JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Rearranged 6/6/5/6-Fused Triterpenoid Acids from the Stems of *Kadsura coccinea*

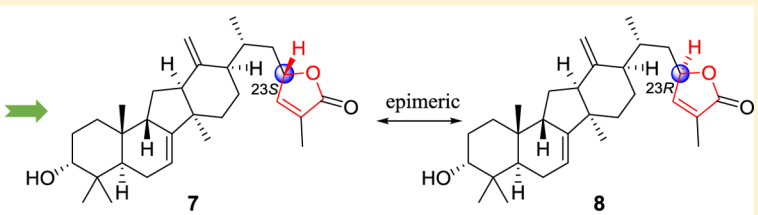
Zheng-Xi Hu,[†,‡] Kun Hu,[‡] Yi-Ming Shi,[‡] Wei-Guang Wang,[‡] Xue Du,[‡] Yan Li,[‡] Yong-Hui Zhang,[*,†] Jian-Xin Pu,[*,‡] and Han-Dong Sun[‡]

[†]Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China

[‡]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

Supporting Information

Kadsura coccinea



ABSTRACT: Fourteen new rearranged 6/6/5/6-fused triterpenoid acids, namely, kadcoccine acids A–N (**1**–**14**), were isolated from an EtOAc-soluble extract of the stems of *Kadsura coccinea*. Their structures were characterized mainly by analyzing 1D and 2D NMR and HRESIMS data and were shown to feature a rare 14(13→12)-*abeo*-lanostane skeleton. Compounds **7** and **8** represented the first examples of a 5-substituted 2(5*H*)-furanone motif on the C-17 side chain of this skeleton. The absolute configurations of C-23 for compounds **1**, **7**, and **8** were determined by comparison of their experimental electronic circular dichroism spectra. All the isolates were screened for their in vitro cytotoxicity against six human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, SW-480, and HeLa), and compounds **2** and **8** exhibited weak inhibitory effects with IC_{50} values ranging from 3.11 to 7.77 μM.

Historically, members of the genus *Kadsura* (Schisandraceae) were economically and medicinally valuable plants,[1] and the stems and roots of *Kadsura* species have been commonly applied in traditional Chinese medicines (TCMs) for the treatment of blood deficiency, numb hands and feet, arthralgia, and irregular menstruation.[2] Most notably, *Kadsura interior* has been used as a major ingredient in one of the TCMs, colloquially called "Dian-ji-xue-teng", and is recorded in the Chinese Pharmacopeia.[3] Previous phytochemical investigations on this genus revealed that it was a rich source of lignans and triterpenoids, many of which showed a wide array of biological activities, including antitumor,[4] antilipid peroxidative,[5] anti-HIV,[6] antihepatitis,[7] and neuroprotective activities.[8]

Kadsura coccinea (Lem.) A. C. Smith, a climbing plant distributed in southwest China, has been widely used to treat cancer and dermatosis and as an anodyne.[9] As part of a systematic search for naturally occurring bioactive agents from this species,[10−16] some schinortriterpenoids (SNTs) and cycloartane-related triterpenoids were obtained from *K. coccinea* collected in the Honghe Prefecture of Yunnan Province, and some rearranged lanostane-related triterpenoids with diverse skeletal types, such as 6/6/5-, 6/6/6-, 6/6/5/5-, 6/6/5/6-, 2,3-*seco*-6/6/5/6-, and 3,4-*seco*-6/6/5/6-fused ring systems, were obtained from *K. coccinea* collected in the Ziyuan Prefecture of Guangxi Province. Also reported were six lanostane-related triterpenoids, kadcoccinones A–F, possessing 6/6/5/6-, 6/6/9-, 18(13→12)-*abeo*-6/6/6/5-, and 6/6/6/5-fused ring systems from *K. coccinea* collected in the Menglun district of Yunnan Province. In the present work, an EtOAc-soluble extract of the stems of *K. coccinea* was investigated, resulting in the isolation of 14 new rearranged 6/6/5/6-fused triterpenoid acids, kadcoccine acids A–N (**1**–**14**). All the isolates are structurally characterized by a rare 14(13→12)-*abeo*-6/6/5/6-fused rearranged lanostane-type triterpenoid skeleton, which occurred mainly in the C/D-ring systems via a Wagner–Meerwein rearrangement reaction.[1] Only 11 triterpenoids possessing this unique tetracyclic carbocyclic core have been reported to date.[1,14,15] Importantly, a pair of C-23 epimeric molecules (**7** and **8**) represented the first examples of rearranged 6/6/5/6-fused triterpenoids featuring a 5-substituted 2(5*H*)-furanone motif on the C-17 side chain. In this report, the isolation and structure identification of new compounds **1**–**14**, as well as their in vitro cytotoxicity, are discussed.

Received: June 3, 2016
Published: October 5, 2016

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590−2598

Chart 1

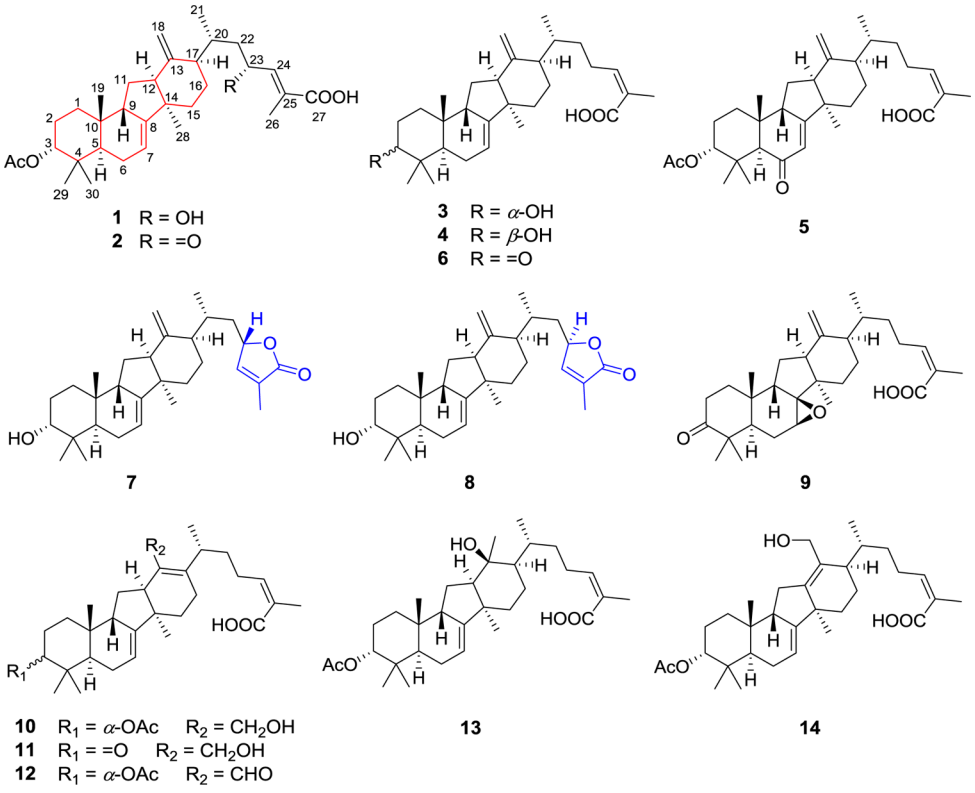


Table 1. ^{1}H NMR Spectroscopic Data of Kadcoccine Acids A–G (1–7) (δ in ppm, J in Hz)

no.	1[a,d]	2[b,d]	3[a,d]	4[a,d]	5[c,d]	6[c,d]	7[a,d]
1	1.01, m; 1.76, m	1.02, m; 1.55, m	1.16, m; 2.33, m	1.39, m; 1.45, m	1.11, m; 1.84, m	1.57, m; 1.61, m	1.15, m; 2.35, m
2	1.61, m; 1.76, m	1.56, m; 1.83, m	1.76, m; 2.02, m	1.84, m; 1.90, m	1.68, m; 1.91, m	2.26, m; 2.73, m	1.80, m; 2.05, m
3	4.84, br s	4.59, br s	3.68, br s	3.51, dd (4.1, 11.4)	4.80, br s		3.69, br s
5	1.52, dd (5.0, 10.8)	1.26, m	1.82, m	1.10, m	2.69, s	1.39, dd (3.8, 11.2)	1.81, m
6	1.92, m	1.84, m	2.05, m	2.09, m		1.96, m; 2.06, m	2.04, m
7	5.52, m	5.35, m	5.59, m	5.55, m	5.92, br d (1.2)	5.51, br d (6.9)	5.57, m
9	2.66, br t (10.3)	2.18, m	2.52, br t (10.4)	2.47, m	2.91, m	2.51, br t (10.3)	2.46, br t (10.3)
11	1.77, m	1.38, m; 1.48, m	1.66, m; 1.85, m	1.56, m	1.78, m; 1.96, m	1.46, m; 1.53, m	1.61, m; 1.84, m
12	2.25, m	2.10, m	2.15, dd (4.3, 12.4)	2.20, m	2.26, m	2.18, m	2.13, dd (3.5, 12.3)
15	1.23, m; 1.98, m	1.05, m; 1.58, m	1.24, m; 1.86, m	1.24, m; 1.82, m	1.25, m; 1.81, m	1.24, m; 1.79, m	1.20, m; 1.77, m
16	1.63, m; 1.97, m	1.55, m; 1.69, m	1.61, m; 1.94, m	1.62, m; 1.92, m	1.61, m; 1.89, m	1.63, m; 1.90, m	1.58, m; 1.74, m
17	2.00, m	1.82, m	1.95, m	1.96, m	1.97, m	1.96, m	1.94, m
18	4.92, br s	4.65, d (2.2); 4.72, d (2.2)	4.78, d (2.6); 4.83, d (2.6)	4.83, d (2.4); 4.92, d (2.4)	4.85, d (2.1); 4.90, d (2.1)	4.83, d (2.2); 4.91, d (2.2)	4.78, br s; 4.85, br s
19	1.04, s	0.97, s	1.17, s	1.11, s	1.20, s	1.18, s	1.18, s
20	2.51, m	1.72, m	1.95, m	1.92, m	1.88, m	1.90, m	1.94, m
21	1.29, d (6.4)	0.96, m	1.09, d (5.5)	1.12, m	1.12, d (6.4)	1.11, d (6.1)	1.07, d (3.7)
22	1.18, m; 2.23, m	1.55, m; 2.23, m	1.25, m; 1.83, m	1.25, m; 1.84, m	1.26, m; 1.81, m	1.27, m; 1.83, m	1.47, m; 1.90, m
23	5.04, m		2.84, m; 2.90, m	2.82, m; 2.91, m	2.80, m; 2.92, m	2.82, m; 2.91, m	5.08, m
24	7.54, br d (8.1)	6.79, s	6.03, t (7.1)	6.02, t (7.1)	6.02, t (7.0)	6.03, t (7.3)	7.21, m
26	2.19, s	1.83, s					
27			2.19, s	2.18, s	2.17, s	2.19, s	1.89, s
28	1.17, s	1.00, s	1.15, s	1.15, s	1.09, s	1.14, s	1.12, s
29	0.88, s	0.92, s	1.00, s	1.16, s	1.43, s	1.08, s	1.00, s
30	0.92, s	0.83, s	1.26, s	1.31, s	1.34, s	1.22, s	1.26, s
−OAc	2.12, s	2.00, s			2.13, s		

[a]Recorded at 600 MHz in pyridine-d_5. [b]Recorded at 600 MHz in $CDCl_3$. [c]Recorded at 400 MHz in pyridine-d_5. [d]"m" means overlapped or multiplet with other signals.

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590−2598

Table 2. ^{1}H NMR Spectroscopic Data of Kadcoccine Acids H−N (8−14) (δ in ppm, J in Hz)

no.	8[a,c]	9[b,c]	10[a,c]	11[a,c]	12[a,c]	13[a,c]	14[a,c]
1	1.20, m; 2.33, m	1.40, m; 1.56, m	1.09, m; 1.76, m	1.57, m	1.04, m; 1.68, m	1.13, m; 1.84, m	1.11, m; 1.79, m
2	1.75, m; 2.02, m	2.18, m; 2.70, m	1.59, m; 1.76, m	2.19, m; 2.65, m	1.55, m; 1.72, m	1.68, m; 1.85, m	1.64, m; 1.79, m
3	3.67, br s		4.81, br s		4.78, br s	4.84, br s	4.83, br s
5	1.81, m	1.55, m	1.47, m	1.34, dd (3.6, 11.8)	1.45, dd (3.9, 11.7)	1.53, m	1.51, dd (4.1, 11.0)
6	2.03, m	1.89, m	1.92, m	1.94, m; 2.03, m	1.91, m	1.93, m	1.94, m
7	5.56, m	3.36, m	5.58, br d (5.4)	5.55, m	5.59, m	5.50, m	5.71, m
9	2.57, br t (10.4)	2.53, br t (10.4)	2.40, m	2.43, m	2.20, m	2.12, m	2.11, m
11	1.65, m; 1.84, m	1.64, m; 1.70, m	1.89, m	1.62, m; 1.75, m	1.64, m; 2.04, m	1.55, m; 2.18, m	2.18, m; 2.84, m
12	2.15, dd (3.9, 12.2)	2.33, dd (6.1, 11.8)	2.68, br d (9.3)	2.65, m	2.80, br d (8.9)	2.03, m	
15	1.19, m; 1.77, m	1.28, m; 1.97, m	1.46, m; 1.81, m	1.45, m; 1.79, m	1.37, m; 1.80, m	1.37, m; 1.68, m	1.56, m; 1.65, m
16	1.55, m; 1.70, m	1.55, m; 1.95, m	2.02, m	2.02, m	2.21, m	1.54, m; 1.96, m	1.80, m; 1.85, m
17	1.88, m	1.98, m				1.86, m	2.64, m
18	4.79, d (2.3); 4.85, d (2.3)	4.84, d (2.2); 4.93, d (2.2)	4.44, d (12.0); 4.80, d (12.0)	4.40, d (12.0); 4.79, d (12.0)	10.53, s	1.46, s	4.69, br s
19	1.24, s	1.29, s	1.03, s	1.14, s	1.00, s	1.05, s	0.95, s
20	2.21, m	1.95, m	3.19, m	3.19, m	3.72, m	2.37, m	2.55, m
21	1.10, d (6.5)	1.11, d (6.0)	1.08, d (6.5)	1.08, d (6.8)	1.08, m	1.27, d (6.9)	1.11, d (6.7)
22	1.32, m; 1.71, m	1.25, m; 1.83, m	1.55, m; 1.66, m	1.55, m; 1.67, m	1.58, m; 1.71, m	1.43, m; 1.93, m	1.28, m; 1.80, m
23	5.05, m	2.77, m; 2.93, m	2.78, m	2.78, m	2.74, m	2.86, m; 2.96, m	2.85, m
24	7.10, m	6.02, t (7.3)	6.08, t (6.8)	6.10, t (7.2)	6.02, t (7.3)	6.12, t (7.1)	6.06, t (7.0)
27	1.86, s	2.18, s	2.13, s	2.14, s	2.13, s	2.15, s	2.07, s
28	1.13, s	0.79, s	1.14, s	1.13, s	1.08, s	1.34, s	1.16, s
29	0.98, s	0.99, s	0.87, s	1.03, s	0.84, s	0.91, s	0.86, s
30	1.25, s	1.21, s	0.89, s	1.18, s	0.88, s	0.89, s	0.90, s
−OAc			2.01, s		1.99, s	2.06, s	2.06, s

[a]Recorded at 600 MHz in pyridine-d_5. [b]Recorded at 500 MHz in pyridine-d_5. [c]"m" means overlapped or multiplet with other signals.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white, amorphous powder, and its molecular formula was deduced to be $C_{32}H_{48}O_5$ based on the HRESIMS ion at m/z 535.3391 ([M + Na]$^+$, calcd for 535.3394) and ^{13}C NMR data. The ^{1}H NMR data (Table 1) showed the characteristic signals attributable to one methyl doublet at δ_H 1.29 (d, J = 6.4 Hz, H_3-21); six methyl singlets at δ_H 1.04 (H_3-19), 2.19 (H_3-26), 1.17 (H_3-28), 0.88 (H_3-29), 0.92 (H_3-30), and 2.12 (−OAc); two oxygenated methines at δ_H 4.84 (br s, H-3) and 5.04 (m, H-23); and four olefinic protons at δ_H 5.52 (m, H-7), 4.92 (2H, br s, H_2-18), and 7.54 (br d, J = 8.1 Hz, H-24). Examination of the ^{13}C NMR (Table 3) and DEPT spectroscopic data disclosed 32 carbon resonances corresponding to one acetoxy group, six methyls (one secondary and five tertiary), eight methylenes (one olefinic), nine methines (two oxygenated and two olefinic), six quaternary carbons (three olefinic), and one carboxyl group. The presence of three double bonds and two carbonyls accounted for five of the nine indices of hydrogen deficiency required by its molecular formula, illustrating the presence of a tetracyclic ring system for **1**.

Analysis of the NMR and HRESIMS data of **1** implied that its structural features were similar to those of the known compound kadcoccinone A,[15] whose absolute configuration was confirmed by single-crystal X-ray crystallographic analysis. The corresponding ^{1}H−^{1}H COSY and HMBC correlations (Figure 1) of **1** further confirmed its 2D structure and indicated that the structures of these two compounds were consistent with a 14(13→12)-*abeo*-6/6/5/6-fused rearranged lanostane-type triterpenoid skeleton, in which the configurations of the A/B/C/D-ring systems remained intact, differing only in the absolute configurations of C-23. This assumption was confirmed by comparison of the electronic circular dichroism (ECD) spectra (Figure S8, Supporting Information), showing an opposite Cotton effect at approximately 214 nm ascribable to the adjacent α,β-unsaturated hydroxycarbonyl unit. Therefore, the absolute configuration of C-23 in **1**, kadcoccine acid A, was established as *R*.

Compound **2**, obtained as a white, amorphous powder, had a molecular formula of $C_{32}H_{46}O_5$ based on its HRESIMS analysis (m/z 509.3261, [M − H]$^-$, calcd for 509.3272), which was two mass units less than that of **1**. Comparison of the NMR spectroscopic data of **2** with those of **1** (Tables 1 and 3) revealed that they possessed identical carbocyclic cores, with the only difference being at C-23 (δ_C 66.6 for **1**, 207.7 for **2**), showing that the hydroxy group was replaced by a carbonyl group in **2** and giving rise to the H-24 olefinic signal resonating as a singlet at δ_H 6.79 (s, H-24). The ROESY spectrum (Figure S15, Supporting Information) showed that the relative configuration of **2** was identical to that of **1**. Accordingly, the structure of **2**, kadcoccine acid B, was defined as shown.

Compounds **3** and **4**, obtained as white, amorphous powders, were assigned the same molecular formula $C_{30}H_{46}O_3$ (eight indices of hydrogen deficiency) by analyzing the HRESIMS and

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590−2598

Table 3. ^{13}C NMR Spectroscopic Data of Kadcoccine Acids A−N (1−14) (δ in ppm)

no.	1[a,c]	2[b,c]	3[a,c]	4[a,c]	5[a,e]	6[a,e]	7[a,c]	8[a,c]	9[a,d]	10[a,c]	11[a,c]	12[a,c]	13[a,e]	14[a,e]
1	30.8, CH_2	30.3, CH_2	30.6, CH_2	36.1, CH_2	30.7, CH_2	36.4, CH_2	30.7, CH_2	30.6, CH_2	37.1, CH_2	31.1, CH_2	36.4, CH_2	30.9, CH_2	31.4, CH_2	31.5, CH_2
2	23.4, CH_2	22.7, CH_2	26.8, CH_2	28.7, CH_2	22.8, CH_2	35.5, CH_2	26.8, CH_2	26.8, CH_2	34.9, CH_2	23.5, CH_2	35.5, CH_2	23.4, CH_2	23.5, CH_2	23.6, CH_2
3	79.1, CH	79.1, CH	76.1, CH	79.1, CH	79.4, CH	215.7, C	76.0, CH	76.1, CH	215.3, C	79.0, CH	215.8, C	78.9, CH	79.1, CH	78.9, CH
4	36.7, C	36.2, C	37.9, C	39.5, C	36.3, C	48.0, C	37.9, C	37.9, C	47.9, C	36.8, C	48.0, C	36.8, C	36.8, C	37.0, C
5	41.0, CH	40.3, CH	40.3, CH	45.9, CH	51.9, CH	47.5, CH	40.3, CH	40.3, CH	44.6, CH	41.8, CH	48.4, CH	41.9, CH	40.8, CH	43.3, CH
6	23.3, CH_2	22.6, CH_2	23.6, CH_2	23.8, CH_2	200.8, C	24.4, CH_2	23.6, CH_2	23.5, CH_2	23.8, CH_2	23.3, CH_2	24.5, CH_2	23.3, CH_2	23.3, CH_2	23.3, CH_2
7	115.1, CH	114.5, CH	115.9, CH	115.4, CH	120.1, CH	114.9, CH	116.0, CH	115.8, CH	55.8, CH	115.5, CH	115.2, CH	116.7, CH	114.6, CH	118.0, CH
8	152.1, C	151.1, C	152.0, C	151.9, C	171.2, C	152.2, C	151.7, C	151.9, C	71.8, C	153.0, C	153.3, C	152.0, C	153.2, C	153.2, C
9	51.6, CH	51.0, CH	52.1, CH	51.8, CH	52.2, CH	51.2, CH	52.1, CH	52.0, CH	46.9, CH	53.1, CH	52.7, CH	53.8, CH	53.4, CH	57.9, CH
10	35.1, C	34.4, C	35.4, C	35.5, C	34.5, C	35.1, C	35.4, C	35.3, C	34.4, C	35.1, C	35.1, C	35.1, C	35.0, C	35.3, C
11	32.0, CH_2	30.7, CH_2	31.9, CH_2	31.7, CH_2	28.7, CH_2	31.1, CH_2	31.7, CH_2	32.1, CH_2	28.7, CH_2	32.1, CH_2	31.6, CH_2	34.1, CH_2	28.5, CH_2	32.8, CH_2
12	51.2, CH	50.7, CH	51.5, CH	51.3, CH	50.7, CH	51.3, CH	51.3, CH	51.2, CH	49.1, CH	46.9, CH	46.9, CH	43.0, CH	58.2, CH	146.0, C
13	152.1, C	150.1, C	151.9, C	151.9, C	150.3, C	151.5, C	151.1, C	151.5, C	150.3, C	135.4, C	135.4, C	138.8, C	76.0, C	130.5, C
14	44.5, C	43.8, C	44.6, C	44.5, C	44.8, C	44.5, C	44.5, C	44.4, C	43.0, C	41.9, C	41.9, C	41.4, C	43.5, C	42.3, C
15	31.1, CH_2	30.4, CH_2	31.1, CH_2	31.1, CH_2	30.3, CH_2	31.1, CH_2	31.0, CH_2	31.1, CH_2	26.8, CH_2	34.8, CH_2	34.7, CH_2	33.9, CH_2	32.5, CH_2	34.1, CH_2
16	25.5, CH_2	24.8, CH_2	25.5, CH_2	25.5, CH_2	25.0, CH_2	25.4, CH_2	25.5, CH_2	25.4, CH_2	24.3, CH_2	21.5, CH_2	21.4, CH_2	23.4, CH_2	21.8, CH_2	19.8, CH_2
17	49.4, CH	48.2, CH	48.8, CH	48.7, CH	48.2, CH	48.6, CH	48.8, CH	48.8, CH	49.1, CH	137.8, C	137.9, C	163.4, C	48.3, CH	39.6, CH
18	112.3, CH_2	112.0, CH_2	112.1, CH_2	112.1, CH_2	112.7, CH_2	112.1, CH_2	112.4, CH_2	112.2, CH_2	113.3, CH_2	61.1, CH_2	61.0, CH_2	191.2, CH	20.6, CH_3	61.9, CH_2
19	23.8, CH_3	23.4, CH_3	24.3, CH_3	24.3, CH_3	25.6, CH_3	23.1, CH_3	24.3, CH_3	24.2, CH_3	22.4, CH_3	24.2, CH_3	23.3, CH_3	24.2, CH_3	23.9, CH_3	24.0, CH_3
20	28.4, CH	27.9, CH	31.6, CH	31.6, CH	32.0, CH	31.8, CH	29.6, CH	29.3, CH	31.8, CH	35.3, CH	35.3, CH	33.9, CH	33.5, CH	34.8, CH
21	19.3, CH_3	21.1, CH_3	19.1, CH_3	19.1, CH_3	19.1, CH_3	19.2, CH_3	20.0, CH_3	18.9, CH_3	19.2, CH_3	20.0, CH_3	20.0, CH_3	20.0, CH_3	21.7, CH_3	19.4, CH_3
22	41.7, CH_2	42.4, CH_2	34.1, CH_2	34.0, CH_2	33.9, CH_2	34.1, CH_2	38.4, CH_2	38.4, CH_2	34.4, CH_2	35.7, CH_2	35.7, CH_2	35.2, CH_2	35.0, CH_2	32.2, CH_2
23	66.6, CH	207.7, C	27.4, CH_2	27.4, CH_2	27.4, CH_2	27.5, CH_2	81.1, CH	79.9, CH	27.6, CH_2	29.2, CH_2	29.2, CH	28.9, CH_2	28.9, CH_2	29.2, CH_2
24	146.9, CH	147.5, CH	142.6, CH	142.7, CH	142.5, CH	142.6, CH	150.1, CH	150.9, CH	142.6, CH	143.0, CH	143.2, CH	142.0, CH	143.0, CH	143.3, CH
25	128.1, C	131.9, C	129.6, C	129.5, C	129.7, C	129.6, C	130.2, C	129.7, C	129.6, C	129.3, C	129.2, C	129.9, C	129.3, C	129.2, C
26	13.7, CH_3	11.1, CH_3	171.1, C	171.1, C	171.2, C	171.1, C	174.6, C	174.6, C	171.1, C	171.4, C	170.9, C	170.7, C	171.3, C	171.0, C
27	171.4, C	172.1, C	22.2, CH_3	22.2, CH_3	22.2, CH_3	22.2, CH_3	11.2, CH_3	11.2, CH_3	22.1, CH_3	22.1, CH_3	22.0, CH_3	22.0, CH_3	22.0, CH_3	22.0, CH_3
28	24.7, CH_3	23.9, CH_3	24.6, CH_3	24.6, CH_3	23.9, CH_3	24.6, CH_3	24.5, CH_3	24.7, CH_3	21.3, CH_3	26.8, CH_3	27.1, CH_3	27.2, CH_3	28.8, CH_3	27.3, CH_3
29	23.0, CH_3	22.6, CH_3	23.7, CH_3	17.3, CH_3	22.6, CH_3	23.1, CH_3	23.7, CH_3	23.7, CH_3	22.6, CH_3	23.1, CH_3	23.1, CH_3	23.0, CH_3	23.0, CH_3	23.2, CH_3
30	28.7, CH_3	28.2, CH_3	30.0, CH_3	29.8, CH_3	28.5, CH_3	26.5, CH_3	30.0, CH_3	30.0, CH_3	25.7, CH_3	28.8, CH_3	26.6, CH_3	28.8, CH_3	28.8, CH_3	29.0, CH_3
−OAc	170.8, C	171.0, C			170.6, C					170.8, C		170.7, C	170.7, C	170.7, C
	21.5, CH_3	21.4, CH_3			21.5, CH_3					21.5, CH_3		21.4, CH_3	21.5, CH_3	21.5, CH_3

[a]Recorded in pyridine-d_5. [b]Recorded in $CDCl_3$. [c]Recorded at 150 MHz. [d]Recorded at 125 MHz. [e]Recorded at 100 MHz.

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590−2598

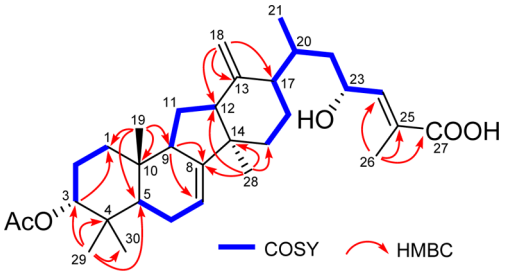


Figure 1. $^{1}H-^{1}H$ COSY and HMBC correlations of compound **1**.

^{13}C NMR data. The ^{1}H and ^{13}C NMR spectroscopic data of **3** and **4** (Tables 1 and 3) were closely related to those of kadcoccinone B,[15] with the only difference being the presence of a hydroxy group at C-3 in **3** and **4** rather than an acetoxy group in kadcoccinone B. This conclusion was confirmed by the HMBC correlations (Figures S23 and S32, Supporting Information) of H_3-29 and H_3-30 with C-3 (δ_C 76.1 for **3**, 79.1 for **4**). The chemical shift of C-5 (δ_C 40.3) in **3** was close to that of kadcoccinone B (δ_C 41.1), indicating the presence of a γ-steric compression effect between HO-3 and H-5α; thus, HO-3 was determined to be α-oriented in **3**, which was supported by the ROESY correlations (Figure 3) of H-3/H_3-29, H-3/H_3-30, H-3/H-2β, H-3/H-2α, H_3-29/H-2β, H-2β/H_3-19β, and H-5α/H_3-30. On the contrary, the chemical shift of C-5 (δ_C 45.9) in **4** was significantly different ($\Delta\delta_C$ 4.8 ppm) relative to that of kadcoccinone B, indicating the absence of a γ-steric compression effect of HO-3/H-5α, and thus, HO-3 was determined to be β-oriented in **4**. This was supported by the ROESY correlations (Figure 3) of H-3/H-5α, H-3/H_3-30, H-5α/H_3-30, and H-3/H-2α, but there was no ROESY correlation of H-3/H-2β. Consequently, the structures of **3** and **4** were identified as C-3 epimers, and these compounds were named kadcoccine acids C and D, respectively.

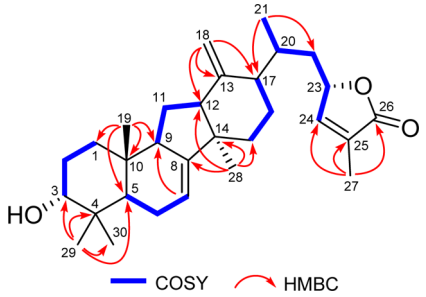


Figure 2. $^{1}H-^{1}H$ COSY and HMBC correlations of compound **7**.

Compound **5** had a molecular formula of $C_{32}H_{46}O_5$ based on the m/z 511.3414 $[M + H]^+$ ion in the positive HRESIMS and ^{13}C NMR data, suggesting 10 indices of hydrogen deficiency. Comparison of the 1D and 2D NMR spectra of **5** with those of kadcoccinone B[15] indicated that these compounds possessed identical scaffolds and substitution patterns, with the only difference that **5** had a carbonyl group replacing an sp^3 methylene function. Furthermore, the UV spectrum (Figure S45, Supporting Information) of **5** displayed an absorption maximum at approximately 239 nm, consistent with an α,β-unsaturated hydroxycarbonyl group. The significant downfield shifts of C-5 (δ_H 2.69, s; δ_C 51.9), C-7 (δ_C 120.1), and C-8 (δ_C 171.2) in conjunction with the HMBC correlations (Figure S41, Supporting Information) from H-5 and H-7 to C-6 (δ_C 200.8) demonstrated that a conjugated carbonyl group was attached to C-6. The ROESY spectrum (Figure S43, Supporting Information) of **5** revealed that the relative configurations of the stereogenic carbons were similar to those of kadcoccinone B. Therefore, the structure of **5**, kadcoccine acid E, was established as shown.

Compound **6** was isolated as a white, amorphous powder, and its HRESIMS data revealed a positive molecular ion peak at m/z 453.3361 ($[M + H]^+$, calcd for 453.3363), corresponding to a molecular formula of $C_{30}H_{44}O_3$, which was two mass units less than that of **3** and **4**. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 3) of **6** strongly resembled those of **3** and **4**, except for the presence of a carbonyl group (δ_C 215.7) in **6** instead of the oxygenated methines (δ_C 76.1 and 79.1) in **3** and **4**, respectively. In the HMBC spectrum (Figure S50, Supporting Information), H_3-29 (δ_H 1.08) and H_3-30 (δ_H 1.22) correlated with C-3 (δ_C 215.7), C-4 (δ_C 48.0), and C-5 (δ_C 47.5), demonstrating that the carbonyl group was located at C-3. Similar ROESY correlations (Figure S52, Supporting Information) showed that the relative configuration of **6** was the same as that of **4**. Accordingly, the structure of **6**, kadcoccine acid F, was determined as shown.

Compounds **7** and **8** were determined to have the same molecular formula, $C_{30}H_{44}O_3$, according to the ^{13}C NMR and HRESIMS data, indicative of nine indices of hydrogen deficiency. The NMR spectroscopic data (Tables 1–3) of **7** and **8** revealed similar structures, with the largest variation being the chemical shifts of C-23 ($\Delta\delta_C$ 1.2 ppm), implying that these compounds are a pair of C-23 epimers. Comparison of the NMR spectroscopic data of compounds **7** and **3** revealed that they possessed identical A/B/C/D-ring systems and differed only as far as the C-17 side chain was concerned. In comparison to **3**, an additional degree of unsaturation, a deshielded oxymethine (δ_C 81.1, C-23), and the molecular formula for **7** indicated the presence of a ring system on the side chain. A 5-substituted 2(5*H*)-furanone motif was inferred by the $^{1}H-^{1}H$ COSY correlations (Figure 2) of H_3-21/H-20/H-22/H-23/H-24 and the HMBC correlations (Figure 2) of H_3-27 (δ_H 1.89) with C-24 (δ_C 150.1), C-25 (δ_C 130.2), and C-

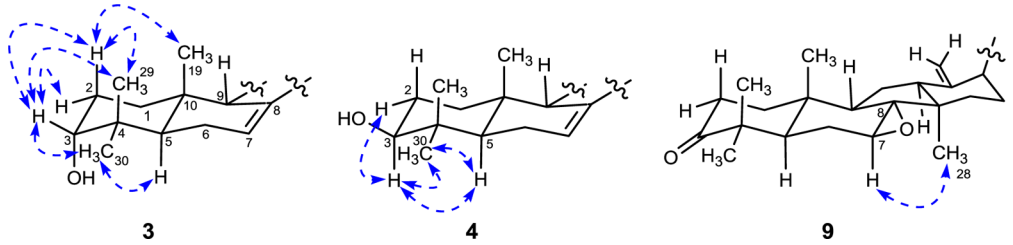


Figure 3. ROESY correlations of compounds **3**, **4**, and **9**.

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590–2598

Table 4. Cytotoxicity of Compounds 1−14 against Human Tumor Cell Lines[a]

compound	HL-60	SMMC-7721	A-549	MCF-7	SW-480	HeLa
2	3.11	11.61	13.00	4.61	5.99	7.77
7	10.40	25.02	27.07	15.60	20.14	12.32
8	7.36	>40	>40	13.62	25.42	18.57
cisplatin[b]	1.93	11.83	12.40	18.34	18.10	8.93
paclitaxel[b]	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008

[a]Results are expressed as IC_{50} values in μM. Cell lines: HL-60, acute leukemia; SMMC-7721, hepatic cancer; A-549, lung cancer; MCF-7, breast cancer; SW-480, colon cancer; HeLa, cervical cancer. [b]Cisplatin and paclitaxel were used as positive controls.

26 (δ_C 174.6). To verify their absolute configurations, the experimental ECD spectra of **7** and **8** were measured in MeOH. As expected, compounds **7** and **8** displayed opposite Cotton effects (CEs) (Figures S64 and S73, Supporting Information) at approximately 214 nm (involving $\pi-\pi^*$ transitions), due to the 5-substituted 2(5*H*)-furanone motif, which matched well with the experimental ECD curves of kadcoccinone A[15] and **1**, respectively, as well as the reported cases,[17] indicating the 23*S* and 23*R* configurations for **7** and **8**, respectively. Hence, the structures of **7** and **8** were elucidated and the compounds were named kadcoccine acids G and H, respectively.

Compound **9** was obtained as a white, amorphous powder. The molecular formula $C_{30}H_{44}O_4$, with nine indices of hydrogen deficiency, was established from the HRESIMS (*m*/*z* 491.3136, $[M + Na]^+$, calcd for 491.3132) and ^{13}C NMR data. The ^1H and ^{13}C NMR signals (Tables 2 and 3) were similar to those of **6**, suggesting that these two compounds shared identical carbocyclic skeletons and substitution patterns, except for the replacement of the $\Delta^{7,8}$ double bond (δ_C 114.9 and 152.2) in **6** by a 7,8-oxirane moiety in **9**, as indicated by the resonances at δ_C 55.8 (C-7) and 71.8 (C-8) and a resonance attributed to H-7 at δ_H 3.36. This conclusion was supported by the $^1H-^1H$ COSY correlations (Figure S79, Supporting Information) of H-5/H-6/H-7 and the HMBC correlation (Figure S78, Supporting Information) of H_3-28 with C-8. The 7,8-oxirane functional group was determined to be β-oriented based on the ROESY correlation (Figure 3) between H-7 (δ_H 3.36) and H_3-28 (δ_H 0.79). The structure of **9**, kadcoccine acid I, was thus defined as shown.

Compound **10** was isolated as a white, amorphous powder, and the HRESIMS data showed a sodium adduct ion at *m*/*z* 535.3391 ($[M + Na]^+$, calcd for 535.3394), consistent with the molecular formula $C_{32}H_{48}O_5$. The ^1H and ^{13}C NMR spectroscopic data (Tables 2 and 3) of **10** implied that its structure was similar to that of kadpolysperin A,[2] except for an oxygenated methylene (δ_C 61.1) in **10** replacing a methyl group at C-18. This conclusion was confirmed by the HMBC correlations (Figure S87, Supporting Information) from H_2-18 (δ_H 4.44 and 4.80) to C-12 (δ_C 46.9), C-13 (δ_C 135.4), and C-17 (δ_C 137.8). Additionally, the ROESY correlations (Figure S89, Supporting Information) disclosed that the orientations of the substituents in **10** were in accordance with those of kadpolysperin A. Therefore, the structure of **10**, kadcoccine acid J, was defined as shown.

Compound **11**, obtained as a white, amorphous powder, gave the molecular formula $C_{30}H_{44}O_4$ based on the $[M + Na]^+$ ion at *m*/*z* 491.3128 in the HRESIMS analysis. The similarities of its 1D NMR data (Tables 2 and 3) with those of **10** revealed that these compounds were structural analogues. The main difference involved the absence of an acetyl group at C-3 (δ_C 79.0) in **10** and the presence of a carbonyl group at C-3 (δ_C 215.8) in **11**. This conclusion was supported by the HMBC correlations of H_3-29 (δ_H 1.03) and H_3-30 (δ_H 1.18) with C-3 (δ_C 215.8) (Figure S97, Supporting Information). Thus, the structure of **11**, kadcoccine acid K, was determined as shown.

Compound **12** gave a molecular formula of $C_{32}H_{46}O_5$, based upon its ^{13}C NMR and HRESIMS data at *m*/*z* 533.3240 ($[M + Na]^+$, calcd for 533.3237). The 1D NMR spectroscopic data (Tables 2 and 3) of **12** were comparable to those of **10**, with the major differences observed for the signals of C-12 ($\Delta\delta_C$ 3.9 ppm), C-13 ($\Delta\delta_C$ 3.4 ppm), C-17 ($\Delta\delta_C$ 25.6 ppm), and C-18 ($\Delta\delta_C$ 131.1 ppm), revealing that these compounds differed in that **12** had a formyl group at C-18 rather than the oxygenated methylene group present in **10**. The UV spectrum (Figure S111, Supporting Information) showed an absorption maximum at 253 nm attributed to an α,β-unsaturated aldehyde motif, which was supported by the HMBC signals (Figure S106, Supporting Information) of H-18 (δ_H 10.53) with C-12 (δ_C 43.0), C-13 (δ_C 138.8), and C-17 (δ_C 163.4) and of H_3-21 (δ_H 1.08) with C-17. Thus, the structure of **12**, kadcoccine acid L, was determined as shown.

Compound **13** was obtained as a white, amorphous powder, and its HRESIMS analysis showed a positive ion at *m*/*z* 537.3551 ($[M + Na]^+$, calcd for 533.3550), in accordance with the molecular formula $C_{32}H_{50}O_5$. Comparison of the NMR data of **13** (Tables 2 and 3) with those of kadcoccinone B[15] suggested that these compounds shared identical carbon scaffolds, except that an exocyclic $\Delta^{13,18}$ double bond (δ_C 151.8 and 112.3) was replaced by an oxygenated tertiary carbon (δ_C 76.0, C-13) and a methyl singlet (δ_H 1.46, δ_C 20.6; C-18), correlating with C-12 (δ_C 58.2), C-13 (δ_C 76.0), and C-17 (δ_C 48.3) in the HMBC experiment (Figure S116, Supporting Information) of **13**. The ROESY correlations (Figure S118, Supporting Information) of H-12α/H_3-18, H_3-18/H-17α, and H_3-18/H-16α (δ_H 1.96) proved that H_3-18 was α-oriented, in accordance with neokadsuranic acid C.[18] Therefore, the structure of **13**, kadcoccine acid M, was determined as shown.

Compound **14** showed a deprotonated molecular ion at *m*/*z* 511.3422 ($[M - H]^-$, calcd for 511.3429) in the HRESIMS analysis, corresponding to the same molecular formula, $C_{32}H_{48}O_5$, as **10**. Its NMR spectroscopic data (Tables 2 and 3) were comparable to those of **10**, indicating that these compounds had structural similarities and that the major distinction was the replacement of the $\Delta^{13,17}$ double bond in **10** by a $\Delta^{12,13}$ double bond in **14**. This was corroborated by the HMBC correlations (Figure S126, Supporting Information) of H_2-18 (δ_H 4.69) with C-12 (δ_C 146.0), C-13 (δ_C 130.5), and C-17 (δ_C 39.6), of H_3-28 (δ_H 1.16) with C-12, and of H_3-21 (δ_H 1.11) with C-17. On the basis of a ROESY spectrum and biosynthesis considerations, **14** was assigned the same relative configuration as that of **10**. Accordingly, the structure of **14** was defined, and this compound was named kadcoccine acid N.

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590−2598

From a biogenetic point of view, kadcoccine acids A–N (**1**–**14**), structurally characterized as possessing a rare 14(13→12)-*abeo*-6/6/5/6-fused tetracyclic rearranged skeleton, might possibly be traced back to the lanostane-type triterpenoids.[1] Compounds **7** and **8** represented the first examples of a 5-substituted 2(5*H*)-furanone group on the C-17 side chain. Assignment of the absolute configurations on the side chains of Schisandraceae triterpenoids had always been a challenge. By comparison of the experimental ECD spectra with the known compound kadcoccinone A, the absolute configurations at C-23 of compounds **1**, **7**, and **8** were determined, providing a useful reference for the determination of absolute configurations on the side chains of this class of triterpenoids.

The structure–activity relationships of compounds **1**–**14** were assessed in terms of their cytotoxicity against six human tumor cell lines, namely, HL-60 (acute leukemia), SMMC-7721 (hepatic cancer), A-549 (lung cancer), MCF-7 (breast cancer), SW-480 (colon cancer), and HeLa (cervical cancer), using the MTS method as previously reported[19] with cisplatin and paclitaxel as positive controls. Among the test substances (Table 4), compounds **2** and **8** showed moderate selective inhibitory effects, with IC_{50} values ranging from 3.11 to 7.77 μM, and the remaining 12 compounds (**1**, **3**–**7**, and **9**–**14**) were inactive (IC_{50} > 10 μM). Comparison of **1** and **2** suggested that a C-23 carbonyl group rather than a hydroxy group may enhance the cytotoxic potency.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. Experimental ECD spectra were recorded on a Chirascan instrument. A Tenor 27 FT-IR spectrometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were performed on Bruker AM-400, DRX-500, and DRX-600 spectrometers using tetramethylsilane as the internal standard. All chemical shifts (δ) were expressed in ppm relative to the solvent signals. HRESIMS data were recorded on an API QSTAR Pulsar 1 spectrometer. Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China) and Lichroprep RP-C_{18} gel (40–63 μm, Merck, Darmstadt, Germany). Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS column. Semipreparative HPLC was performed on an Agilent 1200 liquid chromatograph with a Zorbax SB-C_{18} (Agilent, 9.4 mm × 250 mm) column. Thin-layer chromatography was performed on precoated TLC plates (200–250 μm thickness, silica gel 60 F_{254}, Qingdao Marine Chemical, Inc.). Fractions were monitored by TLC, and spots were visualized by spraying heated silica gel plates with 10% H_2SO_4 in EtOH.

Plant Material. The stems of *K. coccinea* were collected in the Menglun district of Yunnan Province, People's Republic of China, in September 2009 and authenticated by Prof. Xi-Wen Li at the Kunming Institute of Botany. A voucher specimen (KIB 20090901) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried stems of *K. coccinea* (9 kg) were powdered and extracted with 70% aqueous acetone (3 × 45 L, 3 days each) at room temperature. Evaporation of the solvent under reduced pressure afforded a crude extract (810 g), which was suspended in H_2O (12 L) and partitioned successively with EtOAc and *n*-BuOH, respectively. The EtOAc residue (450 g) was subjected to silica gel CC (200–300 mesh, 3 kg) eluted with $CHCl_3$/acetone (1:0–0:1 gradient system) to give six fractions (Fr.1–Fr.6). Fr.2 (150 g) was chromatographed on RP-C_{18} silica gel CC (30–100% MeOH/H_2O) to yield seven main fractions (Fr.2-1–Fr.2-7). Fr.2-6 (40 g) was further fractionated into nine subfractions (Fr.2-6-1–Fr.2-6-9) by RP-C_{18} silica gel CC with a stepwise gradient elution of MeOH/H_2O (65–100%). Subfraction Fr.2-6-7 (6 g) was separated by silica gel CC (petroleum ether/acetone, 5:1–2:1) to give nine major subfractions (Fr.2-6-7A–Fr.2-6-7I) based on TLC analysis. Subfractions Fr.2-6-7C (550.3 mg), Fr.2-6-7D (450.3 mg), and Fr.2-6-7H (640.0 mg) were respectively separated by preparative HPLC with the same gradient elution (75–100% MeCN/H_2O in 25 min, 15 mL/min), followed by semipreparative HPLC (MeCN/H_2O, 75:25, 3.0 mL/min), to afford compounds **5** (10.0 mg), **7** (4.0 mg), **8** (4.1 mg), **9** (18.5 mg), and **11** (5.3 mg). Fr.2-6-8 (25 g) was fractioned by silica gel CC eluted with petroleum ether/acetone (5:1–2:1) to give eight minor subfractions (Fr.2-6-8A–Fr.2-6-8H). Semipreparative HPLC analysis (MeOH/H_2O, 85:15, 3.0 mL/min) of subfraction Fr.2-6-8C (2 g) afforded compounds **2** (71.2 mg), **4** (29.8 mg), and **12** (4.4 mg). Fr.2-6-8F (1.6 g) was chromatographed by performing repeated silica gel CC eluted with a mixed gradient of petroleum ether/$CHCl_3$/2-propanol (15:15:1) and further purified by semipreparative HPLC to afford compounds **1** (6.9 mg), **10** (9.4 mg), **13** (18.9 mg), and **14** (8.1 mg). A portion (650 mg) of Fr.2-7 (65 g) was applied to semipreparative HPLC (MeCN/H_2O, 90:10, 3.0 mL/min) to give compounds **3** (56.3 mg) and **6** (25.2 mg).

*Kadcoccine acid A (**1**):* white, amorphous powder; $[\alpha]_D^{22}$ −59 (MeOH, *c* 0.3); UV (MeOH) λ_{max} (log ε) 204 (4.15) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 214 (−2.45) nm; IR (KBr) ν_{max} 3450, 2956, 2930, 2870, 1731, 1714, 1637, 1550, 1451, 1406, 1383, 1247, 1051, 1028, 607 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 535.3391 $[M + Na]^+$ (calcd for $C_{32}H_{48}O_5Na$, 535.3394).

*Kadcoccine acid B (**2**):* white, amorphous powder; $[\alpha]_D^{22}$ −73 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 204 (4.25), 244 (3.71) nm; IR (KBr) ν_{max} 3441, 2956, 2928, 2872, 1765, 1731, 1707, 1609, 1383, 1452, 1246, 1114, 1048, 602 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 509.3261 $[M - H]^-$ (calcd for $C_{32}H_{45}O_5$, 509.3272).

*Kadcoccine acid C (**3**):* white, amorphous powder; $[\alpha]_D^{23}$ −89 (MeOH, *c* 0.2); UV (MeOH) λ_{max} (log ε) 203 (4.34) nm; IR (KBr) ν_{max} 3440, 2955, 2927, 2869, 1692, 1635, 1457, 1380, 1252, 1202, 1158, 1062, 984, 890, 577 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 455.3518 $[M + H]^+$ (calcd for $C_{30}H_{47}O_3$, 455.3520).

*Kadcoccine acid D (**4**):* white, amorphous powder; $[\alpha]_D^{23}$ −107 (MeOH, *c* 0.2); UV (MeOH) λ_{max} (log ε) 204 (4.40) nm; IR (KBr) ν_{max} 3441, 2955, 2931, 2869, 1691, 1634, 1457, 1378, 1252, 1201, 1075, 1019, 995, 586 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 455.3519 $[M + H]^+$ (calcd for $C_{30}H_{47}O_3$, 455.3520).

*Kadcoccine acid E (**5**):* white, amorphous powder; $[\alpha]_D^{26}$ −119 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 203 (4.09), 239 (4.08) nm; IR (KBr) ν_{max} 3438, 2959, 2931, 2874, 1729, 1711, 1679, 1643, 1458, 1379, 1245, 1185, 1049, 585 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 511.3414 $[M + H]^+$ (calcd for $C_{32}H_{47}O_5$, 511.3418).

*Kadcoccine acid F (**6**):* white, amorphous powder; $[\alpha]_D^{23}$ −133 (MeOH, *c* 0.2); UV (MeOH) λ_{max} (log ε): 203 (4.39) nm; IR (KBr) ν_{max} 3439, 2957, 2928, 2868, 1706, 1691, 1633, 1457, 1383, 1260, 1196, 1112, 581 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 453.3361 $[M + H]^+$ (calcd for $C_{30}H_{45}O_3$, 453.3363).

*Kadcoccine acid G (**7**):* white, amorphous powder; $[\alpha]_D^{26}$ −31 (MeOH, *c* 0.2); UV (MeOH) λ_{max} (log ε) 204 (4.33) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 214 (+10.97) nm; IR (KBr) ν_{max} 3440, 2956, 2928, 2870, 1754, 1631, 1453, 1384, 1103, 1061, 1025, 584 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 475.3182 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_3Na$, 475.3183).

*Kadcoccine acid H (**8**):* white, amorphous powder; $[\alpha]_D^{26}$ −43 (MeOH, *c* 0.3); UV (MeOH) λ_{max} (log ε) 204 (4.31) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 214 (−7.19) nm; IR (KBr) ν_{max} 3451, 2956, 2928, 2870, 1755, 1633, 1459, 1383, 1101, 1060 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m*/*z* 475.3183 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_3Na$, 475.3183).

*Kadcoccine acid I (**9**):* white, amorphous powder; $[\alpha]_D^{26}$ −74 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 203 (4.00) nm; IR (KBr) ν_{max} 3441, 2932, 2874, 1706, 1633, 1551, 1458, 1430, 1383, 1265, 1198, 1113, 586 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3;

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590–2598

HRESIMS *m/z* 491.3136 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_4Na$, 491.3132).

*Kadcoccine acid J (**10**):* white, amorphous powder; $[\alpha]_D^{21}$ −122 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 204 (4.10) nm; IR (KBr) ν_{max} 3445, 2956, 2927, 2869, 1732, 1714, 1643, 1632, 1596, 1458, 1415, 1384, 1246, 1030, 608 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 535.3391 $[M + Na]^+$ (calcd for $C_{32}H_{48}O_5Na$, 535.3394).

*Kadcoccine acid K (**11**):* white, amorphous powder; $[\alpha]_D^{26}$ −166 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 203 (4.27) nm; IR (KBr) ν_{max} 3441, 2958, 2929, 2867, 1705, 1635, 1569, 1457, 1428, 1383, 1245, 1195, 1112, 1004 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 491.3128 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_4Na$, 491.3132).

*Kadcoccine acid L (**12**):* white, amorphous powder; $[\alpha]_D^{26}$ −159 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 203 (4.06), 253 (3.95) nm; IR (KBr) ν_{max} 3443, 2957, 2929, 2869, 1729, 1639, 1558, 1458, 1418, 1381, 1247, 1052, 1031, 605 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 533.3240 $[M + Na]^+$ (calcd for $C_{32}H_{46}O_5Na$, 533.3237).

*Kadcoccine acid M (**13**):* white, amorphous powder; $[\alpha]_D^{20}$ −33 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 203 (3.67) nm; IR (KBr) ν_{max} 3440, 2955, 2928, 2870, 1719, 1631, 1564, 1458, 1419, 1382, 1246, 1184, 1168, 1056, 1028 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 537.3551 $[M + Na]^+$ (calcd for $C_{32}H_{50}O_5Na$, 537.3550).

*Kadcoccine acid N (**14**):* white, amorphous powder; $[\alpha]_D^{21}$ −152 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 204 (4.25) nm; IR (KBr) ν_{max} 3441, 2956, 2926, 2855, 1732, 1717, 1632, 1552, 1457, 1422, 1383, 1246, 1030, 606 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 511.3422 $[M - H]^-$ (calcd for $C_{32}H_{47}O_5$, 511.3429).

Cytotoxicity Assays. The following human tumor cell lines were used: HL-60 (acute leukemia), SMMC-7721 (hepatic cancer), A-549 (lung cancer), MCF-7 (breast cancer), SW-480 (colon cancer), and HeLa (cervical cancer). All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere containing 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)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) (Sigma, St. Louis, MO, USA).[19] Briefly, 100 μL of adherent cells was seeded into each well of a 96-well cell culture plate, and the cells were allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before addition of the test compound, 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 incubation, MTS (100 μg) was added to each well and the incubation continued at 37 °C for 4 h. 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 490 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated with Reed and Muench's method.[20]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00508.

1D and 2D NMR, HRESIMS, IR, and UV spectra of compounds **1**−**14** and experimental ECD spectra of compounds **1**, **7**, and **8** (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail (Y.-H. Zhang): zhangyh@mails.tjmu.edu.cn. Tel: 86-27-83692892. Fax: 86-27-83692762.

*E-mail (J.-X. Pu): pujianxin@mail.kib.ac.cn. Tel: 86-871-65223616. Fax: 86-871-65216343.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was supported financially by the National Natural Science Foundation of China (Nos. 81373290 and 21322204).

■ REFERENCES

(1) (a) Xiao, W.-L.; Li, R.-T.; Huang, S.-X.; Pu, J.-X.; Sun, H.-D. *Nat. Prod. Rep.* **2008**, *25*, 871−891. (b) Shi, Y.-M.; Xiao, W.-L.; Pu, J.-X.; Sun, H.-D. *Nat. Prod. Rep.* **2015**, *32*, 367−410.

(2) Dong, K.; Pu, J.-X.; Du, X.; Su, J.; Li, X.-N.; Yang, J.-H.; Zhao, W.; Li, Y.; Sun, H.-D. *Tetrahedron* **2012**, *68*, 4820−4829.

(3) Liu, J.-S.; Qi, Y.-D.; Lai, H.-W.; Zhang, J.; Jia, X.-G.; Liu, H.-T.; Zhang, B.-G.; Xiao, P.-G. *Phytomedicine* **2014**, *21*, 1092−1097.

(4) (a) Chen, D.-F.; Zhang, S.-X.; Kozuka, M.; Sun, Q.-Z.; Feng, J.; Wang, Q.; Mukainaka, T.; Nobukuni, Y.; Tokuda, H.; Nishino, H.; Wang, H.-K.; Morris-Natschke, S.-L.; Lee, K.-H. *J. Nat. Prod.* **2002**, *65*, 1242−1245. (b) Wang, N.; Li, Z.-L.; Song, D.-D.; Li, W.; Pei, Y.-H.; Jing, Y.-K.; Hua, H.-M. *Planta Med.* **2012**, *78*, 1661−1666. (c) Wang, N.; Li, Z.-L.; Song, D.-D.; Li, W.; Fu, H.-W.; Koike, K.; Pei, Y.-H.; Jing, Y.-K.; Hua, H.-M. *J. Nat. Prod.* **2008**, *71*, 990−994.

(5) Yang, X.-W.; Miyashiro, H.; Hattori, M.; Namba, T.; Tezuka, Y.; Kikuchi, T.; Chen, D.-F.; Xu, G.-J.; Hori, T.; Extine, M.; Mizuno, H. *Chem. Pharm. Bull.* **1992**, *40*, 1510−1516.

(6) Pu, J.-X.; Yang, L.-M.; Xiao, W.-L.; Li, R.-T.; Lei, C.; Gao, X.-M.; Huang, S.-X.; Li, S.-H.; Zheng, Y.-T.; Huang, H.; Sun, H.-D. *Phytochemistry* **2008**, *69*, 1266−1272. (b) Sun, R.; Song, H.-C.; Wang, C.-R.; Shen, K.-Z.; Xu, Y.-B.; Gao, Y.-X.; Chen, Y.-G.; Dong, J.-Y. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 961−965.

(7) (a) Kuo, Y.-H.; Li, S.-Y.; Huang, R.-L.; Wu, M.-D.; Huang, H.-C.; Lee, K.-H. *J. Nat. Prod.* **2001**, *64*, 487−490. (b) Ban, N.-K.; Thanh, B.-V.; Kiem, P.-V.; Minh, C.-V.; Cuong, N.-X.; Nhiem, N.-X.; Huong, H.-T.; Anh, H.-T.; Park, E.; Sohn, D.-H.; Kim, Y.-H. *Planta Med.* **2009**, *75*, 1253−1257.

(8) (a) Yang, J.-H.; Zhang, H.-Y.; Wen, J.; Du, X.; Chen, J.-H.; Zhang, H.-B.; Xiao, W.-L.; Pu, J.-X.; Tang, X.-C.; Sun, H.-D. *J. Nat. Prod.* **2011**, *74*, 1028−1035. (b) Dong, K.; Pu, J.-X.; Zhang, H.-Y.; Du, X.; Li, X.-N.; Zou, J.; Yang, J.-H.; Zhao, W.; Tang, X.-C.; Sun, H.-D. *J. Nat. Prod.* **2012**, *75*, 249−256.

(9) Hu, X.; Zhang, W.-K.; Zhu, Q.-S. *Zhong Hua Ben Cao*; Shanghai Scientific & Techincal Publishers: Shanghai, China, 1999; Vol. 2, p 895.

(10) Gao, X.-M.; Pu, J.-X.; Huang, S.-X.; Lu, Y.; Lou, L.-G.; Li, R.-T.; Xiao, W.-L.; Chang, Y.; Sun, H.-D. *J. Nat. Prod.* **2008**, *71*, 1182−1188.

(11) Gao, X.-M.; Pu, J.-X.; Xiao, W.-L.; Huang, S.-X.; Lou, L.-G.; Sun, H.-D. *Tetrahedron* **2008**, *64*, 11673−11679.

(12) Liang, C.-Q.; Shi, Y.-M.; Luo, R.-H.; Li, X.-Y.; Gao, Z.-H.; Li, X.-N.; Yang, L.-M.; Shang, S.-Z.; Li, Y.; Zheng, Y.-T.; Zhang, H.-B.; Xiao, W.-L.; Sun, H.-D. *Org. Lett.* **2012**, *14*, 6362−6365.

(13) Liang, C.-Q.; Shi, Y.-M.; Li, X.-Y.; Luo, R.-H.; Li, Y.; Zheng, Y.-T.; Zhang, H.-B.; Xiao, W.-L.; Sun, H.-D. *J. Nat. Prod.* **2013**, *76*, 2350−2354.

(14) Liang, C.-Q.; Shi, Y.-M.; Wang, W.-G.; Hu, Z.-X.; Li, Y.; Zheng, Y.-T.; Li, X.-N.; Du, X.; Pu, J.-X.; Xiao, W.-L.; Zhang, H.-B.; Sun, H.-D. *J. Nat. Prod.* **2015**, *78*, 2067−2073.

(15) Hu, Z.-X.; Shi, Y.-M.; Wang, W.-G.; Li, X.-N.; Du, X.; Liu, M.; Li, Y.; Xue, Y.-B.; Zhang, Y.-H.; Pu, J.-X.; Sun, H.-D. *Org. Lett.* **2015**, *17*, 4616−4619.

(16) Hu, Z.-X.; Shi, Y.-M.; Wang, W.-G.; Tang, J.-W.; Zhou, M.; Du, X.; Zhang, Y.-H.; Pu, J.-X.; Sun, H.-D. *Org. Lett.* **2016**, *18*, 2284−2287.

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590−2598

(17) (a) Tanaka, R.; Wada, S.; Aoki, H.; Matsunaga, S.; Yamori, T. *Helv. Chim. Acta* **2004**, *87*, 240−249. (b) Wang, G.-W.; Lv, C.; Yuan, X.; Ye, J.; Jin, H.-Z.; Shan, L.; Xu, X.-K.; Shen, Y.-H.; Zhang, W.-D. *Phytochemistry* **2015**, *116*, 221−229. (c) Shi, Y.-M.; Wang, L.-Y.; Zou, X.-S.; Li, X.-N.; Shang, S.-Z.; Gao, Z.-H.; Liang, C.-Q.; Luo, H.-R.; Li, H.-L.; Xiao, W.-L.; Sun, H.-D. *Tetrahedron* **2014**, *70*, 859−868.
(18) Li, L.-N.; Xue, H.; Kangouri, K.; Ikeda, A.; Omura, S. *Planta Med.* **1989**, *55*, 294−296.
(19) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(20) Reed, L.-J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493−497.

DOI: 10.1021/acs.jnatprod.6b00508
J. Nat. Prod. 2016, 79, 2590−2598