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nor-Oleanene type triterpene glycosides from the leaves of *Acanthopanax japonicus*

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Abstract

Three new (**1–3**) and two known (**4–5**) triterpene glycosides were isolated from the leaves of *Acanthopanax japonicus* (Araliaceae) and elucidated structurally by mass, 1D, and 2D NMR spectroscopy. All the compounds possessed a *nor*-oleanene triterpene skeleton as the aglycone. The structures of **1–5** were established as 28-*O*-α-L-rhamno-pyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester of 3β-hydroxy- 30-*nor*-olean-12,20(29)-diene-23,28-dioic acid, designated as acanjaposide A, 3β- hydroxy-23-oxo-30-*nor*-olean-12,20(29)-diene-28-oic acid, named acanjaposide B, 3β,20α-dihydroxy-23-oxo-30-*nor*-olean-12-en-28-oic acid, named acanjaposide C, and nipponoside E, a known saponin, respectively. © 2002 Published by Elsevier Science Ltd.

Keywords: *Acanthopanax japonicus*; Araliaceae; Leaves; *nor*-Oleanene glycoside; Acanjaposide A, B and C

1. Introduction

In traditional oriental medication, the root and stem bark portions of *Acanthopanax* have been used as a tonic and prophylactic herbal drug. The leaves and roots of this plant have been also taken as health supplements in Korea. In the course of studies on the phytochemical constituents of *Acanthopanax* species, we previously reported the isolation of triterpene glycosides from the leaves of *Acanthopanax koreanum*, NAKAI *A. trifoliatus* (L.) MERR. (Chang et al., 1998; Yook et al., 1998; Chang et al., 1999), *A. divaricatus* SEEM. var. *albeofructus* YOOK (Oh et al., 2000), *A. senticosus* HARM. forma *inermis* HARM. (Park et al., 2000), and *A. divaricatus* SEEM. var. *sachunenesis* YOOK (Park et al., 2001). Among the *Acanthopanax* species, *Acanthopanax japonicus* FRANCH et. SAVART (= *Acanthopanax nipponicus* Makino) is only found in Japan as an endemic species. We report herein the isolation and structure determination of four *nor*-oleanene triterpene glycosides (**1–4**) from the leaves of *Acanthopanax japonicus*.

2. Results and discussion

The methanolic extract of the leaves of *Acanthopanax japonicus* gave three new triterpene saponins (**1–3**) and two known triterpene saponins (**4**, **5**), nipponoside E [3β,20α,23-trihydroxy-30-*nor*-olean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl -(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] and nipponoside D [3β,20α,29-trihydroxy-olean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside], respectively, isolated previously from the same species through various chromatographic isolation techniques (Miyakoshi et al., 1999).

Compound **1**, a white powder, had a molecular formula of $C_{47}H_{72}O_{19}$ as determined by analysis to positive HRFABMS (high resolution fast atom bombardment mass spectroscopy, *m*/*z* 963.4573 $[M+Na]^+$) and its ^{13}C NMR spectrum. The ^1H NMR spectrum (in pyridine-d_5) showed signals for four tertiary methyl groups (δ 1.01, 1.10, 1.16, 1.65), one secondary methyl group [δ 1.70 (*d*, *J* = 6.1 Hz)], three anomeric protons [δ 4.96 (*d*, *J* = 7.9 Hz), 5.84 (*b rs*), 6.18 (*d*, *J* = 7.9 Hz)], and three olefinic protons. The ^{13}C NMR spectrum showed 47 signals, of which 29 were assigned to a triterpenoid moiety and 18 to three sugar moieties. Acid hydrolysis of **1** afforded

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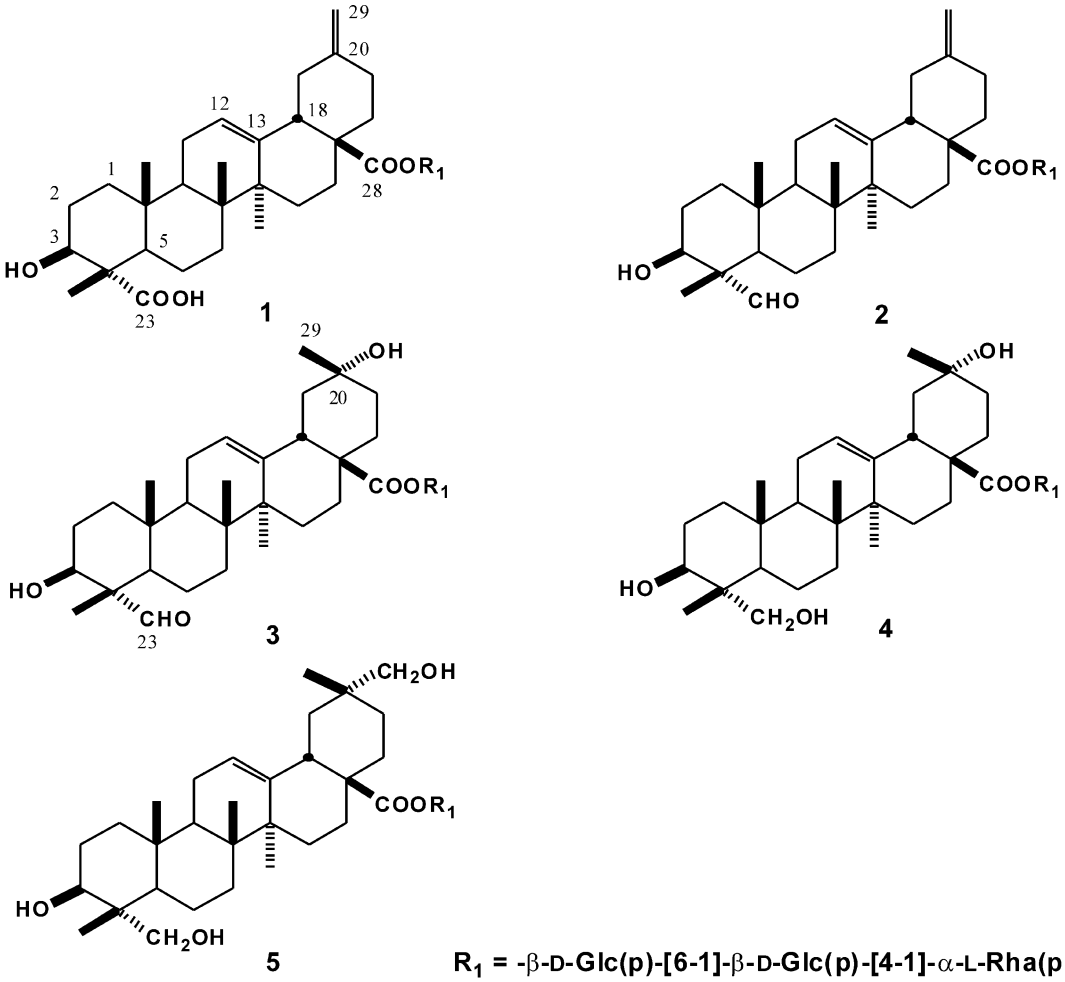

the sugar moieties of L-rhamnose and D-glucose. The assignments of all protons and carbons signals (Table 1) of the respective sugar components and the sequence of the oligosaccharide chain were determined by DEPT, COSY, HMQC, and HMBC spectra of **1**. In the HMBC spectrum of **1**, cross peaks between the inner Glc H-1 at δ 6.18 and the C-28 carboxylic carbon at δ 175.9, inner Glc C-6 at δ 69.4 and outer Glc H-1 δ 4.96, and inner Glc H-4 (δ 4.40) and the terminal rhamnose anomeric carbon (δ 102.9) were observed. β-Anomeric configuration for the two glucose moieties are proposed bases on their large $^{3}J_{H1,H2}$ coupling constants (7.9 and 7.9 Hz). The ^{13}C NMR chemical shift of C-5 (δ 70.4) of rhamnose indicated an α-orientation of its anomeric center (Nishimura et al., 1999). Therefore, the sugar moiety of **1** was determined to be 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester specifically present in the *Acanthopanax* species.

The sapogenol **1a,** obtained by alkali hydrolysis of **1**, exhibited a molecular ion peak at *m*/*z* 470 by EI mass spectral analysis, and its molecular formula was determined as $C_{29}H_{42}O_5$ by examination of the ^{13}C NMR spectrum. The ^{1}H NMR spectrum in pyridine-d_5 displayed signals due to four tertiary methyl groups, a pair of geminal olefinic protons [δ 4.75 (1H, *br s*), and δ 4.80 (1H, *br s*)], a trisubstituted olefinic proton [δ 5.51 (1H, *dd*, *J* = 3.4, 3.4 Hz)], and one oxygen-bearing proton [δ 4.17 (1H, *dd*, *J* = 7.3, 10.3 Hz)]. The ^{13}C NMR spectrum suggested the presence of two carboxyl groups, a trisubstituted and a disubstituted double bond, one oxygen-bearing methine carbon, three methine carbons, ten methylene carbons, and four methyl carbons as listed in Table 2. On the basis of the 1D and 2D NMR data, the trisubstituted double bond signals at δ 122.8 and 144.2 were assigned to C-12 and C-13, respectively, in the Δ^{12}-oleanene skeleton, a disubstituted double bond having geminal protons at C-20 (δ 149. 1) and C-29 (δ 107.1), and a hydroxyl group at C-3 (δ 75.5) with a β-configuration (Mahato et al., 1994). The correlation cross peaks between H_3-24 and H_3-25 and H-2 observed in the NOESY spectrum of **1a** suggested an α-orientation of C-23. The structure of **1a** was determined to be 3β-hydroxy-30-*nor*-olean-12,20(29)-diene-23,28-dioic acid by the above evidence and other NMR spectral data. Therefore, **1** was elucidated as 3β-hydroxy-30-*nor*-olean-12,20(29)-diene-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, and named as acanjaposide A.

Table 1
^{13}C and ^{1}H NMR spectral data for the sugar moieties of compound **1** in pyridine-d_5. (500 MHz, δ in ppm, J in Hz)

Position	δ_C	δ_H
C-28 *O*-inner Glc		
1	95.9 *d*	6.18 *d* (7.9)
2	73.9 *d*	4.09 *m*
3	78.8 *d*	4.19 *m*
4	70.9 *d*	4.27 *m*
5	78.1 *d*	4.05 *m*
6	69.4 *t*	4.31 *m*, 4.64 *m*
Outer Glc′		
1′	105.0 *d*	4.96 *d* (7.9)
2′	75.7 *d*	3.94 *dd* (8.4, 8.5)
3′	76.6 *d*	4.14 *m*
4′	78.4 *d*	4.40 *dd* (9.2, 9.8)
5′	77.3 *d*	3.66 *ddd* (2.4, 3.1, 9.2)
6′	61.5 *t*	4.08 *m*, 4.21 *m*
Terminal Rha		
1	102.9 *d*	5.84 *s*
2	72.7 *d*	4.68 *m*
3	72.9 *d*	4.55 *dd* (3.4, 9.5)
4	74.1 *d*	4.33 *dd* (9.2, 9.4)
5	70.4 *d*	4.95 *m*
6	18.6 *q*	1.70 *d* (6.1)

The second new compound, named acanjaposide B (**2**), showed an ion peak cluster due to $[M+Na]^+$ at m/z 947.4622 in the positive HRFABMS, corresponding to the molecular formula $C_{47}H_{72}O_{18}$ this also being consistent with the ^{13}C NMR spectroscopic data. The ^{1}H NMR spectrum revealed signals for four tertiary methyl groups (δ 0.94, 1.07, 1.21, and 1.38), a secondary methyl group, [δ 1.71 (*d*, $J=6.1$ Hz)], three anomeric protons [δ 4.97 (*d*, $J=7.9$ Hz), 5.85 (*br s*), and 6.20 (*d*, $J=7.9$ Hz)], a set of geminal olefinic protons [δ 4.70 (1H, br s), δ 4.76 (1H, *br s*)], and a trisubstituted olefinic proton [δ 5.46 (1H, *dd*, $J=3.4$, 3.4 Hz)]. It was verified by sugar analysis and its NMR spectral data data that the sugar moiety of **2** was the same as that of **1**. In the ^{13}C NMR spectral data, the signal at δ 207.4 was assigned to an aldehyde carbon at C-23, which was supported by comparison of the NMR spectral data with the A-ring moiety of the 3β-hydroxy-23-oxo-oleanene named gypsogenin (Nie et al., 1989) as well as the 2D NMR spectroscopic data.

These results led to the assignment of **2** as 3β-hydroxy-23-oxo- 30-*nor*-olean-12,20(29)-diene-28-oic acid, named acanjaposide B.

The positive HRFABMS of acanjaposide C (**3**) showed an ion peak cluster due to $[M+Na]^+$ at m/z 965.4715, and the ^{13}C NMR spectral data were also coincident with the molecular formula $C_{47}H_{74}O_{19}$. The ^{1}H NMR spectrum showed signals for five methyl groups (δ 0.93, 1.09, 1.21, 1.36, and 1.47), a secondary methyl group, [δ 1.71 (*d*, $J=6.7$ Hz)], three anomeric protons [δ 4.98 (*d*, $J=7.9$ Hz), 5.85 (*br s*), and 6.25 (*d*, $J=7.9$ Hz)], and a trisubstituted olefinic proton [δ 5.50 (1H, *dd*, $J=3.7$, 3.7 Hz)]. The ^{13}C NMR spectrum of the aglycone **3b**, obtained by alkaline hydrolysis, was similar to that of **2,** except for signals due to the E-ring, which showed a tertiary carbonyl carbon signal (δ 69.7). Thus, **3b** is suggested to be a 30-*nor*-oleanene having a tertiary hydroxyl group at C-20. Furthermore, correlation cross peaks from H_3-29 to H-18, and H_3-24 to H_3-25 and H-2 in the NOESY spectrum of **3b** suggested that the configurations of the two tertiary methyl groups of C-29 and C-24 were both in the β-orientations. All the other data for the 1D and 2D NMR spectra of **3** supported the proposed structure, and the ^{13}C NMR spectrum of the E-ring moiety of this triterpenoid was superimposable on that of nipponoside E (**4**), (Miyakoshi et al., 1999). It was assumed from the sugar analysis of **3** that the sugar chain was composed of L-rhamnose and D-glucose, and it was determined as 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester from its 1D and 2D NMR spectral data. Thus, acanjaposide C (**3**) was assigned as 3β,20α-dihydroxy-23-oxo-30-*nor*- olean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

These *nor*-oleanene triterpene glycosides are characteristic constituents in the leaves of *A. japonicus*, although other kinds of 30-*nor*-olean-12,20(29)-diene glycosides, named ciwuijianosides A_2, B, C_1, C_2, D_2 and E, have previously been found in leaves of *Acanthopanax senticosus* (Shao et al., 1988, 1989). This common structural feature might be a use from a chemotaxomic viewpoint. Interestingly, in of previous investigation by Miyakoshi et al. (1999), the structure of nipponoside E (**4**) was also elucidated as a *nor*-oleanene (*A. japonicus* = *A. nipponicus*). It would be considered that the difference in reports of these nor-oleanene triterpenes is caused by variations of plant constituents in different regional localities about the same plant.

Previously, the genus *Acanthopanax* was classified into three groups phytochemically by the types of their triterpenoids: oleanene, lupane, and 3,4-*seco*-lupane type (Miyakoshi et al., 1999; Park et al., 2000). These data would also contribute to construction of a chemotaxonomic classification dendrogram. Moreover, a RAPD study of the *Acanthopanax* species is in progress as an investigation of the genetic relationships of various *Acanthopanax* species at the DNA level may supplement ongoing chemotaxonomic studies.

3. Experimental

3.1. General procedures

Optical rotations were measured on a JASCO DIP-1000 polarimeter. ^{1}H and ^{13}C NMR spectra were run on

Table 2
^{13}C NMR data for compounds **1**, **1a**, **2**, **3**, **3a, 4** and **5** in pyridine-d_5 (δ in ppm, 500 MHz)[a]

	1	1°	2	3	3a	4	5
Aglycone							
C-1	39.2 *t*	39.1 *t*	38.5 *t*	38.6 *t*	38.5 *t*	38.8 *t*	38.8 *t*
C-2	27.9 *t*	27.8 *t*	27.0 *t*	27.1 *t*	27.0 *t*	27.6 *t*	27.6 *t*
C-3	75.4 *d*	75.5 *d*	71.7 *d*	71.7 *d*	71.7 *d*	73.5 *d*	73.5 *d*
C-4	54.6 *s*	54.5 *s*	56.3 *s*	56.3 *s*	56.3 *s*	42.9 *s*	42.9 *s*
C-5	52.1 *d*	51.9 *d*	47.9 *d*	48.0 *d*	47.9 *d*	48.6 *d*	48.6 *d*
C-6	21.8 *t*	21.7 *t*	21.0 *t*	21.1 *t*	21.0 *t*	18.6 *t*	18.6 *t*
C-7	33.0 *t*	33.0 *t*	32.4 *t*	32.5 *t*	32.5 *t*	32.9 *t*	32.8 *t*
C-8	40.4 *s*	40.1 *s*	40.2 *s*	40.3 *s*	40.1 *s*	39.9 *s*	39.9 *s*
C-9	48.5 *d*	48.3 *d*	47.7 *d*	47.7 *d*	47.7 *d*	48.1 *d*	48.2 *d*
C-10	37.0 *s*	36.8 *s*	36.1 *s*	36.1 *s*	36.1 *s*	37.2 *s*	37.2 *s*
C-11	23.9 *t*	23.8 *t*	23.7 *t*	23.8 *t*	23.8 *t*	23.8 *t*	23.9 *t*
C-12	123.3 *d*	122.8 *d*	123.1 *d*	122.8 *d*	122.4 *d*	123.1 *d*	122.9 *d*
C-13	143.6 *s*	144.2 *s*	143.5 *s*	143.7 *s*	144.4 *s*	143.7 *s*	144.3 *s*
C-14	42.2 *s*	42.1 *s*	42.1 *s*	42.2 *s*	42.2 *s*	42.2 *s*	42.1 *s*
C-15	28.3 *t*	28.3 *t*	28.2 *t*	28.3 *t*	28.2 *t*	28.3 *t*	28.4 *t*
C-16	23.6 *t*	23.7 *t*	23.5 *t*	23.5 *t*	23.7 *t*	23.6 *t*	23.4 *t*
C-17	47.4 *s*	47.0 *s*	47.3 *s*	47.1 *s*	46.7 *s*	47.1 *s*	47.5 *s*
C-18	47.7 *d*	47.9 *d*	47.5 *d*	44.1 *d*	44.4 *d*	44.1 *d*	41.1 *d*
C-19	41.8 *t*	42.0 *t*	41.7 *t*	47.8 *t*	48.1 *t*	47.8 *t*	40.9 *t*
C-20	148.5 *s*	149.1 *s*	148.3 *s*	69.7 *d*	69.9 *d*	69.7 *d*	36.4 *s*
C-21	30.2 *t*	30.4 *t*	30.1 *t*	35.9 *t*	36.2 *t*	35.9 *t*	28.8 *t*
C-22	37.7 *t*	38.3 *t*	37.6 *t*	34.4 *t*	35.1 *t*	34.4 *t*	32.0 *t*
C-23	180.8 *s*	180.6 *s*	207.4 *s*	207.4 *s*	207.5 *s*	67.9 *d*	67.9 *d*
C-24	12.4 *q*	12.3 *q*	9.7 *q*	9.7 *q*	9.7 *q*	13.1 *q*	13.1 *q*
C-25	16.2 *q*	16.0 *q*	15.8 *q*	15.8 *q*	15.6 *q*	16.1 *q*	16.1 *q*
C-26	17.6 *q*	17.3 *q*	17.5 *q*	17.5 *q*	17.3 *q*	17.6 *q*	17.6 *q*
C-27	26.2 *q*	26.1 *q*	26.0 *q*	26.0 *q*	26.0 *q*	26.0 *q*	26.0 *q*
C-28	175.9 *s*	179.4 *s*	175.8 *s*	176.4 *s*	179.9 *s*	176.5 *s*	176.5 *s*
C-29	107.5 *t*	107.1 *t*	107.4 *t*	25.7 *q*	25.7 *q*	25.6 *q*	73.7 *t*
C-30	–	–	–	–	–	–	19.7 *q*
C-28 *O*-inner Glc							
1	95.9 *d*		95.7 *d*	95.8 *d*		95.7 *d*	95.7 *d*
2	73.9 *d*		73.8 *d*	73.9 *d*		73.8 *d*	73.9 *d*
3	78.8 *d*		78.6 *d*	78.8 *d*		78.7 *d*	78.7 *d*
4	70.9 *d*		70.8 *d*	70.8 *d*		70.8 *d*	70.8 *d*
5	78.1 *d*		77.9 *d*	78.0 *d*		78.0 *d*	78.0 *d*
6	69.4 *t*		69.2 *t*	69.3 *t*		69.3 *t*	69.2 *t*
Outer glc′							
1′	105.0 *d*		104.9 *d*	105.0 *d*		104.9 *d*	104.9 *d*
2′	75.7 *d*		75.3 *d*	75.3 *d*		75.3 *d*	75.3 *d*
3′	76.6 *d*		76.5 *d*	76.5 *d*		76.5 *d*	76.5 *d*
4′	78.4 *d*		78.3 *d*	78.3 *d*		78.4 *d*	78.3 *d*
5′	77.3 *d*		77.1 *d*	77.2 *d*		77.2 *d*	77.1 *d*
6′	61.5 *t*		61.3 *t*	61.3 *t*		61.3 *t*	61.3 *t*
Terminal Rha							
1	102.9 *d*		102.7 *d*	102.8 *d*		102.7 *d*	102.7 *d*
2	72.7 *d*		72.5 *d*	72.6 *d*		72.6 *d*	72.6 *d*
3	72.9 *d*		72.8 *d*	72.8 *d*		72.7 *d*	72.7 *d*
4	74.1 *d*		74.0 *d*	74.0 *d*		74.0 *d*	74.0 *d*
5	70.4 *d*		70.3 *d*	70.4 *d*		70.4 *d*	70.3 *d*
6	18.6 *q*		18.5 *q*	18.5 *q*		18.5 *q*	18.5 *q*

[a] Multiplicity was deduced from a DEPT experiment.

a Joel α-500 spectrophotometer (500 MHz) in pyridine-d_5 with TMS as an internal standard. HRFABMS were measured with Joel HX-110 instrument and taken on a glycerol matrix containing NaI, and EIMS on Joel JMS-01SG and JMS-DX303HF instruments. Silica gel 60 (0.040–0.063 mm, Merck), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemicals) and Chromatorex ODS (30-50 μm, Fujisilysia Chemicals Ltd) were used for column chromatography. TLC was performed on pre-coated silica gel 60 F254 (Merck) and RP-18 F254S (Merck) plate, and spots were detected by spraying with 20% H_2SO_4, followed by heating at 100 °C. GLC was performed on a HP5890A gas chromatograph with a flame-ionization detector.

3.2. Plant material

Leaves of *Acanthopanax japonicus* were collected at the medicinal plant garden of Kumamoto University on November 1999, and botanically identified by Professor Chang-Soo Yook in the Department of Pharmacognosy in Kyung-Hee University. A voucher specimen has been deposited at the Herbarium of the College of Pharmacy, Kyung-Hee University.

3.3. Extraction and isolation

Dried leaves (240 g) of *Acanthopanax japonicus* were extracted repeatedly with hot MeOH. After removal of solvent by evaporation, the MeOH extract (57.1 g) was refluxed twice in boiling *n*-hexane (900 ml) and evaporated to dryness in vacuo. The residue (52.0 g) was next dissolved in H_2O and passed through a Diaion-HP 20 column, eluted sequentially with H_2O, 50% MeOH, 80% MeOH, and MeOH. The 80% MeOH eluate was concentrated, dried (10.8 g) and applied to a silica gel column with $CHCl_3$-MeOH-H_2O (9:2:0.1→8:2:0.2) as eluent to give five fractions. The fourth fraction was evaporated to dryness in vacuo, and the residue (2.54 g) dissolved in MeOH and passed through Sephadex LH-20 with MeOH as eluant. The fractions containing triterpene glycosides were combined, evaporated to dryness, and subjected to Chromatorex ODS chromatography, using gradient elution from 60% MeOH to 90% MeOH, to give **1** (199.1 mg), **2** (20.2 mg), **3** (84.6 mg), and **4** (73.5 mg).

3.4. Acanjaposide A (*1*)

White powder; $[\alpha]_D^{25}$ +24.8° (*c* 0.60, MeOH); HRFABMS *m/z*: 963.4573 $[M+Na]^+$, calculated for 963.4566 $[C_{47}H_{72}O_{19}+Na]^+$; ^{1}H NMR (pyridine-$d_5$) δ 1.01 (3H, *s*, H_3-25), 1.10 (3H, *s*, H_3-26), 1.16 (3H, *s*, H_3-27), 1.32 (1H, *ddd*, *J* = 2.4, 3.2,12.2 Hz, H-7*a*), 1.65 (3H, s, H_3-24), 1.70 (3H, *d*, *J* = 6.1 Hz, Rha H_3-6), 1.78 (1H, *dd*, *J* = 6.9,10.5 Hz, H-9), 2.27 (1H, *ddd*, *J* = 3.3, 13.7, 13.7 Hz, H-15*a*), 2.55 (1H, *dd*, *J* = 13.7, 13.7 Hz, H-19b), 3.10 (1H, *dd*, *J* = 4.9, 13.4 Hz, H-18), 4.68 (1H, *br s*, H-29*a*), 4.75 (1H, *br s*, H-29*b*), 4.96 (1H, *d*, *J* = 7.9 Hz, outer Glc H-1), δ 5.46 (1H, *dd*, *J* = 3.4, 3.4 Hz, H-12), 5.84 (1H, *s*, Rha H-1), 6.18 (1H, *d*, *J* = 7.9 Hz, inner Glc H-1); ^{13}C NMR: Table 2.

3.5. Acanjaposide B (*2*)

White solid; $[\alpha]_D^{25}$ +18.9° (*c* 0.82, MeOH); HRFABMS *m/z*: 947.4622 $[M+Na]^+$, calculated for 963.4566 $[C_{47}H_{72}O_{18}+Na]^+$; ^{1}H NMR (pyridine-$d_5$) δ 0.94 (3H, *s*, H_3-25), 1.07 (3H, *s*, H_3-26), 1.21 (3H, *s*, H_3-27), 1.38 (3H, *s*, H_3-24), 1.71 (3H, *d*, *J* = 6.1 Hz, Rha H_3-6), 2.59 (1H, *ddd*, *J* = 13.4, 13.4 Hz, H-19*a*), 3.13 (1H, *dd*, *J* = 4.3, 13.4 Hz, H-18), 4.70 (1H, *br s*, H-29*a*), 4.76 (1H, *br s*, H-29*b*), 4.97 (1H, *d*, *J* = 7.9 Hz, outer Glc H-1), 5.46 (1H, *dd*, *J* = 3.4, 3.4 Hz, H-12), 5.85 (1H, *s*, Rha H-1), 6.20 (1H, *d*, *J* = 7.9 Hz, inner Glc H-1);^{13}C NMR: Table 2.

3.6. Acanjaposide C (*3*)

White powder; $[\alpha]_D^{25}$ +6.5° (*c* 0.85, MeOH); HRFABMS *m/z* : 965.4715 $[M+Na]^+$, calculated for 965.4722 $[C_{47}H_{74}O_{19}+Na]^+$; ^{1}H-NMR (pyridine-$d_5$) δ 0.93 (3H, *s*, H_3-25), 1.09 (3H, *s*, H_3-26), 1.21 (3H, *s*, H_3-27), 1.36 (3H, *s*, H_3-24), 1.47 (3H, *s*, H_3-29), 1.55 (1H, d*dd*, *J* = 3.1, 3.1, 13.4 Hz, H-1*a*), 1.71 (3H, *d*, *J* = 6.7 Hz, Rha H_3-6), 2.40 (1H, *dd*, *J* = 13.8, 13.8 Hz, H-19*a*), 3.24 (1H, *dd*, *J* = 4.3, 13.8 Hz, H-18), 4.98 (1H, *d*, *J* = 7.9 Hz, outer Glc H-1), 5.50 (1H, *dd*, *J* = 3.7, 3.7 Hz, H-12), 5.85 (1H, *s*, Rha H-1), 6.25 (1H, *d*, *J* = 7.9 Hz, inner Glc H-1); ^{13}C NMR: Table 2.

3.7. Alkaline hydrolysis of *1* and *3*

Compounds **1** and **3** (40.1 and 43.0 mg, respectively) were hydrolyzed in 3% KOH in MeOH (3 ml) for 1 h at 80 °C followed by neutralization with 5% HCl in MeOH. After removal of solvent by evaporation, the residue was purified using Diaion HP-20P CC (30%MeOH→MeOH) and silica gel CC (hexane–ethylacetate = 1:1→0:1) to give **1a** (16.0 mg), the aglycone of **1**, and **3a** (16.2 mg), the aglycone of **3**.

3.8. Compound *1a*

White powder, $[\alpha]_D^{20}$ +85.2° (*c* 0.50, MeOH); EIMS *m/z* (rel. int.): 470 $[M]^+$ (3.7), 452 $[M-H_2O]^+$(1.8), 424 $[M-COOH-H]^+$ (6.6), 408 (4.9), 364 (3.5), 232 (39.7), 219 (38.7), 187 (base peak), 175 (39.3), 159 (25.1) 145 (26.5), 131 (28.6), 119 (30.2), 105 (47.5), 91 (27.8) ; ^{1}H NMR (pyridine-d_5) δ 0.97 (3H, *s*, H_3-25), 1.00 (3H, *s*, H_3-26), 1.20 (3H, *s*, H_3-27), 1.37 (1H, overlapped, H-7*a*), 1.65 (3H, *s*, H_3-24), 1.82 (1H, *dd*, *J* = 8.6, 8.6 Hz, H-9), 2.24 (1H, *dd*, *J* = 3.4, 13.6 Hz, H-19*a*), 2.62 (1H, *dd*, *J* = 13.6, 13.6 Hz, H-19*b*), 3.23 (1H, *dd*, *J* = 4.9, 13.4 Hz, H-18), 4.17 (1H, *dd*, *J* = 7.3, 10.3 Hz, H-3), 4.75 (1H, br *s*, H-29*a*), 4.80 (1H, *br s*, H-29*b*), 5.51 (1H, *dd*, *J* = 3.4, 3.4 Hz, H-12); ^{13}C NMR: Table 2.

3.9. Compound *3a*

White powder, $[\alpha]_D^{20}$ +62.7° (*c* 0.50, MeOH); EIMS *m/z* (rel. int.): 470 $[M-2H]^+$ (2.7), 452 $[M-H_2O]^+$(9.9), 436 $[M-2H_2O]^+$ (4.9), 424 $[M-H_2O-CHO-H]^+$ (4.9), 408 (9.5), 250 (11.0), 232 (76.8), 219 (51.9), 187 (base peak), 175 (44.7), 173 (56.4), 159 (39.9), 145 (38.8), 131 (38.0), 119 (42.3), 105 (64.1), 91 (37.6) ; ^{1}H NMR (pyridine-d_5) δ 0.89 (3H, *s*, H_3-25), 0.99 (3H, *s*, H_3-26), 1.26 (3H, *s*, H_3-27), 1.36 (3H, *s*, H_3-24), 1.59 (3H, *s*, H_3-29),

1.74 (1H, *dd*, $J=8.4$, 8.4 Hz, H-9), 2.46 (1H, *dd*, $J=13.7$, 13.7 Hz, H-19*b*), 3.37 (1H, *dd*, $J=3.1$, 14.0 Hz, H-18), 4.10 (1H, *dd*, $J=7.9$, 8.5 Hz, H-3), 5.56 (1H, *br s*, H-12); ^{13}C NMR: Table 2.

3.10. Acid hydrolysis of *1*, *2* and *3*

Each compound (3 mg) was individually hydrolyzed with aqueous 2 N HCl (2 ml) for 4 h at 80 °C, followed by neutralization with 2 N aqueous NaOH and extraction with $CHCl_3$. The H_2O layer of each reaction mixture was concentrated to dryness in vacuo. Each residue was then dissolved in dry pyridine, to which L-cysteine methyl ester hydrochloride was added, with each mixture being heated for 2 h at 60 °C, and concentrated under N_2 stream. Trimethylsilylimidazole was then added to each residue, and each mixture was heated for 1 h at 60 °C. After concentrating the solvent under N_2, the residue was extracted with *n*-hexane and H_2O, the organic layer was injected onto a gas chromatograph; OV-17 (0.32 mm×30 cm); detector, FID; column temperature, 230 °C; carrier gas, He. D-Glucose and L-rhamnose (molar ratio 2:1, respectively) were detected from **1–3**.

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