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Unusual cycloartane triterpenoids from *Kadsura ananosma*

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ABSTRACT

Five 3,4-*seco*-cycloartane triterpenoids were isolated from the stems of *Kadsura ananosma*, two of which had rearranged 5/6 consecutive carbocycle rings C/D (trivially named ananosins A (**1**) and B (**2**)), one had a migrated CH_3-18 (named ananosins C (**3**)), and two were analogs, ananosins D (**4**) and E (**5**). Their structures were characterized by comprehensive spectroscopic analysis, especially using 2D NMR spectra. A biogenetic pathway to **1** was proposed. These 5 compounds, together with 5 known analogs isolated from the same origin, were evaluated for their cytotoxicity against HL-60, SMMC-7721, A-549, PANC-1, and SK-BR-3 human cancer cells, but were inactive.

1. Introduction

A considerable number of studies have been performed on plants of the Schisandraceae family, which only contains two genera, *Schisandra* and *Kadsura*; yet, this is one of the most important groups of medicinal plants. Indeed, the roots and stems of many *Kadsura* species have been both widely and long used in Chinese folk medicine as agents for analgesia and in promoting blood circulation (Chen, 2007). Some *Kadsura* species are also rich sources of dibenzocyclooctadiene lignans (Liu and Li, 1995; Ookawa et al., 1995; Gao et al., 2008a,b; Shen et al., 2009), as well as lanostane (Li et al., 2008; Wang et al., 2008) and cycloartane triterpenoids (Sy and Brown, 1999; Wang et al., 2006); some species also have beneficial pharmacological properties, including platelet aggregation inhibiting (Jiang et al., 2005), antitumor (Chen et al., 2002), anti-HIV (Chen et al., 1996, 1997), and cytotoxic activities (Chen et al., 2004; Pu et al., 2005, 2006). Spiro-benzofuran-type dibenzocyclooctadiene lignans are also major bioactive substances in *Kadsura* medicinal plants, with strong pharmacological effects as regards promoting blood circulation. Interestingly, there are characteristic chemical compositions in *Kadsura* species that differ substantially from those in *Schisandra* species (Chen, 2007; Jiang et al., 2005).

As a liana indigenous to Yunnan Province, China, *Kadsura ananosma* Kerr had no reported applications in Chinese folk medicine (Liu, 1996), although previous phytochemical studies on of *K. ananosma* seeds mainly found a series of dibenzocyclooctadiene lignans (Yang et al., 2011a,b), while studies on the stems established that it mainly produced nor-triterpenoids (Yang et al., 2010a), longipedlactone (Yang et al., 2010b) and lanostane triterpenoids, dibenzocyclooctadiene lignans, and sesquiterpenoids (Zou et al., 1993; Chen et al., 2001, 2004, 2006a,b), respectively. Further examination of its stems herein led to isolation of five new 3,4-*seco*-cycloartane triterpenoids (**1**–**5**), and five known compounds. Interestingly, the C and D rings of **1** and **2** were rearranged to 5/6 consecutive carbocycle systems with an exocyclic double bond on the D ring, thereby markedly differing from previously isolated 6/5 consecutive carbocycles of known cycloartane triterpenoids. Compound **3** possessed the typical 6/5 consecutive carbocycles (rings C/D) of a cycloartane skeleton, although the CH_3-18 moiety had migrated from C-13 to C-12, which had never been observed before in cycloartane triterpenoids. All triterpenoids were evaluated as regards cytotoxicity activities against human tumor cell lines of HL-60 (*acute leukemia*), SMMC-7721 (*liver cancer*), A-549 (*lung cancer*), PANC-1 (*pancreatic cancer*) and SK-BR-3 (*colon cancer*) by the MTT method (Reed and Muench, 1938), but no activity was noted. In this paper, the isolation, structure elucidation, cytotoxicity evaluation and proposed biogenetic pathway are reported herein.

2. Results and discussion

Phytochemical study of the ethyl acetate extract of *K. ananosma* led to isolation of two unusual rearranged cycloartane triterpenoids, ananosins A (**1**) and B (**2**), three new cycloartane triterpenoids, ananosins C–E (**3**–**5**), together with five known ones,

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including schinalactone C (He et al., 2010), kadsulactone A (Chen et al., 1990), kadcoccilactone Q (Gao et al., 2008b), kadsuphilactone B (Shen et al., 2005), and schisandronic acid (Pu et al., 2007). The structures of these known compounds were identified by comparing their spectroscopic data with those reported in the literature.

Ananosin A (**1**) was obtained as an amorphous powder. Its HRESIMS analysis indicated that it has a molecular formula of $C_{32}H_{44}O_7$ (*m/z* 563.2976 [M+Na]$^+$, calcd 563.2984), suggesting 11° of unsaturation. Its UV spectrum showed absorption maxima at 236 and 204 nm, suggesting the occurrence of a conjugated system. The IR absorptions indicated presence of a hydroxy group (3439 cm^{-1}) and two lactone functional groups (1745 and 1720 cm^{-1}). The ^{13}C NMR and HSQC spectra of **1** displayed 32 carbon signals, including four singlet and one doublet methyls, nine methylenes (one olefinic), seven methines (two oxygenated and one olefinic), nine quaternary carbons (two carbonyls, two oxygenated and two olefinic), and an acetyl group (169.5, C, and 21.1, CH_3) (Tables 1 and 2). Apart from two double bonds and three carbonyl groups, the remaining elements of unsaturation suggested that **1** is probably a hexacyclic triterpene.

Careful analysis of the NMR spectroscopic data indicated that **1** had the same C/D/E rings as longipedlactone B (Pu et al., 2006). Furthermore, the ^{1}H NMR spectrum supported the presence of a cycloartane-type skeleton with typical high-field AB doublets due to non-equivalent hydrogens at C-19 (δ_H 0.76, d, J = 4.8 Hz, and δ_H 1.63, d, J = 4.8 Hz) in the cyclopropane ring (Shen et al., 2005), this being further confirmed by HMBC correlations of H_2-19 with C-5 (C), C-8 (CH), C-9 (C), C-10 (C), and C-11 (CH_2), respectively. The location of an acetyl group and a hydroxy group were also confirmed at C-6 and C-4, by HMBC correlations from H-6 (δ_H 5.31) to the acetyl carbonyl (δ_C 169.5), and from OH (δ_H 7.10) to the C-4, C-5, CH_3-29 and CH_3-30. A six-membered lactone was unprecedentedly formed by coupling of carbonyl C-3 and C-5 through an oxygen atom, this being deduced by the downfield shift of the ^{13}C NMR signal for C-5 (δ_C 85.2) and by combining the remaining elements of unsaturation of **1**. Observed ROESY correlations between H-6 and H_2-7, H_3-29, but no correlation between H-6 and H-8, showed that AcO-6 is β-oriented. The stereochemistry of the oxygen atom located at C-5 is thus assigned as having an α-orientation, this being deduced from the ROESY correlation of H-2α–H-11α, and no correlation observed for H-2β with H-19α. The rest of the relative configuration of **1** was determined to be the same as longipedlactone B from further analysis of the ROESY spectrum (Fig. 2). Therefore, structure **1** was determined as shown in Fig. 1, and named ananosin A.

Ananosin B (**2**) was isolated as an amorphous powder, and had a molecular formula of $C_{33}H_{48}O_7$, as determined by HRESIMS ([M+Na]$^+$ *m/z* 579.3308, calcd 579.3297), requiring 10° of unsaturation. The ^{1}H, ^{13}C NMR and DEPT spectra of **2** (Tables 1 and 2) were very similar to **1**, except for the signals ascribed to ring A. The NMR spectroscopic data of **2** showed a methoxy group (δ_C 51.3, q) located at C-3, which was confirmed by HMBC correlations of H-1, H-2 and H_3 of OCH_3 with C-3 (δ_C 174.5, s). The location of a hydroxy group was established at C-4 based on HMBC correlations from H-5, H_3-29 and H_3-30 to C-4 (δ_C 74.4, s). The HMBC correlation of H-6 (δ_H 5.75) with

Table 1
^{1}H NMR spectroscopic data of compounds **1**–**5** in pyridine-d_5 (δ in ppm, J in Hz).

No.	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
1α	1.17 (m)	1.42 (overlap)	1.39 (m)	1.49 (overlap)	1.78 (overlap)
1β	3.31 (m)	3.34 (m)	3.27 (m)	3.53 (m)	3.39 (m)
2α	2.57 (m)	2.39 (m)	2.58 (m)	2.48 (m)	2.67 (m)
2β	2.83 (dd, 22.4, 8.0)	3.25 (m)	2.86 (overlap)	2.80 (dd, 20.6, 5.2)	3.26 (m)
5		2.20 (overlap)			2.19 (overlap)
6α	5.31 (d, 1.7)	5.75 (br s)	5.28 (br s)	5.34 (br s)	0.72 (m)
6β					1.76 (overlap)
7α	1.84 (overlap)	1.06 (t, 10.6)	1.91 (overlap)	1.80 (m)	1.02 (overlap)
7β	1.73 (m)	2.03 (overlap)	1.81 (m)	1.80 (m)	1.22 (m)
8	2.02 (overlap)	1.98 (overlap)	1.88 (overlap)	2.30 (m)	1.34 (overlap)
11α	2.12 (overlap)	2.02 (overlap)	2.34 (m)	5.45 (d, 12.6)	1.94 (m)
11β	1.84 (overlap)	2.02 (overlap)	0.98 (overlap)		1.69 (m)
12α	2.28 (overlap)	1.76 (overlap)	2.11 (m)	6.11 (d, 12.6)	1.34 (overlap)
12β		1.62 (overlap)			1.34 (overlap)
15α	1.64 (overlap)	1.62 (overlap)	1.47 (overlap)	1.23 (overlap)	1.62 (m)
15β	1.22 (m)	1.22 (m)	1.25 (m)	1.23 (overlap)	1.62 (m)
16α	1.34 (m)	1.38 (m)	2.27 (m)	1.25 (overlap)	1.28 (overlap)
16β	1.66 (overlap)	1.62 (overlap)	2.14 (m)	1.25 (overlap)	2.33 (m)
17	1.84 (overlap)	1.85 (m)		1.72 (m)	1.79 (overlap)
18α	5.10 (s)	5.09 (s)	1.31 (d, 7.1)	0.94 (s)	1.01 (s)
18β	4.90 (s)	4.88 (s)			
19α	0.76 (d, 4.8)	0.60 (d, 3.9)	0.69 (d, 4.8)	1.06 (d, 4.8)	0.59 (d, 4.2)
19β	1.63 (d, 4.8)	1.47 (d, 3.9)	1.65 (d, 4.8)	1.88 (d, 4.8)	1.80 (d, 4.2)
20	2.32 (m)	2.34 (overlap)	2.82 (overlap)	2.00 (overlap)	2.05 (overlap)
21	0.98 (d, 6.8)	0.95 (d, 6.7)	0.96 (d, 7.1)	1.06 (d, 6.6)	1.08 (d, 6.7)
22	4.63 (m)	4.62 (m)	4.32 (m)	4.41 (m)	5.40 (m)
23α	2.08 (overlap)	2.03 (overlap)	2.21 (m)	2.04 (overlap)	2.55 (m)
23β	2.21 (m)	2.20 (overlap)	2.38 (m)	2.22 (m)	2.55 (m)
24	6.45 (br d, 6.3)	6.45 (br d, 6.1)	6.50 (br d, 6.3)	6.54 (br d, 6.5)	7.30 (t, 7.2)
27	1.92 (s)	1.93 (s)	1.91 (s)	1.95 (s)	2.17 (s)
28	0.85 (s)	0.91 (s)	0.98 (s)	0.82 (s)	0.95 (s)
29	1.46 (s)	1.47 (s)	1.47 (s)	1.49 (s)	1.44 (s)
30	1.66 (s)	1.62 (s)	1.68 (s)	1.68 (s)	1.42 (s)
CH_3O-3		3.57 (s)			
AcO-6	2.02 (s)	2.00 (s)	2.10 (s)	2.14 (s)	
AcO-22					2.02 (s)

[a] Recorded at 400 MHz.
[b] Recorded at 500 MHz.

Table 2
^{13}C NMR spectroscopic data of compounds **1–5** in pyridine-d_5 (δ in ppm).

No.	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]
1	27.3 (t)	32.7 (t)	25.1 (t)	25.1 (t)	31.1 (t)
2	30.4 (t)	32.1 (t)	31.0 (t)	30.8 (t)	33.1 (t)
3	171.6 (s)	174.5 (s)	171.8 (s)	171.4 (s)	176.8 (s)
4	76.7 (s)	74.4 (s)	76.9 (s)	76.7 (s)	74.9 (s)
5	85.2 (s)	47.4 (d)	85.3 (s)	84.9 (s)	45.4 (d)
6	73.6 (d)	72.1 (d)	72.9 (d)	73.5 (d)	25.3 (t)
7	23.7 (t)	28.7 (t)	24.7 (t)	25.1 (t)	26.0 (t)
8	40.1 (d)	41.4 (d)	41.3 (d)	38.9 (d)	48.6 (d)
9	28.5 (s)	28.0 (s)	21.8 (s)	25.6 (s)	22.4 (s)
10	23.9 (s)	25.9 (s)	23.3 (s)	29.2 (s)	27.1 (s)
11	34.2 (t)	35.2 (t)	35.0 (t)	129.9 (d)	27.2 (t)
12	54.9 (d)	55.1 (d)	28.7 (d)	136.3 (d)	36.1 (t)
13	149.3 (s)	149.4 (s)	148.5 (s)	49.1 (s)	45.7 (s)
14	47.2 (s)	46.0 (s)	49.8 (s)	49.6 (s)	48.6 (s)
15	33.4 (t)	33.5 (t)	41.1 (t)	33.8 (t)	33.1 (t)
16	27.5 (t)	27.4 (t)	29.2 (t)	27.1 (t)	26.6 (t)
17	46.1 (d)	46.8 (d)	133.7 (s)	43.7 (d)	49.3 (d)
18	109.0 (t)	109.2 (t)	23.3 (q)	16.8 (q)	18.3 (q)
19	27.7 (t)	29.6 (t)	30.1 (t)	29.3 (t)	31.3 (t)
20	37.3 (d)	37.3 (d)	37.3 (d)	39.9 (d)	39.8 (d)
21	13.4 (q)	13.4 (q)	15.6 (q)	13.4 (q)	12.7 (q)
22	79.6 (d)	79.7 (d)	80.0 (d)	80.3 (d)	75.8 (d)
23	24.6 (t)	24.6 (t)	28.5 (t)	23.7 (t)	27.7 (t)
24	140.1 (d)	140.0 (d)	139.6 (d)	140.2 (d)	137.9 (d)
25	128.0 (s)	128.0 (s)	128.2 (s)	128.1 (s)	130.8 (s)
26	166.2 (s)	166.1 (s)	165.7 (s)	166.3 (s)	170.1 (s)
27	17.2 (q)	17.1 (q)	17.2 (q)	17.2 (q)	12.8 (q)
28	24.4 (q)	24.6 (q)	22.0 (q)	21.2 (q)	19.5 (q)
29	26.6 (q)	31.0 (q)	26.7 (q)	26.8 (q)	31.7 (q)
30	28.5 (q)	28.1 (q)	28.3 (q)	28.2 (q)	26.6 (q)
CH_3O-3		51.3 (q)			
AcO-6	169.5 (s)	170.2 (s)	169.6 (s)	169.5 (s)	
	21.1 (q)	21.1 (q)	21.2 (q)	21.0 (q)	
AcO-22					170.2 (s)
					20.9 (q)

[a] Recorded at 100 MHz.
[b] Recorded at 125 MHz.

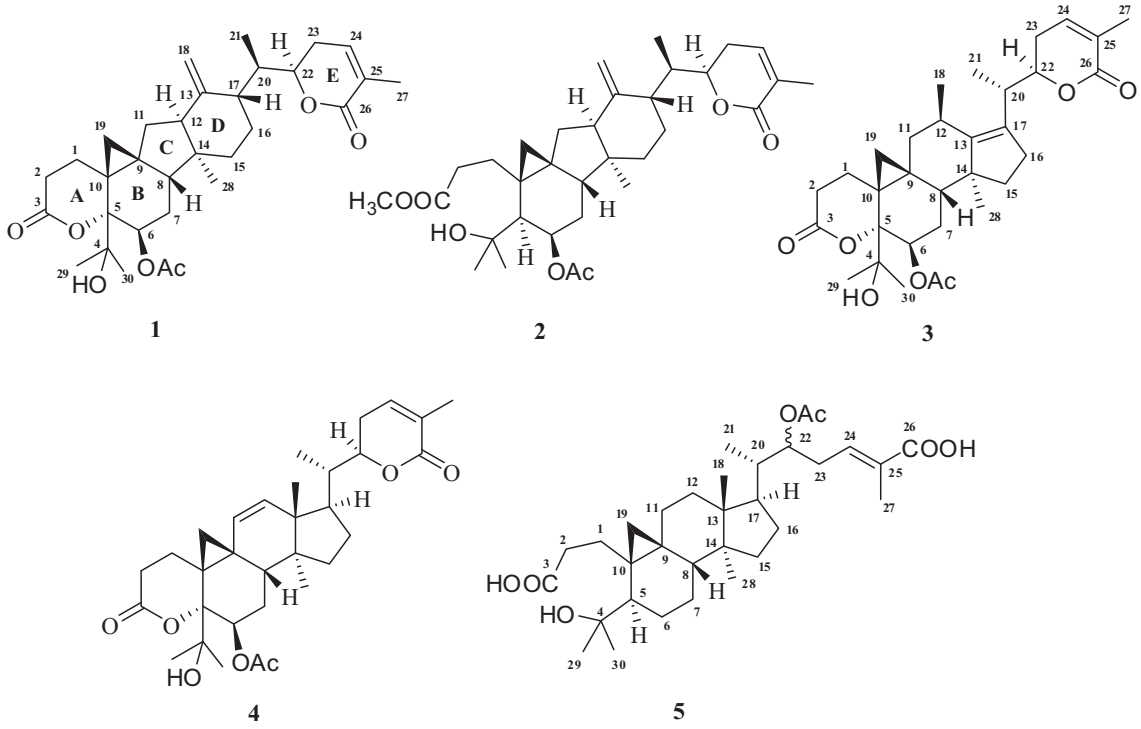


Fig. 1. Structures of compounds **1–10**.

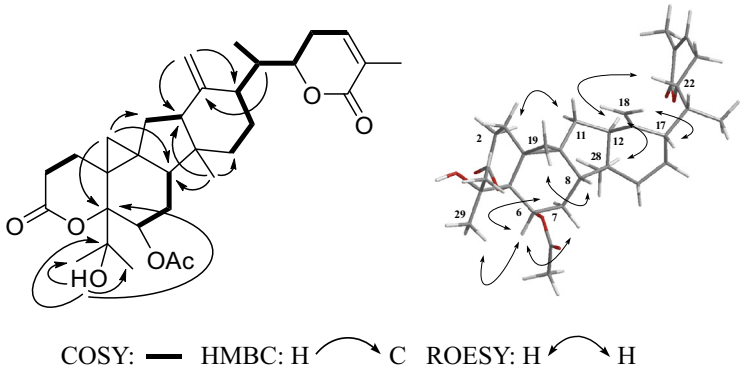


Fig. 2. Key COSY, HMBC and ROESY correlations of **1**.

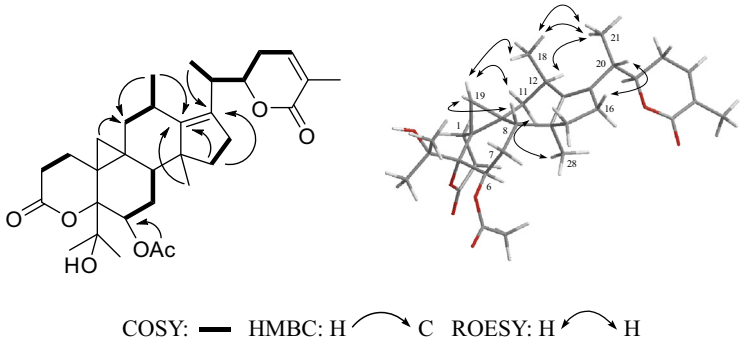


Fig. 3. Key COSY, HMBC and ROESY correlations of **3**.

an acetyl carbonyl (δ_C 170.2) indicated that an acetyl group was located at C-6. The β-configuration of AcO-6 was thus deduced on the basis of ROESY correlations (H-6/H-5α, H-6/CH_3-29α, H-6/H-7α, and H-6/H-7β). The rest of the relative stereochemistry of **2** was assigned as being the same as that for **1** from further analysis of the ROESY spectrum and its spectroscopic data. Thus, structure **2** as shown in Fig. 1, named as ananosin B, was unambiguously determined.

Ananosin C (**3**) was isolated as an amorphous powder. Its molecular formula, $C_{32}H_{44}O_7$, was established from HRESIMS ($[M+Na]^+$ m/z 563.2989, calcd 563.2984) to be the same as **1**. Carefully analyzing the 2D NMR spectroscopic data, and comparison with that of **1**, compound **3** was deduced to retain 6/5 consecutive carbocycle rings C/D as a typical cycloartane triterpenoid, which was also in accord with the observation of a striking upfield shift of the signal of C-12 from δ_C 54.9 in **1** to δ_C 28.7 in **3**. The location of CH_3-18 was confirmed at C-12, based on the HMBC correlations of CH_3-18 with C-11, C-12 and C-13, indicating that CH_3-18 had migrated from C-13 to C-12; moreover, the coupling constant value J = 7.1 Hz of H_3-18 further confirmed the above conclusion. The HMBC correlations of both H_3-30 and H-15 with C-13 (δ_C 148.5, s), and both H-15 and H_3-21 with C-17 (δ_C 133.7, s), established that a double bond was formed between C-13 and C-17. In the ROESY spectrum, the correlations from H-8 to H-19β, from H-19α to H_3-18, and from H-8 to H_3-18 indicated that H-8 and CH_3-18 were β-oriented. The correlations from H_3-21 to H-12α and H_3-18, and from H-20 to H-16β, also indicated CH_3-21 as being α-oriented (Fig. 3). The rest of the relative configuration of **3** was determined to be the same as for **1** from further analysis of the ROESY spectrum. Consequently, structure **3** (ananosin C) as shown in Fig. 1, was established as shown.

Ananosin D (**4**) was obtained as an amorphous powder and established to have a molecular formula of $C_{32}H_{44}O_7$ by HRESIMS ($[M+Na]^+$ m/z 563.2986, calcd 563.2984). Precise comparison of the ^1H and ^{13}C NMR spectra of **3** and **4** indicated that the latter was a cycloartane triterpenoid. The singlet resonance of H_3-18 in **4** established that CH_3-18 had not migrated from C-13 to C-12 as in **3**. This was further confirmed by HMBC correlations from H_3-18 to C-12, C-13, C-14 and C-17, respectively. The location of the bi-substituted double bond was assigned as at C-11 and C-12 on the basis of the HMBC correlations from H-11 (δ_H 5.45, d, J = 12.6 Hz) to C-8, C-13 and C-19, and from H-12 (δ_H 6.11, d, J = 12.6 Hz) to C-9, C-14 and C-17. The HMBC correlations observed from H-6 (δ_H 5.34) to acetyl carbonyl (δ_C 169.5) indicated that an acetyl group was located at C-6. ROESY correlations were observed from CH_3-18 to H-8 and H_2-19, which established that CH_3-18 was in a β-orientation. The rest of the relative stereochemistry of **4** was assigned as being the same as **3** from further analysis of the ROESY spectrum and its spectroscopic data. Therefore, structure **4** was determined as shown in Fig. 1, and this has been named ananosin D.

Ananosin E (**5**) was isolated as a white powder and had a $[M+Na]^+$ ion peak at m/z 569.3460 in its HRESIMS, this being consistent with the molecular formula of $C_{32}H_{50}O_7$ (calcd 569.3454), requiring 8° of unsaturation. The ^1H NMR spectra displayed mutually coupled doublets at δ_H 0.59 (d, J = 4.2 Hz) and 1.80 (d, J = 4.2 Hz) which indicated presence of a cycloartane-type skeleton. Detailed comparison of the NMR spectroscopic data of **5** with those of lancifoic acid A (Xiao et al., 2006) established that these two compounds were identical except for an *O*-acetyl group located at C-22 in **5**. This was confirmed by the HMBC correlation of the proton signal at δ_H 5.40 (H-22) with the resonance δ_C 170.2 (acetyl carbonyl). The ROESY correlation of CH_3-27 with H-23, and absence of a ROESY correlation of CH_3-27 with H-24, supported the

E-type olefinic bond between C-24 and C-25. The relative configuration of C-22 was not determined due to free rotation of the side-chain. Accordingly, ananosin E was assigned as **5**, and the structure was determined as shown in Fig. 1.

All triterpenoids were tested for their cytotoxicity against the HL-60, SMMC-7721, A-549, PANC-1 and SK-BR-3 human tumor cell lines by the MTT method (Reed and Muench, 1938), but no activity was noted with IC_{50} values more than 40 μM.

A plausible biogenetic pathway for ananosin A (**1**) was proposed on the basis of schinalactone C isolated from the same plant. Hydroxylation of schinalactone C at C-12 could result in intermediate **A**, which might then be followed by a Wagner–Meerwein rearrangement through intermediate **B** and **C** to give **D** (Pu et al., 2006). Then, hydroxylation at C-5 (intermediate **E**) and lactonization between C-3 and C-5 could yield F, which could then undergo successive hydroxylation and acetylation at C-6 to finally generate ananosin A (**1**) (Scheme 1).

3. Concluding remarks

In this paper, studies on the chemical constituents of *K. ananosma* stems resulted in isolation of two unusual cycloartane triterpenoids (**1** and **2**), whose obvious differences to that of typical cycloartane triterpenoids indicated rings C and D were rearranged to 5/6 consecutive carbocycle systems with an exocyclic double bond in D ring; this had never been discovered before in cycloartane triterpenoids. Therefore, the above compounds can be considered as characteristic constituents in *K. ananosma* for chemotaxonomic studies.

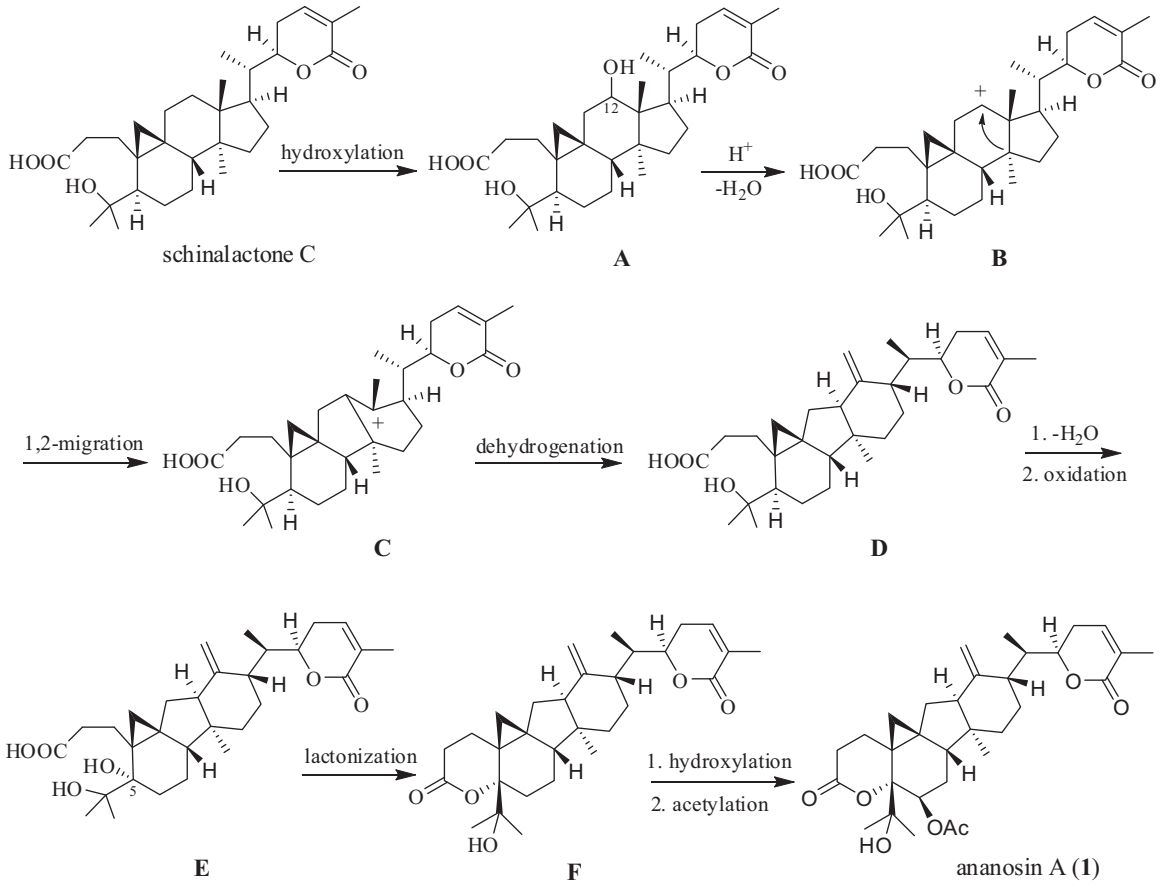
4. Experimental

4.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight and VG Autospec-3000 spectrometers, respectively. Column chromatography (CC) was performed with silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, PR China) and MCI gel (75–150 mm, Mitsubishi Chemical Corporation, Tokyo, Japan). Semi-preparative HPLC was carried out with an Agilent 1100 liquid chromatograph equipped with a Zorbax SB-C18 (9.4 mm × 25 cm) column. Fractions were monitored by TLC ($CHCl_3$, acetone and MeOH solvent system) with spots visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

4.2. Plant material

K. ananosma stems were collected in the Simao County of Yunnan Province, People's Republic of China, in August 2006, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (KIB 08102009) is deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.



Scheme 1. Proposed biogenetic pathway to ananosin A (**1**).

4.3. Extraction and isolation

Air-dried and powdered stems of *K. ananosma* (10 kg) were extracted with Me_2CO–H_2O (40 L × 3, 70:30, v/v, each 2 days) at room temperature and concentrated *in vacuo* to yield a residue (860 g), which was partitioned between H_2O and EtOAc. The EtOAc extract (300 g) was subjected to MCI–gel CHP 20P CC, eluted with a gradient of MeOH–H_2O. The MeOH:H_2O (90:10, v/v) fraction (245 g) so obtained was subjected to silica gel (200–300 mesh, 3.0 kg) CC, eluting with a $CHCl_3$–Me_2CO gradient system (9:1, 8:2, 2:1, 1:1, 0:1), to give fractions 1–5. Fraction 2 (38 g) was applied to a silica gel column, eluted in a step gradient manner with petroleum ether–Me_2CO (5:1, 2:1, 0:1) to afford three subfractions 2.1–2.3. Semi-preparative HPLC (MeOH–H_2O, 65:35) of subfraction 2.2 (230 mg) yielded **1** (4 mg), **2** (9 mg), **4** (3 mg), **6** (3 mg), **7** (32 mg) and **9** (6 mg), respectively. Fraction 3 (20 g) was subjected to silica gel CC, which was eluted with $CHCl_3$–MeOH (60:1, 30:1, 15:1, 5:1) to obtain four main subfractions 3.1–3.4. Subfraction 3.3 (307 mg) was finally purified by semi-preparative HPLC (MeOH–H_2O, 60:40) to yield **3** (3 mg), **5** (7 mg), **8** (10 mg), and **10** (200 mg).

4.3.1. Ananosin A (1)

White amorphous powder; $[\alpha]_D^{26.5}$ −0.6 (*c* 0.32, $CHCl_3$–MeOH, 1:1); UV (MeOH) λ_{max} (log ε) nm: 236 (3.01), 204 (2.99), 195 (3.03); IR (KBr) ν_{max} cm^{-1}: 3439, 2968, 2933, 1745, 1720, 1640, 1234, 1213; positive ESIMS *m/z* 563 (100) $[M+Na]^+$; positive HRESIMS *m/z* 563.2976 $[M+Na]^+$ (calcd for $C_{32}H_{44}O_7Na$, 563.2984); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.2. Ananosin B (2)

White amorphous powder; $[\alpha]_D^{21.5}$ +0 (*c* 0.13, $CHCl_3$–MeOH, 1:1); UV (MeOH) λ_{max} (log ε) nm: 237 (2.79), 207 (2.87), 199 (2.92); IR (KBr) ν_{max} cm^{-1}: 3449, 2968, 2931, 1734, 1721, 1640, 1245, 1228; positive ESIMS *m/z* 579 (100) $[M+Na]^+$; positive HRESIMS *m/z* 579.3308 $[M+Na]^+$ (calcd for $C_{33}H_{48}O_7Na$, 579.3297); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.3. Ananosin C (3)

White amorphous powder; $[\alpha]_D^{22.5}$ +79.6 (*c* 0.09, $CHCl_3$–MeOH, 1:1); UV (MeOH) λ_{max} (log ε) nm: 256 (3.03), 250 (3.05), 237 (3.10), 193 (3.02); IR (KBr) ν_{max} cm^{-1}: 3437, 2970, 2935, 1746, 1721, 1235, 1215; positive ESIMS *m/z* 563 (100) $[M+Na]^+$; positive HRESIMS *m/z* 563.2989 $[M+Na]^+$ (calcd for $C_{32}H_{44}O_7Na$, 563.2984); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.4. Ananosin D (4)

White amorphous powder; $[\alpha]_D^{26.2}$ +13.8 (*c* 0.18, $CHCl_3$–MeOH, 1:1); UV (MeOH) λ_{max} (log ε) nm: 236 (2.95), 217 (2.85), 214 (2.87), 199 (2.96); IR (KBr) ν_{max} cm^{-1}: 3450, 2978, 2920, 1745, 1724, 1384, 1215; positive ESIMS *m/z* 563 (100) $[M+Na]^+$; positive HRESIMS *m/z* 563.2986 $[M+Na]^+$ (calcd for $C_{32}H_{44}O_7Na$, 563.2984); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.5. Ananosin E (5)

White amorphous powder; $[\alpha]_D^{22.5}$ +32.6 (*c* 0.09, $CHCl_3$–MeOH, 1:1); UV (MeOH) λ_{max} (log ε) nm: 236 (3.30), 205 (3.14), 192 (3.16); IR (KBr) ν_{max} cm^{-1}: 3432, 2954, 2930, 1744, 1723, 1708, 1242; positive ESIMS *m/z* 569 (100) $[M+Na]^+$; positive HRESIMS *m/z* 569.3460 $[M+Na]^+$ (calcd for $C_{32}H_{50}O_7Na$, 569.3454); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4. Cytotoxicity assay

The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, PANC-1 and SK-BR-3. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO). Briefly, adherent cells (100 μL) were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with initial density of 1×10^5 cells/mL in medium (100 μL). Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with 10-hydroxy camptothecin (Sigma) as positive control. After incubation, MTT (100 μg) was added to each well, with the incubation continued for 4 h at 37 °C. Cells were lysed with 20% SDS and 50% DMF (100 μL) after removal of medium (100 μL). The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method (Reed and Muench, 1938).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2014.10.014.

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