (C-3), 52.5 (C-4) ,44.1 (C-5), 209.8 (C-23)
25	1.18 (3H, s)	33.1 (C-1), 44.1 (C-5), 50.6 (C-9)
26	0.86 (3H, s)	34.6 (C-7), 50.6 (C-9), 42.9 (C-14)
27	0.95 (3H, s)	41.8 (C-8), 38.4 (C-13), 42.9 (C-14), 30.1 (C13)
28		
29	4.73 (1H, brs), 4.86 (1H, brs)	47.5 (C-19), 19.4 (C-30)
30	1.70 (3H, s)	47.5 (C-19), 150.9 (C-20), 110.1 (C-29)
C-28-O-inner glc		
1	6.36 (1H, d, 8.0)	175.0 (C-28)
2	4.10 (m*)	95.3 (g-1), 78.7 (g-3)
3	4.19 (m*)	74.0 (g-2), 70.8 (g-4)
4	4.31 (m*)	78.7 (g-3)
5	4.11 (m*)	95.3 (g-1), 69.4 (g-6)
6	4.32 (m*), 4.69 (1H, d, 11.4)	77.2 (g-5), 105.1 (g-1')
glc'(1→6)glc		
1	4.95 (1H, d, 7.9)	69.5 (g-6)
2	3.95 (1H, t-like, 10.0)	75.3 (g-1'), 76.5 (g-3')
3	4.14 (m*)	78.3 (g-4')
4	4.39 (1H, t-like, 10.0)	76.5 (g-3'), 77.2 (g-5'), 102.7 (r-1)
5	3.66 (1H, d, 11.1)	61.4 (g-6)
6	4.11 (m*), 4.22 (m*)	
rha(1→4)glc'		
1	5.82 (1H, brs)	78.0 (g-4'), 72.6 (r-2), 72.3 (r-3), 70.3 (r-5)
2	4.70 (1H, brs)	72.8 (r-3)
3	4.55 (1H, dd, 4.6, 12.0)	74.1 (r-4)
4	4.29 (1H, d, 9.1)	72.8 (r-3), 18.6 (r-6)
5	4.94 (m*)	18.6 (r-6)
6	1.69 (3H, d, 6.6)	74.1 (r-4), 70.3 (r-5)

All assignments of NMR were comfirmed by ^{1}H-^{1}H COSY, HMQC, HMBC spectra. a): *J* values (in Hz) in parentheses; glc: β-D-glucopyranosyl; rha: α-L-rhamnopyranosyl. s: singlet; d: doublet; t; triplet; q: quarterplet; brs: broad singlet; dd: double doublet; m: multiplet; * Overlapped signals.

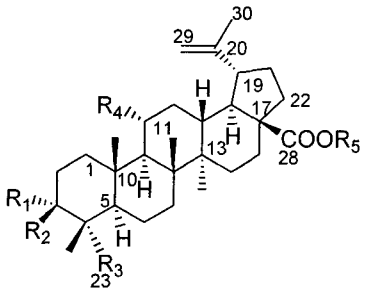
spectrum (500 MHz, in pyridine-d_5) showed signals due to five tertiary methyl groups at δ 0.87, 0.95, 1.20, 1.46 and 1.70 ppm, one secondary methyl group at δ ppm 1.69 (3H, d, J = 6.3 Hz), three anomeric protons due to two hexosyl residues at δ ppm 4.95 (1H, d, J = 7.9 Hz) and 6.34 (1H, d, J = 7.9 Hz) and one methylpentosyl residue at δ ppm 5.84 (1H, brs), and two olefinic protons at δ 4.72 (1H, brs), 4.85 (1H, brs), respectively. ^{13}C-NMR and DEPT spectrum (125 MHz, in pyridine-d_5) (as listed in Table I) suggested 48 total carbons, including one ester carboxyl group, an carboxylic carbon, five tertiary methyl groups, one secondary methyl group, one 1,1-disubsituted double bond, one oxygen bearing methine carbon besides hexosyl residues Thus, compound **1** was deduced to be a lupane-triterpene glycoside. Moreover, Its NMR data were identical with those of acankoreoside A isolated from the leaves of *Acanthopanax koreanum* (Chang *et al*, 1998). Therefore, compound **1** was determined as acankoreoside A.

Compound **2** was a white powder, mp 222~224°C(dil. MeOH), $[\alpha]_D$-40.8°(c=0.5 in EtOH), gave a positive reaction in the Liebermann-Burchard and the Molish tests. ^{1}H-NMR spectrum (500 MHz, in pyridine-d_5) showed signals due to five tertiary methyl groups at δ 1.01, 1.18, 1.22, 1.25 and 1.64 ppm, one secondary methyl group at δ ppm 1.71 (3H, d, J = 6.0 Hz), three anomeric protons due to two hexosyl residues at δppm 4.95 (1H, d, J = 8.0 Hz) and 6.35 (1H, d, J = 7.9 Hz) and one methylpentosyl residue at δ ppm 5.82 (1H, brs), and two olefinic protons at δ 4.63 (1H, brs) and 4.81 (1H, brs), respectively. ^{13}C-NMR and DEPT spectrum (125 MHz, in pyridine-d_5) suggested 48 carbons, including one ester carboxyl group, an aldehyde carbonyl group, five tertiary methyl groups, one secondary methyl group, one 1,1-disubsituted double bond, two oxygen bearing methine carbons besides hexosyl residues (listed in Table I). Thus, compound **2** was deduced to be a lupane-triterpene glycoside. Moreover, Its NMR data were good agreement with those of acankoreoside D isolated from *A. koreanum*.(Chang *et al*, 1999). Therefore, compound **2** was determined as acankoreoside D.

Compound **3** obtained as a white powder, gave a positive reaction in the Liebermann-Burchard and the Molish tests. mp 230~234°C (from dil. MeOH); $[\alpha]_D$-34.4°(c=1.0 in MeOH); Its IR spectrum showed the absorption for hydroxyl groups (3416 cm^{-1}), ester carbonyl group (1721 cm^{-1}) and double bond (1639 cm^{-1}). The HR-FAB-MS spectrum provided a formula of $C_{48}H_{76}O_{18}$ with a peak at 939.4999[M-H]$^-$ (cald. for $C_{48}H_{76}O_{18}$-H: 939.4955), as well as [M-H-sugar residue]$^-$ at m/z 469, which was also confirmed by ^{13}C-NMR and DEPT spectral data. The ^{1}H-NMR spectrum (500 MHz in pyridine-d_5, δ ppm) showed signals due to five tertiary methyl groups at δ 0.86, 0.95, 1.12, 1.18 and 1.70, one secondary methyl group at δ1.69 (3H, d, J = 6.3 Hz), three anomeric protons due to two hexosyl residues at δ 4.95 (1H, d, J = 7.9 Hz) and δ 6.36(1H, d, J = 8.0 Hz), and one methylpentosyl residue at δ 5.82 (1H, brs), two olefinic protons at δ4.73 (1H, brs) and δ 4.86 (1H, brs), respectively (Table II). The chemical shift at δ 6.36 assignable to a hexosyl anomeric proton and the IR absorption at 1721 cm^{-1} suggested the presence of an ester glycosyl linkage. The carbon signals observed in the ^{13}C-NMR spectrum (Table I) suggested the presence of total 48 carbons, including one aldehyde group at 209.8, one ester carboxyl group at 175.0 and one 1,1-disubstituted double bond at 110.1 and 150.9, one oxygen-bearing methiene carbon at 73.0 and three anomeric carbons at 95.3, 102.7 and 105.1. Therefore, **3** was deduced to be a lupane-triterpene glycoside.

Moreover, the heteronuclear multiple bond correlations (HMBC) from inner glucose H-1 at δ 6.36 (1H, d, J = 8.0 Hz) to C-28 at δ 175.0 (s) of the aglycone, from outer glucose H-1 at δ 4.95 (1H, d, J = 7.9 Hz) to inner glucose C-6 at δ 69.4(t), and from rhamnose H-1 at δ 5.82 (1H, brs) to outer glucose C-4 at δ 78.3 (d) were observed. These evidences suggested the sequence of the sugar linkage of **3**. Furthermore, its NMR data were very similar to those of **2**, except for the absence of one oxygen-bearing methine carbon signal of C-11 at δ 69.8 (d) observed in **3** and the presence of one methylene carbon signal at δ 21.1 (t) in **3**. Therefore, **3** was hydrolyzed with 2 N HCl to give an aglycone **5**, 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 **3** was found to be composed of β-D-glucopyranose and α-L-rhamnopyranose. Measurements of 2D-NMR spectra of ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectroscopy enabled the respective signals to be assigned (Table I and Table II). Moreover, the carbon signals due to this sugar moiety were identical with those of compounds **1** and **2**.

The ^{1}H-NMR spectrum (500 MHz, pyridine-d_5) of **5** displayed signals due to five teriary methyl groups, two olefinic protons and one oxygen bearing methine protons. The carbon signals observed on the ^{13}C-NMR spectrum (Table I) suggested the presence of one carboxyl group, one 1,1-disubstituted double bond, an aldehyde carbonyl group, one oxygen bearing methine carbons, six methine carbons, ten methylene carbons and five methyl carbons. All the ^{13}C-NMR data of **5** were very similar to those of aglycone of acankoreoside D (Chang *et al*, 1999), except for significant upfield shifts of C-9 (-5.3 ppm), C-11 (-47.5 ppm), C-12 (-11.5 ppm) of **5** were observed than those of the aglycone of acankoreoside D isolated from *A. koreanum*, owing to an effect of dehydroxylation at C-11 position in **5**. Measurements of 2D-NMR spectra of **5** enabled the respective signals to be assigned. Based on the above data, **5** was identified as the 3α-hydroxy-lup-20(29)-en-23-al-



	R_1	R_2	R_3	R_4	R_5
1	OH	H	COOH	H	S
2	OH	H	CHO	OH	S
3	OH	H	CHO	H	S
4	H	OH	CHO	H	H
5	OH	H	CHO	H	H

S = -β-D-glc^6-β-D-glc^4-α-L-rha

Fig. 1. The structures of compounds **1~5**

28-oic acid. To our best knowledge, this sapogenol has not been reported yet. On the other hand, alkaline hydrolysis of **3** gave a sapogenol **4** different from **5**, which showed a double double signal (J = 4.9 Hz, 11.2 Hz) at δ4.16 assigned to H-3α, and then the carbon chemical shift of C-3 in **4** was also downfield shift than that of **5.** Therefore, the structure of **4** was deduced to 3β-hydroxy-lup-20 (29)-en-23-al-28-oic acid. According to the reference (Chang *et al*, 1999), we assumed that the α-orientation of hydroxyl group at C-3 in **5** was also converted in β-orientation by alkali reaction.

Based on the above data and chemical features, the structure of **3** was determined to be 3α-hydroxy-lup-23-al-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyransyl ester. This compound appears to be found for the first time in the plant kingdom and we called it wujiapioside A.

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