New Oleanene Glycosides from the Leaves of *Acanthopanax japonicus*

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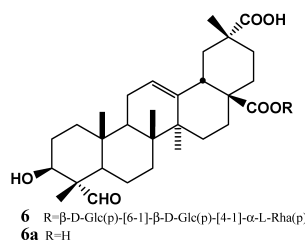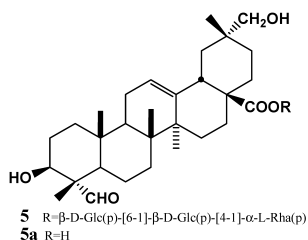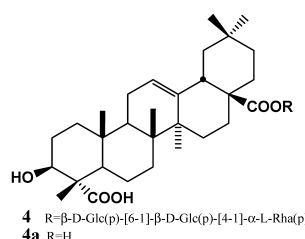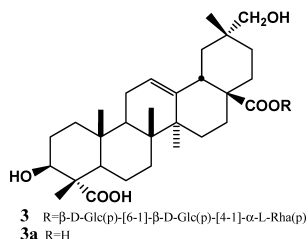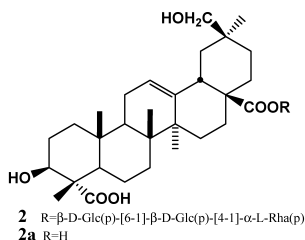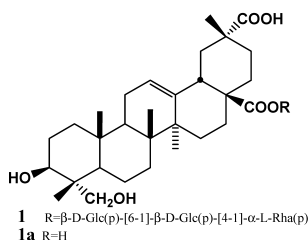
The structures of 6 new oleanene glycosides (1—6) isolated from the leaves of *Acanthopanax japonicus* Franch *et.* Savart (Araliaceae) were elucidated by mass, 1D, and 2D NMR spectroscopy. The structures of 1—6 were established as 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester of 3β,23-dihydroxy-olean-12-en-28,29-dioic acid, 3β,30-dihydroxy-olean-12-en-23,28-dioic acid, 3β,29-dihydroxy-olean-12-en-23,28-dioic acid (=dianic aicd), 3β-dihydroxy-olean-12-en-23,28-dioic acid (=gypsogenic acid), 3β,29-dihydroxy-23-oxo-olean-12-en-28-oic acid, and 3β-hydroxy-23-oxo-olean-12-en-28,29-dioic acid, designated acanjaposide D (1), E (2), F (3), G (4), H (5), and I (6), respectively.

Key words   *Acanthopanax japonicus*; Araliaceae; oleanene-type glycoside; acanjaposide D—I

The *Acanthopanax* genus is an important herbal drug used to strengthen sinew and bone, and to disperse water swelling in traditional oriental medicine. The leaves and roots of this genus, as well as stem bark, have been generally taken as a health food in Korea. Earlier,[1] we reported on the isolation and characterization of *nor*-oleanene type glycosides (acanjaposides A—C) from the methanolic extract of the leaves of *Acanthopanx japonicus* Franch *et.* Savart (Araliaceae) distributed in Japan as an endemic species in our course of phytochemical studies on the genus of *Acanthopanax*. Further studies on this extract led to the isolation of 6 new glycosides of oleanene derivatives, based on their spectroscopic data including HR-MS, ^{1}H- and ^{13}C-NMR spectroscopic data, designated acanjaposide D (1), E (2), F (3), G (4), H (5), and I (6).

Compound 1, acanjaposide D, was obtained as a white amorphous powder of molecular formula $C_{48}H_{76}O_{20}$ as determined by positive HR-FAB-MS. It exhibited a molecular ion peak at *m/z* 995.4826 due to $[M+Na]^+$ (Calcd for $C_{48}H_{76}O_{20} \cdot Na$: 995.4828). The ^{1}H-NMR spectrum of 1 revealed signals for 5 tertiary methyl groups [δ 1.00, 1.05, 1.13, 1.18, 1.43 (3H, s)], a secondary methyl group [δ 1.68 (3H, d, *J*=6.1 Hz)], 1 trisubstituted olefinic proton [δ 5.49 (1H, br s)], a methine proton [δ 3.30 (1H, dd, *J*=3.2, 13.7 Hz)] and 3 anomeric protons [δ 6.27 (1H, d, *J*=8.6 Hz), 5.82 (1H, s), 4.98 (1H, d, *J*=7.9 Hz)]. The ^{13}C-NMR spectrum of compound 1 showed 48 carbon signals, of which 30 were assigned to a triterpene moiety and 18 to three sugar moieties. Alkaline hydrolysis of 1 yielded 1a as a white amorphous powder, which had a molecular ion peak at *m/z* 502 and characteristic fragment ion peaks at *m/z* 278 and 233 (produced by a retro Diels-Alder cleavage of C-ring in its EI-MS).[2,3] The ^{1}H-NMR spectrum of 1a showed signals for 5 tertiary methyl groups [δ 0.97, 1.05, 1.05, 1.24, 1.57 (3H, s)], an olefinic proton (δ 5.58 br s), an oxygen-bearing methine proton [δ 4.18 (1H, dd, *J*=5.5, 11.0 Hz)], 2 oxygen-bearing methylene protons [δ 3.71 (1H, d, *J*=11.0 Hz) and 4.16 (1H, d, *J*=11.0 Hz)] and a methine proton [δ 3.44 (1H, dd, *J*=3.7, 13.7 Hz)]. The ^{13}C-NMR spectrum of 1a showed 30 carbon signals, assignable to 5 methyl carbons, 11 methylene carbons, 5 methine carbons, and 9 quaternary carbons by DEPT and HMQC spectra. Among 2 carbonyl groups, C-28 (δ 180.6) showed a glycosylation-induced shift (Table 1). These findings suggested that aglycone 1a was a Δ^{12}-unsaturated oleanene glycoside of $C_{30}H_{46}O_6$, and that 1 was a 28-*O*-monodesmoside with tri-saccharides.[4] Acid hydrolysis of 1 afforded the sugar moieties of L-rhamnose and D-glucose which were identified by GLC.[1] The identity of the single sugar moiety and the sequence of the oligosaccharide chain were determined by HMBC, HMQC and ^{1}H–^{1}H COSY. The 2 glucose moieties were ascertained to have β-anomeric configuration from $^3J_{H1-H2}$ coupling constants [δ 6.27 (1H, d, *J*=8.6 Hz, Glc H-1), 4.98 (1H, d, *J*=7.9 Hz, Glc′ H-1)], and the anomeric configuration of the rhamnose moiety was determined to be the α-form from ^{13}C-NMR chemical shifts of the C-3 and C-5 rhamnosyl moieties (δ 72.7, 70.3, respectively).[5] Therefore, the sugar moiety was determined to be 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl ester, which is common in the *Acanthopanax* species.[6—12] The proton signal at δ 4.18 (1H, dd, *J*=5.2, 11.0 Hz) was assigned to H-3 by 2D-NMR, and correlated with δ_C 73.6 in the HMQC spectrum and the cross

1 R=β-D-Glc(p)-[6-1]-β-D-Glc(p)-[4-1]-α-L-Rha(p)
1a R=H

2 R=β-D-Glc(p)-[6-1]-β-D-Glc(p)-[4-1]-α-L-Rha(p)
2a R=H

3 R=β-D-Glc(p)-[6-1]-β-D-Glc(p)-[4-1]-α-L-Rha(p)
3a R=H

4 R=β-D-Glc(p)-[6-1]-β-D-Glc(p)-[4-1]-α-L-Rha(p)
4a R=H

5 R=β-D-Glc(p)-[6-1]-β-D-Glc(p)-[4-1]-α-L-Rha(p)
5a R=H

6 R=β-D-Glc(p)-[6-1]-β-D-Glc(p)-[4-1]-α-L-Rha(p)
6a R=H

peak between H-24 (δ 1.05) and C-3 (δ 73.6) in the HMBC spectrum. The hydroxyl group at C-3 appeared to have a β-configuration based on its coupling constants in the ^{1}H-NMR spectrum.[13] The proton signals at δ 3.71 and 4.16 were found to originate from the H-23 of oxymethylene group at C-4 based on the HMBC correlation between H-24 (δ 1.05) and C-23 (δ 68.1), and the functional group was concluded to be in the α-configuration according to ^{13}C-NMR spectral data (δ_{C-23} 68.1, δ_{C-24} 13.1).[1] Two carboxylic carbon signals at 180.6 and 181.0 were assigned to C-28 and C-29, respectively, by their HMBC and HMQC. These assignments were confirmed by NOESY spectrum, which showed a NOE correlation between the tertiary methyl proton signal (δ 1.43, H-30) and the methine proton signal at 3.30 (dd, $J=3.7$, 13.7 Hz, H-18). This assignment was ascertained by comparison with the ^{13}C-NMR spectrum of serratagenic acid.[14,15] Accordingly, the structure of **1a** was determined as 3β,23-dihydroxy-olean-12-ene-28,29-dioic acid.

Therefore, **1** was determined to be 3β,23-dihydroxy-olean-12-ene-28,29-dioic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside, and was termed acanjaposide D.

The second new compound (**2**), called acanjaposide E, showed a quasimolecular ion peak at m/z 996 due to [M+Na]$^+$ by FAB-MS and its ^{13}C-NMR spectrum showed the presence of 48 carbon signals (CH$_3$×6, –CH$_2$–×11, >CH–×3, >C<×6, CH$_2$–O×3, >CH–O×13, O–CH–O× 3, >C=CH–×1, and CO×2). These data indicated that acanjaposide E (**2**) had the molecular formula C$_{48}$H$_{76}$O$_{20}$. This assignment is consistent with HR-FAB-MS spectrum showing m/z 995.4821 (Calcd for C$_{48}$H$_{76}$O$_{20}$·Na: 995.4828). Alkaline hydrolysis of **2** afforded **2a**, a novel aglycone, and we verified by sugar analysis and by ^{1}H- and ^{13}C-NMR that the sugar moiety was the same as that of **1**. The HR-FAB-MS spectrum of **2a** had a quasimolecular ion peak at m/z 525.3187 due to [M+Na]$^+$, which corresponds to the molecular formula C$_{30}$H$_{46}$O$_6$. Moreover, the ^{13}C-NMR resonance signals attributable to the carbons of the A, B, C and D-rings of the aglycone (**2a**) matched those of gypsogenic acid (**4a**) (Table 1). Furthermore, although the ^{13}C-NMR spectrum of the E-ring of **2a** differed from that of nipponoside D (3β,23,29-trihydroxy-olean-12-en-28-oic acid glycoside) previously isolated from this species,[1] the observed ^{13}C-NMR data of C-29 and C-30 were in good agreement with methyl querotaroate[15] and spinoside C2.[9] The above results and their ^{13}C-NMR data indicated that **2a** was 30-hydroxygypsogenic acid with an axial oxymethylene group at C-20. This conclusion was confirmed by 2D-NMR and NOESY, which showed a NOE correlation between H-30a [δ 3.92 (1H, $J=10.4$ Hz)] to H-18 [δ 3.44 (1H, dd, $J=6.4$, 11.9 Hz)] and H-24 [δ 1.64 (3H, s)] to H-25 [δ 0.96 (3H, s)]. Thus, **2** was identified as 3β,30-dihydroxy-olean-12-en-23,28-dioic acid (30-hydroxy gypsogenic acid) 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside, and called acanjaposide E.

Compound **3**, acanjaposide F, had a quasimolecular ion peak at 995.4835 due to [M+Na]$^+$ in the HR-FAB-MS, corresponding to the molecular formula C$_{48}$H$_{76}$O$_{20}$ (Calcd for C$_{48}$H$_{76}$O$_{20}$·Na: 995.4828). The ^{13}C-NMR spectrum of **3** showed 48 carbon signals, 30 of which were assigned to the aglycone moiety and 18 to an oligosaccharide, which ap-

peared to be the same as that of **1** based on 1D and 2D-NMR data which included ^{1}H–^{1}H COSY and HMBC experiments. Sugar analysis confirmed that the oligosaccharide was 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside, like that of acanjaposide E (**1**).

When compound **3** was subjected to alkali hydrolysis with 3% KOH it yielded the sapogenin (**3a**) which contained five tertiary methyl groups [δ 0.98, 1.04, 1.22, 1.27, 1.64 (3H, s)], one oxymethylene group [δ 3.59 (2H, s)], one oxymethine proton [δ 4.68 (1H, dd, $J=7.9$, 7.9 Hz)], a tri-substituted olefinic proton [δ 5.54 (1H, br s)], and a methine proton [δ 3.42 (1H, dd, $J=3.4$, 13.6 Hz)]. The molecular formula of **3a**, C$_{30}$H$_{46}$O$_6$, was established by positive FAB-MS (m/z 525, [M+Na]$^+$) and EI-MS, which showed a molecular ion at m/z 486 and fragments at m/z 264 and 219 produced by retro Diels-Alder cleavage of the C-ring in olean-12-ene.

NMR analysis and MS spectral data demonstrated that compound **3a** was a 3β,29-dihydroxyl-olean12-en-23,28-dioic acid, which is an analogue of gypsogenic acid with a hydroxyl group attached at C-29 (=29-hydroxyl gypsogenic acid), called dianic acid.[16] Therefore, compound **3** was identified as 3β,29-hydroxy-olean-12-en-23,28-dioic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside, and termed acanjaposide F.

In the HR-FAB-MS, compound **4** exhibited a quasimolecular ion at m/z 979.4905 due to [M+Na]$^+$ corresponding to the molecular formula C$_{48}$H$_{76}$O$_{19}$. Since this compound was suspected to be a triterpenoidal saponin due to the positive Lieberman–Burchard reaction and by the ^{13}C-NMR spectral analysis, **4** was also subjected to alkali hydrolysis and yielded the aglycone **4a**, which showed a quasimolecular ion peak at 509 due to [M+Na]$^+$ by positive FAB-MS, and a molecular ion (C$_{30}$H$_{46}$O$_6$) at m/z 486 along with fragments at m/z 248 and 203 in EI-MS. Consequently, **4a** was identified as gypsogenic acid based on ^{1}H- and ^{13}C-NMR spectral data (Table 1).[16] It was ascertained by sugar analysis and its NMR spectral data that the sugar moiety of **4** was the same as that of **1**. Thus, compound **4** was identified as gypsogenic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside, and called acanjaposide G.

Acanjaposide H (**5**), a white powder, produced quasimolecular ion at m/z 979.4884 due to [M+Na]$^+$ in the positive HR-FAB-MS, and its molecular formula was determined to be C$_{48}$H$_{76}$O$_{19}$ from this MS and its NMR data (Calcd for C$_{48}$H$_{76}$O$_{19}$Na: 979.4878).

^{1}H- and ^{13}C-NMR spectrum of **5** exhibited three anomeric proton signals at δ 6.25 (d, $J=7.9$ Hz), 4.99 (d, $J=7.9$ Hz), and 5.85 (br s) and anomeric carbon signals at δ 95.6, 104.9, and 102.7, respectively. The ^{13}C-NMR spectrum showed 48 carbon signals of which 30 were assigned to the aglycone moiety and 18 to the oligosaccharide. The 1D and 2D-NMR data including ^{1}H–^{1}H COSY and HMBC experiments, and sugar analysis allowed the assignments of its sugar components and sequence to 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside, as for acanjaposide E (**1**).

Alkali hydrolysis of **5** afforded the novel sapogenin (**5a**), which showed 5 tertiary methyl groups [δ 0.90, 1.00, 1.22, 1.30, 1.35 (all 3H, s)], 1 oxymethylene proton [δ 3.60 (2H, s)], 1 oxymethine proton [δ 4.06 (1H, dd, $J=7.3$, 8.6 Hz)], a tri-substituted olefinic proton [δ 5.23 (1H, br s)], and a me-

Table 1. ^{13}C-NMR Data for Compounds **1—6** and Their Algycone in Pyridine-d_5 (125 MHz)

C	1	1a	2	2a	3	3a	4	4a	5	5a	6	6a
1	38.8 t	38.8 t	39.1 t	39.1 t	39.1 t	39.0 t	39.0 t	39.0 t	38.5 t	38.5 t	38.5 t	38.5 t
2	27.5 t	27.6 t	27.8 t	27.8 t	27.8 t	27.8 t	27.7 t	27.7 t	27.1 t	27.0 t	27.0 t	27.0 t
3	73.4 d	73.6 d	75.5 d	75.5 d	75.5 d	75.5 d	75.5 d	75.5 d	71.7 d	71.7 d	71.6 d	71.6 d
4	42.8 s	42.9 s	54.5 s	54.5 s	54.4 s	54.4 s	54.4 s	54.4 s	56.3 s	56.3 s	56.3 s	56.3 s
5	48.5 d	48.7 d	51.9 d	52.0 d	51.9 d	52.0 d	51.9 d	51.9 d	48.0 d	48.0 d	47.9 d	48.0 d
6	18.5 t	18.6 t	21.7 t	21.7 t	21.7 t	21.7 t	21.7 t	21.7 t	21.1 t	21.0 t	21.0 t	21.0 t
7	32.8 t	32.9 t	33.0 t	33.0 t	32.9 t	33.0 t	32.9 t	33.0 t	32.5 t	32.7 t	32.4 t	32.5 t
8	39.9 s	39.8 s	40.3 s	40.0 s	40.3 s	40.1 s	40.2 s	40.1 s	40.2 s	40.1 s	40.2 s	40.1 s
9	48.1 d	48.1 d	48.4 d	48.4 d	48.4 d	48.4 d	48.3 d	48.4 d	47.7 d	47.7 d	47.6 d	47.7 d
10	37.2 s	37.2 s	36.9 s	36.9 s	36.9 s	36.9 s	36.8 s	36.7 s	36.1 s	36.2 s	36.1 s	36.2 s
11	23.8 t	23.9 t	23.8 t	23.8 t	23.9 t	23.8 t	23.8 t	23.9 t	23.8 t	23.8 t	23.7 t	23.8 t
12	123.5 d	123.3 d	122.9 d	122.5 d	122.8 d	122.4 d	122.7 d	122.4 d	122.6 d	122.3 d	123.2 d	122.9 d
13	143.6 s	144.3 s	144.1 s	144.8 s	144.4 s	145.0 s	144.1 s	144.9 s	144.4 s	145.1 s	143.7 s	144.1 s
14	42.1 s	42.2 s	42.2 s	42.2 s	42.1 s	42.1 s	42.1 s	42.2 s	42.2 s	42.2 s	42.1 s	42.2 s
15	28.2 t	28.3 t	28.4 t	28.4 t	28.3 t	28.3 t	28.2 t	28.3 t	28.3 t	28.3 t	28.1 t	28.2 t
16	23.4 t	23.9 t	23.7 t	24.0 t	23.4 t	23.7 t	23.3 t	23.7 t	23.4 t	23.7 t	23.4 t	23.8 t
17	47.0 s	46.6 s	47.2 s	46.7 s	47.5 s	47.1 s	47.0 s	46.6 s	47.5 s	47.1 s	46.9 s	46.6 s
18	40.8 d	41.1 d	41.3 d	41.6 d	41.1 d	41.4 d	41.6 d	42.0 d	41.2 d	41.5 d	40.8 d	41.1 d
19	40.8 t	41.0 t	41.7 t	42.0 t	41.0 t	41.3 t	46.2 t	46.5 t	40.9 t	41.2 t	40.8 t	41.0 t
20	42.3 s	42.6 s	35.7 d	35.9 d	36.4 d	36.6 d	30.7 d	31.0 d	36.4 s	36.6 s	42.3 s	42.6 s
21	29.1 t	29.3 t	29.5 t	29.6 t	28.8 t	29.1 t	33.9 t	34.2 t	28.8 t	29.1 t	29.1 t	29.3 t
22	31.7 t	32.4 t	32.3 t	33.1 t	32.0 t	32.7 t	32.4 t	33.2 t	32.0 t	32.6 t	31.6 t	32.3 t
23	67.9 t	68.1 t	180.7 s	180.7 s	180.7 s	180.6 s	180.7 s	180.3 s	207.3 q	207.3 q	207.4 q	207.3 q
24	13.1 q	13.1 q	12.3 q	12.3 q	12.3 q	12.2 q	12.2 q	12.3 q	9.7 q	9.7 q	9.6 q	9.6 q
25	16.1 q	16.0 q	16.1 q	16.0 q	16.1 q	16.0 q	16.1 q	16.0 q	15.8 q	15.7 q	15.8 q	15.7 q
26	17.6 q	17.5 q	17.5 q	17.4 q	17.5 q	17.3 q	17.4 q	17.3 q	17.5 q	17.4 q	17.4 q	17.4 q
27	26.0 q	26.1 q	26.1 q	26.2 q	26.1 q	26.1 q	26.0 q	26.2 q	26.1 q	26.2 q	26.0 q	26.1 q
28	176.4 s	180.6 s	176.6 s	180.3 s	176.6 s	180.2 s	176.5 s	178.9 s	176.6 s	180.3 s	176.3 s	179.9 s
29	181.1 s	181.0 s	28.3 q	28.4 q	73.7 t	73.9 t	33.0 q	33.2 q	73.6 t	73.9 t	181.1 s	181.1 s
30	19.9 q	20.0 q	65.4 t	65.6 t	19.7 q	19.7 q	23.6 q	23.8 q	19.7 q	19.8 q	19.9 q	20.0 q
28-*O*-glc												
1′	95.7 d		95.7 d		95.7 d		95.6 d		95.6 d		95.7 d	
2′	73.8 d		73.8 d		73.9 d		73.8 d		73.9 d		73.8 d	
3′	78.6 d		78.7 d		78.7 d		78.6 d		78.7 d		78.7 d	
4′	70.8 d		70.9 d		70.8 d		70.8 d		70.8 d		70.7 d	
5′	77.9 d		77.9 d		78.0 d		78.0 d		78.0 d		78.0 d	
6′	69.2 t		69.4 t		69.2 t		69.1 t		69.2 t		69.2 t	
6′-*O*-glc												
1″	104.8 d		105.0 d		104.9 d		104.8 d		104.9 d		104.9 d	
2″	75.2 d		75.3 d		75.3 d		75.3 d		75.3 d		75.3 d	
3″	76.5 d		76.5 d		76.5 d		76.5 d		76.5 d		76.5 d	
4″	78.3 d		78.4 d		78.3 d		78.3 d		78.3 d		78.2 d	
5″	77.1 d		77.2 d		77.2 d		77.1 d		77.2 d		77.1 d	
6″	61.2 t		61.4 t		61.3 t		61.5 t		61.3 t		61.3 t	
4″-*O*-rha												
1	102.7 d		102.8 d		102.7 d		102.7 d		102.7 d		102.7 d	
2	72.5 d		72.6 d		72.6 d		72.5 d		72.6 d		72.5 d	
3	72.7 d		72.8 d		72.8 d		72.7 d		72.8 d		72.7 d	
4	73.9 d		74.0 d		74.0 d		73.9 d		74.0 d		73.9 d	
5	70.3 d		70.4 d		70.3 d		70.3 d		70.3 d		70.3 d	
6	18.5 q		18.5 q		18.5 q		18.6 q		18.5 q		18.5 q	

Multiplicities were obtained from DEPT experiments.

thine proton [δ 3.42 (1H, dd, $J=3.4$, 13.9 Hz)]. The EI-MS spectrum of **5a** showed a molecular ion at m/z 486 and fragments at m/z 264 and 219. On comparison of the ^{13}C-NMR spectra of **5** with acanjaposide B (3β-hydroxyl-23-oxo-olean-12, 20(29)-dien-28-oic acid glycoside)[1] and acanjaposide F(**3**), the signals due to A/B/C/D ring carbons were found the same as those of acanjaposide B, and the signals due to E ring carbons were the same as those of **3**. Furthermore, the correlation cross peaks from H-30 [δ 1.09 (3H, s)] to H-18 [δ 3.31 (1H, dd, $J=3.2$, 12.0 Hz)], and from H-24 [δ 1.36 (3H, s)] to H-25 [δ 0.94 (3H, s)] were observed in the NOESY spectrum of **5**. Therefore, acanjaposide H (**5**) was

determined to be 3β-hydroxyl-23-oxo-olean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **6** (acanjaposide I) exhibited a quasimolecular ion at m/z 993.4661 due to [M+Na]$^+$ corresponding to the molecular formula $C_{48}H_{74}O_{20}$ in the HR-FAB-MS. The ^{13}C-NMR spectrum of **6** showed 48 signals, which were similar to those of **5** except for an aglycone E-ring moiety assignable to C-17, C-20, and C-29 (Table 1). The carbon signals of the E-ring moiety, including the signal at δ 181.0 ascribable to carboxylic acid were similar to those of acanjaposide D (**1**). Its aglycone **6a**, obtained by alkali hydrolysis, showed a qua-
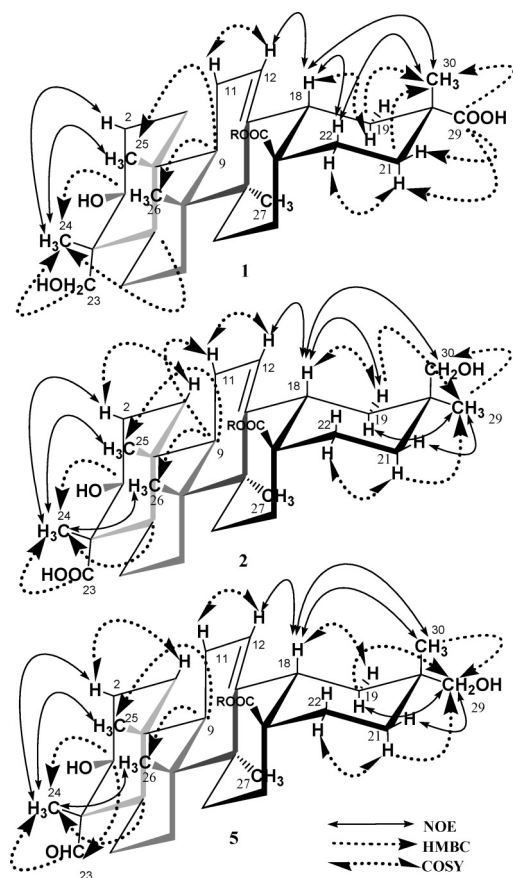
Fig. 1. Selected COSY, HMBC and NOE Data

simolecular ion at m/z 523 due to [M+Na]$^+$ in the positive FAB-MS, and a molecular ion peak at m/z 502 along with characteristic fragment peaks at m/z 264 and 233 in EI-MS corresponding to the molecular formula $C_{30}H_{44}O_6$. Thus, **6a** was putatively identified as a 3β-hydroxyl-23-oxo-olean-12-en-28,29-dioic acid by NMR. Its sugar moiety was the same as that of compounds **1**—**5** based on sugar analysis and NMR data. Therefore, acanjaposide I (**6**) was established as 3β-hydroxyl-olean-12-en-28,29-dioic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside.

From a chemotaxonomic point of view, it was of interest to note that the various oleanene analogues possessing a dicarboxylic acid (**1, 2, 4, 5, 6**), or an aldehyde (**3, 4**) of β-amyrin skeleton, have been found only in the *Acanthopanax* species.[6—12]

Experimental

General Experimental Procedures The equipment used, the plant material, the extraction and isolation of the saponins, and methods used for alkaline and acidic hydrolysis, sapogenin mixture chromatography, and sugar characterizations were as described previously.[1] Compounds **1**—**6** were obtained from the 80% MeOH fraction of Diaion-HP 20 described previously,[1] followed by silica gel column chromatography using CHCl$_3$–MeOH–H$_2$O (8 : 2 : 0.2→7 : 3 : 0.2) as eluent and reverse-phase column chromatography using a 50% to 100% MeOH gradient. In addition to acanjaposide A, B, and C previously reported, acanjaposide D (**1**, 53.5 mg), E (**2**, 19.5 mg), F (**3**, 467.4 mg), G (**4**, 250.9 mg), H (**5**, 425.3 mg), and I (**6**, 110.1 mg) were obtained during the present study.

Acanjaposide D (**1**): A white powder; $[\alpha]_D^{25}$ −12.0° (c=0.61, MeOH). Positive HR-FAB-MS m/z: 995.4826 (M+Na$^+$, Calcd for $C_{48}H_{76}O_{20}$·Na:

995.4828). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.00 (3H, s, H-25), 1.05 (3H, s, H-24), 1.13 (3H, s, H-26), 1.18 (3H, s, H-27), 1.31 (1H, m, H-7b), 1.43 (3H, s, H-30), 1.68 (3H, d, J=6.1 Hz, Rha H-6), 2.23 (1H, m, H-15a), 2.53 (1H, dd, J=14.0, 14.0 Hz, H-19a), 3.30 (1H, dd, J=3.2, 13.7 Hz, H-18), 3.68 (1H, d, J=10.3 Hz, H-23b), 3.62 (1H, d, J=10.7 Hz, Glc H-5), 3.68 (1H, d, J=10.3 Hz, H-23b), 4.98 (1H, d, J=7.9 Hz, Glc′ H-1), 5.49 (1H, br s, H-12), 5.82 (1H, s, Rha H-1), 6.27 (1H, J=8.6 Hz, Glc H-1). ^{13}C-NMR: Table 1.

Acanjaposide E (**2**): A white powder; $[\alpha]_D^{25}$ −3.6° (c=1.08, MeOH). Positive FAB-MS m/z: 996 (M+Na$^+$). Positive HR-FAB-MS m/z 995.4821 (Calcd for $C_{48}H_{76}O_{20}$·Na: 995.4828). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.00 (3H, s, H-25), 1.11 (3H, s, H-26), 1.14 (3H, s, H-29), 1.25 (3H, s, H-27), 1.65 (3H, s, H-24), 1.69 (3H, d, J=6.1 Hz, Rha H-6), 2.29 (1H, ddd, J=3.1, 13.4, 13.4 Hz, H-15a), 3.34 (1H, dd, J=6.7, 11.6 Hz, H-18), 3.63 (1H, d, J=10.7 Hz, Glc H-5), 3.80 (1H, d, J=10.4 Hz, H-23b), 3.84 (1H, d, J=10.4 Hz, H-23a), 4.98 (1H, d, J=7.9 Hz, Glc′ H-1), 5.44 (1H, br s, H-12), 5.84 (1H, s, Rha H-1), 6.22 (1H, J=8.5 Hz, Glc H-1). ^{13}C-NMR: Table 1.

Acanjaposide F (**3**): A white powder, $[\alpha]_D^{25}$ +1.9° (c=0.60, MeOH). Positive HR-FAB-MS m/z: 995.4835 (M+Na$^+$, Calcd for $C_{48}H_{76}O_{20}$·Na: 995.4828). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.02 (3H, s, H-25), 1.09 (3H, s, H-30), 1.14 (3H, s, H-26), 1.22 (3H, s, H-27), 1.65 (3H, s, H-24), 1.71 (3H, d, J=6.1 Hz, Rha H-6), 2.09 (1H, dd, J=13.7, 13.9 Hz, H-19a), 2.30 (1H, ddd, J=2.4, 12.8, 13.4 Hz H-15a), 3.30 (1H, dd, J=3.2, 13.7 Hz, H-18), 3.52 (2H, s, H-29), 3.63 (1H, d, J=10.7 Hz, Glc H-5), 4.99 (1H, d, J=7.9 Hz, Glc′ H-1), 5.47 (1H, br s, H-12), 5.85 (1H, s, Rha H-1), 6.25 (1H, J=7.9 Hz, Glc H-1). ^{13}C-NMR: Table 1.

Acanjaposide G (**4**): A white powder; $[\alpha]_D^{25}$ +1.5° (c=1.15, MeOH). Positive HR-FAB-MS m/z: 979.4905 (M+Na$^+$, Calcd for $C_{48}H_{76}O_{19}$·Na: 979.4879). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.87 (3H, s, H-29), 0.89 (3H, s, H-30), 1.02 (3H, s, H-25), 1.12 (3H, s, H-26), 1.20 (3H, s, H-27), 1.56 (1H, br d, J=13.5 Hz, H-6), 1.66 (3H, s, H-24), 1.70 (3H, d, J=6.1 Hz, Rha H-6), 2.28 (1H, ddd, J=3.4, 13.8, 13.8 Hz, H-15a), 3.18 (1H, dd, J=3.2, 12.9 Hz, H-18), 3.62 (1H, d, J=10.7 Hz, Glc H-5), 4.99 (1H, d, J=7.9 Hz, Glc′ H-1), 5.44 (1H, br s, H-12), 5.85 (1H, s, Rha H-1), 6.23 (1H, J=8.6 Hz, Glc H-1). ^{13}C-NMR: Table 1.

Acanjaposide H (**5**): A white powder; $[\alpha]_D^{25}$ +3.4° (c=0.54, MeOH). Positive HR-FAB-MS m/z: 979.4884 (M+Na$^+$, Calcd for $C_{48}H_{76}O_{19}$·Na: 979.4878). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.94 (3H, s, H-25), 1.09 (3H, s, H-30), 1.10 (3H, s, H-26), 1.25 (3H, s, H-27), 1.36 (3H, s, H-24), 1.57 (1H, br d, J=13.4 Hz H-1b), 1.70 (3H, d, J=6.1 Hz, Rha H-6), 2.14 (1H, dd, J=14.7, 14.7 Hz, H-19a), 2.30 (1H, ddd, J=3.1, 13.4, 13.4 Hz H-15a), 3.31 (1H, dd, J=3.2, 12.0 Hz, H-18), 3.55 (2H, s, H-29), 3.63 (1H, d, J=10.7 Hz, Glc H-5), 4.99 (1H, d, J=7.8 Hz, Glc′ H-1), 5.44 (1H, br s, H-12), 5.85 (1H, s, Rha H-1), 6.26 (1H, J=7.9 Hz, Glc H-1). ^{13}C-NMR: Table 1.

Acanjaposide I (**6**): A white powder; $[\alpha]_D^{25}$ +3.6° (c=0.56, MeOH). Positive HR-FAB-MS m/z: 993.4661 (M+Na$^+$, Calcd for $C_{48}H_{74}O_{20}$·Na: 993.4671). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.94 (3H, s, H-25), 1.09 (3H, s, H-26), 1.22 (3H, s, H-27), 1.36 (3H, s, H-24), 1.46 (3H, s, H-30), 1.58 (1H, br d, J=12.8 Hz H-1b), 1.71 (3H, d, J=6.1 Hz, Rha H-6), 2.28 (1H, ddd, J=2.4, 12.2, 13.5 Hz H-15a), 2.54 (1H, dd, J=13.7, 14.0 Hz, H-19a), 3.32 (1H, dd, J=4.3, 14.0 Hz, H-18), 3.62 (1H, d, J=10.7 Hz, Glc H-5), 4.99 (1H, d, J=7.9 Hz, Glc′ H-1), 5.49 (1H, br s, H-12), 5.85 (1H, s, Rha H-1), 6.26 (1H, J=7.9 Hz, Glc H-1). ^{13}C-NMR: Table 1.

Alkali Hydrolysis of Compounds 1—6 These compounds (34.4 mg, 15.0 mg, 48.0 mg, 64.8 mg, 52.3 mg, and 66.1 mg, respectively) were hydrolyzed with 2 ml of 3% KOH, and then neutralized and purified by column chromatography[1] to give compounds **1a** (17.3 mg), **2a** (8.0 mg), **3a** (20.1 mg), and **4a** (31.4 mg), **5a** (16.0 mg), and **6a** (31.1 mg), as their respective aglycone.

Sugar Analysis Each compound (about 3 mg) was hydrolyzed with 2 ml of 2 N HCl in H$_2$O for 4 h at 80 °C, which was followed by neutralization with 2 N NaOH in H$_2$O 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 °C to 250 °C at 15 °C/min. The retention times of persilylated glucose and rhamnose were found to be 10.8 and 7.01 min, respectively, when compared with the standard solutions prepared by the same reaction from the standard monosaccharides.

Acanjapogenin D (**1a**): White powder; $[\alpha]_D^{25}$ +56.2° (c=0.55, MeOH).

EI-MS m/z (%): 502 (M$^+$, 2), 488 (4), 278 (27), 264 (74), 233 (100), 206 (24), 201 (50), 187 (34), 175 (26), 159 (11), 119 (12), 105 (12). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.97 (3H, s, H-25), 1.05 (3H, s, H-24), 1.05 (3H, s, H-26), 1.24 (3H, s, H-27), 1.57 (3H, s, H-30), 1.76 (1H, m, H-9), 2.31 (1H, ddd, $J=3.9$, 13.4, 13.4 Hz, H-21a), 2.58 (1H, dd, $J=13.7$, 14.0 Hz, H-19a), 3.44 (1H, dd, $J=3.7$, 13.7 Hz, H-18), 3.71 (1H, d, $J=11.0$ Hz, H-23b), 4.16 (1H, d, $J=11.0$ Hz, H-23a), 4.18 (1H, dd, $J=5.2$, 11.0 Hz, H-3), 5.58 (1H, br s, H-12). ^{13}C-NMR: Table 1.

Acanjapogenin E (**2a**): White powder; $[\alpha]_D^{25}$ +55.1° ($c=0.50$, MeOH). Positive HR-FAB-MS m/z: 525.3192 (M+Na$^+$, Calcd for C$_{30}$H$_{46}$O$_6 \cdot$Na: 525.3192). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.96 (3H, s, H-25), 1.02 (3H, s, H-26), 1.20 (3H, s, H-29), 1.30 (3H, s, H-27), 1.64 (3H, s, H-24), 2.04 (1H, ddd, $J=6.1$, 6.1, 11.6 Hz, H-5), 3.44 (1H, dd, $J=6.4$, 11.9 Hz, H-18), 3.92 (1H, d, $J=10.4$ Hz, H-30b), 3.99 (1H, d, $J=10.4$ Hz, H-30a), 4.67 (1H, dd, $J=7.9$, 7.9 Hz, H-3), 5.51 (1H, br s, H-12). ^{13}C-NMR: Table 1.

Acanjapogenin F (**3a**): White powder; $[\alpha]_D^{25}$ +73.5° ($c=0.68$, MeOH). Positive FAB-MS m/z: 525 (M+Na$^+$, C$_{30}$H$_{44}$O$_6 \cdot$Na). EI-MS m/z (%): 502 (M$^+$, 1), 264 (36), 233 (100), 219 (21), 201 (78), 187 (42), 173 (24), 159 (31), 131 (29), 119 (27), 105 (42). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.98 (3H, s, H-25), 1.04 (3H, s, H-26), 1.22 (3H, s, H-30), 1.27 (3H, s, H-27), 1.40 (1H, br d, $J=12.2$ Hz, H-21b), 1.51 (1H, dd, $J=3.4$, 13.2 Hz, H-19b), 1.64 (3H, s, H-24), 3.42 (1H, dd, $J=3.4$, 13.6 Hz, H-18), 3.59 (2H, br s, H-29), 4.68 (1H, dd, $J=7.9$, 7.9 Hz, H-3), 5.54 (1H, br s, H-12). ^{13}C-NMR: Table 1.

Acanjapogenin G (**4a**): White powder; $[\alpha]_D^{25}$ +52.6° ($c=0.46$, MeOH). Positive FAB-MS m/z: 509 (M+Na$^+$, C$_{30}$H$_{46}$O$_5 \cdot$Na). EI-MS m/z (%): 486 (M$^+$, 1), 441 (4), 248 (78), 203 (100), 189 (22), 175 (24), 161 (13), 147 (17), 133 (36), 119 (32), 105 (34). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.94 (3H, s, H-29), 0.98 (3H, s, H-25), 1.01 (3H, s, H-30), 1.02 (3H, s, H-26), 1.13 (1H, br d, $J=12.2$ Hz, H-15b), 1.25 (3H, s, H-27), 1.63 (3H, s, H-24), 3.29 (1H, dd, $J=3.7$, 13.7 Hz, H-18), 4.67 (1H, dd, $J=6.9$, 8.6 Hz, H-3), 5.50 (1H, br s, H-12). ^{13}C-NMR: Table 1.

Acanjapogenin H (**5a**): White powder; $[\alpha]_D^{25}$ +63.4° ($c=0.50$, MeOH). Positive FAB-MS m/z: 509 (M+Na$^+$, C$_{30}$H$_{46}$O$_5 \cdot$Na). EI-MS m/z (%): 486 (M$^+$, 2), 456 (5), 441 (2), 264 (51), 233 (100), 219 (15), 201 (67), 187 (32), 175 (19), 159 (22), 119 (29), 105 (33). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.90 (3H, s, H-25), 1.00 (3H, s, H-26), 1.22 (3H, s, H-30), 1.30 (3H, s, H-27), 1.35 (3H, s, H-24), 1.57 (1H, br d, $J=12.8$ Hz, H-1b), 3.44 (1H, dd, $J=3.4$, 13.9 Hz, H-18), 3.60 (2H, s, H-29), 4.06 (1H, dd, $J=7.3$, 8.6 Hz, H-3), 5.23 (1H, br s, H-12). ^{13}C-NMR: Table 1.

Acanjapogenin I (**6a**): White powder; $[\alpha]_D^{25}$ +49.2° ($c=0.48$, MeOH), Positive FAB-MS m/z: 523 (M+Na$^+$, C$_{30}$H$_{44}$O$_6 \cdot$Na). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.90 (3H, s, H-25), 0.99 (3H, s, H-26), 1.28 (3H, s, H-27), 1.35 (3H, s, H-24), 1.58 (3H, s, H-30), 2.32 (1H, ddd, $J=3.2$, 12.5, 14.0 Hz, H-21a), 2.59 (1H, dd, $J=14.0$, 14.0 Hz H-19a), 3.43 (1H, dd, $J=3.7$, 14.0 Hz, H-18), 4.07 (1H, dd, $J=6.7$, 7.9 Hz, H-3), 5.55 (1H, br s, H-12). ^{13}C-NMR: Table 1.

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