Lupane-triterpene Carboxylic Acids from the Leaves of *Acanthopanax trifoliatus*

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Two new lupane-triterpene carboxylic acids, called acantrifoic acid A (1) and acantrifoside C (2) have been isolated from the leaves of *Acanthopanax trifoliatus*. Based on extensive 1D and 2D NMR spectroscopic data, their chemical structures were determined as 3α-acetoxy-30-hydroxylup-20(29)-ene-23,28-dioic acid and 3α-acetoxy-30-hydroxylup-20(29)-ene-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Key words *Acanthopanax trifoliatus*; Araliaceae; lupane-triterpene carboxylic acid; acantrifoic acid A; acantrifoside C

Acanthopanax trifoliatus (L.) MERR., (Araliaceae) is distributed in North Vietnam and used in the folk medicines of Southeast Asia[1,2] as a drug with ginseng-like activity. Lupane-triterpene carboxylic acids and a lupane-triterpene glycoside have been reported from the leaves of *A. trifoliatus*.[3—5] Herein we describe the isolation and the structures of two new compounds, a lupane-triterpene carboxylic acid and a lupane-triterpene glycoside from the same source. Based on spectroscopic data, the chemical structures of constituents were determined as 3α-acetoxy-30-hydroxylup-20(29)-ene-23,28-dioic acid, called acantrifoic acid A (**1**) and 3α-acetoxy-30-hydroxylup-20(29)-ene-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, which we called acantrifoside C (**2**).

Results and Discussion

Repeated column chromatography on silica gel of the dichloromethane extract (see experimental) of the dried leaves of *A. trifoliatus* yielded a new lupane-triterpene carboxylic acid **1**. Compound **1** formed white crystals and produced a carbonyl IR absorption peak at 1747 cm^{-1}. HR-FAB-MS gave a molecular ion at *m/z* 567.3300 $[M+Na]^+$ providing the formula $C_{32}H_{48}O_7$ (Calcd for $C_{32}H_{48}O_7Na$: 567.3298). The ^{1}H-NMR spectrum of **1** (Table 1) showed signals due to four tertiary methyl groups [δ 0.83 (3H, s), 0.96 (3H, s), 1.06 (3H, s) and 1.37 (3H, s)], two olefinic protons [δ 5.08 (1H, br s) and (5.48 (1H, br s)], one methine proton [δ 5.42 (br s)], two protons for a primary alcohol group [δ 4.45 (2H, s)] and one acetoxy group [δ 1.90 (3H, s)]. The ^{13}C-NMR and DEPT spectra revealed 32 carbon signals, including two carboxyl groups at δ 178.8 and 177.7, one ester carbonyl group at δ 170.0, one double bond at δ 157.0 and 106.0, one oxygen bearing methine carbon at δ 76.0, one hydroxy methylene carbon at δ 64.5 and five methyl carbons at δ 15.2, 16.6, 16.8, 17.5 and 21.2. The ^{1}H- and ^{13}C-NMR signals of **1** were very similar to those of 3α-acetoxylup-20(29)-ene-23,28-dioic acid,[6)] except for the signals of C-19, C-20, C-21, C-29 and C-30 positions (Table 1). This suggested that **1** is a related compound to 3α-acetoxylup-20(29)-ene-23,28-dioic acid.[6,8)] In order to determine the locations of acetoxy, carboxylic and hydroxy group at C-3, C-23 and C-30 positions, 2D NMR spectra of **1** were taken using HMQC, NOESY and HMBC techniques. The C–H long-range correlations between methyl protons CH_3-24 (δ_H 1.37) and carbons C-3 (δ_C 76.0)/C-4 (δ_C 50.2)/C-23 (δ_C 177.7), between proton H-3 (δ_H 5.42) and acetoxy carbon (δ_C 170.0)/(C-4 (δ_C 50.2)/C-23 (δ_C 177.7), between acetoxy protons (δ_H 1.90) and acetoxy carbon (δ_C 170.0), between protons H-19 (δ_H 3.50)/H_2-29 (δ_H 5.08 and 5.48) and carbon C-30 (δ_C 64.5), and between protons H-30 (δ_H 4.45) and carbons C-19 (δ_C 43.5)/C-20 (δ_C 157.0)/C-29 (δ_C 106.0) were observed in the HMBC spectrum. The NOE correlations between methyl protons CH_3-24 (δ_H 1.37) and CH_3-25 (δ_H 0.83) were observed in the NOESY spectrum of **1**. This evidence confirmed that acetoxy, carboxylic and hydroxyl groups were connected to C-3, C-23 and C-30 positions, respectively. Furthermore, chemical shift at δ_C 76.0 (C-3) and δ_H 5.42 (1H, t, J_{ax+bx}=5.4 Hz, H-3β) confirmed the location of 3α-acetoxy group of **1**.[6—9)] Based on the other HOMO COSY, HMQC, NOESY and HMBC spectral data and direct comparison with those reported data of 3α-acetoxylup-20(29)-ene-23,28-dioic acid,[6,8)] the structure of **1** was determined to be 3α-acetoxy-30-hydroxylup-20(29)-ene-23,28-dioic acid and was called acantrifoic acid A.

Compound **2** was obtained as a white powder from the water fraction of the dried leaves of *A. trifoliatus* (see experimental). Its IR spectrum showed strong absorptions due to hydroxyl groups at 3410 cm^{-1}, carboxyl groups at 1720 cm^{-1}, and C–O–C groups at 1060 cm^{-1}. The negative FAB-mass spectrum produced a molecular ion at *m/z* 1013 $[M-H]^-$. The negative HR-FAB-MS showed an ion at 1013.4951 $[M-H]^-$ and provided the formula $C_{50}H_{77}O_{21}$ (Calcd for $C_{50}H_{77}O_{21}$: 1013.4956). The ^{1}H- and ^{13}C-NMR spectral data of the aglycone of **2** were very similar to those of **1** (Table 1), except for the presence of 18 signals of three sugars including anomeric protons at δ 6.31 (1H, d, J=7.9 Hz), δ 4.94 (1H, d, J=7.8 Hz) and δ 5.80 (1H, br s), three anomeric carbons at δ 95.2, δ 105.0 and δ 102.7, two primary alcohol groups (δ_C 61.4, δ_H 4.25/4.38) and (δ_C 69.4, δ_H 4.27/4.65), and one methyl group [δ_C 18.5, δ_H 1.68 (3H, d, J=6.0 Hz)] in the NMR spectrum of **2**. This suggested that **2** is a related compound of **1**. The NMR spectral data of the glycosyl moiety of **2** were also similar to those of the re-

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1** and **2** (Measured in Pyridine-d_5)

Pos.	1		2	
	δ_C (ppm)[a)]	δ_H (ppm)[b)]	δ_C (ppm)[a)]	δ_H (ppm)[b)]
1	33.4 t	2.20 (m), 2.54 (m)	33.4 t	2.20 (m), 2.54 (m)
2	22.8 t	1.65 (m), 1.80 (m)	22.9 t	1.65 (m), 1.80 (m)
3	76.0 d	5.42 (1H, br s)	76.0 d	5.43 (1H, br s)
4	50.2 s	—	50.2 s	—
5	46.0 d	2.32 (1H, m)	46.0 d	2.32 (1H, m)
6	21.6 t	1.39 (m), 1.50 (m)	21.6 t	1.39 (m), 1.50 (m)
7	34.5 t	1.35*	34.5 t	1.33*
8	41.7 s	—	41.7 s	—
9	51.0 d	1.58 (1H, d, 11.5)	51.0 d	1.60 (1H, d, 11.5)
10	37.1 s	—	37.1 s	—
11	21.1 t	1.39*	21.0 t	1.40*
12	27.1 t	1.70*	27.1 t	1.70*
13	38.5 d	2.65 (1H, ddd, 11.6, 11.6, 2.8)	38.5 d	2.65 (1H, ddd, 11.6, 11.6, 2.8)
14	42.9 s	—	42.9 s	—
15	30.2 t	1.20 (m), 1.90 (m)	30.1 t	1.20 (m), 1.90 (m)
16	32.7 t	1.53 (m), 2.52 (m)	32.7 t	1.53 (m), 2.55 (m)
17	56.6 s	—	56.6 s	—
18	50.1 d	1.93 (m)	50.1 d	1.93 (m)
19	43.5 d	3.50 (1H, ddd, 11.2, 11.2, 3.6)	43.1 d	3.50 (1H, ddd, 11.2, 11.2, 3.6)
20	157.0 s	—	156.6 s	—
21	33.0 t	1.52 (m), 2.00 (m)	32.7 t	1.52 (m), 2.00 (m)
22	37.4 t	1.59 (m), 2.35 (m)	37.6 t	1.59 (m), 2.35 (m)
23	177.7 s	—	178.1 s	—
24	17.5 q	1.37 (3H, s)	17.5 q	1.37 (3H, s)
25	16.6 q	0.83 (3H, s)	16.6 q	0.83 (3H, s)
26	16.8 q	0.96 (3H, s)	16.9 q	0.96 (3H, s)
27	15.2 q	1.06 (3H, s)	15.0 q	1.06 (3H, s)
28	178.8 s	—	175.0 s	—
29	106.0 t	5.08 (1H, br s) 5.48 (1H, br s)	106.0 t	5.08 (1H, br s) 5.48 (1H, br s)
30	64.5 t	4.45 (2H, s)	64.5 t	4.45.(2H, s)
$CH_3\underline{C}O$	170.0 s	—	170.0 s	—
$\underline{CH_3}CO$	21.2 q	1.90 (3H, s)	21.2 q	1.90 (3H, s)
C-28 O-inner glc				
1			95.2 d	6.31 (1H, d, 7.9)
2			74.0 d	4.32 (m)*
3			78.4 d	4.10 (m)*
4			70.8 d	4.30 (m)*
5			78.2 d	4.07 (m)*
6			69.4 t	4.27 (m), 4.65 (1H, d, 11.5)
Glc′(1→6)glc				
1′			105.0 d	4.94 (1H, d, 7.8)
2′			75.4 d	3.92 (1H, dd, 7.8, 5.5)
3′			76.6 d	4.15 (m)*
4′			78.7 d	4.21 (t, 9.0)
5′			77.2 d	3.63 (ddd, 9.0, 6.0, 2.8)
6′			61.4 t	4.25 (m)*, 4.38 (m)*
Rha(1→4)glc′				
1″			102.7 d	5.80 (1H, br s)
2″			72.6 d	4.66 (br s)
3″			72.8 d	4.54 (m)*
4″			74.0 d	4.10 (m)*
5″			70.4 d	4.93 (m)*
6″			18.5 q	1.68 (3H, d, 6.0)

All assignments were confirmed by [1]H–[1]H chemical shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra. Glc, β-D-glucopyranosyl; rha, -α-L-rhamnopyranosyl; *a*) 100 MHz; *b*) 400 MHz; * Overlapped signals.

ported compound.[5,12)] Acid hydrolysis of **2** yielded **1**, β-D-glucose and α-L-rhamnose. Morever, in order to determine the location of the glycosyl moiety, 2D NMR spectra of **2** were taken by means of HMQC and HMBC techniques. The C–H long-range correlations between proton H-1 of inner glucopyranosyl (δ_H 6.31) and carbon C-28 of the aglycone (δ_C 175.0), between proton H-1 of outer glucopyranosyl (δ_H 4.94) and carbon C-6 of inner glucopyranosyl (δ_C 69.4), and between proton H-1 of rhamnopyranosyl (δ 5.80) and carbon C-4 of outer glucopyranosyl (δ_C 78.7) were also observed in the HMBC spectrum. This evidence suggested a sequence of sugar linkages resembling those reported previously for glycosides of *Acanthopanax* species.[5,10—12)] Based on the above data, the structure of **2** was determined to be 3α-acetoxy-30-

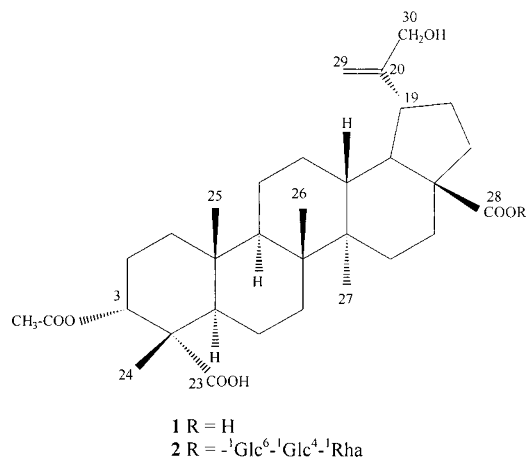

Fig. 1. Structures of Compounds **1** and **2**

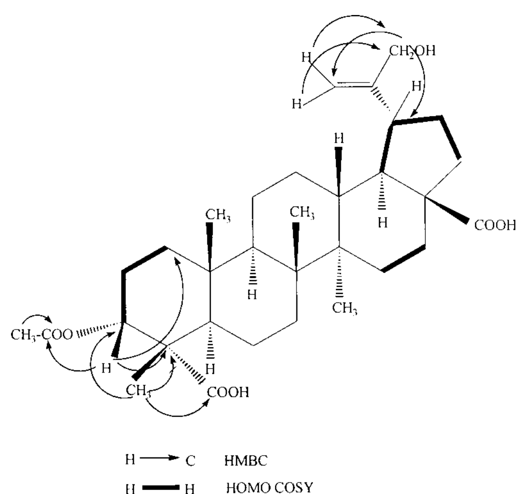

Fig. 2. Selected H–C Long-Range Correlations in HMBC Spectrum and H–H Correlations in COSY Spectrum of Compound **1**

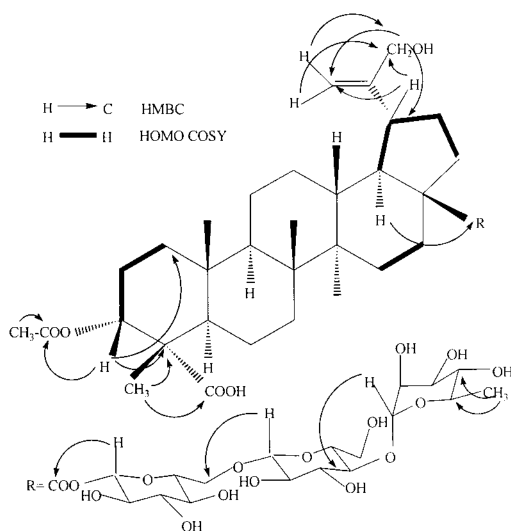

Fig. 3. Selected H–C Long-Range Correlations in HMBC Spectrum and H–H Correlations in COSY Spectrum of Compound **2**

hydroxylup-20(29)-ene-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, which we called acantrifoside C.

Experimental

Melting points were determined using a Kofler micro-hotstage; IR spectra were obtained on a Hitachi 270-30 type spectrometer with 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. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) were recorded on a Bruker AM400 FT-NMR spectrometer and TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70—230 mesh and 230—400 mesh, Merck), YMC * GEL ODS-A and Dianion HP-20 resin. HPLC was performed using an ODS column (Waters, NOVA-Pak C_{18}, 3.9×300 mm).

Plant Material *A. trifoliatus* was collected in Langson province, Vietnam in January 2001 and identified by Prof. Dr. Tran Minh Hoi, Institute of Ecology, Biological Resources, NCST of Vietnam. Voucher specimens (No. 2539) are deposited at the herbarium of the Institute of Natural Product 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 give MeOH extract (250 g), which was suspended in water and then partitioned with dichloromethane. The dichloromethane fraction (56 g) was then subjected to repeated chromatography on a silica gel column, using hexane–acetone (5 : 1) as eluent and repeatedly recrystallized from MeOH to yield **1** (30.0 mg) as white crystals.

The residue of 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% H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, 100% MeOH). The 20% MeOH and 40% MeOH fractions were combined and chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (70 : 30 : 4) as eluent. Repeated CC on a YMC RP-18 column using a MeOH–H_2O (7 : 3) system yielded **2** (45.0 mg).

3α-Acetoxy-30-hydroxylup-20(29)-ene-23,28-dioic Acid (**1**): White crystals, mp 278—279 °C, $[\alpha]_D^{25}$ $-12.9°$ (c=0.51, MeOH); IR (KBr) cm^{-1}: 3400 (br, OH), 3070 and 1645 (>C=CH_2), 1747 (>C=O); FAB-MS *m/z*: 543 $[M-H]^-$ (negative); HR-FAB-MS *m/z* 567.3300 $[M+Na]^+$ (Calcd 567.3298 for $C_{32}H_{48}O_7Na$); ^{1}H- and ^{13}C-NMR: see Table 1.

3α-Acetoxy-30-hydroxylup-20(29)-ene-23,28-dioic Acid 28-*O*-α-L-Rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl Ester (**2**): White powder, mp 217—218 °C, $[\alpha]_D^{25}$ $-19.5°$ (c=0.51, MeOH); IR (KBr) cm^{-1}: 3410 (br, OH), 3070 and 1645 (>C=CH_2), 1720 (>C=O), 1060 (C–O–C); FAB-MS *m/z*: 1013 $[M-H]^-$, HR-FAB-MS *m/z*: 1013.4951 $[M-H]^-$ (Calcd 1013.4956 for $C_{50}H_{77}O_{21}$); ^{1}H- and ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 2 A solution of **2** (30.0 mg in 5.0 ml 10% HCl) was refluxed for 5 h. The reaction solution was concentrated under reduced pressure and the residue was diluted with 30 ml H_2O. The solution was neutralized with Ag_2CO_3 and extracted with EtOAc. The EtOAc extract was evaporated and chromatographed on a Sephadex LH-20 column (*ca.* 30 g) using MeOH (800 ml) as eluent to afford aglycon **1** (12.0 mg). The H_2O layer was concentrated, filtered and passed through a NOVA-Pak C_{18} cartridge (Waters) and then repeatedly separated by HPLC {HPLC conditions: mobile phase: MeCN–H_2O (3 : 1), flow rate: 0.6 ml/min, detection: refractive index (RI)} to afford D-glucose (6.5 mg) and L-rhamnose (3.5 mg). The optical rotation values of the above monosaccharides indicated these sugars to be β-D-glucose {$[\alpha]_D^{25}$ +52.5° (c=0.70, H_2O); lit. value[13] +52.7°} and α-L-rhamnose {$[\alpha]_D^{25}$ +8.0° (c=0.35, H_2O); lit. value[13] +8.2°}.

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