Ananosic Acids B and C, Two New 18(13→12)-*abeo*-Lanostane Triterpenoids from *Kadsura ananosma*

Ye-Gao Chen,† Li-Na Hai,‡ Xin-Rong Liao,‡ Guo-Wei Qin,§ Yu-Yuan Xie,§ and Fathi Halaweish*,⊥

Department of Chemistry, Yunnan Normal University, Kunming 650092, People's Republic of China, Department of Traditional Chinese Herbs, Yunnan Institute of Traditional Chinese Medicine, Kunming 650011, People's Republic of China, Shanghai Institute of Materia Medica, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, People's Republic of China, and Department of Chemistry and Biochemistry, South Dakota State University, Brookings, South Dakota 57007

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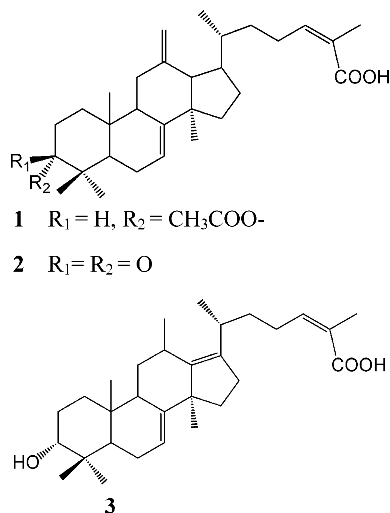
Two new 18(13→12)-*abeo*-lanostane triterpenoid acids, ananosic acids B (**1**) and C (**2**), were isolated from the stems of *Kadsura anaosma.* Their structures were elucidated by spectral studies and chemical transformation. Compounds **1** and **2** were evaluated for cytotoxicity using CCRF-CEM leukemia cells and HeLa cells.

A considerable number of studies have been performed on plants of the family Schisandraceae, which contains only two genera, *Schisandra* and *Kadsura*. These investigations have yielded dibenzocyclooctadiene lignans, lanostane triterpenoid acids, and lactones with pharmacological properties, including antihepatitis, antihepatotoxic, antioxidant, antitumor, and anti-HIV activities.[1−5] *Kadsura ananosma* Kerr is a plant indigenous to Yunnan Province, People's Republic of China.[6] Previously, a triterpenoid acid, a lignan, and four sesquiterpenoids were isolated from this species.[7,8] In the course of a search for bioactive natural products, we have reinvestigated this plant and isolated two new 18(13→12)-*abeo*-lanostane triterpenoid acids, ananosic acids B (**1**) and C (**2**), from the stems. The isolation and structure elucidation of compounds **1** and **2** and their cytotoxicity for two cell lines are reported herein.

A CH_2Cl_2 extract of *K. ananosma* stems was dissolved in diethyl ether and extracted with 1 M Na_2CO_3. The aqueous layer was acidified with HCl and extracted with EtOAc. Repeated column chromatography of the obtained acidic portion yielded ananosic acids B (**1**) and C (**2**).



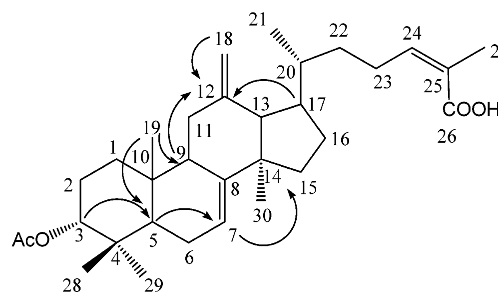


Figure 1. HMBC correlations of **1**.

Ananosic acid B (**1**) was obtained as a white amorphous powder. Its HREIMS exhibited a molecular ion peak at *m*/*z* 496.3565 [M^+] (calcd for $C_{32}H_{48}O_4$). The IR spectrum showed absorption bands at 3444 (OH), 1727 (ester), 1691 (carboxyl group), and 1636 (double bond) cm^{-1}. The ^1H NMR (Table 1), ^{13}C NMR (Table 2), and DEPT spectra revealed the presence of six methyls, nine methylenes, eight methines, and seven quaternary carbons besides an acetyl group (δ 2.06, 3H, s; 170.7 s, 21.3 q). This indicated that **1** is an acetylated triterpenoid, which was supported by a significant peak at *m*/*z* 436 ($M^+ - 60$) in the EIMS. The ^1H NMR spectrum showed signals for a vinyl proton and methyl group [δ 6.10 (1H, t, $J = 7.1$ Hz) and 1.92 (3H, d, $J = 0.8$ Hz)], a secondary methyl [δ 0.93 (3H, d, $J = 6.1$ Hz)] and four tertiary methyl groups at δ 1.07, 1.03, 0.99, and 0.90 (each 3H, s), which closely resembled those of ananosic acid A (**3**), the initial 18(13→12)-*abeo*-lanostane triterpenoid acid isolated from this plant.[7] The signal at δ 4.67 (1H, br s) indicated that an acetoxy group was attached to C-3 with α-orientation. The signal at δ 5.42 (1H, brs) showed there was a trisubstituted double bond located at C-7, C-9 (11), or C-5.[6,8] In the HMBC spectrum (Figure 1), the olefinic proton signal at δ 5.42 was correlated with carbon signals at δ 44.0 (C-14) and 40.3 (C-5), suggesting the trisubstituted double bond was at C-7.[8] Comparison of the ^1H NMR spectrum of **1** with that of **3** revealed that an exomethylene group occurred between C-12 (δ 151.0 s) and C-18 [δ 111.3; 4.76, 4.69 (each 1H, d, $J = 2.3$ Hz)], which was supported by correlations between C-12 and H-18, H-9 (δ 2.25) and H-17 (δ 1.88) in the HMBC spectrum. Thus, **1** was elucidated as 3α-acetoxy-18(13→12)-*abeo*-lanost-7(8),12(18),24(*Z*)-trien-26-oic acid. The ^1H and ^{13}C NMR spectral data were fully assigned by various NMR techniques, including DEPT, $^1H-^1H$ COSY, HSQC, and HMBC.

* To whom correspondence should be addressed. Tel: (605) 688-4269. Fax: (605) 688-6364. E-mail: Fathi_Halaweish@sdstate.edu.
† Yunnan Normal University.
‡ Yunnan Institute of Traditional Chinese Medicine.
§ Shanghai Institute of Materia Medica.
⊥ South Dakota State University.

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Table 1. ^{1}H NMR Data of Compounds **1** and **2** ($CDCl_3$, J in Hz)

position	1	2
1	1.56 (1H, m), 1.44 (1H, m)	1.71 (1H, m), 1.68 (1H, m)
2	1.88 (1H, m), 1.62 (1H, m)	2.74 (1H, m), 2.25 (1H, m)
3	4.67 (1H, brs)	
5	1.35 (1H, m)	1.30 (1H, m)
6	1.88 (1H, m), 1.62 (1H, m)	2.13 (1H, m), 2.03 (1H, m)
7	5.42 (1H, brs)	5.40 (1H, brd, J = 7.0)
9	2.25 (1H, m)	2.35 (1H, m)
11	1.68 (1H, m), 1.55 (1H, m)	1.58 (1H, m), 1.47 (1H, m)
13	2.16 (1H, m)	2.13 (1H, m)
15	1.67 (1H, m), 1.12 (1H, m)	1.58 (1H, m), 1.14 (1H, m)
16	1.77 (1H, m), 1.55 (1H, m)	1.65 (1H, m), 1.45 (1H, m)
17	1.88 (1H, m)	1.87 (1H, m)
18	4.76 (1H, d, J = 2.3)	4.70 (1H, d, J = 2.0)
	4.69 (1H, d, J = 2.3)	4.65 (1H, d, J = 2.0)
19	1.03 (3H, s)	1.16 (3H, s)
20	1.70 (1H, m)	1.70 (1H, m)
21	0.93 (3H, d, J = 6.1)	0.89 (3H, d, J = 6.4)
22	1.69 (1H, m), 1.17 (1H, m)	1.68 (1H, m), 1.20 (1H, m)
23	2.55 (2H, m)	2.52 (2H, m)
24	6.10 (1H, t, J = 7.1)	6.08 (1H, t, J = 7.2)
27	1.92 (3H, d, J = 0.8)	1.90 (3H, s)
28	0.90 (3H, s)	1.07 (3H, s)
29	0.99 (3H, s)	1.08 (3H, s)
30	1.07 (3H, s)	1.04 (3H, s)
CH_3COO-	2.06 (3H, s)	

Table 2. ^{13}C NMR Data for Compounds **1** and **2** ($CDCl_3$)

carbon	1	2	carbon	1	2
1	30.3 t	35.8 t	17	47.8 d	47.5 d
2	22.6 t	34.8 t	18	111.3 t	111.3 t
3	78.8 d	216.8 s	19	23.4 q	22.6 q
4	36.1 s	47.5 s	20	31.0 d	31.2 d
5	40.3 d	46.7 d	21	18.3 q	18.3 q
6	22.6 t	23.6 t	22	33.2 t	33.2 t
7	114.5 d	113.9 d	23	26.7 t	26.7 t
8	151.2 s	151.5 s	24	146.6 d	146.8 d
9	51.0 d	50.4 d	25	126.3 s	126.2 s
10	34.0 s	34.3 s	26	173.0 s	173.3 s
11	30.9 t	30.4 t	27	20.6 q	20.6 q
12	151.0 s	150.3 s	28	28.2 q	25.7 q
13	50.6 d	50.4 d	29	22.5 q	22.6 q
14	44.0 s	43.6 s	30	23.8 q	23.9 q
15	30.1 t	30.2 t	CH_3COO-	21.3 q	
16	24.6 t	24.5 t		170.7 s	

Anānosic acid C (**2**), obtained as an amorphous powder, was assigned the molecular formula $C_{30}H_{44}O_3$, as revealed by its HREIMS (*m/z* 452.3300). It showed features in the NMR spectra very similar to those of **1**. The only structural difference occurred in the substitution at C-3. Its ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectra suggested a keto group (δ 216.8) at C-3 in **2**, which was confirmed by a chemical transformation. Hydrolysis of **1** with KOH and then oxidation with CrO_3 in pyridine gave **2** (identified by TLC and ^{1}H NMR). Thus, **2** was elucidated as 3-oxo-18-(13→12)-*abeo*-lanost-7(8),12(18),24(*Z*)-trien-26-oic acid. The structure proposed for **2** is compatible with all the ^{1}H and ^{13}C NMR data obtained. Compounds **1** and **2** are the second and third examples of 18(13→12)-*abeo*-lanostane triterpenoids found in nature.

The cytotoxic activity of compounds **1**–**3** was examined in vitro using CCRF-CLM leukemia cells and showed IC_{50} values of 49.6, 45.2, and 45.4 μg/mL, respectively. Compounds **1**–**3** showed IC_{50} values of 0.54, 0.48, and 0.46 μg/mL, respectively, toward HeLa cells in vitro.

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a Polax-2L polarimeter. IR spectra were recorded as KBr pellets on a Bio-Rad FTS-135 spectrophotometer. NMR spectra were measured on a Bruker DRX 500 spectrometer with TMS as internal standard and $CDCl_3$ as solvent. EIMS were determined on a VG Autospec-3000 spectrometer, and HREIMS were acquired on a MASPEC II system. Silica gel (200–300 mesh, Qingdao) was used for column chromatography and silica gel plates (GF_{254}, Yantai Institute of Chemical Technology) for analytical TLC. Spots were observed under UV light and visualized by spraying with 15% H_2SO_4 followed by heating.

Plant Material. The stem bark of *Kadsura ananosma* was collected from Men-Na County of Yunnan Province, People's Republic of China, in February 1998, and identified by Mr. Hong Wang, a botanist of Xi-Shuang-Bang-Na Botanical Garden, Chinese Academy of Sciences, where a voucher specimen (No. 9802015) is deposited.

Extraction and Isolation. The dried, powered stem bark of *K. ananosma* (6.0 kg) was extracted exhaustively with CH_2Cl_2. The CH_2Cl_2 extract was concentrated in vacuo to give a residue (500 g), of which 400 g was dissolved in 1 L of diethyl ether and extracted with 1 N Na_2CO_3 (250 mL × 4). The aqueous layer was acidified with HCl to pH 3 and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield 130 g of a residue. The latter was subjected to silica gel column chromatography eluting with 400 mL portions of a solvent gradient system with increasing polarity from petroleum ether to ethyl acetate to afford eight fractions (A–I). Fractions D and G were subjected to repeated column chromatography to yield ananosic acid B (**1**, 140 mg) and ananosic acid C (**2**, 60 mg), respectively.

Ananosic acid B (1): amorphous powder, $[\alpha]_D$ −55.0° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3444, 3060–2700, 1728, 1691, 1570, 1376, 1246, 1078, 987 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS (70 eV) *m/z* 496 [M$^+$] (100), 481 (29), 436 (96), 421 (82), 313 (60), 253 (82), 240 (45), 213 (26), 173 (44), 107 (46); HREIMS *m/z* 496.3565 (calcd for $C_{32}H_{48}O_4$, 496.3553).

Ananosic acid C (2): amorphous powder, $[\alpha]_D$ −62.0° (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3095–2900, 1725, 1695, 1570, 1376, 1246, 1078, 987 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS (70 eV) *m/z* 452 [M$^+$] (100), 437 (41), 421 (7), 311 (22), 269 (88), 256 (50), 244 (47), 229 (21), 173 (27), 105 (61), 95 (71); HREIMS *m/z* 452.3300 (calcd for $C_{30}H_{44}O_3$, 452.3290).

Hydrolysis and Oxidation of 1. A solution of **1** (14.6 mg) in 0.5 mol/L KOH (4 mL) was refluxed for 1 h. The reaction mixture was acidified with 5% HCl to pH 7 and extracted with

diethyl ether. The organic layer was evaporated and dissolved in anhydrous pyridine (2 mL) and then added to 50 mg of CrO_3. The mixture was stirred at 20 °C for 3 h, diluted with 5% HCl, and extracted with diethyl ether. The organic layer was washed with H_2O to pH 7 and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified, yielding 5 mg of **2**.

Cytotoxicity Testing. All compounds were solublized in DMSO (Sigma, St. Louis, MO) and stored at −20 °C. Cytotoxicity assays (IC_{50} μg/mL) were carried out against murine leukemia (ATCC: CCRF-CEM) and Hela (ATCC-17) cells. The methodology for this in vitro cytotoxicity screening was conducted using a previously published protocol. Cytotoxicity was determined by measuring cell viability, using doxorubicin as positive control.[9,10]

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