Two 3,4-seco-Lupane Triterpenes from Leaves of *Acanthopanax divaricatus* var. *albeofructus*

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Two 3,4-seco-lupane triterpenoids (**1**, **2**) were isolated from the leaves of *Acanthopanax divaricatus* var. *albeofructus* (Araliaceae). Based on the spectroscopic data, the chemical structures of **1** and **2** were characterized as 24-hydroxychiisanogenin and 22 α -hydroxychiisanogenin, respectively. **1** was a new compound and **2** was isolated for the first time from the natural source, although it had been obtained as an enzymatically hydrolyzed artifact from 22 α -hydroxychiisanoside.

Key words *Acanthopanax divaricatus* var. *albeofructus*; Araliaceae; 3,4-seco-lupane triterpene; 24-hydroxychiisanogenin; 22 α -hydroxychiisanogenin

In previous papers, we reported the isolation and characterization of two lupane triterpenoids, chiisanogenin and chiisanoside, from the leaves of *Acanthopanax divaricatus* SEEMANN var. *albeofructus* C. S. YOOK *et al.* T. H. KIM and two lignoids, *l*-sesamin and *l*-savinin, from the root bark of this plant,^{1,2)} and five new lupane triterpenoids, acantrifoside A, and acankoreosides A, B, C and D, from *A. trifoliatum*³⁾ and *A. koreanum*.^{3–5)} In a continuing study on *Acanthopanax* species, we report here the isolation and structural determination of two additional 3,4-seco-lupane triterpenoids, named 24-hydroxychiisanogenin (**1**) and 22 α -hydroxychiisanogenin (**2**), as minor components of the leaves of *A. divaricatus* var. *albeofructus*.

24-Hydroxychiisanogenin (**1**), obtained as colorless needles, mp 250–252 °C (MeOH), $[\alpha]_D^{25} +61.0^\circ$ (MeOH), showed absorption bands due to hydroxyl groups at 3425 cm^{–1}, carbonyl groups at 1711 cm^{–1} and C=C groups at 1643 cm^{–1} in the IR spectrum. The negative FAB-MS exhibited a molecular ion peak due to $[M-H]^-$ at m/z 499 (C₃₀H₄₄O₆–H). The ¹H-NMR spectrum (in pyridine-*d*₅) showed signals due to four tertiary methyl groups at δ 1.00, 1.04, 1.05 and 1.67, four olefinic protons at δ 4.62 (1H, br s), 4.91 (1H, br s), 5.12 (1H, br s) and 5.58 (1H, br s), two oxygen-bearing methine protons at δ 3.69 (1H, d, $J=7.8$ Hz) and 4.62 (1H, d, $J=6.8$ Hz), and two oxygen-bearing methylene protons at δ 4.43 (1H, d, $J=14.5$ Hz) and 4.62 (1H, d, $J=14.5$ Hz), as illustrated in Table 1. Signals due to one tertiary methyl group appeared at δ 1.67 and two exomethylene protons with geminal coupling suggested the presence of an isopropenyl group. Moreover, a total of thirty carbon signals observed in the ¹³C-NMR spectrum (Table 1) and distortionless enhancement by polarization transfer (DEPT) experiment suggested the presence of two carboxyl groups, two disubstituted double bonds, two oxygen-bearing methine carbons, one oxygen-bearing methylene carbon, five methine carbons, eight methylene carbons and four methyl carbons. All ¹³C-NMR data of **1** were very similar to those of chiisanogenin (**3**) isolated from the leaves of this plant, except for the absence of one tertiary methyl carbon signal of C-24 observed in **3** and the presence of one oxygen-bearing methylene car-

bon signal at δ 67.2 in **1**. Furthermore, taking into consideration the molecular ion peaks of both compounds, it was suggested that one tertiary methyl carbon at C-24 in **3** was replaced by one oxygen-bearing methylene carbon in **1**. This conclusion was supported by a significant downfield shift of the C-4 signal (5.2 ppm) by an α -effect of hydroxylation shift, upfield shifts of the C-5 signal (4.2 ppm) and C-23 signal (2.9 ppm) by β -effect, and downfield effects of the C-6 (1.8 ppm) and C-10 (0.5 ppm). Moreover, the ¹H-NMR signals of olefinic protons at δ 5.12 (1H, br s, H-23a) and 5.58 (1H, br s, H-23b) shifted downfield (0.12 and 0.48 ppm, respectively). Measurements of two-dimensional (2D) NMR spectra of **1** enabled the respective signals to be assigned. Based on the above spectroscopic data, **1** was determined to be 1(*R*)-1 α ,11 α ,24-trihydroxy-3,4-seco-lupa-4(23),20(30)-dien-3,28-dioic acid 3,11-lactone.

22 α -Hydroxychiisanogenin (**2**), obtained as colorless needles, mp 243–245 °C (MeOH), $[\alpha]_D^{25} +92.1^\circ$ (MeOH), showed absorption bands due to hydroxyl groups at 3462, 3377 cm^{–1}, carbonyl groups at 1697 cm^{–1} and C=C groups at 1643 cm^{–1} in the IR spectrum. The negative FAB-MS also exhibited a molecular ion peak due to $[M-H]^-$ at m/z 499 (C₃₀H₄₄O₆–H). The ¹H-NMR spectrum (in pyridine-*d*₅) showed signals due to five tertiary methyl groups, four olefinic protons and three oxygen-bearing methine protons,

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Table 1. ^1H - and ^{13}C -NMR Data for Compounds **1** and **2** in Pyridine- d_5 (δ in ppm, 400 MHz)

C	1		2	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	70.9 d	3.69 (1H, d, $J=8.1$ Hz, H-1 β)	70.4 d	3.66 (1H, d, $J=8.0$ Hz, H-1 β)
2	38.4 t	3.07 (1H, d, $J=14.5$ Hz, H-2 α)	38.7 t	3.05 (1H, d, $J=14.9$ Hz, H-2 α)
		2.75 (1H, dd, $J=8.1, 14.5$ Hz, H-2 β)		2.76 (1H, dd, $J=8.0, 14.9$ Hz, H-2 β)
3	172.8 s	—	173.1 s	—
4	152.9 s	—	147.6 s	—
5	45.4 d	2.74 ^{a)}	49.4 d	2.86 (1H, d, $J=12.9$ Hz, H-5 α)
6	26.9 t	1.56, ^{a)} 1.88 ^{a)}	25.1 t	1.06, ^{a)} 1.37 ^{a)}
7	32.6 t	1.12, ^{a)} 1.34 ^{a)}	32.4 t	1.15, ^{a)} 1.34 ^{a)}
8	41.6 s	—	42.1 s	—
9	44.2 d	2.73 (1H, d, $J=9.5$ Hz, H-9 α)	44.0 s	2.70 ^{a)}
10	44.6 s	—	44.1 s	—
11	75.5 d	4.62 (1H, d, $J=6.8$ Hz, H-11 β)	75.4 d	4.58 ^{a)}
12	33.5 t	1.73, ^{a)} 2.58 ^{a)}	33.6 t	1.80, ^{a)} 1.64 ^{a)}
13	35.3 d	2.88 (1H, m, H-13 β)	35.0 d	3.01 ^{a)}
14	42.2 s	—	41.6 s	—
15	29.6 t	1.10, ^{a)} 1.73 ^{a)}	29.0 t	1.17, ^{a)} 1.74 ^{a)}
16	32.9 t	1.49, ^{a)} 2.56 ^{a)}	26.9 t	2.30 (1H, m), 2.43 ^{a)}
17	56.3 s	—	62.4 s	—
18	49.6 d	1.63 ^{a)}	49.6 d	1.68 ^{a)}
19	47.8 d	3.48 (1H, m, H-19 β)	47.9 d	3.55 (1H, m, H-19 β)
20	150.5 s	—	150.9 s	—
21	31.0 t	1.47, ^{a)} 2.20 ^{a)}	41.8 t	1.69, ^{a)} 2.57 ^{a)}
22	37.3 t	1.51, ^{a)} 2.21 ^{a)}	75.2 d	4.71 (1H, d, $J=3.2$ Hz, H-22 β)
23	111.0 t	5.12 (1H, br s, H-23 α)	113.8 t	4.95 (1H, br s, H-23 α)
		5.58 (1H, br s, H-23 β)		5.06 (1H, br s, H-23 β)
24	67.2 t	4.43 (1H, $J=14.2$ Hz, H-24 α)	23.5 q	1.80 (3H, s)
		4.62 (1H, $J=14.9$ Hz, H-24 β)		
25	18.6 q	1.05 (3H, s)	19.0 q	0.94 (3H, s)
26	17.9 q	1.00 (3H, s)	17.8 q	0.99 (3H, s)
27	13.7 q	1.04 (3H, s)	13.7 q	1.11 (3H, s)
28	178.7 s	—	178.4 s	—
29	18.9 q	1.67 (3H, s)	18.7 q	1.88 (3H, s)
30	110.6 t	4.62 (1H, br s, H-30 α)	110.9 t	4.62 (1H, br s, H-30 α)
		4.91 (1H, br s, H-30 β)		4.98 (1H, br s, H-30 β)

All assignments of ^1H and ^{13}C signals were made by ^1H - ^1H COSY (Correlation Spectroscopy), HMQC (^1H -Detected Multiple Quantum Coherence Spectrum) and HMBC (^1H -Detected Multiple-bond Heteronuclear Multiple Quantum Coherence Spectrum) spectra. a) Overlapped signals.

as illustrated in Table 1. Signals due to two tertiary methyl groups appeared at δ 1.80 and 1.88, and four exomethylene protons with geminal coupling suggested the presence of an isopropenyl group. Moreover, a total of thirty carbon signals observed in the ^{13}C -NMR spectrum (Table 1) indicated the presence of two carboxyl, two disubstituted double bonds, three oxygen-bearing methine carbons, five methine carbons, seven methylene, and five methyl carbons. The ^{13}C -NMR data of **2** were also very similar to that of **3**, except for signals due to the DE ring moiety. One methylene carbon signal at δ 37.3 in **3** was replaced by one oxygen-bearing methine carbon signal at δ 75.2 in **2**. The position of one oxygen-bearing methine carbon was deduced at C-22 by comparison of chemical shifts of the ^{13}C -NMR data of **2** with that of **3**: significant downfield shifts at C-21 (10.8 ppm) and C-17 (6.1 ppm) and an upfield shift at C-16 (5.7 ppm). Moreover, this location was verified by ^1H -detected multiple-bond heteronuclear quantum coherence spectrum (HMBC), that is, one oxygen-bearing methine proton at δ 4.71 (1H, d, $J=3.2$ Hz) had cross peaks with C-18, C-19 and C-28, and the configuration at H-21 was determined to be β since H-21 possessed cross peaks with H-19 β , not with H-18 α , in the nuclear Overhauser and exchange spectroscopy (NOESY). Therefore, hydroxylation occurred at C-22 with an α -con-

figuration, and **2** was characterized as 22 α -hydroxychisanogenin.

To the best of our knowledge, **1** was a new compound and **2** was isolated for the first time from a natural source, although the latter had previously been obtained as an enzymatically hydrolyzed artifact from 22 α -hydroxychisanoside.⁶⁾

Experimental

Melting points (uncorrected) were measured using a Boetius micromelting point apparatus. Optical rotations were determined on a JASCO DIP-1000 KUY 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 and negative ion mode using JEOL JMS-DX300 and JMS-DX303HF instruments. NMR spectra were measured in pyridine- d_5 on a JEOL λ -400 spectrometer, and chemical shifts were relative to tetramethylsilane (TMS). Column chromatography (CC) was carried out on silica gel 60 (0.040–0.063 mm, Merck). TLC was performed on precoated silica-gel 60F₂₅₄ (Merck) and RP-18F_{254S} plates (Merck).

Plant Material The leaves of *A. divaricatus* var. *albeofructus* were harvested at Cheonan-gun, Chungcheongnam-do Province of Korea, in September 1995 and were botanically identified by one of the authors, Prof. Yook, C. S.; the specimen has been deposited in the Herbarium of the College of Pharmacy, Kyung-Hee University.

Isolation The dried leaves of *A. divaricatus* var. *albeofructus* (500 g) were repeatedly extracted with 70% MeOH to give an extract (121 g), a part of which (37 g) was chromatographed on Diaion HP-20P (Mitsubishi Chem.

Ind. Co., Ltd., Japan) by elution with H₂O, 20%, 40%, 60%, 80% MeOH and MeOH, successively. A fraction eluted with 80% MeOH was subsequently chromatographed on silica gel (Merck Co.) using the gradient elution of CHCl₃–MeOH–H₂O (10:1:0→7:3:0.5) to give six fractions. Fraction 1 was chromatographed on a reverse phase column, Chromatorex ODS (30–50 μ m, Fuji Silysia Chem. Ind. Co., Ltd., Japan), using gradient elution from 65% MeOH to 80% MeOH to give **1** (32 mg) and **2** (610 mg).

24-Hydroxychiisanogenin (**1**): Colorless needles, mp 250–252 °C (from MeOH); $[\alpha]_D^{28} +61.0^\circ$ ($c=0.30$ in MeOH); IR $\nu_{\max}$ (KBr) cm⁻¹: 3425 (OH), 2947 (aliphatic CH), 1711 (carbonyl), 1643 (C=C); negative FAB-MS m/z ; 499 [M–H]⁻; ¹H- and ¹³C-NMR: see Table 1.

22 α -Hydroxychiisanogenin (**2**): Colorless needles, mp 243–245 °C (from MeOH); $[\alpha]_D^{28} +92.1^\circ$ ($c=1.03$ in MeOH); IR $\nu_{\max}$ (KBr) cm⁻¹: 3462, 3377 (OH), 2942 (aliphatic CH), 1697 (carbonyl), 1643 (C=C); negative FAB-MS m/z ; 499 [M–H]⁻; ¹H- and ¹³C-NMR: see Table 1.

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