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Kadpolysperins A–N, lanostane triterpene acids possessing rich structure types from *Kadsura polysperma*

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ABSTRACT

Fourteen new lanostane triterpene acids (**1**–**14**), and five known analogues (**15**–**19**) were isolated from the stems of *Kadsura polysperma*. Compound **1**, with a rearranged tetracyclic skeleton, is an important biogenic precursor of longipedlactone skeleton. Compounds **2**–**6** are members of 18(13→12)-*abeo*-lanostane triterpene acids. The structures of new compounds were elucidated by spectroscopic evidence. Selected compounds were evaluated for their in vitro cytotoxicity against human tumor HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines.

1. Introduction

The stems and roots of *Kadsura* species (Schisandraceae) are commonly used in China as folk medicines for the treatment of blood deficiency, numb hands and feet, arthralgia, and irregular menstruation.[1] Phytochemical investigations of plants belonging to the genus *Kadsura* have indicated that it is rich in dibenzocyclooctadiene lignans,[2–6] lanostane, and cycloartane triterpenes,[7–10] and some of these compounds have shown antitumor,[11] anti-HIV,[12] anti-hepatitis B,[13] and PAF inhibitory activities.[14]

Recently, we have initiated a program to discover structurally unique and bioactive natural products from different *Kadsura* species, which resulted in a series of structural intriguing triterpenes, mainly involving kadlongilactone,[15,16] longipedlactone,[17,18] and nortriterpenoid[19] skeletons. In order to identify new natural compounds with interesting bioactivities, *Kadsura polysperma* Yang collected in the Emei Mountain of Sichuan Province, People's Republic of China was phytochemically investigated. Previous chemical investigations of this plant collected in Chongqing city, P.R. China, just led to the isolation of four triterpene lactones, polysperlactones A, and B, heteroclitalactone D, and schisanlactone E.[20] In this study, 19 lanostane triterpene acids were isolated, including 14 new ones, kadpolysperins A–N (**1**–**14**), and 5 known triterpene acids. Especially, kadpolysperin A owned a lanostane triterpene skeleton with its C and D rings rearranged to 5/6 consecutive carbocycle systems, and only four triterpenes with this skeleton were reported.[21] A class of 18(13→12)-*abeo*-lanostanoids, kadpolysperins B–G (**2**–**6**) and ananosic acids A–D (**16**–**19**)[22–24] were also obtained in this plant. In this paper, the isolation, structure elucidation, and plausible biogenetic pathway are described, and cytotoxic evaluation of selected compounds is reported.

2. Results and discussion

A 70% aqueous acetone extract of the stems of *K. polysperma* was partitioned between EtOAc and H_2O. The EtOAc solubles were dried and subjected to silica gel, MCI CHP-20 gel, Sephadex LH-20, and Lichroprep RP-18 gel column chromatography (CC) and semi-preparative HPLC to afford 14 new compounds, kadpolysperins A–N (**1**–**14**), together with 5 known lanostane triterpene acids, manwuweizic acid (**15**),[25] ananosic acids A–D (**16**–**19**). The structures of the known compounds were determined by comparing spectroscopic data with literature values.

Kadpolysperin A (**1**) was isolated as a white oil and gave a molecular formula of $C_{32}H_{48}O_4$, as determined by HRESIMS ($[M-H]^-$ *m*/*z* 495.3476, calcd 495.3474), requiring nine degrees of unsaturation. The ^1H NMR spectrum showed signals for one secondary methyl (δ_H 0.94, d, *J*=6.8 Hz), seven tertiary methyls (δ_H 0.90, 0.97, 0.97, 1.04, 1.63, 1.91, and 2.07), two olefinic proton signals (δ_H 5.42, and 6.09),

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one oxygenated methine proton (δ_H 4.68). The ^{13}C NMR and DEPT spectra exhibited 32 carbon signals, consisting of 7 methyls, 8 methylenes, 7 methine carbons (including 1 oxidized, and 2 olefinic carbons), 8 quaternary carbons (including 4 olefinic, and 1 carboxylic group), and an acetyl group (170.8, C, and 21.3, CH_3). Apart from three double bonds and two carbonyl groups, the remaining elements of unsaturation in **1** were assumed to be a tetracyclic skeleton. These data were consistent with the molecular formula obtained from the HRESIMS and suggested that **1** is a tetracyclic triterpenoid. Analysis of the NMR spectroscopic data of **1** showed this compound to have close structural resemblance to (24*Z*)-3-oxo-14(13→12)-*abeo*-lanosta-8,13(17),24-*trien*-26-oic acid[21] and own a lanostane triterpene skeleton with its C and D rings rearranged to 5/6 consecutive carbocycle systems. The HMBC correlations between H-3 (δ_H 4.68, br s) with C-1, C-5, C-29, and the acetyl carbonyl (δ_C 170.8) permitted the location of acetyl group at C-3. HMBC correlations from H_3-28 (δ_H 1.04, s) to C-8, and from H-7 to C-5, C-9, and C-14 determined double bond in **1** formed between C-7 and C-8. These were the only differences between these two compounds.

Acetyl group at position C-3 was proved to be α-orientation (typical shifts observed for carbons of the A-ring).[26] ROESY correlations from H_3-28 to H-12, H-15α, H-16α, and from H-9 to H_3-19 enabled the placements of H-12, H_3-28 in an α-orientation, and H-9 in a β-orientation, respectively (Fig. 2). The α-orientation of the H_3-21 is also deduced by the key correlation between H_3-18 and H-20 as shown in computer-generated 3D drawing (Fig. 2) and by comparing NMR data of **1** with those of the above similar compound. The C-24 (25) double bond geometry is in the *Z* configuration, due to the ROESY correlation between H-24 and H_3-27. The other details of the relative configuration of **1** were determined to be the same as the above known compound (Fig. 1). Therefore, the structure of **1** was determined as shown and given the name as kadpolysperin A.

Kadpolysperin B (**2**) was isolated as a yellow oil. The HRESIMS of **2** gave a $[M+Na]^+$ ion peak at *m*/*z* 505.3293 (calcd 505.3293), which is consistent with the molecular formula $C_{31}H_{46}O_4$. The ^1H NMR spectrum (Table 1) displayed a total of six methyl signals, including two secondary methyl (δ_H 0.95, d, *J*=7.0 Hz, H_3-21, and δ_H 1.16, d, *J*=6.8 Hz, H_3-18), two tertiary methyls (δ_H 0.89, H_3-19, and δ_H 0.97, H_3-28), and two olefinic methyls (δ_H 1.75, H_3-29, and δ_H 1.91, H_3-27), and two proton signals of an sp^2 exomethylene (δ_H 4.67, H-30a, and δ_H 4.89, H-30b). The ^{13}C NMR and DEPT spectra (Table 2) revealed 31 carbon signals, consisting of 7 methyls (including a OCH_3 group), 10 methylenes (including an exomethylene), 4 methines (including an olefinic carbon), and 10 quaternary carbons (including 6 olefinic and

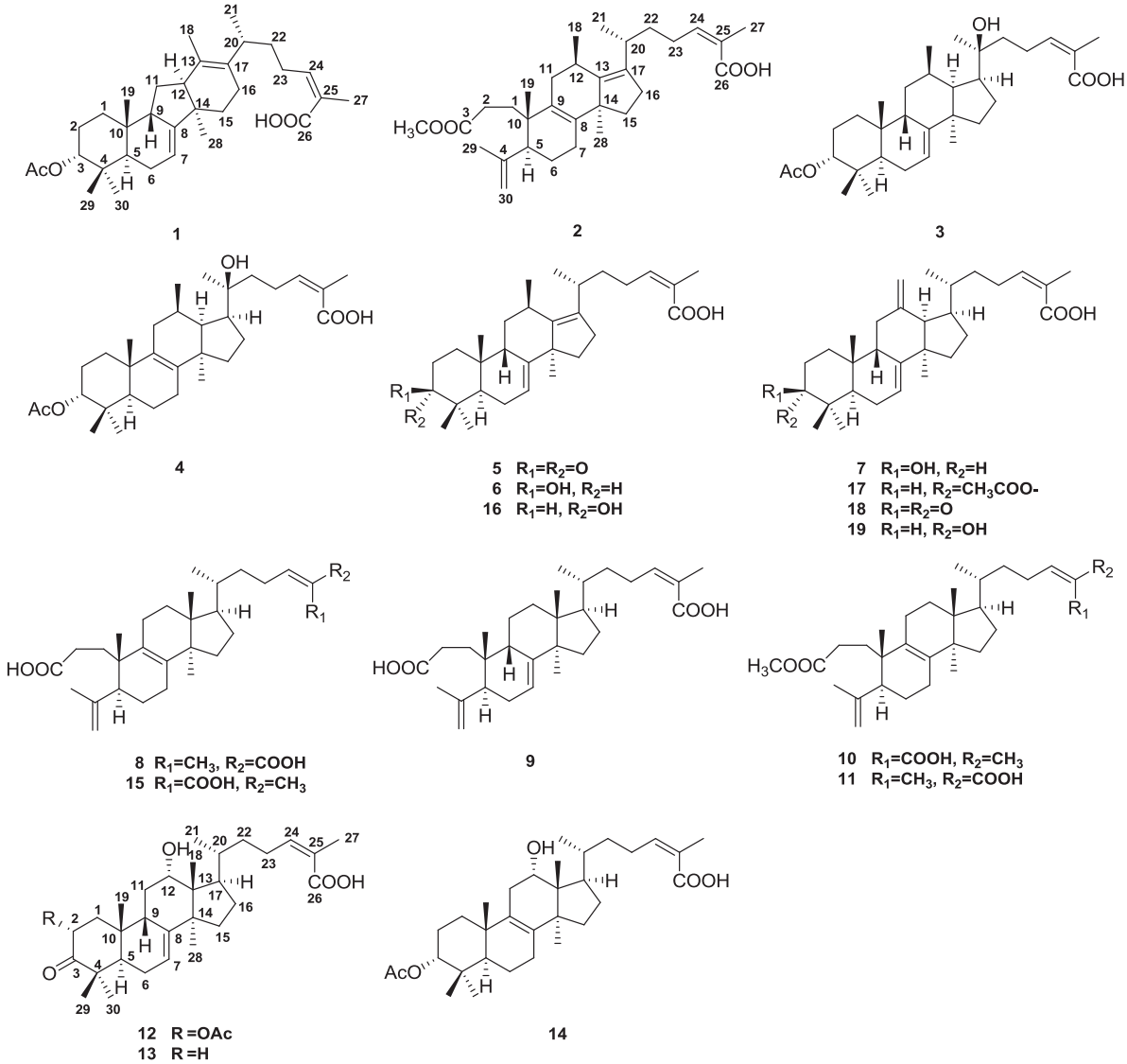


Fig. 1. The structures of compounds **1**–**19**.

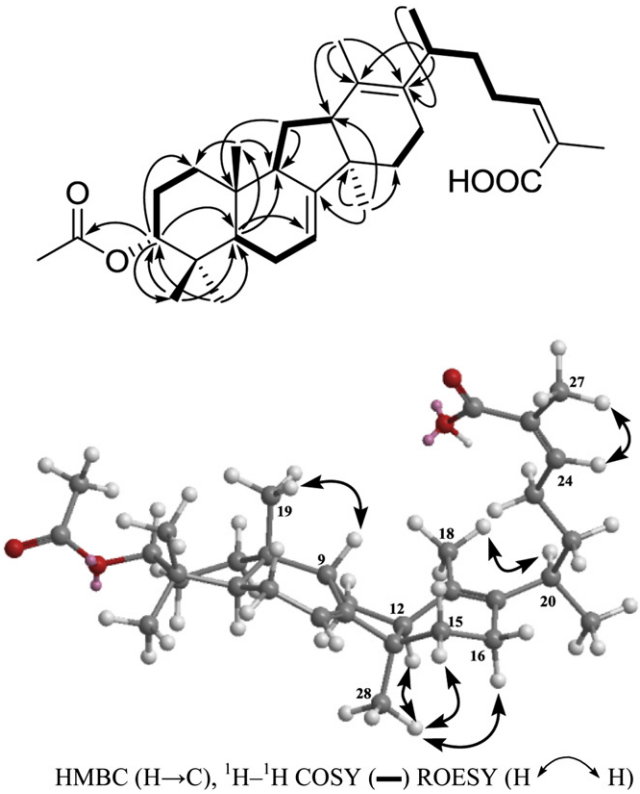


Fig. 2. Key HMBC, ^{1}H–^{1}H COSY, and ROESY correlations of **1**.

Table 1
^{1}H NMR data of compounds **1**–**5** in $CDCl_3$ (δ in ppm, J in Hz)

H	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1α	1.69 (m)	1.86 (overlap)	1.83 (overlap)	1.49 (overlap)	1.76 (overlap)
1β	1.13 (m)	1.74 (overlap)	1.80 (overlap)	1.33 (overlap)	1.53 (m)
2α	1.60 (overlap)	2.35 (overlap)	1.87 (overlap)	1.88 (overlap)	2.73 (m)
2β	1.59 (overlap)	2.00 (overlap)	1.65–1.67 (overlap)	1.65 (overlap)	2.21 (overlap)
3	4.68 (brs)		4.67 (br s)	4.68 (br s)	
5	1.30 (brs)	2.17 (overlap)	1.58 (overlap)	1.48 (overlap)	1.57 (overlap)
6α	1.88 (overlap)	1.65 (overlap)	1.92 (overlap)	1.62 (overlap)	2.01 (m)
6β	1.84 (m)	1.61 (overlap)	1.85 (overlap)	1.56 (overlap)	2.01 (m)
7α	5.42 (brs)	2.01 (overlap)	5.40 (br d, 3.8)	1.61 (overlap)	5.50 (t, 3.5)
7β		1.97 (overlap)		1.43 (overlap)	
9	2.13 (dd, 11.4, 9.7)		1.86 (overlap)		1.77 (overlap)
11α	1.53 (m)	2.06 (overlap)	1.36 (overlap)	1.96 (overlap)	1.50 (overlap)
11β	1.44 (m)	1.78 (overlap)	1.36 (overlap)	1.94 (overlap)	1.20 (overlap)
12	1.89 (overlap)	2.52 (overlap)	1.64 (overlap)	1.89 (overlap)	2.10 (m)
13			1.71 (overlap)	1.66 (overlap)	
15α	1.30 (m)	1.66 (overlap)	1.63 (overlap)	1.48 (overlap)	1.60 (overlap)
15β	1.57 (m)	1.45 (overlap)	1.56 (overlap)	1.39 (m)	1.45 (overlap)
16α	1.84 (m)	2.23 (overlap)	1.68 (overlap)	1.97–2.01 (overlap)	2.14 (m)
16β	1.77 (m)	2.07 (overlap)	2.04 (overlap)	2.12 (overlap)	2.32 (m)
17			2.06 (overlap)	1.99 (overlap)	
18	1.63 (s)	1.16 (d, 6.8)	0.97 (overlap)	0.80 (d, 6.6)	1.20 (d, 7.0)
19	0.97 (s)	0.89 (s)	0.92 (s)	0.96 (s)	1.05 (s)
20	2.72 (m)	2.80 (m)			2.89 (m)
21	0.94 (d, 6.8)	0.95 (d, 7.0)	1.19 (s)	1.21 (s)	0.92 (d, 6.7)
22α	1.43 (m)	1.53 (overlap)	1.59 (overlap)	1.58 (overlap)	1.45 (overlap)
22β	1.39 (m)	1.45 (overlap)	1.59 (overlap)	1.58 (overlap)	1.45 (overlap)
23α	2.43 (m)	2.46 (m)	2.61 (m)	2.75–2.70 (m)	2.64 (m)
23β	2.37 (m)	2.46 (m)	2.61 (m)	2.60 (m)	2.64 (m)
24	6.09 (t, 6.9)	6.13 (t, 7.3)	6.11 (t, 7.4)	6.13 (t, 7.4)	6.13 (t, 7.1)
27	1.91 (s)	1.91 (s)	1.92 (s)	1.94 (s)	1.90 (s)
28	1.04 (s)	0.97 (s)	1.17 (s)	1.04 (s)	1.04 (s)
29	0.97 (s)	1.75 (s)	0.97 (s)	0.93 (s)	1.09 (s)
30	0.90 (s)	4.67 (br s)	0.87 (s)	0.89 (s)	1.08 (s)
		4.89 (br s)			
OCH_3		3.68 (s)			
OAc	2.07 (s)		2.07 (s)	2.09(s)	

[a] Recorded at 600 MHz.
[b] Recorded at 400 MHz.

Table 2
^{13}C NMR data of compounds **1**–**7** in $CDCl_3$ (δ in ppm)

Carbon	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[a]
1	30.3 (t)	32.2 (t)	27.8 (t)	29.7 (t)	34.9 (t)	33.4 (t)	35.3 (t)
2	22.7 (t)	29.5 (t)	22.4 (t)	23.1 (t)	34.9 (t)	27.2 (t)	27.4 (t)
3	78.8 (d)	174.8 (s)	78.9 (d)	77.9 (d)	216.9 (s)	79.6 (d)	79.7 (d)
4	36.1 (s)	147.3 (s)	35.9 (s)	36.6 (s)	47.3 (s)	38.3 (s)	38.5 (s)
5	40.9 (d)	47.1 (d)	36.3 (d)	45.9 (d)	43.7 (d)	41.7 (d)	45.0 (d)
6	22.5 (t)	24.5 (t)	22.9 (t)	25.2 (t)	23.9 (t)	23.3 (t)	23.0 (t)
7	114.6 (d)	25.2 (t)	115.7 (d)	18.3 (t)	116.8 (d)	117.1 (d)	114.5 (d)
8	151.9 (s)	139.2 (s)	145.9 (s)	133.1 (s)	146.9 (s)	146.6 (s)	151.4 (s)
9	52.2 (d)	130.5 (s)	45.4 (d)	134.1 (s)	45.8 (d)	46.1 (d)	51.0 (d)
10	34.3 (s)	40.7 (s)	34.7 (s)	37.0 (s)	34.5 (s	34.6 (s)	34.8 (s)
11	30.8 (t)	34.5 (t)	33.0 (t)	31.6 (t)	38.7 (t)	39.3 (t)	31.0 (t)
12	50.1 (d)	29.9 (d)	30.0 (d)	31.0 (d)	30.2 (d)	30.3 (d)	151.0 (s)
13	128.6 (s)	143.7 (s)	55.3 (d)	50.2 (d)	145.0 (s)	145.5 (s)	50.6 (d)
14	41.2 (s)	52.5 (s)	50.1 (s)	45.7 (s)	54.2 (s)	54.2 (s)	43.8 (s)
15	34.0 (t)	36.9 (t)	40.8 (t)	38.2 (t)	36.8 (t)	36.7 (t)	30.4 (t)
16	20.3 (t)	27.4 (t)	30.6 (t)	26.1 (t)	29.4 (t)	29.4 (t)	24.7 (t)
17	133.5 (s)	134.3 (s)	51.6 (d)	50.4 (d)	136.4 (s)	136.0 (s)	47.9 (d)
18	18.0 (q)	21.2 (q)	21.6 (q)	15.1 (q)	19.5 (q)	19.5 (q)	111.4 (t)
19	23.4 (q)	22.6 (q)	21.8 (q)	18.1 (q)	20.6 (q)	21.5 (q)	23.7 (q)
20	34.5 (d)	31.9 (d)	75.0 (s)	74.1 (s)	31.3 (d)	31.3 (d)	31.1 (d)
21	18.6 (q)	19.7 (q)	25.4 (q)	25.1 (q)	19.9 (q)	20.0 (q)	18.4 (q)
22	34.4 (t)	35.1 (t)	37.2 (t)	40.8 (t)	35.1 (t)	35.1 (t)	33.3 (t)
23	28.3 (t)	28.5 (t)	24.4 (t)	24.7 (t)	28.5 (t)	28.5 (t)	26.9 (t)
24	147.0 (d)	147.1 (d)	146.2 (d)	146.0 (d)	147.2 (d)	147.1 (d)	147.0 (d)
25	127.0 (s)	125.7 (s)	126.3 (s)	126.2 (s)	126.0 (s)	125.6 (s)	126.0 (s)
26	171.3 (s)	172.5 (s)	172.1 (s)	172.4 (s)	173.1 (s)	171.6 (s)	171.7 (s)
27	20.6 (q)	20.5 (q)	20.5 (q)	20.5 (q)	20.5 (q)	20.5 (q)	20.9 (q)
28	26.1 (q)	23.0 (q)	27.6 (q)	28.5 (q)	20.1 (q)	20.0 (q)	24.0 (q)
29	22.6 (q)	22.7 (q)	21.9 (q)	21.8 (q)	22.2 (q)	15.3 (q)	16.0 (q)
30	28.2 (q)	113.8 (t)	27.5 (q)	27.5 (q)	25.1 (q)	28.0 (q)	28.7 (q)
OCH_3		51.5 (q)					
OAc	170.8 (s)		170.7 (s)	170.9 (s)			
	21.3 (q)		21.2 (q)	21.3 (q)			

[a] Recorded at 150 MHz.
[b] Recorded at 125 MHz.

2 carboxylic groups). These data were consistent with the elemental formula obtained from the HRESIMS and suggested that **2** is a lanostane triterpene. Comparing with normal lanostane triterpene acid, the main differences were H_3-18 signal at δ_H 1.16 (3H, d, J=6.8 Hz) appeared as a secondary methyl instead of C-13 tertiary methyl signal, it was likely that H_3-18 migrates from C-13 to C-12. The HMBC correlations between H_3-18 and C-12, C-11, C-13 and the key ^1H–^1H COSY correlations of H_3-18/H-12/H_2-11 confirmed the above deduction (Fig. 3). The presence of a carboxylic group (δ_C 174.8, C-3), an exomethylene (δ_C 113.8, C-30), and an olefinic carbon (δ_C 147.3, C-4) in **2**, indicating compound **2** is a derivative of a 3,4-*seco*-lanostane triterpene. This was further confirmed by HMBC correlations (Fig. 3) from H_2-30 to C-4, C-5, and C-29 and from H_2-1 and H_2-2 to C-3. In addition, the HMBC correlations were observed from H_3-19 (δ_H 0.89) to C-9 (δ_C 130.5), from H_3-28 (δ_H 0.97) to C-8 (δ_C 139.2), from H_3-21 (δ_H 0.95) to C-17 (δ_C 134.3), and from H_3-18 (δ_H 1.16) to C-13 (δ_C 143.7), indicating the presence of C-8(9) and C-13(17) double bonds. The HMBC correlation between OCH_3 (δ_H 3.68) and C-3 of **2** indicated that the methyl ester group was located at C-3. The ROESY correlations of H_3-18 with H-20β, and H-12 with H_3-28 indicated that CH_3-18 is β-oriented, and the correlation between H-24 and H_3-27 confirmed that the double bond geometry adopts the Z configuration (Fig. 3). Therefore, the structure of **2** was assigned, named as kadpolysperin B.

Kadpolysperin C (**3**) was obtained as a yellow oil. Its HRESIMS ($[M+Na]^+$ m/z 537.3550, calcd 537.3555) indicated its molecular formula to be $C_{32}H_{50}O_5$. The IR spectrum showed absorption bands at 3436 (hydroxyl), 1727 (ester), and 1633 (double bond) cm^{-1}. The 1D NMR spectra revealed the presence of seven methyls, eight methylenes, eight methines, and seven quaternary carbons besides an acetyl group (δ 2.07, 3H, s; 170.7 s, 21.2 q). The ^1H NMR spectrum showed signals for two olefinic protons [δ 6.11 (1H, t, J=7.4 Hz) and

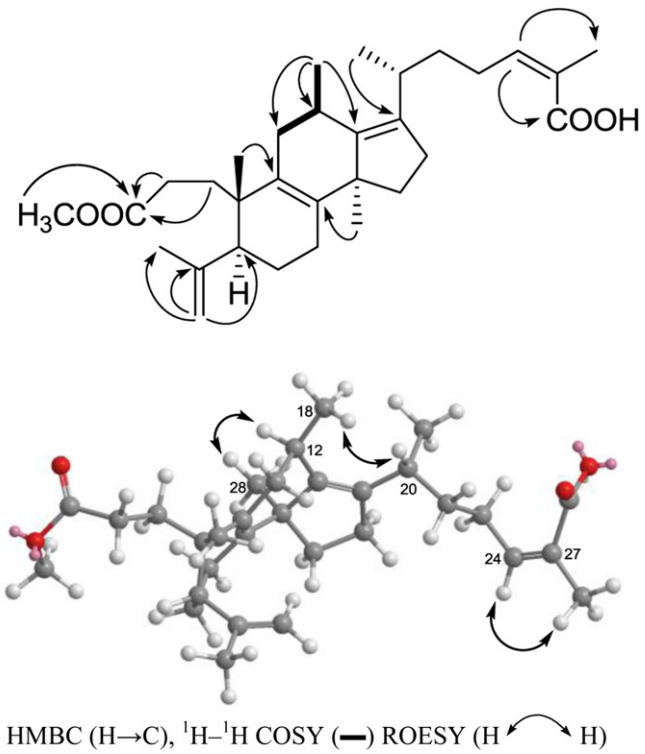


Fig. 3. Key HMBC, ^1H–^1H COSY, and ROESY correlations of **2**.

5.40 (1H, br d, J=3.8 Hz)], one oxygenated methine proton (δ 4.67, br s), a secondary methyl (δ 0.97, overlap), and seven tertiary methyl groups at δ 0.87, 0.92, 0.97, 1.17, 1.19, 1.92, and 2.07 (each 3H, s). Comparison of the NMR data of **3** and those of **2** indicated that **3** is also an 18(13→12)-*abeo*-lanostane triterpene. The C-13 tertiary methyl signal disappeared in **3**, instead appearing as a secondary methyl signal at δ_H 0.97. Meanwhile, the HMBC correlations from H_3-18 to C-11, C-12, and C-13, and the $^1H-^1H$ COSY correlations of H_3-18/H-12/H_2-11 confirmed H_3-18 migrating from C-13 to C-12. The olefinic proton signal (δ_H 5.40, H-7) showed that there was a trisubstituted double bond located at C-7 (8), due to the HMBC correlation between H-7 and C-14. A hydroxyl group was attached to C-20, this suggestion was clearly supported by HMBC correlations of H-13, H-16, H_3-21, H-22 with C-20. The correlation observed from H-3 (δ_H 4.67) to an acetyl carbonyl (δ_C 170.7, s) indicated that the acetyl group is located at C-3. In the ROESY spectrum, the correlations between H-3 and H_3-29, H_3-30, H-2α, H-2β and the correlation of H-3 with H-5 were absent indicated that the acetyl group is α-oriented. The ROESY correlations of H-13 with H_3-28 and H-17, of H_3-28 with H-15α and H-16α suggested that H-13 is α-orientation. The H_3-18 is inferred to be β, and C-24(25) double bond to be the *Z*-type olefinic bond, judging from ROESY correlations from H-9 to H_3-18 and H_3-19, and from H-24 to H_3-27. The C-20 configuration of **3** was established to be *S* on the basis of comparison of the ^{13}C NMR chemical shifts of C-20, 21 and 22 with those of dammarenediol I (20*R*), dammarenediol II (20*S*),[27] and (2β,9β,10α,16α,20β,24*Z*)-2-(β-D-glucopyranosyloxy).[28] Furthermore the ROESY correlations of H_3-21 with H-12, H-13, and H-17 confirmed the above deduction. Thus, the structure of **3** was determined to be as shown in Fig. 1.

Kadpolysperin D (**4**) was isolated as a yellow oil and had the same molecular formula of $C_{32}H_{50}O_5$ as that of **3** by HRESIMS ($[M+Na]^+$ m/z 537.3563, calcd 537.3555). Detailed comparison of NMR data of **4** with those of **3** indicated that these two compounds were almost identical, except for an evident absence of an olefinic proton in **4**. The HMBC correlation of H_3-19 with C-9 and H_3-28 with C-8 revealed that the double bond is located at C-8(9). ROESY correlations suggested the same relative configuration as that of **3**. Thus, the structure of **4**, named as kadpolysperin C, was unambiguously determined.

Kadpolysperin E (**5**) was obtained as a yellow oil. Its molecular formula was established as $C_{30}H_{44}O_3$ by HRESIMS ($[M+Na]^+$ m/z 475.3190, calcd 475.3188), suggesting nine degrees of unsaturation. The IR spectrum indicated the presence of 3430 cm^{-1} (hydroxyl) and 1707 cm^{-1} (carbonyl) functional groups. The ^1H NMR (Table 1) signals revealed five tertiary methyl groups [δ_H 1.04, 1.05, 1.08, 1.09, and 1.90 (each 3H, s)], and two secondary methyl [δ_H 0.92 (3H, d, J=6.7 Hz) and 1.20 (3H, d, J=7.0 Hz)], two olefinic protons [δ_H 5.50 (1H, t, J=3.5 Hz), and δ_H 6.13 (1H, t, J=7.1 Hz)]. The ^{13}C NMR and DEPT spectra (Table 2) exhibited 30 carbon signals, consisting of 7 methyls, 8 methylenes, 6 methines and 9 quaternaries (including 4 olefinic, 1 carbonyl, and 1 carboxyl carbons). These data were consistent with the elemental formula obtained from the HRESIMS and suggested that **5** is a tetracyclic triterpene acid. Analyzing the NMR data of **5** indicated that **5** was similar to **16**. Differences between **5** and **16** were the absence of an oxygenated methine (δ_C 76.9, CH, C-3) in **16** and the presence of a keto group (δ_C 216.9, C, C-3) in **5**. The HMBC correlations between H_3-29, H_3-30, H_2-1 and C-3 (δ_C 216.9) confirmed that the keto group is located at C-3. The ROESY correlations of H_3-18 with H-20β and H-12 with H_3-28α indicated that H_3-18 is β-oriented. The correlations between H-24 and H_3-27 indicated that the double bond geometry is in the *Z* configuration. Thus, **5** was unambiguously elucidated, named as kadpolysperin E.

Kadpolysperin F (**6**) was obtained as a yellow oil, and the molecular formula was $C_{30}H_{46}O_3$ based on its HRESIMS data ($[M+Na]^+$ m/z 475.3190). Careful analysis of its ^1H and ^{13}C NMR data indicated that **6** and **16** might be C-3 epimers. The ROESY correlations of H_3-30 with H-3, and H-5 with H-3 supported that the hydroxyl at C-3 is β-oriented. This deduction was fully confirmed by the abnormal upfield chemical shift of C-29 from δ_C 22.5 in **16** to δ_C 15.3 in **6** due to the γ-steric compression effect between hydroxyl at C-3 and H-29 in **6**. The rest of the relative stereochemistry of **6** was assigned as being the same as those of **16** from the further analysis of the ROESY spectrum. Consequently, the structure of **6**, kadpolysperin F, was elucidated as shown.

Kadpolysperin G (**7**) was obtained as a yellow oil and had the molecular formula $C_{30}H_{46}O_3$, based on its HRESIMS. The NMR data (Tables 2 and 3) revealed the presence of six methyls, nine methylenes, eight methines and seven quaternary carbons, indicating that **7** was a lanostane triterpene. Extensive analysis of the NMR spectroscopic data of **7** showed a close resemblance to **19**, being a 18(13→12)-*abeo*-lanostene triterpene acid. The ROESY correlations of H-3 with H_3-30 and H-5 confirmed HO-3 is β-oriented. This was the only difference between **7** and **19**. Thus, **7** was determined to be kadpolysperin G shown in Fig. 1.

Kadpolysperin H (**8**) was obtained as a white, amorphous powder, and the molecular formula $C_{30}H_{46}O_4$ was assigned through its HRESIMS (m/z 493.3293, $[M+Na]^+$), requiring eight degrees of unsaturation. The ^1H and ^{13}C NMR data of **8** were similar to those of **15**, except for the upfield chemical shift of C-27 from δ_C 20.4 in **15** to δ_C 11.9 in **8**. The ROESY correlations of H_3-27 with H-23α indicated that the double bond geometry is in the *E* configuration. The relative configuration of **8** was determined otherwise to be the same as that of **15**. Thus, the structure of kadpolysperin H was proposed as **8**.

Kadpolysperin I (**9**) was isolated as a white, amorphous powder. The molecular formula, $C_{30}H_{46}O_4$, was deduced from the HRESIMS ($[M+Na]^+$ m/z 493.3292, calcd 492.3293). Detailed comparison of the NMR data of **9** with those of 24(*E*)-3, 4-*seco*-9β*H*-lanosta-4(28),7,24-triene-3,26-dioic acid[29] indicated that these two compounds are closely comparable. The only significant difference was that C-24 double bond geometry is in the *Z* configuration in **9** and is in the *E* configuration in the above known compound. The ROESY correlations of H_3-27 with H-24 indicated this deduction, and the remaining relative configuration was confirmed to be the same as that of 24(*E*)-3,4-*seco*-9β*H*-lanosta-4(28),7,24-triene-3,26-dioic acid. Thus, the structure of kadpolysperin H was elucidated as **9**.

Kadpolysperins J and K (**10** and **11**) were obtained as identical molecular formula of $C_{31}H_{48}O_4$, requiring eight degrees of unsaturation, based on accurate mass measurement and NMR data. Comparison of the spectroscopic data of **10** with those of **15** revealed that they were quite similar, except for the evidence for an evident methoxy group (δ_C 51.5, q) in **10**. The HMBC correlation of δ_H 3.65 (3H, s, OCH_3) with δ_C 174.8 (s, C-3) revealed that the methoxy group is located at C-3. ROESY correlations suggested the same relative configuration as that of **15**. Thus, the structure of **10**, named as kadpolysperin J, was unambiguously determined.

Side-by-side comparison of their 1D and 2D NMR data, indicate that **10** and **11** possess the same planar structure. The only difference is the strong ROESY correlation of H_3-27 and H-24 showed in **10**, but disappeared in **11**, and correlation of H_3-27 and H-23α in **11** further confirmed that the double bond geometry is in the *E* configuration. Accordingly, kadpolysperin K was assigned as **11**.

Kadpolysperin L (**12**) was obtained as a white, amorphous powder; its molecular formula was determined to be $C_{32}H_{48}O_6$ by HRESIMS. The ^1H NMR spectrum of **12** (Table 4) showed the presence of two olefinic protons (δ_H 5.59, 6.08), two oxygenated methine protons (δ_H 3.99, 5.63), five tertiary methyl groups, one secondary methyl group, one vinylic methyl, and one acetoxy group. The ^{13}C NMR spectral data of **12** (Table 6) revealed double bond geometry adopts the *Z* configuration. Hence, compound **12** (kadpolysperin L) was elucidated as shown.

Table 3
^{1}H NMR data of compounds **6**–**10** (400 MHz, δ in ppm, J in Hz)

H	**6**[a]	**7**[a]	**8**[b]	**9**[b]	**10**[a]
1α	1.50 (overlap)	1.37 (overlap)	1.71 (overlap)	2.00 (overlap)	1.75 (overlap)
1β	1.21 (overlap)	1.37 (overlap)	1.71 (overlap)	1.89 (m)	1.75 (overlap)
2α	1.66 (overlap)	1.59 (overlap)	2.31 (m)	2.55 (overlap)	2.37 (m)
2β	1.60 (overlap)	1.59 (overlap)	1.94–1.97 (overlap)	2.55 (overlap)	2.00 (overlap)
3	3.22 (t, 7.7)	3.13 (t, 7.7)			
5	1.20 (overlap)	0.88 (overlap)	2.06–2.08 (m)	2.23 (m)	2.14 (br s)
6α	2.04 (overlap)	1.95 (m)	1.61 (m)	2.36 (m)	1.74 (overlap)
6β	1.90 (overlap)	1.92 (overlap)	1.42 (overlap)	2.00 (overlap)	1.55 (m)
7α	5.48 (d, 4.0)	5.33 (br d, 6.4)	1.79 (overlap)	5.28 (br s)	2.00 (overlap)
7β			1.20 (m)		2.00 (overlap)
9	1.65 (overlap)	2.21 (m)		2.61 (overlap)	
11α	1.53 (overlap)	1.45 (m)	1.87 (overlap)	1.64 (overlap)	2.12 (m)
11β	1.22 (overlap)	1.39 (overlap)	1.87 (overlap)	1.50 (overlap)	1.91 (overlap)
12α	2.09 (m)		1.59 (m)	1.77 (overlap)	1.80 (overlap)
12β			1.68 (overlap)	1.60 (overlap)	1.73 (overlap)
13		2.09 (m)			
15α	1.58 (overlap)	1.53 (overlap)	1.46 (overlap)	1.50 (overlap)	1.59 (overlap)
15β	1.43 (m)	1.09 (m)	1.07 (overlap)	1.43 (overlap)	1.21 (m)
16α	2.11 (overlap)	1.73 (m)	2.00 (m)	1.99 (overlap)	1.94 (overlap)
16β	1.26 (overlap)	1.53 (overlap)	1.90 (overlap)	1.28 (overlap)	1.32 (m)
17		1.84 (m)	1.39 (overlap)	1.52 (overlap)	1.51 (m)
18	1.23 (d, 6.8)	4.65 (d, 2.2)	0.62 (s)	0.76 (s)	0.73 (s)
		4.60 (d, 2.2)			
19	0.86 (s)	0.91 (s)	0.84 (s)	0.90 (s)	0.95 (s)
20	2.19 (overlap)	1.64 (overlap)	1.32 (m)	1.46 (overlap)	1.42 (m)
21	0.95 (d, 6.7)	0.84 (d, 6.3)	0.82 (d, 5.6)	0.95 (d, 6.1)	0.93 (d, 6.5)
22α	1.47 (overlap)	1.65 (overlap)	1.44 (overlap)	1.62 (overlap)	1.53 (overlap)
22β	1.47 (overlap)	1.13 (m)	1.05 (m)	1.23 (overlap)	1.13 (m)
23α	2.45 (m)	2.54 (m)	2.12 (m)	2.85 (m)	2.57 (m)
23β	2.45 (m)	2.50 (m)	1.98 (m)	2.74 (m)	2.46 (m)
24	6.16 (t, 6.9)	6.02 (t, 6.7)	6.73 (t, 7.1)	6.03 (t, 7.4)	6.08 (t, 7.1)
27	1.94 (s)	1.86 (s)	1.76 (s)	2.13 (s)	1.92 (s)
28	1.05 (s)	0.98 (s)	0.80 (s)	1.03 (s)	0.92 (s)
29	0.88 (s)	0.81 (s)	1.63 (s)	1.82(s)	1.76 (s)
30	1.03 (s)	0.96 (s)	4.77 (s)	4.97 (s)	4.90 (s)
			4.60 (s)	4.97 (s)	4.68 (s)
OCH_3					3.65 (s)

[a] Measured in $CDCl_3$.
[b] Measured in C_5D_5N.

Kadpolysperin M (**13**) was obtained as a white, amorphous powder. The HRESIMS at m/z 493.3291 $[M+Na]^+$ (calcd 493.3293) revealed a quasi-molecular ion consistent with a molecular formula of $C_{30}H_{46}O_4$. The ^{1}H and ^{13}C NMR data for **13** were similar to those of **12**, with the only difference being that one acetoxy group at C-2 occurred in **12** absent. The HMBC correlations and the molecular formula of **13** confirmed the above deduction. The ROESY correlation of H_3-18 with H-12 was used to place HO-12 in an α-orientation. The other ROESY correlations suggested the same relative configuration as those of **12**. Therefore, compound **13** was assigned as kadpolysperin M, as shown.

Kadpolysperin N (**14**) obtained as a white, amorphous powder had the molecular formula $C_{32}H_{50}O_5$ as revealed by its HRESIMS data (m/z 537.3560 $[M+Na]^+$ calcd 537.3555). Extensive analysis of the 2D NMR spectral data led to the establishment of a structure of lanostane triterpene acid was the same as **13**. C-12 in **14** was substituted by a hydroxyl group, and a double bond was assigned to C-8 and C-9 on the basis of the HMBC correlations from H_3-18 to C-12 (δ_C 73.3, s), from H_3-28 to C-8 (δ_C134.6, s), and from H_3-19 to C-9 (δ_C133.0, s). The correlation observed from H-3 (δ_H 4.65, d, J=2.5 Hz) to an acetyl carbonyl (δ_C170.8, s) indicated that the acetyl group is located at C-3. The ROESY correlation of H_3-29 with H-3 was used to position acetyl group in an α-orientation. ROESY correlations from H_3-18 to H-12 enabled the placement of HO-12 in an α-orientation. Therefore, the structure of **14** was established as kadpolysperin N, as shown.

Compounds **1**, **4**, **5**, **8**–**11**, **13**–**16**, **18**, and **19** were assayed for their cytotoxicity against the HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines by the MTT method.[30] The results are summarized in Table 5. Compounds **1** and **11** showed weak cytotoxicity against HL-60 cell lines, the others were inactive with IC_{50} >40 μM. Due to limitations in the amounts available, compounds **2**, **3**, **6**, **7**, **12**, and **17** were not tested in the bioassay used.

A plausible biogenetic pathway for kadpolysperin A (**1**) was proposed on the basis of kadpolysperin N (**14**) isolated from the same plant, *K. polysperma*, Scheme 1. The protonation of C-9 of kadpolysperin N formed key intermediate A, which underwent the deprotonation of C-7 to produce intermediate B. The intermediate B release of one molecular H_2O to give key intermediate C, which further rearranged to form the kadpolysperin A (**1**) through a Wagner–Meerwein rearrangement.[17]

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tensor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400, DRX-500 and Avance III 600 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in parts per million with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm×25 cm) column. Column chromatography was performed with silica gel

Table 4
^{1}H NMR data of compounds **11**–**14** in $CDCl_3$ (400 MHz, δ in ppm, J in Hz)

H	11	12	13	14
1α	1.73 (overlap)	1.97 (overlap)	1.72 (m)	1.48 (overlap)
1β	1.73 (overlap)	1.77 (m)	1.58 (overlap)	1.48 (overlap)
2α	2.37 (m)		2.53 (m)	1.86 (m)
2β	1.99 (overlap)	5.63 (m)	2.43 (m)	1.65 (overlap)
3				4.65 (d, 2.5)
5	2.13 (overlap)	1.24 (overlap)	1.43–1.41 (m)	1.49 (overlap)
6α	1.79 (overlap)	2.07 (m)	1.89 (overlap)	1.61 (overlap)
6β	1.54 (m)	1.91 (overlap)	1.71 (m)	1.49 (overlap)
7α	2.00 (overlap)	5.59 (m)	5.66 (br d, 7.7)	2.03 (overlap)
7β	2.00 (overlap)			2.03 (overlap)
9		2.35 (m)	2.13 (m)	
11α	2.11 (overlap)	2.26 (m)	2.21 (m)	2.62–2.60 (overlap)
11β	1.92 (overlap)	1.39 (m)	1.60 (overlap)	2.09–2.06 (overlap)
12α	1.80 (overlap)			
12β	1.73 (overlap)	3.99 (dd, 8.4,7.8)	3.90 (dd, 8.6,7.2)	4.00 (d, 8.1)
15α	1.59 (overlap)	1.51 (overlap)	2.32 (m)	1.64 (overlap)
15β	1.21 (m)	1.51 (overlap)	1.37 (overlap)	1.16 (m)
16α	1.93 (overlap)	2.04 (m)	2.06 (overlap)	2.00 (overlap)
16β	1.31 (m)	1.51 (overlap)	1.31 (m)	1.36 (m)
17	1.51 (m)	2.00 (m)	2.06 (m)	2.04 (overlap)
18	0.73 (s)	0.89 (s)	0.68 (s)	0.61 (s)
19	0.95 (s)	1.32 (s)	0.93 (s)	0.97 (s)
20	1.43 (m)	1.43 (m)	1.41 (m)	1.43 (m)
21	0.93 (d, 6.5)	1.00 (d, 6.6)	0.99 (d, 6.5)	1.02 (d, 6.5)
22α	1.56 (overlap)	1.57 (overlap)	1.18 (overlap)	1.56 (overlap)
22β	1.17 (m)	1.19 (overlap)	1.18 (overlap)	1.20 (m)
23α	2.25 (m)	2.58 (m)	2.57 (m)	2.59 (overlap)
23β	2.11 (m)	2.48 (m)	2.45 (m)	2.45 (m)
24	6.87 (m)	6.08 (t, 6.9)	6.06 (t, 6.7)	6.09 (t, 6.6)
27	1.84 (s)	1.92 (s)	1.90 (s)	1.92 (s)
28	0.92 (s)	1.12 (s)	1.09 (s)	1.10 (s)
29	1.76 (s)	1.20 (s)	1.07 (s)	0.91 (s)
30	4.90 (s)	1.13 (s)	1.07 (s)	0.86 (s)
	4.68 (s)			
OAc		2.17 (s)		2.06(s)
OCH_3	3.65 (s)			

Table 5
IC_{50} values (μM) of compounds **1** and **11** for human tumor cell lines

Compound[a]	HL-60	SMMC-7721	A-549	MCF-7	SW-480
1	12.97	>40	>40	>40	>40
11	18.18	>40	>40	>40	>40
DDP(MW300)[b]	1.04	14.75	13.61	16.95	19.68

[a] Other compounds in selected ones were inactive (IC_{50} >40 μM) for all cell lines.
[b] DDP (cisplatin) was used a positive control.

(200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μM, Merck, Darmstadt, Germany), and MCI gel (75–150 μM, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

3.2. Plant material

The stems of *K. polysperma* were collected in Emei Country of Sichuan Province, People's Republic of China, in August 2009, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (KIB 08102009) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried and powdered stems of *K. polysperma* (10.5 kg) were extracted with 70% aqueous Me_2CO (40 L×3) at room temperature and concentrated in vacuo to yield a residue, which was partitioned between H_2O and EtOAc. The EtOAc extract (330 g) was chromatographed on silica gel (100–200 mesh, 3.0 kg) column chromatography, eluting with a $CHCl_3$/Me_2CO gradient system (40:1, 20:1, 9:1, 8:2, 7:3, 6:4, 1:1), to give fractions 1–8. Fraction 4 (18 g) was subjected to RP-18 column chromatography (40–100% gradient CH_3OH/H_2O) to afford subfractions 4.1–4.6. Fraction 4.6 (800 mg) was chromatographed on silica gel (petroleum ether/Me_2CO, 30:1–2:1) to give six subfractions. Fraction 4.6.1 (60 mg) was chromatographed by semipreparative HPLC (90% CH_3OH/H_2O) to give **2** (2 mg), **10** (20 mg), and **11** (3 mg). Fraction 4.6.2 (250 mg) was subjected to chromatography over silica gel (petroleum ether/EtOAc, 30:1–1:1) to give subfractions 4.6.2.1–4.6.2.6. Subfraction 4.6.2.3 (32 mg) was separated by semipreparative HPLC (80% CH_3OH/H_2O) to yield **1** (3 mg), **5** (14 mg), **17** (2 mg), and **18** (10 mg). Fraction 4.6.3 (200 mg) was applied to a silica gel column (eluted with petroleum ether/EtOAc, 20:1), and then semipreparative HPLC, with 80% CH_3OH/H_2O, to yield **6** (3 mg), **7** (2 mg), **16** (10 mg), and **19** (12 mg). Fraction 5 (10 g) was subjected to RP-18 column chromatography (40–100% gradient CH_3OH/H_2O), with final purification by semipreparative HPLC (70% CH_3OH/H_2O), to yield **3** (3 mg), **4** (5 mg), **8** (5 mg), **9** (2 mg), and **15** (6 mg). Compounds **12** (3 mg), **13** (5 mg), and **14** (3.5 mg) were obtained from fraction 6 (22 g) after repeated silica gel column chromatography (petroleum ether/Me_2CO, 20:1), followed by semipreparative HPLC (65% CH_3OH/H_2O).

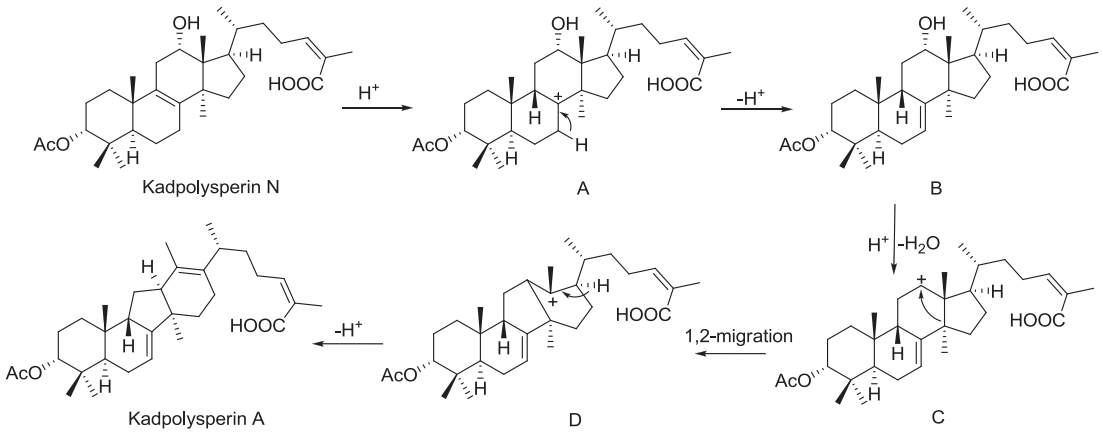
*3.3.1. Kadpolysperin A (**1**).* Yellow oil; $[\alpha]_D^{15}$ −104.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.49) nm; IR (KBr) ν_{max} 3432, 2926, 1734, 1629, 1375, 1245, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS *m*/*z* 495 (100) $[M-H]^-$, negative HRESIMS *m*/*z* 495.3476 $[M-H]^-$ (calcd for $C_{32}H_{47}O_4$, 495.3474).

*3.3.2. Kadpolysperin B (**2**).* Yellow oil; $[\alpha]_D^{22}$ −35.8 (*c* 0.05, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 204 (4.84) nm; IR (KBr) ν_{max}

Table 6
^{13}C NMR Data of Compounds **8**–**14** (δ in ppm)

Carbon	**8**[a]	**9**[b]	**10**[c]	**11**[c]	**12**[c]	**13**[c]	**14**[d]
1	32.3 (t)	29.6 (t)	32.5 (t)	32.4 (t	42.2 (t)	33.6 (t)	30.5 (t)
2	29.4 (t)	30.0 (t)	29.4 (t)	29.4 (t)	72.1 (d)	34.1 (t)	23.2 (t)
3	176.6 (s)	176.9 (s)	174.8 (s)	174.8 (s)	208.7 (s)	219.5 (s)	77.9 (d)
4	147.0 (s)	150.3 (s)	147.2 (s)	147.3 (s)	48.4 (s)	46.8 (s)	36.7 (s)
5	46.3 (d)	45.6 (d)	46.7 (d)	46.7 (d)	50.1 (d)	53.0 (d)	45.3 (d)
6	23.6 (t)	30.0 (t)	23.9 (t)	23.9 (t)	23.6 (t)	22.7 (t)	17.9 (t)
7	27.5 (t)	118.1 (d)	25.8 (t)	25.8 (t)	121.7 (d)	122.3 (d)	25.9 (t)
8	138.2 (s)	147.1 (s)	139.0 (s)	139.0 (s)	147.7 (s)	147.7 (s)	134.6 (s)
9	129.2 (s)	39.2 (d)	129.3 (s)	129.3 (s)	46.5 (d)	43.9 (d)	133.0 (s)
10	39.8 (s)	36.6 (s)	40.3 (s)	40.3 (s)	36.7 (s)	35.7 (s)	36.7 (s)
11	25.4 (t)	18.9 (t)	21.6 (t)	21.6 (t)	33.9 (t)	32.0 (t)	32.6 (t)
12	30.6 (t)	34.2 (t)	31.0 (t)	31.0 (t)	76.0 (d)	74.8 (d)	73.3 (d)
13	43.9 (s)	43.9 (s)	44.3 (s)	44.3 (s)	48.2 (s)	48.5 (s)	49.6 (s)
14	50.2 (s)	51.8 (s)	50.7 (s)	50.7 (s)	53.0 (s)	51.9 (s)	49.5 (s)
15	30.5 (t)	34.4 (t)	30.9 (t)	30.9 (t)	34.3 (t)	34.0 (t)	31.9 (t)
16	21.2 (t)	28.5 (t)	28.0 (t)	28.0 (t)	28.5 (t)	28.2 (t)	27.7 (t)
17	49.8 (d)	53.2 (s)	50.3 (d)	50.2 (d)	44.8 (d)	44.1 (d)	43.0 (d)
18	15.5 (q)	21.8 (q)	15.9 (q)	15.9 (q)	21.9 (q)	19.8 (q)	16.2 (q)
19	21.8 (q)	24.3 (q)	22.2 (q)	22.2 (q)	24.4 (q)	22.9 (q)	18.7 (q)
20	35.8 (d)	36.4 (d)	36.4 (d)	36.3 (d)	36.0 (d)	35.9 (d)	36.0 (d)
21	18.0 (q)	18.5 (q)	18.5 (q)	18.5 (q)	17.9 (q)	17.9 (q)	17.7 (q)
22	34.5 (t)	36.2 (t)	35.8 (t)	34.7 (t)	35.3 (t)	35.4 (t)	35.6 (t)
23	25.2 (t)	27.1 (t)	26.8 (t)	25.8 (t)	27.1 (t)	27.0 (t)	26.9 (t)
24	141.8 (d)	142.5 (d)	147.3 (d)	145.6 (d)	146.7 (d)	146.6 (d)	146.7 (d)
25	127.7 (s)	128.6 (s)	125.8 (s)	126.3 (s)	125.8 (s)	126.0 (s)	125.8 (s)
26	170.5 (s)	170.6 (s)	173.4 (s)	174.8 (s)	172.1 (s)	172.7 (s)	171.9 (s)
27	11.9 (q)	21.4 (q)	20.6 (q)	12.0 (q)	20.5 (q)	20.5 (q)	20.5 (q)
28	24.6 (q)	27.5 (q)	25.1 (q)	25.1 (q)	31.3 (q)	27.0 (q)	24.5 (q)
29	22.7 (q)	26.0 (q)	22.9 (q)	22.9 (q)	22.4 (q)	20.8 (q)	21.7 (q)
30	113.2 (t)	112.1 (t)	113.7 (t)	113.7 (t)	25.5 (q)	28.4 (q)	27.5 (q)
OCH_3			51.5 (q)	51.5 (q)			
OAc					170.3 (s)		170.8 (s)
					20.7 (q)		21.3 (q)

[a] Recorded in C_5D_5N+$CDCl_3$, 100 MHz.
[b] Recorded in C_5D_5N, 125 MHz.
[c] Recorded at $CDCl_3$, 100 MHz.
[d] Recorded at $CDCl_3$, 125 MHz.



Scheme 1. Hypothetical biogenetic pathway of **1**.

3440, 2926, 1710, 1638, 1630 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m*/*z* 505 (100) [M+Na]$^+$; positive HRESIMS *m*/*z* 505.3293 [M+Na]$^+$ (calcd for $C_{31}H_{46}O_4Na$, 505.3293).

*3.3.3. Kadpolysperin C (**3**).* Yellow oil; $[\alpha]_D^{22}$ −127.2 (*c* 0.07, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 205 (4.66) nm; IR (KBr) ν_{max} 3436, 2926, 1727, 1633, 1375, 1246, 1184, 1030, cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m*/*z* 537 (100) [M+Na]$^+$; positive HRESIMS *m*/*z* 537.3550 [M+Na]$^+$ (calcd for $C_{32}H_{50}O_5Na$, 537.3555).

*3.3.4. Kadpolysperin D (**4**).* Yellow oil; $[\alpha]_D^{22}$ −30.0 (*c* 0.13, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 204 (4.50) nm; IR (KBr) ν_{max} 3437, 2955, 1723, 1376, 1247, 1181, 1022 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m*/*z* 537 (100) [M+Na]$^+$; positive HRESIMS *m*/*z* 537.3563 [M+Na]$^+$ (calcd for $C_{32}H_{50}O_5Na$, 537.3555).

*3.3.5. Kadpolysperin E (**5**).* Yellow oil; $[\alpha]_D^{23}$ −108.6 (*c* 0.14, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 205 (3.36) nm; IR (KBr) ν_{max} 3430, 2930, 1707, 1634, 1456, 1379, 1242, 1119 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m*/*z* 475 (100) [M+Na]$^+$; positive HRESIMS *m*/*z* 475.3190 [M+Na]$^+$ (calcd for $C_{30}H_{44}O_3Na$, 475.3188).

*3.3.6. Kadpolysperin F (**6**).* Yellow oil; $[\alpha]_D^{22}$ −91.1 (*c* 0.06, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 204 (4.75) nm; IR (KBr) ν_{max}

3439, 2926, 1708, 1656, 1629, 1460, 1250, 1066, 1033 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 2; positive ESIMS *m/z* 477 (100) $[M+Na]^+$; positive HRESIMS *m/z* 477.3346 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3344).

*3.3.7. Kadpolysperin G (**7**).* Yellow oil; $[\alpha]_D^{25}$ −62.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.27) nm; IR (KBr) ν_{max} 3437, 2928, 1724, 1633, 1458, 1378, 1287, 1075 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 2; negative ESIMS *m/z* 453 (100) $[M-H]^-$; negative HRESIMS *m/z* 453.3371 $[M-H]^-$ (calcd for $C_{30}H_{45}O_3$, 453.3368).

*3.3.8. Kadpolysperin H (**8**).* White powder; $[\alpha]_D^{22}$ +39.8 (*c* 0.12, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 203 (4.56) nm; IR (KBr) ν_{max} 3431, 2929, 1697, 1639, 1451, 1376, 1251 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 6; positive ESIMS *m/z* 493 (100) $[M+Na]^+$; positive HRESIMS *m/z* 493.3196 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3293).

*3.3.9. Kadpolysperin I (**9**).* White powder; $[\alpha]_D^{20}$ −39.8 (*c* 0.12, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 205 (4.50) nm; IR (KBr) ν_{max} 3431, 2953, 1695, 1635, 1456, 1377, 1273 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 6; positive ESIMS *m/z* 493 (100) $[M+Na]^+$; positive HRESIMS *m/z* 493.3292 $[M+Na]^+$ (calcd for $C_{30}H_{44}O_3Na$, 493.3293).

*3.3.10. Kadpolysperin J (**10**).* White powder; $[\alpha]_D^{21}$ +23.5 (*c* 0.13, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 204 (4.44) nm; IR (KBr) ν_{max} 3432, 2930, 1736, 1638, 1457, 1377, 1282, 1201 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 6; positive ESIMS *m/z* 507 (100) $[M+Na]^+$; positive HRESIMS *m/z* 507.3443 $[M+Na]^+$ (calcd for $C_{31}H_{48}O_4Na$, 507.3450).

*3.3.11. Kadpolysperin K (**11**).* White powder; $[\alpha]_D^{20}$ +18.9 (*c* 0.10, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 204 (4.51) nm; IR (KBr) ν_{max} 3432, 2928, 1737, 1639, 1451, 1378, 1282, 1201 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 4 and 6; positive ESIMS *m/z* 507 (100) $[M+Na]^+$; positive HRESIMS *m/z* 507.3463 [M+Na] (calcd for $C_{31}H_{48}O_4Na$, 507.3450).

*3.3.12. Kadpolysperin L (**12**).* White powder; $[\alpha]_D^{21}$ +11.9 (*c* 0.16, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 204 (4.43) nm; IR (KBr) ν_{max} 3433, 2932, 1747, 1724, 1639, 1459, 1379, 1237, 1151, 1034 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 4 and 6; positive ESIMS *m/z* 551 (100) $[M+Na]^+$; positive HRESIMS *m/z* 551.3344 $[M+Na]^+$ (calcd for $C_{31}H_{48}O_4Na$, 551.3348).

*3.3.13. Kadpolysperin M (**13**).* White powder; $[\alpha]_D^{23}$ +31.1 (*c* 0.17, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 203 (4.40) nm; IR (KBr) ν_{max} 3439, 2932, 1706, 1633, 1457, 1381, 1252, 1157, 1020 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 4 and 6; positive ESIMS *m/z* 493 (100) $[M+Na]^+$; positive HRESIMS *m/z* 493.3291 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3293).

*3.3.14. Kadpolysperin N (**14**).* White powder; $[\alpha]_D^{21}$ +10.8 (*c* 0.27, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε) 203 (4.28) nm; IR (KBr) ν_{max} 3434, 2948, 1720, 1639, 1458, 1376, 1247, 1183, 1038 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 4 and 6; positive ESIMS *m/z* 537 (100) $[M+Na]^+$; positive HRESIMS *m/z* 537.3560 $[M+Na]^+$ (calcd for $C_{31}H_{48}O_4Na$, 537.3555).

3.4. Cytotoxicity bioassay

The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7 and SW-480. 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).[30] Briefly, 100 μL of adherent cells was 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 an initial density of 1×10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS/50% DMF after removal of 100 μL of medium. 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.[31]

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.03.116. These data include MOL files and InChiKeys of the most important compounds described in this article.

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