Two New Lupane-Triterpene Glycosides from Leaves of *Acanthopanax koreanum*

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Two new lupane-triterpene glycosides, acankorenside A (1) and B (2), were isolated from the leaves of *Acanthopanax koreanum* Nakai (Araliaceae). Based on spectroscopic data, the chemical structures of 1 and 2 were determined as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and 3α,11α,23,23-tetrahydroxy-lup-20(29)-en-28-oic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, respectively.

**Key words** *Acanthopanax koreanum*; Araliaceae; leaf; lupane-triterpene glycoside; acankorenside A and B

The roots and bark of *Acanthopanax* species (Araliaceae) are used as a tonic and sedative as well as a drug with ginseng-like activity. *Acanthopanax koreanum* Nakai is indigenous to Korea. 1)

Although lignan and diterpene derivatives have been reported in the stems and root bark of *A. koreanum*, 2)–6) few constituents have so far been isolated or characterized from the leaves of this plant.

In a previous paper, 7) we described the isolation and characterization of a lupane-triterpene glycoside (acantrifoside A) from the leaves of *Acanthopanax koreanum* and *A. trifoliatum*. As part of a continuing study on this crude drug, we report here the isolation and structural determination of two new lupane-triterpene glycosides, named acankorenside A (1) and B (2), as major components of the leaves of *A. koreanum*.

Acantrifoside A (1), obtained as a white powder, mp 225–228°C (dil. MeOH), [α]D 23417 cm⁻¹ and an ester carbonyl group at 1724 cm⁻¹ in the IR spectrum. The HR-FAB-MS provided a molecular ion peak for C₃₅H₆₆O₁₉, with a cluster ion peak at 979.4872 [M + Na]⁺ (Calcd for C₃₅H₆₆O₁₉Na: 979.4878). The ¹H-NMR spectrum (in pyridine-d₅) showed signals due to five tertiary methyl groups at δ 0.87, 0.95, 1.20, 1.46 and 1.70, one secondary methyl group at δ 1.69 (3H, d, J = 6.1 Hz), three anomic protons due to two hexosyl residues at δ 4.95 (1H, d, J = 7.9 Hz) and 6.34 (1H, d, J = 7.9 Hz) and one methylpentosyl residue at 5.84 (1H, brs) as listed in Table 1.

Therefore, 1 was deduced to be a triterpene glycoside. The chemical shift of the hexosyl anomeric proton signal appeared at δ 6.34 and the IR absorption at 1724 cm⁻¹ of 1 suggested that the sapogenol possessed an ester group, with a hexosyl moiety attached. Therefore, 1 was saponified in 0.5 M aqueous KOH to give an aglycone (3), mp 259–262°C, [α]D 3.1° (EtOH). It exhibited absorption bands due to a hydroxyl group at 3390 cm⁻¹ and a carbonyl group at 1702 cm⁻¹ in the IR spectrum. The positive FAB-MS of 3 exhibited a molecular ion peak due to [M−H]⁻ at m/z 587 (C₃₀H₄₆O₁₂⁻−H). The ¹H-NMR spectrum (pyridine-d₅) of 3 displayed signals due to five tertiary methyl groups, two olefinic protons and one oxygen-bearing methine proton (Table 1). The carbon signals observed in the ¹C-NMR spectrum (Table 2) suggested the presence of two carboxyl groups, one monosubstituted double bond, one oxygen-bearing methine carbon, five methine carbons, ten methylene carbons and five methyl carbons.

Based on the above data, 3 was identified as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid. 9) On the other hand, acid hydrolysis of 1 with 2N HCl gave a mixture of sugars and a sapogenol, which was identical with 3. The sugar mixture was derivatized to give the trimethylsilyl ethers of the corresponding methyl 2-(polyhydroxyalkyl)-thiazolidine-4(R)-carboxylates and analyzed by gas liquid chromatography (GLC) to show that it was composed of glucose and rhamnose. From the above facts and the coupling constants of anomic protons, 1 was found to be composed of β-D-glucopyranosyl and α-L-rhamnopyranosyl moieties. Measurements of ¹H, ²H and ¹³C two dimensional (2D) NMR spectra enabled the respective signals to be assigned.

Furthermore, heteronuclear multiple bond correlations (HMBC) from inner gICl H-1 at δ 5.36 (1H, d, J = 7.9 Hz) to C-28 at δ 174.9 (s) of the aglycone, from outer gICl H-1' at δ 4.95 (1H, d, J = 7.9 Hz) to inner gICl C-6 at δ 69.4 (t), and from rH-1 to δ 5.84 (1H, brs) to outer gICl C-4' at δ 78.2 (d) were observed. This evidence suggested the sequence of the sugar linkages of 1. Moreover, the carbon signals due to this sugar moiety were superimposable on those of chiisanoside isolated from *Acanthopanax chiisanensis* and *A. divaricatus* by Tanaka et al. 9–11)

Consequently, the structure of 1 was determined as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Acantrifoside B (2), obtained as a white powder, mp 220–223°C (dil. MeOH), [α]D 37.5° (EtOH). The negative FAB-MS exhibited a molecular ion peak due to [M−H]⁻ at m/z 957 (C₄₈H₇₂O₁₉-H). The ¹H-NMR spectrum (pyridine-d₅) showed signals due to five tertiary methyl groups, one secondary methyl group, three anomic protons due to two hexosyl residues and one methylpentosyl residue (Table 1). Therefore, taking into consideration the molecular formula, 2 was also deduced to be a triterpene glycoside. The chemical shift at δ 6.32 assignable to a hexosyl anomeric proton suggested

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the presence of an ester glycosyl linkage. Therefore, 2 was saponified with aqueous 0.5 M KOH to give an aglycone (4), mp 191—196 °C, [x]D = —3.1° (EtOH).

The positive FAB-MS of 4 exhibited a molecule ion peak due to [M + H]+ at m/z 489 (C14H24O6 + H). The 1H-NMR spectrum (pyridine-d5) of 4 displayed signals due to five tertiary methyl groups, two olefinic protons, two protons form a hydroxymethyl group and two oxygen-bearing methine protons as listed in Table 2. The carbon signals observed in the 13C-NMR spectrum (Table 2) suggested the presence of one carboxyl group, one monosubstituted double bond, a hydroxymethyl group, two oxygen-bearing methine carbons, five methine carbons, nine methane carbons and five methyl carbons.

Based on the above data, 4 was identified as the known 3x,11x,23-trihydroxy-lup-20(29)-en-28-oic acid. 12

1H- and 13C-signals and HMBC correlations of the sugar moiety in 2 were almost identical with those of 1, suggesting that the sequence of sugar linkages of 2 is the same as that of 1.

Consequently, the structure of 2 was determined as 3x,11x,23-tri-hydroxy-lup-20(29)-en-28-oic acid 28-O-alpha-L-rhamnopyranosyl-(1→4)-beta-D-glucopyranosyl-(1→6)-beta-D-glucopyranosyl ester.
chromatography (n-hexane:acetone=1:1). The obtained aglycone fraction was recrystallized from MeOH to give 3 (40 mg). 3. Colorless needles. mp 259—262 °C (EtOH); [α]D20 3.31 (c = 0.36 in EtOH); IR νmax cm⁻¹: 3300 (OH), 2946 (aliphatic CH), 1702 (ester carbonyl), 1643 (C=O); positive FAB-MS m/z: 487 [M+H]+; 1H- and 13C-NMR: see Tables 1 and 2.

Acid Hydrolysis of 1 Compound 1 (100 mg) was hydrolyzed with 4 ml 2N HCl in H2O for 4 h at 80 °C. The reaction mixture was neutralized with 2N NaOH in H2O and extracted with CHCl3. The organic layer was evaporated to give a residue, which was purified using Silica-gel column chromatography (n-hexane:acetone=1:1). The obtained aglycone fraction was recrystallized from MeOH to give 3 (32 mg). On the other hand, the aqueous layer was concentrated to dryness in vacuo. The remaining residue was dissolved in dry pyridine and combined with l-cystein methyl ester hydrochloride. The reaction mixture was then heated for 2 h at 60 °C and concentrated to dryness under a N2 gas stream. The residue was combined with trimethylsilyl-imidazole and heated for 1 h at 60 °C. The reaction mixture was concentrated to dryness under a N2 gas stream. The residue was extracted with n-hexane and H2O, and the organic layer was analyzed by GLC: column: OV-17 (0.32 mm x 30 m), detector: FID, column temp.: 230 °C, detector temp.: 270 °C, injector temp.: 270 °C, carrier gas: He (2.2 kg/cm²). Two peaks were observed at tR (min): 457” (l-Rha) and 712” (l-Glc). The standard monosaccharides were subjected to the same reaction and GLC analysis under the same conditions.

Alkaline Hydrolysis of 2 Compound 2 (130 mg) was hydrolyzed with 6 ml 0.5M KOH in MeOH for 1 h at 70 °C. The obtained aglycone fraction was recrystallized from MeOH to give 4 (46 mg). 4. Colorless needles. mp 101—106 °C (EtOH); [α]D20 37.5 °C (c = 0.37 in EtOH); IR νmax cm⁻¹: 3430 (OH), 2939 (aliphatic CH), 1727 (ester carbonyl), 1641 (C=O); Negative FAB-MS m/z: 957 [M-H]+; 1H- and 13C-NMR: see Tables 1 and 2.

Acid Hydrolysis of 2 Compound 2 (80 mg) was treated with acid in the same manner as I to give an aglycone 4 (27 mg) and a sugar mixture which was derivatized to the corresponding cysteine derivatives and detected as t-thiobimyl and d-glucosyl derivatives.

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