

A New 24-Nor-Lupane-Glycoside of *Acanthopanax trifoliatum*

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A new 24-nor-lupane glycoside was isolated from the leaves of *Acanthopanax trifoliatum*. Based on spectroscopic data its chemical structure was determined as 24-nor-11 α -hydroxy-3-oxo-lup-20(29)-en-28-oic acid 28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl ester.

Key words: *Acanthopanax trifoliatum*, Araliaceae, 24-Nor-lupane-glycoside, Acantrifoside B

INTRODUCTION

Acanthopanax trifoliatum (L.) Merr., (Araliaceae) is a medicinal plant that mainly occurs in Asian subtropical countries, including China, Japan, Korea, Laos and Vietnam. In Vietnam, the species is concentrated in the border mountainous regions of the North, in Caobang, Langson, Laocai and Laichau provinces, where it is used in folk medicine (Chi, 1997; Loi, 2001) as a drug with ginseng-like activity. In China, decoctions of the leaves and young shoots are prescribed for the treatment of tuberculosis and lung hemorrhage, and as a tonic to improve general weakness (Perry, 1980). Previously, a number of lupane-triterpene compounds have been isolated from the leaves of this plant (Ty *et al.*, 1984 and 1985; Lischewsky *et al.*, 1985; Yook *et al.*, 1998). During the course of our continuing work on *Acanthopanax* species, we isolated and determined the structure of a new 24-nor-lupane-triterpene glycoside from the leaves of *A. trifoliatum*. Based on spectroscopic data, its chemical structure was determined to be 24-nor-11 α -hydroxy-3-oxo-lup-20(29)-en-28-oic acid 28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl ester, which we named acantrifoside B (1).

MATERIALS AND METHODS

General experimental procedures

M.p.'s were determined using a Kofler micro-hotstage;

IR spectra were obtained on a Hitachi 270-30 type spectrometer from KBr discs. Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter. FAB-MS and HR FAB-MS were obtained using a JEOL JMS-DX 300 spectrometer. ¹H-NMR (300 MHz) and ¹³C-NMR (75 MHz) were recorded on a Bruker DRX300 spectrometer and chemical shifts are referenced to δ using TMS as an internal standard. Column chromatography (CC) was performed on silica gel 60, YMC RP-18 resin or Dianion HP-20 resin.

Plant material

The leaves of *A. trifoliatum* were collected in Langson province, Vietnam in January 2001 and identified by Prof. Dr. Tran Minh Hoi, Institute of Ecology, Biological Resources, NCST of Vietnam. Two voucher specimens (No 2539) are deposited at the herbarium of the Institute of Natural Products Chemistry, NCST, Vietnam, and at the herbarium of the College of Pharmacy, Chungnam National University, Korea.

Extraction and isolation

Dried and powdered leaves (3.7 kg) were extracted three times with hot MeOH. The combined solutions were evaporated under reduced pressure to yield a MeOH extract (250 g), which was suspended in water and partitioned with dichloromethane. The water fraction was adsorbed on highly porous polymer resin (Dianion HP-20, Mitsubishi Chem. Ind. Co. Ltd, Tokyo, Japan) and eluted with water containing increasing concentrations of MeOH (100% H₂O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, and 100% MeOH). The 20% MeOH and 40%

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Table I ^1H and ^{13}C -NMR spectral data of compound **1**

Cs	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$
1	43.9 (t)	1.36 (m)
2	38.4 (t)	2.09 (dd, 8.7, 6.9 Hz)
3	217.4 (s)	–
4	46.2 (d)	2.46 (m)
5	54.5 (s)	1.61 (m)
6	23.2 (t)	1.46 (m)/ 1.60 (m)
7	35.0 (t)	1.38 (m)/ 1.46 (m)
8	43.5 (s)	–
9	55.8 (d)	1.20 (d, 7.7 Hz)
10	39.4 (s)	–
11	70.8 (d)	4.0 (dt, 10.7, 5.5 Hz)
12	37.6 (t)	2.07 (m)
13	38.4 (d)	2.25 (m)
14	43.5 (s)	–
15	32.9 (t)	1.19 (m)/ 1.55 (m)
16	32.9 (t)	2.44 (m)
17	58.0 (s)	–
18	50.2 (d)	1.82 (m)
19	48.3 (d)	3.09 (m)
20	151.4 (s)	–
21	31.3 (t)	2.07 (m)
22	37.6 (t)	2.07 (m)
23	12.7 (q)	1.04 (d, 6.5 Hz)
24	–	–
25	14.4 (q)	1.30 (s)
26	17.7 (q)	1.10 (s)
27	14.9 (q)	1.11 (s)
28	176.4 (q)	–
29	111.0 (q)	4.59 (1H, br s)/ 4.73 (1H, br s)
30	19.8 (q)	1.77 (s)
C-2 α O-nner glc		
1	95.5 (d)	5.55 (d, 6.0 Hz)
2	73.9 (d)	3.92 (m)
3	78.4 (d)	3.66 (m)
4	71.1 (d)	4.19 (m*)
5	78.2 (d)	3.54 (m*)
6	69.6 (t)	4.21 (1H, d, 10.2 Hz), 3.92 (1H, m)
Glc(1 \rightarrow 6)glc		
1	104.6 (d)	4.47 (d, 7.8 Hz)
2	75.4 (d)	3.35 (t, 8.5 Hz)
3	76.8 (d)	3.56 (m*)
4	79.7 (d)	3.63 (m*)
5	77.0 (d)	3.58 (m*)
6	63.0 (t)	3.75 (1H, m), 3.90 (1H, m)
Rha 1 \rightarrow 4)glc'		
1	103.1 (d)	4.86 (d, 4.1 Hz)
2	72.5 (d)	3.85 (m)
3	72.3 (d)	3.71 (dd, 9.3, 3.2 Hz)
4	74.1 (d)	3.56 (m)
5	71.2 (d)	3.92 (m)
6	18.0 (q)	1.36 (d, 3.8 Hz)

Glc, β -D-gucopyranosyl; Rha, α -L-rhamnopyranosyl; ^aIn methanol-d₄; ^b75 MHz; ^c300 MHz; *Overlapped signals; All assignments of ^1H and ^{13}C signals were confirmed by ^1H - ^1H COSY and HMQC spectrum.

MeOH fractions were combined and chromatographed on a silica gel column using CHCl_3 -MeOH- H_2O (70:30:4) as eluant. This was followed by CC on a YMC RP-18 column using a MeOH- H_2O (7:3) eluant to yield **1** (1.9 g).

24-Nor-11 α -hydroxy-3-oxo-lup-20(29)-en-28-oic acid 28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl ester (**1**)

A white powder, m.p. 218–220°C, $[\alpha]_{\text{D}}^{25}$ 20.0° (c 2.00, MeOH); IR $^{\text{KBr}}$ ν_{max} cm^{-1} : 3,414 (br, OH), 2,985 (C-H), 1,736 ($>\text{C}=\text{O}$), 1,693 ($-\text{COO}$), 1,645 ($>\text{C}=\text{CH}_2$), 1,060 (C-O-C); FAB-MS (m/z): 925 $[\text{M}-\text{H}]^-$, HR FAB-MS (m/z): 925.4771 $[\text{M}-\text{H}]^-$ (Calcd. 925.4800 for $\text{C}_{47}\text{H}_{74}\text{O}_{18}$); ^1H - and ^{13}C -NMR: see Table I.

RESULTS AND DISCUSSION

Compound **1** was obtained as white powder from water fraction of the leaves of the MeOH extract of *A. trifoliatum*, and showed hydroxyl absorbance bands at 3,414 cm^{-1} , carbonyl absorbance at 1,736 cm^{-1} , and carboxyl at 1,693 cm^{-1} in the IR spectrum (KBr). The negative HR FAB-MS spectrum produced a molecular ion at m/z 925.4771 $[\text{M}-\text{H}]^-$ providing the formula $\text{C}_{47}\text{H}_{74}\text{O}_{18}$ (Calcd. for $\text{C}_{47}\text{H}_{74}\text{O}_{18}$: 925.4800). The negative FAB-mass spectrum also exhibited a molecular ion due to $[\text{M}-\text{H}]^-$ at m/z 925. The ^1H -NMR spectrum of **1** (Table I) confirmed the presence of four tertiary methyl groups [δ 1.10 (s), 1.11 (s), 1.30 (s) and 1.77 (s)]; two secondary methyl groups [δ 1.04 (d, J = 6.5 Hz) and 1.36 (d, J = 3.8 Hz)], three anomeric protons [δ 4.47 (1H, d, J = 7.8 Hz), 4.86 (1H, d, J = 4.1 Hz), and 5.55 (1H, d, J = 6.0 Hz)], two primary alcohol functions [3.92 (1H, m), 4.21 (1H, d, J = 10.2 Hz), $\text{H}_{\text{CH}_2\text{OH}}\text{-glc}$; and 3.75 (1H, m), 3.90 (1H, m) $\text{H}_{\text{CH}_2\text{OH}}\text{-glc}$] and two olefinic protons [δ 4.59 (br s) and 4.73 (br s)]. The ^{13}C -NMR and DEPT spectrum showed 47 carbon signals, including six methyl groups (δ 12.7, 14.4, 14.9, 17.7, 18.0 and 19.8), 12 methylene groups, 22 methine groups (16 oxygenated) and 7 quaternary carbon groups (2 oxygenated). In the ^{13}C -NMR spectrum, chemical shifts confirmed the presence of one carbonyl group at δ 217.4 ($>\text{C}=\text{O}$, C-3), monosubstituted double bonds at δ 151.4 and δ 111.0 ($>\text{C}=\text{CH}_2$, C-20 and C-29) and one carboxylate group at δ 176.4 ($>\text{COO}$, C-28). The assignment of signals was made by comparing results with the ^{13}C -NMR data of 24-nor-11 α -hydroxy-3-oxo-lup-20(29)-en-28-oic acid (Lischewski *et al.*, 1985). The shift values of the aglycone moiety of lupane skeleton were in a good agreement, except for the three-unit sugar moiety of **1**. On the other hand, the shift values of δ 95.5, 104.6 and 103.1 were typical for the three anomeric carbons of the inner glucopyranosyl, outer glucopyranosyl and rhamnopyranosyl (terminal) and agreed with that of acantrifoside A isolated from *A. trifoliatum* and

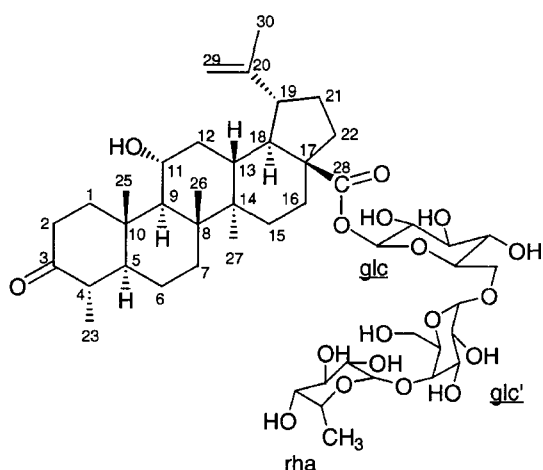


Fig. 1. Chemical structure of compound 1

A. koreanum (Yook *et al.*, 1998). Based on the above spectral data, the structure of 1 was determined as 24-nor-11 α -hydroxy-3-oxo-lup-20(29)-en-28-oic acid 28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl ester, which we named acantrifoside B (1).

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