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Studies on the Constituents of the Leaves of *Acanthopanax divaricatus* forma *flavi-flos*

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Abstract – Four 3,4-*seco*-lupane triterpenoids were isolated from the MeOH extract of the leaves of *Acanthopanax divaricatus* forma *flavi-flos* Yook by using various column chromatography. The chemical structures of isolates were identified as chiisanogenin, chiisanoside, isochiisanoside and 11-deoxyisochiisanoside on the basis of physico-chemical and spectroscopic data (^{1}H-NMR, ^{13}C-NMR, 2D-NMR and FAB-MS). These compounds were isolated for the first time from *A. divaricatus* forma *flavi-flos*.

Keywords – *Acanthopanax divaricatus* forma *flavi-flos*, Araliaceae, *seco*-lupane triterpene

Introduction

Acanthopanax species distributed in Northeast Asia are well known plants as Oriental herbal materials. It has been popular with a tonic medicine like ginseng radix and also been used for strengthener of sinew and bone in the traditional medicine (Oh *et al.*, 2000).

A. divaricatus forma *flavi-flos* is closely related to *A. divaricatus* and has a distinguishable morphological characteristic in the flower, showing big and yellow umbel-shape inflorescence with a long pedicel (Park *et al.*, 2004 a & b).

Some 3,4-*seco*-lupane triterpenes had been reported from *A. divaricatus.* (Matsumoto *et al.*, 1987, Shirasuma *et al.*, 1997). However, there has been no report on the constituents of this plant. In the present study, we isolated four triterpenoids; namely chiisanogenin **(1)**, chiisanoside **(2)**, isochiisanoside **(3)** and 11-deoxyisochiisanoside **(4)**.

Experimental

Instruments and reagents – For column chromatography, silica gel 60 (70-230 Mesh, ASTM), Sephadex LH-20 (25~100 μm, Pharmacia Fine Chemical), ODS (Li Chroprep RP-18, 40~63 μm, Merck) were used. TLC was performed using a pre-coated silica gel plate (60 F_{254}, Merk) and spots were detected by spraying 10% H_2SO_4 (in EtOH) followered by heating at 105 °C Melting points were determined on MEL-TEMP (Lavoratory Devices Inc. U.S.A). NMR spectra were recorded on Varian 200 and 400 in C_5D_5N using TMS as an internal standard. FAB-MS were measured on Jeol JMS-DX 300 (Japan) and JMS-DX 303 HF (Japan).

Plant Material – The leaves of *Acanthopanax divaricatus* forma *flavi-flos* Yook growing in the Medicinal Plant Garden of Kyung Hee University were collected in September 2003 (320 g). The identification of the plant was confirmed by Emeritus Professor Chang Soo Yook of the College of Pharmacy, Kyung Hee University.

Extraction and isolation – Dried leaves of *A. divaricatus* forma *flavi-flos* (320 g) were extracted twice with MeOH for 4 hours. After evaporation under vacuum, the extract (13.77 g) was chromatographed on a silica gel column by using a gradient elution of with $CHCl_3$-MeOH-H_2O (8 : 2 : 0.2 → 6 : 4 : 1) and separated into 10 fractions (Fr. I ~ Fr. X).

Fr. II was secondarily chromatographed on a silicagel column using gradient elution of $CHCl_3$-MeOH-H_2O and chiisanogenin (**1**, 1.07 g) was obtained. Fr. IV was chromatographed on Sephadex with MeOH (100%), silica gel column with $CHCl_3$-MeOH-H_2O (8 : 2 : 0.2 → 7 : 3 : 0.2), and chiisanoside (**2**, 1.19 g) was obtained. Isochiisanoside (**3**, 0.17 g) was obtained by using sephadex with MeOH (100%) and ODS column with MeOH (60% → 70%) on Fr. VIII. Fr. V was chromatographed on sephadex with MeOH (100%), ODS column with MeOH (60% → 100%), silica gel column again with $CHCl_3$-MeOH-H_2O (8 : 2 : 0.2 → 7 : 3 : 0.2), and then 11-deoxyisochisanoside (**4**, 0.06 g) was obtained.

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Table 1. ^{13}C-Chemical shifts (δ) in C_5D_5N

	1	2	3	4
1	70.5	70.3	87.5	85.5
2	38.8	38.7	36.7	38.5
3	173.1	173.1	175.5	174.9
4	147.7	147.6	79.2	81.1
5	49.6	49.5	56.2	56.2
6	25.2	25.0	18.8	18.8
7	32.4	32.0	35.4	34.3
8	41.6	41.6	42.8	42.6
9	44.0	44.0	48.9	43.1
10	44.1	44.0	46.9	47.6
11	75.3	75.1	67.7	23.7
12	33.5	33.3	36.9	25.5
13	35.3	35.1	37.5	38.5
14	42.2	42.0	42.8	41.5
15	29.6	29.4	30.4	30.3
16	32.6	32.1	32.2	32.2
17	56.3	56.7	57.0	56.9
18	49.5	49.5	49.5	49.7
19	47.8	47.5	47.3	47.3
20	150.5	150.1	150.5	150.8
21	31.0	30.6	30.8	30.8
22	37.3	36.7	36.7	36.8
23	113.9	113.9	25.0	24.6
24	23.6	23.5	32.8	32.7
25	18.9	18.5	19.2	19.2
26	17.8	17.9	17.9	17.0
27	13.7	13.6	15.2	14.8
28	178.7	175.0	174.9	174.9
29	19.0	19.1	19.5	19.4
30	110.6	110.7	110.3	110.0
inner Glc-1		95.3	95.3	95.2
2		74.0	73.9	73.8
3		78.7	78.6	78.5
4		70.6	70.8	70.6
5		76.3	76.4	77.0
6		69.2	69.4	69.3
outer Glc-1'		105.0	105.0	104.9
2'		75.1	75.3	75.2
3'		78.0	77.1	76.3
4'		78.5	78.2	78.1
5'		77.1	78.0	77.9
6'		61.1	61.2	61.2
terminal Rha-1		102.6	102.7	102.6
2		72.5	72.7	72.4
3		72.7	72.5	72.6
4		73.9	74.0	74.0
5		70.2	70.3	70.2
6		18.7	18.5	18.4

Chiisanogenin (1) – White amorphous powder. FAB-MS (positive ion mode) *m/z* 507 $[M(C_{30}H_{44}O_5)+Na]^+$. HR FABMS *m/z* 507.3047 (Calcd. $C_{30}H_{44}O_5 \cdot Na$ 507.3086). ^{1}H-NMR (in C_5D_5N) δ : 1.00 (6H, *s*, H-25, 26), 1.08 (3H, *s*, H-27), 1.70 (3H, *s*, H-29), 1.88 (3H, *s*, H-24), 2.75 (1H, *d*, *J*=9.7 Hz, H-9), 2.83 (1H, *dd*, *J*=8.5, 14.8 Hz, H-2β), 3.13 (1H, *d*, *J*=14.6 Hz, H-2α), 3.49 (1H, *m*, H-19), 3.73 (1H, *d*, *J*=8.0 Hz, H-1β), 4.63 (1H, *d*, *J*= 10.0 Hz, H-11), 4.64 (1H, *s*, H-30a), 4.93 (1H, *s*, H-30b), 5.03 (1H, *s*, H-23a), 5.13 (1H, *s*, H-23b), ^{13}C-NMR (in C_5D_5N) δ: Table 1.

Chiisanoside (2) – White amorphous powder. mp; 236 ± 1 °C FAB-MS (positive ion mode) *m/z*: 977 $[M(C_{48}H_{74}O_{19})+Na]^+$. HR FABMS *m/z* 977.4739 (Calcd. $C_{48}H_{74}O_{19} \cdot Na$ 977.4722). ^{1}H-NMR (in C_5D_5N) δ : 1.02 (6H, *s*, H-25, 27), 1.12 (3H, *s*, H-26), 1.64 (3H, *s*, H-29), 1.73 (3H, *d*, *J*= 5.6 Hz, terminal Rha H-6), 1.89 (3H, *s*, H-24), 3.37(1H, *m*, H-19), 4.59 (1H, *s*, H-30a), 4.87 (1H, *s*, H-30b), 4.97 (1H, *d*, *J*=8.9 Hz, outer Glc H-1), 5.04 (1H, *s*, H-23a), 5.17 (1H, *s*, H-23b), 5.91 (1H, *s*, terminal Rha H-1), 6.39 (1H, *d*, *J*= 7.6 Hz, inner Glc H-1), ^{13}C-NMR (in C_5D_5N) δ: Table 1.

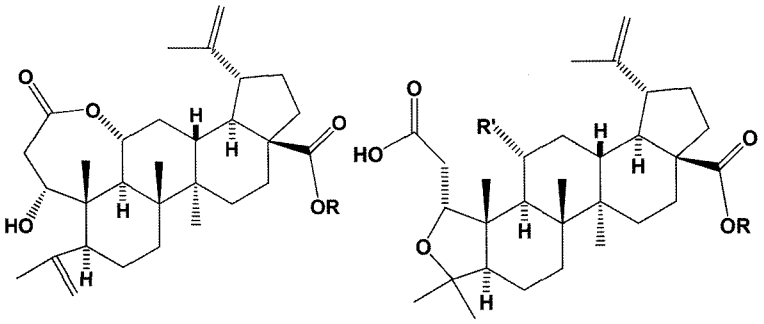
Isochiisanoside (3) – White and amorphous powder. FAB-MS (positive ion mode) *m/z* 995 $[M(C_{48}H_{76}O_{20})+Na]^+$. HR FABMS *m/z* 995.4844 (Calcd. $C_{48}H_{76}O_{20} \cdot Na$ 995.4828). ^{1}H-NMR (in C_5D_5N) δ : 1.15 (3H, *s*, H-27), 1.20 (3H, *s*, H-26), 1.21 (3H, *s*, H-23), 1.38 (3H, *s*, H-25), 1.49 (3H, *s*, H-24), 1.70 (3H, *s*, H-29), 1.70 (3H, *d*, *J*= 5.4 Hz, terminal Rha H-6), 2.83 (1H, *m*, H-16), 3.37 (1H, *m*, H-19), 4.81 (1H, *s*, H-30a), 4.67 (1H, *s*, H-30b), 4.96 (1H, *d*, *J*=7.2 Hz, outer Glc H-1), 5.06 (1H, *br d*, *J*= 11.1, H-1), 5.85 (1H, *s*, terminal Rha H-1), 6.33 (1H, *d*, *J* = 8.1 Hz, inner Glc H-1) ^{13}C-NMR (in C_5D_5N) δ: Table 1.

11-Deoxyisochiisanoside (4) – White and amorphous powder. FAB-MS (positive ion mode) *m/z* 980 $[M(C_{48}H_{76}O_{19})+Na]^+$. HR FABMS *m/z* 979.4888 (Calcd. $C_{48}H_{76}O_{19} \cdot Na$ 979.4879). ^{1}H-NMR (in C_5D_5N) δ : 1.08 (3H, *s*, H-25), 1.08 (3H, *s*, H-27), 1.14 (6H, *s*, H-23, 26), 1.42 (3H, *s*, H-24), 1.69 (3H, *d*, *J*=6.7 Hz, terminal Rha H-6), 1.75 (3H, *s*, H-29), 1,79 (1H, *m*, H-9), 2.06 (1H, *m*, H-15a), 3.36 (1H, *m*, H-19), 4.43 (1H, *m*, H-1), 4.67 (1H, *s*, H-30a), 4.89 (1H, *s*, H-30b), 4.94 (1H, *d*, *J*=7.3 Hz, outer Glc H-1), 5.83 (1H, *s*, terminal Rha H-1), 6.34 (1H, *d*, *J*=8.5 Hz, inner Glc H-1) ^{13}C-NMR (in C_5D_5N) δ: Table 1.

Results and Discussion

In the present study, we isolated four triterpenoidal glycosides, compounds **1**~**4** from *A. divaricatus* forma *flavi-flos.*

Compound **1** was obtained as a white amorphous powder with a molecular formula, of $C_{30}H_{44}O_5$, as determined by



(1) R=H

(2) R=β-glc^6-β-glc^4-α-rha

(3) R=β-glc^6-β-glc^4-α-rha R'=OH

(4) R=β-glc^6-β-glc^4-α-rha R'=H

Fig. 1. Structures of compounds **1-4**.

FABMS. The ^{1}H-NMR spectrum revealed signals for five tertiary methyl groups (δ 1.00 (×2), 1.08, 1.70, 1.88) and four olefinic protons (δ 4.64, 4.93, 5.03, 5.13). ^{13}C-NMR spectrum showed 30 carbon signals which were assigned by DEPT and HMQC techniques as two carboxyl groups, two di-substituted double bonds, two oxygen-bearing methine carbons, five methine carbons, eight methylene carbons and five methyl carbons. From these data, compound **1** was confirmed as *seco*-lupane triterpene. The structure of compound **1** was determined as chiisanogenin by comparison its spectral data with those in the previously published data for chiisanogenin (Oh *et al.*, 2000; Shirasuna *et al.*, 1997; Kasai *et al.*, 1986; Ryoo *et al.*, 2003).

Compound **2** showed a glycosylation-induced shift at C-28 (δ 175) in ^{13}C-NMR and three anomeric protons (δ 6.39, 4.97, 5.91) in ^{1}H-NMR. Compared with compound **1,** compound **2** was a glycoside bond in the skeleton of chiisanogenin. The structure of compound **2** was elucidated as chiisanoside by analysis of 2D NMR spectra and HR-MS and by comparing their spectral data with those previously published data (Ryoo *et al.*, 2003, Hahn *et al.*, 1984, Matsumoto *et al.*, 1987).

Compound **3** and compound **4** were identified as isochiisanoside (Shirasuna *et al.*, 1997; Kasai *et al.*, 1986) and 11-deoxyisochiisanoside (Park *et al.*, 2000), respectively, on the basis of their NMR spectral data, positive FAB-MS and HR-MS.

3,4-*seco*-Lupane triterpenoids are frequently found in some *Acanthopanax* species including *A. divaricatus* var. *albeofructus, A. divaricatus, A. chiisanensis, A. sessiliflorus, A. divaricatus* forma *nambuensis*, and *A. senticosus* forma *inermis*. From the chemotaxonomic point of view, *A. divaricatus* forma *falvi-flos* was classified into a group possessing 3,4-*seco*-lupanes, and thus as a homologue of *A. divaricatus* var. *sachunensis* (Park *et al.*, 2001) or *A. divaricatus* var. *albeofructus* (Oh *et al.*, 2000).

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