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3,4-Secolupane-Type Triterpene Glycosyl Esters from Leaves of *Acanthopanax divaricatus* SEEM

KAZUHIRO MATSUMOTO, RYOJI KASAI, FUJIKO KANAMARU,
HIROSHI KOHDA and OSAMU TANAKA*

*Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,
Kasumi, Minami-ku, Hiroshima 734, Japan*

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From leaves of *Acanthopanax divaricatus* (Araliaceae), two 3,4-secolupane type triterpene glycosyl esters, chiisanoside and isochiisanoside, were isolated, both of which have already been isolated from leaves and stem-bark of *A. chiisanensis*. Besides these compounds, a new glycoside named divaroside and hyperin(querletin-3-*O*- β -D-galactopyranoside) were also isolated from the leaves. Divaroside was established to be the β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester of chiisanogenin.

Keywords—*Acanthopanax divaricatus*; Araliaceae; divaroside; chiisanoside; isochiisanoside; hyperin; 3,4-secolupane-type triterpene; oligo-glycosyl ester

Recently, we reported the isolation and structural determination of three novel 3,4-secolupane-type triterpene glycosyl esters, chiisanoside (**1**) and its two homologues, isochiisanoside (**2**) and the methyl ester of **2**(**3**), from the leaves and stem-bark of *Acanthopanax chiisanensis* NAKAI (Araliaceae),^{1,2)} which is a Korean folk medicine used as a tonic, an anti-rheumatic and an anti-inflammatory. This is the first example of the isolation of 3,4-seco-triterpene glycosides from a natural source.

As a continuation of our work on glycoside constituents of *Acanthopanax* species, the chemical constituents of leaves of *A. divaricatus* SEEM (Japanese plant name: Keyama-ukogi), which grows widely in Japan, have been studied.

A methanolic extract of the leaves of *Acanthopanax divaricatus* (collected in Hiroshima) was defatted with ether and repeatedly chromatographed to give a new glycoside named divaroside (**4**) (yield: 0.03%) together with the known glycosides, chiisanoside (**1**) (yield: 3.0%), isochiisanoside (**2**) (yield: 0.09%) and hyperin (querletin-3-*O*- β -D-galactopyranoside) (**5**) (0.37%). The known glycosides **1** and **2** were identified by comparison of their proton and carbon-13 nuclear magnetic resonance (¹H- and ¹³C-NMR) spectra, electron impact mass spectra (EI-MS) of the trimethylsilyl ether and optical rotation ($[\alpha]_D$) with those of authentic samples, and the identification of **5** was established by comparison of the melting point (mp) and ¹³C-NMR spectrum with reference data.^{3,4)}

Acid hydrolysis of **4** gave D-glucose.⁵⁾ The ¹³C-NMR signals due to the aglycone moiety of **4** (Table I) were observed at almost the same positions as those of **1**, indicating that **4** is a glycosyl ester of chiisanogenin (**6**).²⁾ The ¹H- and ¹³C-NMR spectra showed the presence of two glucosyl units. The EI-MS of the trimethylsilyl ether of **4** exhibited fragment ions at *m/z* 829 [(Glc-Glc)TMS₇], 451[(Glc)TMS₄] and 583[Glc(TMS)₄-OCH₂-CH=O⁺-TMS], a characteristic fragment ion due to aldohexosyl(1 $\rightarrow$ 6)aldohexose.⁶⁾ Inspection of the ¹³C-NMR signals due to the sugar moiety revealed the presence of a β -gentiobiosyl ester moiety in **4** (Table I). Based on these results, **4** is formulated as the β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside of (1*R*)-1,11 α -dihydroxy-3,4-seco-lupa-4(23),20(29)-diene-3,28-dioic acid

Chart 1

TABLE I. ^{13}C Chemical Shifts (δ) in $\text{C}_5\text{D}_5\text{N}$

		1	4			1	4
Aglycone moiety	1	75.0	75.1	Sugar moiety	Glc-1	95.2	95.4
	2	38.6	38.8		-2	73.8	74.1
	3	173.1	173.0		-3	78.4	78.1 ^{b)}
	4	147.6	147.7		-4	70.5	70.9
	5	49.5	49.6		-5	76.3	78.4 ^{b)}
	6	25.1	25.1		-6	69.2	69.5
	7	33.4	33.5		Glc-1'	104.7	105.3
	8	41.6	41.7		-2'	75.2	75.2
	9	44.0	44.1		-3'	76.9	78.4 ^{b)}
	10	44.0	44.1		-4'	78.4	71.5
	11	70.2	70.5		-5'	77.8	78.7
	12	32.2	32.2		-6'	61.3	62.6
	13	35.2	35.2		Rha-1	102.5	
	14	42.1	42.2		-2	72.5 ^{b)}	
	15	30.7	30.8		-3	72.3 ^{b)}	
	16	32.5	32.2		-4	73.8	
	17	56.7	56.8		-5	70.2	
	18	47.5	47.6		-6	18.3	
	19	49.5	49.6				
	20	150.7	150.1				
	21	29.5	29.5				
	22	36.8	36.8				
	23	113.9	113.9				
	24	23.5	23.5				
	25	18.9 ^{a)}	18.9 ^{a)}				
	26	17.9 ^{a)}	18.0 ^{a)}				
	27	13.8	13.8				
	28	175.0	175.1				
	29	18.9 ^{a)}	19.1 ^{a)}				
	30	110.6	110.7				

a, b) These assignments may be interchanged in each column. Glc, $\beta\text{-D}$ -glucopyranosyl; Rha, $\alpha\text{-L}$ -rhamnopyranosyl.

3,11-lactone (=desrhamno-chiisanoside) (Chart 1).

The preliminary investigation of methanolic extracts of leaves of five other Japanese *Acanthopanax* spp. (*A. hypoleucus* MAKINO, *A. japonicus* FR. et SAV., *A. sciadophylloides* FR.

et SAV., *A. senticosus* HARMS and *A. spinosus* MIGUEL) by thin layer chromatography (TLC) indicated the absence of these 3,4-secolupane type triterpene glycosides, suggesting a unique taxonomical position of *A. divaricatus* and *A. chiisanensis* in this genus.

Experimental

General Procedure—Melting points were determined on a Yanaco micro hot stage and are uncorrected. Optical rotations were measured with a Union PM-101 automatic digital polarimeter. Infrared (IR) spectra were taken on a Shimadzu IR-408 spectrometer. NMR spectra were recorded on a JEOL FX-100 instrument using tetramethylsilane (TMS) as an internal standard. For gas liquid chromatography (GLC), a Shimadzu GC-6A apparatus was used. MS were taken on a JEOL JMS-01-SG-2 spectrometer by the direct inlet method; ionization voltage 75 eV. For column chromatography, Kieselgel 60 (70–230 mesh, Merck), LiChroprep RP-8 (40–63 μ m, Merck), Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) and Sephadex LH-20 (Pharmacia) were used. All solvent systems for chromatography were homogeneous.

Extraction and Separation of Glycosides—Dried leaves of *Acanthopanax divaricatus* (148 g) collected in Hiroshima prefecture were extracted with MeOH, and the MeOH extract was concentrated to dryness. The residue (37.8 g) was suspended in H₂O and then washed with Et₂O. The aqueous layer was chromatographed on a column of highly porous polymer (Diaion HP-20) and eluted with H₂O, 60% MeOH, 80% MeOH, MeOH and Me₂CO, successively. The 60% MeOH eluate was chromatographed on a reversed-phase column (LiChroprep RP-8, 65% MeOH) to give crude **5** and **2**. The crude **5** and **2** were purified by column chromatography on Sephadex LH-20 (MeOH) and silica gel (CHCl₃–MeOH–H₂O (6:4:1)) to give **5** (78 mg) and **2** (a white powder, 19 mg), respectively. The 80% MeOH eluate was subjected to chromatography on a LiChroprep RP-8 column (68% MeOH) and then a silica gel column (CHCl₃–MeOH–H₂O) (10:5:1) to give two fractions (fr. 1 and fr. 2). Fraction 1 was purified by high performance liquid chromatography (column, TSK-GEL ODS-120T 21 mm $\times$ 30 cm; solvent, 60% MeOH; flow rate, 7 ml/min; detection, RI) to give **4** (16 mg). Fraction 2 was chromatographed on LiChroprep RP-8 to give **1** (1.4 g).

The identification of **1** and **2** was established by direct comparison (TLC, $[\alpha]_D$, ¹H- and ¹³C-NMR) with authentic samples. The identification of **5** was established by comparison of the melting point and ¹³C-NMR data with reference data.^{3,4)}

Compound **4**: a white powder, $[\alpha]_D^{25} + 30.0^\circ$ ($c = 0.59$, MeOH). Anal. Calcd for C₄₂H₆₄O₁₅·4H₂O: C, 57.26; H, 8.24. Found: C, 57.11; H, 8.24. IR (Nujol) cm⁻¹: 3450 (OH), 1750, 1710 (COOR), 1640, 890 (C=CH₂). ¹H-NMR (C₅D₅N, 100 MHz) δ : 6.22 (1H, d, $J = 8$ Hz, anomeric H of Glc'), 5.01 (1H, d, $J = 8$ Hz, anomeric H of Glc). ¹³C-NMR data are given in Table I.

Acid Hydrolysis of 4 and Identification of the Resulting Monosaccharide⁵⁾—A sample (10 mg) was heated with 3.5% HCl in H₂O–dioxane (1:1, 1 ml) in a sealed micro-tube at 80 °C for 3 h. The reaction mixture was diluted with H₂O and then washed with CHCl₃. The aqueous layer was neutralized with Amberlite MB-3 ion exchange resin and then concentrated to give a sugar fraction. A solution of the sugar fraction (1 mg) in 50 μ l of H₂O was treated with a solution of L-(–)- α -methylbenzylamine (9 mg) and NaBH₃CN (0.6 mg) in 50 μ l of EtOH, and the mixture was kept at 40 °C for 4 h. Then several drops of acetic acid were added, and the whole was concentrated to dryness. The residue was trimethylsilylated with *N*-trimethylsilylimidazole and subjected to GLC analysis (dual flame ionization detector; carrier gas, He 2.5 ml/min; WCOT glass capillary column (0.25 mm i.d. $\times$ 25 m) coated with Carbowax 20M; isothermal, 150 °C; injection temperature, 190 °C; retention time, 46.29 min). D-Glucose was identified by comparison of the retention time with that of an authentic sample.

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