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Kadcoccilactones K–R, triterpenoids from *Kadsura coccinea*

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ABSTRACT

Phytochemical investigation on the stems of *Kadsura coccinea* led to the isolation of 8 new triterpenoids, kadcoccilactones K–R (**1**–**8**), and 10 known analogues. Their structures were elucidated on the basis of extensive spectroscopic analysis. Compounds **1**–**3** characterized with an aromatic ring E in their molecules are rarely naturally occurred kadlongilactone derivatives. Moreover, all compounds were evaluated for their inhibitory activity against K562, Bel-7402, and A549 human tumor cells. Compounds **9** and **10** exhibited potent cytotoxicity against K562, Bel-7402, and A549 cell lines with IC_{50} values less than 0.1, 0.1, and 1.0 μm, respectively.

1. Introduction

Phytochemically, plants of the genus *Kadsura* were a rich source of lignans and triterpenoids.[1–9] Some of them have been proved to be effective as antitumor,[7] anti-HIV,[8] anti-lipid peroxidative,[9] cytotoxic,[4,6] and anti-hepatitis agents.[10] *Kadsura coccinea* (Lem.) A.C. Smith is used in traditional Chinese medicine for treating gastroenteric disorders and rheumatoid arthritis.[11] Chemical investigations of this plant have yielded a number of dibenzocyclooctadiene lignans and triterpenoids.[12–16] In order to find bioactive constituents, we previously reported 10 triterpenoids, named kadcoccilactones A–J, isolated from the stems of this plant.[16] In our continuous chemical investigation of this plant, eight new triterpenoids, named kadcoccilactones K–R (**1**–**8**), were isolated. Among the new ones, compounds **1**–**5** and **6** possessed the rare naturally occurred carbon skeletons same as those of kadlongilactone A (**9**) and longipedlactone A (**12**). Compound **7** was a cycloartane type triterpenoid, which possessed the same carbon skeleton as kadsuphilactone B.[5] Compound **8** showed to be a 3,4-*seco*-lanostane type triterpenoid. In addition, 10 known triterpenoids, kadlongilactones A, B, and D (**9**–**11**),[1,2] longipedlactones A–C and E–F (**12**–**16**),[3] heteroclic acid and schisandronic acid (**17** and **18**),[4] were also isolated. All compounds were evaluated for their cytotoxicity against K562, Bel-7402, and A549 human tumor cells. Compounds **9** and **10** exhibited significant inhibitory activity against K562, Bel-7402, and A549 cell lines with IC_{50} values less than 0.1, 0.1, and 1.0 μm, respectively. In this paper, we reported the isolation and structural elucidation of these eight new triterpenoids as well as the biological activity of all the isolated compounds.

2. Results and discussion

Compound **1** was obtained as colorless crystals. HRESIMS analysis of **1** indicated that it has the molecular formula $C_{31}H_{38}O_7$ (*m*/*z* 545.2527 $[M+Na]^+$), suggesting 13 degrees of unsaturation. The UV spectrum displayed absorption maximum at 287 and 208 nm, which indicated the presence of an extended chromophore. The IR spectrum showed the presence of hydroxyl groups (3441 cm^{-1}), aromatic ring (1612 cm^{-1}), and carbonyl groups (1631 and 1703 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 3) showed 1 methoxyl, 5 methyls, 5 methylenes, 8 methines (2 oxygenated and 3 olefinic), and 12 quarternary carbons (2 carbonyls, 7 olefinic and 2 oxygenated). Apart from five double bonds and two lactones, the remaining elements of the unsaturation in **1** were assumed to be a hexacyclic skeleton. Interpretation of HSQC, ^1H–^1H COSY, and HMBC spectral data provided structure similar to that of kadlongilactone A (**9**),[1] except for the presence of a methoxy group at δ_C 58.5 and more olefinic carbons in **1** (Fig. 1). The methoxy group was located at C-19 according to the HMBC correlation of H-19 (δ_H 3.94) with C-1, C-5, C-8, C-9, C-10, and C-11, and of OMe at δ_H 3.22 with C-19 (δ_C 94.4). The

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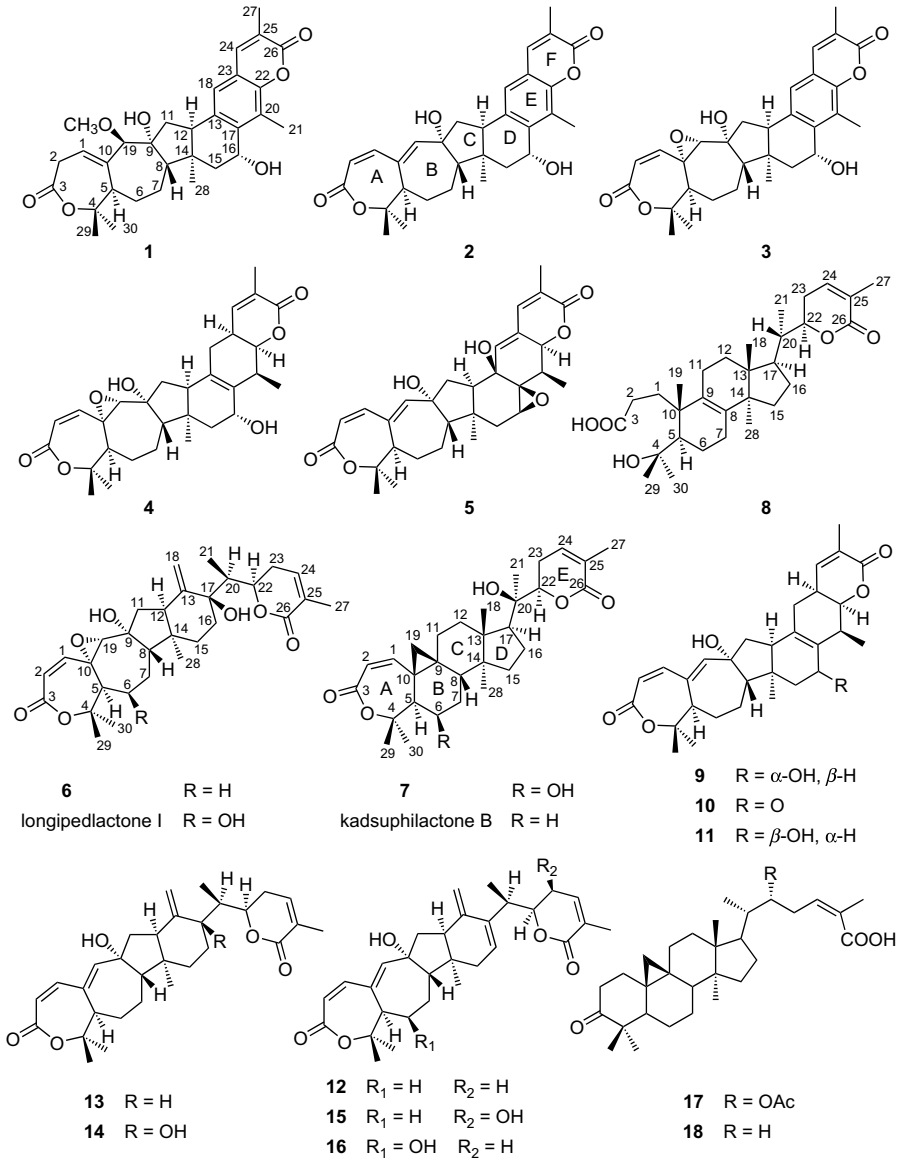

ring E was aromatic in **1**, instead of a six-membered ring with a double bond in **9**. This assignment was in accord with the observation of remarkable downfield shifts of the C-18, C-20, C-22, and C-23 signals from δ_C 32.1, 34.4, 80.1, and 33.3 in **9** to δ_C 125.4, 124.8, 149.6, and 118.2 in **1**, respectively. This was further confirmed by the HMBC correlations of H-16 (δ_H 5.44, s) with C-13, C-17, and C-20, and of H_3-21 (δ_H 2.57, s) with C-17, C-20, and C-22. In addition, HMBC correlations of H-19 (δ_H 3.94, s) and H_2-2 (3.49, dd, J=19.0, 3.6 Hz and 3.38, dd, J=19.0, 6.0 Hz) with C-1 (δ_C 118.9) and C-10 (δ_C 140.9), respectively, positioned a double bond between C-1 and C-10. In the ROESY spectrum of **1**, the correlations from H-16 to H-15α, H-15β, and H_3-21, and no correlation of H-16 with H_3-28 (Fig. 2), indicated that OH-16 should also be α-oriented as that of **9**. ROESY correlations of H-19 with H-5, and of H-8 with H-6β, H-11β, and H-15β suggested H-19 was α-oriented and H-8 was β-oriented. The relative configurations of all other stereocenters of **1** were identical to that of **9** determined by the observed ROESY correlations and by comparison of NMR data of both compounds. Thus, the structure of **1** was fully established and has been accorded the trivial name kadcoccilactone K.

Compound **2** was obtained as white amorphous powder. The molecular formula of **2** was established as $C_{30}H_{34}O_6$ from HRFABMS (m/z 491.2445 $[M+H]^+$), indicating 14 degrees of unsaturation. Analysis of the ^1H and ^{13}C NMR (Tables 1 and 3) and HSQC spectra revealed that the structure of **2** was similar to that of **1**, except for the presence of two more olefinic carbons and the absence of a methoxy group in **2**. HMBC correlations of H-1 (δ_H 6.58, d, J=12.1 Hz) with C-2 (δ_C 119.1, d), C-3 (δ_C 166.5, s), C-5 (δ_C 48.6, d), C-19 (δ_C 147.4, d), and of H-2 (δ_H 6.00, d, J=12.1 Hz) with C-3 and C-10 (δ_C 146.1, s), and of H-19 (δ_H 6.38, s) with C-1 (δ_C 144.0, d), C-5, C-8 (δ_C 59.3, d), C-9 (δ_C 79.0, s), and C-11 (δ_C 50.9, t) suggested two double bonds between C-1 and C-2, and between C-10 and C-19, respectively. The relative configurations of all stereocenters in **2** were established to be identical with those of **1** by their similar ROESY correlations, that is, α-oriented Me-30, Me-28, OH-16, H-12, OH-9, and H-5, β-oriented Me-29 and H-8.

Compound **3** was obtained as white amorphous powder and possessed a molecular formula of $C_{30}H_{34}O_7$ as deduced from its HRESIMS (m/z 529.2213 $[M+Na]^+$) and NMR data. The NMR data of **2** and **3** disclosed that the main structural differences between these two compounds were substituent groups at C-10 and C-19. HMBC correlations of H-2 with C-10 and C-19, of H-5 with C-10 and C-19, and the chemical shifts of C-10 (δ_C 62.5, s) and C-19 (δ_C 67.0, d) suggested an epoxide group between C-10 and C-19 in **3** instead of

Table 1
^{1}H NMR data of compounds **1**–**4** in C_5D_5N[a]

Proton	1	2	3	4
1 α	5.59 (t, 5.0)	6.58 (d, 12.1)	5.78 (d, 12.5)	5.75 (d, 12.5)
2 α	3.38 (dd, 19.0, 6.0)	6.00 (d, 12.1)	6.37 (d, 12.5)	6.30 (d, 12.5)
2 β	3.49 (dd, 19.0, 3.6)			
5	2.66 (d, 10.2)	4.16 (d, 9.6)	3.01 (br d, 10.2)	2.95 (d, 10.3)
6 α	2.12 (overlap)	2.40–2.43 (m)	2.12–2.13 (overlap)	2.07–2.08 (m)
6 β	1.65 (dd, 25.0, 12.5)	1.84–1.87 (m)	1.33–1.35 (m)	1.28–1.31 (m)
7 α	1.94 (dd, 24.3, 12.1)	1.98–2.00 (m)	2.04–2.06 (m)	1.94–1.95 (m)
7 β	1.79–1.81 (m)	1.26–1.28 (m)	1.72–1.74 (m)	1.57–1.59 (m)
8	3.18 (d, 10.2)	1.67 (br d, 13.2)	1.72–1.74 (m)	1.69 (br d, 12.1)
11 α	2.47 (dd, 12.4, 7.8)	2.78 (dd, 12.6, 7.3)	2.78 (dd, 12.5, 6.9)	2.43 (dd, 13.2, 7.7)
11 β	2.58–2.60 (m)	1.90–1.92 (m)	1.98–2.00 (m)	1.54–1.56 (m)
12 α	3.64 (dd, 11.4, 7.8)	3.80 (dd, 12.2, 7.1)	3.81 (dd, 12.2, 6.9)	2.65 (t, 7.5)
15 α	1.40 (dd, 14.4, 3.2)	1.81 (dd, 14.4, 2.6)	1.91 (dd, 14.3, 3.9)	2.01 (br d, 5.4)
15 β	2.40 (dd, 14.4, 3.5)	2.37–2.39 (m)	2.44 (dd, 14.2, 2.7)	
16 α	5.44 (s)			
16 β		5.33 (br s)	5.33 (br s)	4.63 (br s)
18 α	7.17 (s)	7.24 (s)	7.21 (s)	1.96–1.97 (m)
18 β				2.04–2.05 (m)
19	3.94 (s)	6.38 (s)	3.18 (s)	2.98 (s)
20				3.21 (br d, 2.8)
21	2.57 (s)	2.70 (s)	2.70 (s)	1.54 (d, 7.2)
22				4.46 (br d, 2.2)
23 α				2.24–2.28 (m)
24	7.40 (s)	7.36 (s)	7.35 (s)	6.68 (d, 5.4)
27	2.12 (s)	2.12 (s)	2.12 (s)	1.92 (s)
28	1.36 (s)	1.74 (s)	1.69 (s)	1.46 (s)
29	1.37 (s)	1.44 (s)	1.40 (s)	1.35 (s)
30	1.45 (s)	1.46 (s)	1.66 (s)	1.61 (s)
OMe	3.22 (s)			
OH-9		6.75 (s)	6.72 (s)	6.56 (s)

[a] Data were recorded with a Bruker DRX-500 MHz spectrometer, chemical shifts (δ) are in parts per million, J in hertz.

a double bond between C-10 (δ_C 146.1, s) and C-19 (δ_C 147.4, d) in **2** (Tables 1 and 3). ROESY correlations of H-19/H-1, H-19/H-8β, and H-19/H-11β indicated that the epoxy ring of **3** was in α-orientation. The relative configurations of all other stereocenters in **3** were established to be identical with those of **2** by their similar ROESY correlations.

Compound **4** was obtained as white amorphous powder and had the molecular formula $C_{30}H_{38}O_7$ as revealed by its HRESIMS at m/z 533.2521 $[M+Na]^+$ (calcd 533.2515). Detailed analysis of the NMR (Tables 1 and 3) and MS spectra led to the conclusion that the only difference between **4** and **9** was substituents at C-10 and C-19. There is an epoxide group between C-10 and C-19 in **4** instead of a double bond between C-10 (δ_C 145.6, s) and C-19 (δ_C 148.2, d) in **9**. This epoxide group was elucidated by HMBC correlations of H-2 with C-10 and C-19, and of H-5 with C-10 and C-19, as well as the chemical shifts of C-10 (δ_C 62.3, s) and C-19 (δ_C 67.9, d). ROESY correlations of H-19/H-1, H-19/H-8β, and H-19/H-11β indicated that the epoxy ring of **4** was also in α-orientation as that of **3**. The relative stereochemistry of **4** was in agreement with that of **9** due to the similar ROESY correlations and by the comparison of NMR data with both compounds.

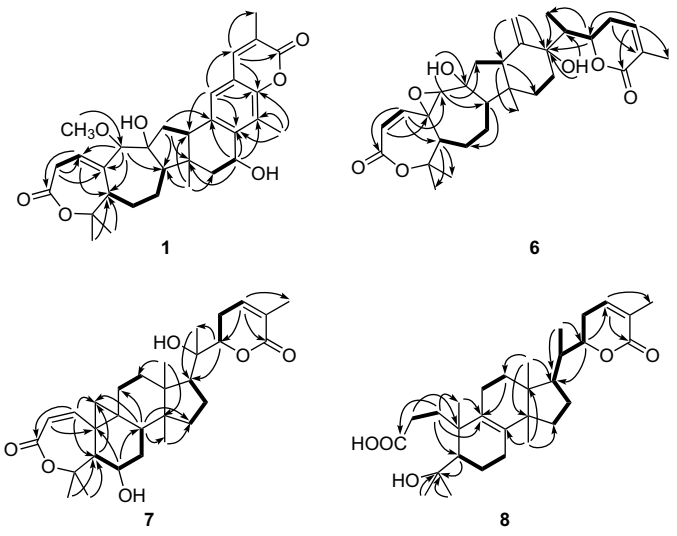

Figure 1. Selected HMBC (→) and ^{1}H–^{1}H COSY (—) correlations of **1** and **6**–**8**.

Compound **5** was obtained as white amorphous powder. The positive HRESIMS showed a quasi-molecular ion at m/z 531.2351 corresponding to $[M+Na]^+$, indicating a molecular formula of $C_{30}H_{36}O_7$. Analysis of the ^{1}H and ^{13}C NMR (Tables 1 and 3) and HSQC spectra revealed that **5** contains 10 quaternary carbons (including 2 α,β-unsaturated carbonyls, 3 olefinic, and 4 oxygenated), 11 methines (5 olefinic and 2 oxygenated), 4 methylenes, and 5 methyls. The UV absorption maximum at 280, 219, and 203 nm indicated the presence of extended chromophore. The NMR spectral features (Tables 2 and 3) of **5** were similar to those of **9**. The difference observed between compounds **5** and **9** was the substituted pattern on ring E and C-16. HMBC correlations of OH-13 (δ_H 6.66, s) with C-13 and C-17 indicated a hydroxyl group at C-13. HMBC correlations of H-24 with C-18 and C-23, of H-22 with C-18 and C-23, and the chemical shifts of C-18 (δ_C 132.1, d) and C-23 (δ_C 129.5, s) suggested a double bond between C-18 and C-23. According to the chemical shifts of C-16 (δ_C 58.7, d) and C-17 (δ_C 63.0, s) and the molecular formula of **5**, an epoxide group should be located between C-16 and C-17. ROESY correlation of OH-13 with H_3-21 positioned OH-13 at β-orientation. H-16 was deduced to be α-oriented by ROESY correlation of H-16 with H-12, of H-16 with H-15α, and the absence of ROESY correlation of H-16 with H_3-21.

Compound **6** was obtained as a white amorphous solid. It was assigned the molecular formula $C_{30}H_{40}O_7$ by its HRESIMS m/z 511.2688 $[M+Na]^+$ (calcd 511.2695), requiring 11 sites of unsaturation.

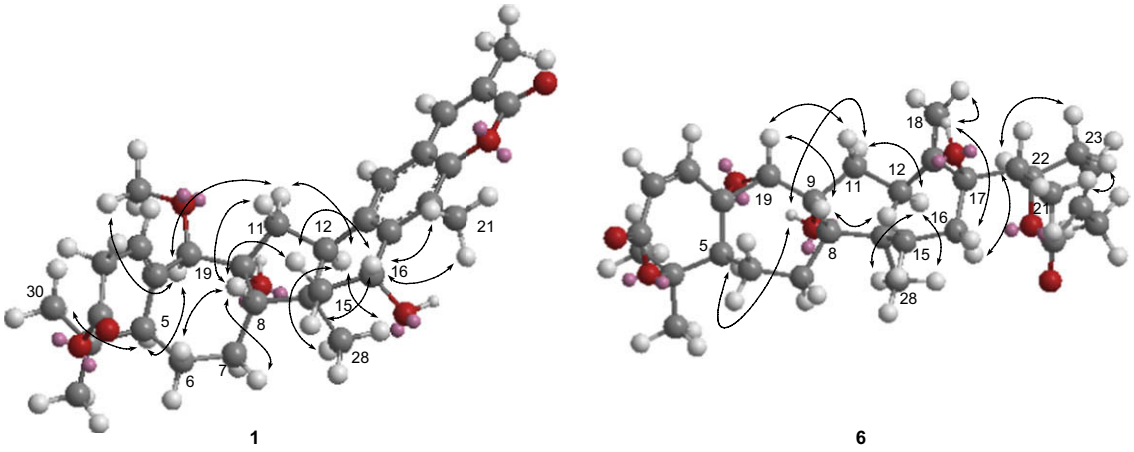

Figure 2. Key ROESY correlations and relative configurations assigned for **1** and **6**.

The IR spectrum showed the presence of hydroxyl groups (3474 cm^{-1}) and two lactone groups (1636 and 1698 cm^{-1}). Analysis of ^{1}H, ^{13}C NMR (Tables 2 and 3), DEPT, and HSQC data revealed the presence of five methyls, seven methylenes (one olefinic), nine methines (two oxygenated and three olefinic), and nine quaternary carbons (two carbonyls, four oxygenated, and two olefinic) in its structure. Apart from three double bonds, an epoxy ring, and two carbonyl groups, the remaining elements of unsaturation in **6** were assumed to be a pentacyclic skeleton. The characteristic EIMS cleavage fragment *m*/*z* 111 $[C_6H_7O_2]^+$ indicated the presence of a six-membered α-methyl-α,β-unsaturated lactone moiety.[3] The NMR spectral features of **6** indicated that the most significant difference between **6** and the compounds mentioned above was the appearance of an exocyclic double bond, which implied a different skeleton. Extensive analysis of ^{1}H–^{1}H COSY, HMBC, and HSQC spectral data led to the establishment of a structure similar to that of longipedlactone I.[3]

Table 2
^{1}H NMR data of compounds **5**–**8** in C_5D_5N[a]

Proton	5	6	7	8
1 α	6.42 (d, 12.4)	5.78 (dd, 12.5, 1.8)	6.13 (d, 12.6)	2.11–2.15 (m)
1 β				3.28–3.34 (m)
2 α	5.91 (d, 12.4)	6.39 (d, 12.5)	6.20 (d, 12.6)	2.79–2.86 (m)
2 β				2.37–2.43 (m)
5	4.06 (d, 9.6)	2.93 (br d, 9.0)	2.37–2.39 (m)	1.79 (br d, 11.0)
6 α	2.13–2.15 (m)	2.09–2.12 (m)	4.50 (br d, 2.4)	2.21–2.24 (m)
6 β	1.19–1.24 (m)	1.40–1.42 (m)		1.59–1.63 (overlap)
7 α	2.28–2.30 (overlap)	1.92–1.94 (m)	1.25–1.29 (m)	1.97–2.00 (overlap)
7 β	1.70 (br d, 11.8)	1.60–1.64 (m)	1.37–1.40 (m)	1.43–1.45 (overlap)
8	2.23–2.26 (overlap)	1.76 (br d, 11.9)	2.51 (dd, 13.3, 4.2)	
11 α	2.23–2.26 (overlap)	2.19–2.22 (m)	2.17–2.20 (m)	2.16–2.20 (m)
11 β	2.63 (dd, 13.2, 5.8)	1.97–1.99 (m)	1.43–1.45 (overlap)	
12 α	1.89 (br d, 10.1)	3.17 (dd, 13.6, 6.6)	1.84–1.86 (m)	1.69–1.72 (overlap)
12 β			1.57–1.60 (m)	1.59–1.63 (overlap)
15 α	2.28–2.30 (overlap)	1.57 (br d, 13.9)	1.31–1.34 (m)	1.18 (t, 10.5)
15 β	2.02 (dd, 14.0, 4.2)	1.53 (dd, 13.9, 3.2)	1.31–1.34 (m)	
16 α	3.36 (d, 4.2)	2.16–2.18 (m)	2.20–2.24 (overlap)	1.29–1.36 (m)
16 β		1.85–1.89 (m)	1.74–1.76 (m)	1.72–1.75 (m)
17			2.20–2.24 (overlap)	1.43–1.45 (overlap)
18	6.10 (s)	5.59 (d, 2.5)	0.96 (s)	0.75 (s)
		5.27 (d, 2.5)		
19 α	6.45 (s)	3.10 (s)	1.18 (d, 3.3)	1.40 (s)
19 β			2.28 (d, 3.3)	
20	2.09–2.12 (m)	2.37 (d, 5.4)		1.97 (d, 5.0)
21	1.53 (d, 7.2)	1.21 (d, 5.0)	1.43 (s)	1.00 (d, 6.6)
22	5.64 (br d, 3.7)	5.08 (dt, 12.9, 3.2)	4.44 (dd, 13.5, 3.6)	4.45 (dd, 9.9, 3.3)
23 α		2.76–2.79 (m)	2.28–2.31 (overlap)	1.97–2.00 (overlap)
23 β		2.49–2.51 (m)		1.69–1.72 (overlap)
24	6.78 (s)	6.48 (d, 6.5)	6.53 (d, 6.2)	6.50 (d, 6.0)
27	1.94 (s)	1.93 (s)	1.92 (s)	1.94 (s)
28	1.62 (s)	1.07 (s)	1.46 (s)	0.96 (s)
29	1.41 (s)	1.36 (s)	1.84 (s)	1.51 (s)
30	1.44 (s)	1.65 (s)	1.66 (s)	1.48 (s)
OMe				
OH-6			6.16 (s)	
OH-9	6.57 (s)	6.54 (s)		
OH-13	6.66 (s)			
OH-17		5.97 (s)		

[a] Data were recorded with a Bruker DRX-500 MHz spectrometer, chemical shifts (δ) are in parts per million, *J* in hertz.

Table 3
^{13}C NMR data of compounds **1**–**8** in C_5D_5N[a]

No.	1	2	3	4	5	6	7	8
1	118.9 (d)	144.0 (d)	144.8 (d)	144.8 (d)	144.2 (d)	145.0 (d)	152.2 (d)	34.2 (t)
2	38.0 (t)	119.1 (d)	126.3 (d)	126.2 (d)	119.2 (d)	126.2 (d)	119.9 (d)	30.7 (t)
3	171.3 (s)	166.5 (s)	165.6 (s)	165.5 (s)	166.4 (s)	165.6 (s)	167.2 (s)	177.6 (s)
4	83.5 (s)	80.4 (s)	81.6 (s)	81.5 (s)	80.2 (s)	81.5 (s)	84.8 (s)	74.5 (s)
5	51.9 (d)	48.6 (d)	49.7 (d)	49.5 (d)	48.4 (d)	49.8 (d)	49.3 (d)	48.8 (d)
6	31.0 (t)	28.3 (t)	26.7 (t)	26.7 (t)	27.7 (t)	26.6 (t)	65.8 (d)	23.6 (t)
7	22.3 (t)	22.9 (t)	25.7 (t)	24.8 (t)	27.5 (t)	25.7 (t)	35.5 (t)	27.2 (t)
8	54.3 (d)	59.3 (d)	59.2 (d)	55.5 (d)	50.8 (d)	55.6 (d)	39.6 (d)	139.0 (s)
9	83.3 (s)	79.0 (s)	77.4 (s)	77.3 (s)	79.6 (s)	78.1 (s)	28.4 (s)	131.8 (s)
10	140.9 (s)	146.1 (s)	62.5 (s)	62.3 (s)	145.5 (s)	62.6 (s)	32.7 (s)	43.2 (s)
11	48.0 (t)	50.9 (t)	50.2 (t)	47.3 (t)	44.0 (t)	48.0 (t)	29.0 (t)	21.3 (t)
12	48.8 (d)	47.6 (d)	48.7 (d)	52.1 (d)	57.6 (d)	54.6 (d)	33.6 (t)	31.6 (t)
13	138.7 (s)	136.3 (s)	135.3 (s)	132.3 (s)	72.8 (s)	153.2 (s)	48.6 (s)	44.9 (s)
14	40.4 (s)	40.1 (s)	39.7 (s)	41.0 (s)	45.8 (s)	43.8 (s)	46.5 (s)	50.9 (s)
15	43.5 (t)	44.8 (t)	44.4 (t)	44.6 (t)	37.5 (t)	34.9 (t)	33.5 (t)	31.5 (t)
16	65.4 (d)	65.2 (d)	65.1 (d)	64.0 (d)	58.7 (d)	35.1 (t)	22.2 (t)	26.9 (t)
17	140.9 (s)	140.1 (s)	139.7 (s)	131.3 (s)	63.0 (s)	75.4 (s)	51.3 (d)	46.7 (d)
18	125.4 (d)	125.3 (d)	125.4 (d)	31.9 (t)	132.1 (d)	115.3 (t)	20.0 (q)	16.2 (q)
19	94.4 (d)	147.4 (d)	67.0 (d)	67.9 (d)	149.7 (d)	66.6 (d)	37.5 (t)	22.4 (q)
20	124.8 (s)	125.0 (s)	125.1 (s)	34.4 (d)	39.4 (d)	43.5 (d)	75.1 (s)	39.8 (d)
21	10.9 (q)	11.2 (q)	11.2 (q)	14.6 (q)	12.7 (q)	9.6 (q)	21.0 (q)	13.6 (q)
22	149.6 (s)	149.4 (s)	149.6 (s)	80.0 (d)	77.2 (d)	79.6 (d)	84.1 (d)	80.4 (d)
23	118.2 (s)	119.3 (s)	119.2 (s)	33.2 (d)	129.5 (s)	27.3 (t)	26.0 (t)	23.6 (t)
24	139.9 (d)	139.7 (d)	139.7 (d)	146.0 (d)	139.0 (d)	141.3 (d)	140.1 (d)	140.1 (d)
25	124.8 (s)	124.9 (s)	125.1 (s)	127.9 (s)	128.2 (s)	127.6 (s)	127.9 (s)	128.1 (s)
26	162.4 (s)	162.3 (s)	162.3 (s)	166.6 (s)	164.9 (s)	166.5 (s)	166.1 (s)	166.3 (s)
27	17.0 (q)	17.0 (q)	17.0 (q)	17.1 (q)	17.3 (q)	17.1 (q)	17.1 (q)	17.2 (q)
28	28.6 (q)	26.7 (q)	26.3 (q)	26.4 (q)	28.4 (q)	25.4 (q)	20.7 (q)	24.9 (q)
29	27.8 (q)	31.4 (q)	29.3 (q)	29.3 (q)	29.3 (q)	29.3 (q)	28.4 (q)	33.6 (q)
30	27.0 (q)	30.4 (q)	25.0 (q)	25.0 (q)	25.8 (q)	24.9 (q)	24.5 (q)	28.3 (q)
OMe	58.5 (q)							

[a] Data were recorded with a Bruker DRX-125 MHz spectrometer, chemical shifts (δ) are in parts per million; assignments were based on DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC experiments.

By contrast, the only difference between **6** and longipedlactone I was the substituent at C-6. There was a hydroxy group at C-6 (δ_C 66.1, d) in longipedlactone I while no substituent at C-6 (δ_C 26.6, t) in **6**. Observed ROESY correlations of OH-17 with H-16β, H-18, and H-21β, and no correlations observed for OH-17 with H-12 and H-16α, showed that OH-17 in **6** was β-oriented. Furthermore, H-19 showed ROESY correlations with H-1, H-8β, and H-11β, indicating an α-orientation of the epoxy ring like that of longipedlactone I. The other relative configurations of **6** were determined to be the same as that of longipedlactone I by ROESY spectrum: α-oriented Me-30, Me-28, H-22, H-20, H-12, OH-9, and H-5, β-oriented Me-29, Me-21, H-19, OH-17, and H-8 (Fig. 2).

Compound **7** was obtained as a white amorphous solid. The molecular formula of **7** was determined as $C_{30}H_{42}O_6$ from its HRESIMS at *m*/*z* 521.2877 $[M+Na]^+$ (calcd 521.2879), indicating 10 degrees of unsaturation. The presence of the seven-membered α,β-unsaturated lactone and a six-membered α,β-unsaturated lactone was revealed from the IR absorption bands (1712 and 1664 cm^{-1})[5] and NMR spectral data (Table 2). The DEPT spectra showed six methyl singlets (δ_C 20.0, 21.0, 17.1, 20.7, 28.4, and 24.5). The mutually coupled doublets at δ_H 1.18 and 2.28 (J=3.3 Hz) indicated a cyclopropane.[5] The location of a tertiary hydroxyl group at C-20 was revealed by HMBC correlations, of which H-22 (δ_H 4.44, dd, J=13.5, 3.6 Hz), H-17 (δ_H 2.20–2.24, overlap), and Me-21 (δ_H 1.43, s) were correlated with C-20 (δ_C 75.1). The NMR, IR, and UV spectra of **7** were similar to those of kadsuphilactone B,[5] except that one hydroxyl group was located at C-6 (δ_C 65.8, d) in **7** instead of no substituent at C-6 (δ_C 24.1, t) in kadsuphilactone B. ROESY correlation of H-6 with H-5 positioned OH-6 at β-orientation. The relative configuration of other stereocenters of **7** was established to be identical to that of kadsuphilactone B from ROESY analysis.

Compound **8** obtained as white amorphous solid had the molecular formula $C_{30}H_{46}O_5$ as revealed by its HRESIMS data (*m*/*z* 509.3234 $[M+Na]^+$), indicating eight degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **8** (Tables 2 and 3) clearly indicated the presence of seven methyls, nine methylenes, five methines (one oxygenated and one olefinic), and nine quaternary carbons (two carbonyls, three olefinic, and one oxygenated). Apart from two double bonds and two carbonyl groups, the remaining elements of unsaturation in **8** were assumed to be a four rings' skeleton. By comparison, compound **8** showed similar structure in rings B, C, D, and E to those of **7**. The chemical shifts of C-8 (δ_C 139.0, s) and C-9 (δ_C 131.8, s) indicated a double bond between

Table 4
Cytotoxic activities of compounds **1**–**18** against tumor cell lines

Compd.	IC_{50} (μM)		
	K562	Bel-7402	A549
1	11.1	26.1	61.6
2	6.1	6.5	51.6
3	38.0	>100	>100
4	0.4	1.2	10.8
5	0.4	0.8	10.0
6	25.9	4.8	>100
7	28.1	50.7	75.9
8	>100	>100	>100
9	8.6×10^{-2}	8.6×10^{-2}	1.0
10	7.3×10^{-2}	5.4×10^{-2}	0.6
11	0.3	0.7	4.7
12	0.7	2.8	7.5
13	0.4	0.8	4.6
14	20.1	14.0	67.6
15	0.4	1.4	4.5
16	0.5	0.9	1.7
17	20.0	13.2	74.1
18	17.8	7.2	50.5
ADR	1.1×10^{-2}	1.0×10^{-2}	2.5×10^{-2}

ADR=Adriamycin (Doxorubicin).

C-8 and C-9. HMBC correlations of H_3-19 (δ_H 1.40, s) with C-1, C-5, C-9, and C-10 located a methyl at C-10, which was a typical character of lanostane triterpenoid.[17] The chemical shifts of C-3 (δ_C 177.6, s) and C-4 (δ_C 74.5, s), together with the molecular formula, suggested 3,4-*seco* ring A and a hydroxy group at C-4. All these data suggested that **8** possessed a 3,4-*seco*-lanostane skeleton. ROESY correlation of H_3-19 with H-11 and the absence of ROESY correlation between H-5 and H_3-19 suggested β-oriented Me-19. The configurations of the rest of the stereocenters were the same as those of compound **7** according to the ROESY correlations.

In addition, all the isolated triterpenoids were tested against K562, Bel-7402, and A549 human tumor cells in vitro using the method previously reported, with ADR as the positive control.[18,19] As shown in Table 4, some compounds showed promising bioactivity against the tested cell lines with IC_{50} values <2.0 μM. Especially, both compounds **9** and **10** exhibited potent cytotoxicity against K562, Bel-7402, and A549 cell lines with IC_{50} values <0.1, 0.1, and 1.0 μm, respectively.

3. Conclusion

In conclusion, this research led to the isolation of 8 new triterpenoids, together with 10 known ones, from the stems of *K. coccinea*. It is the first report of aromatic ring-E containing triterpenoids (**1**–**3**) with kadlongilactone-type skeleton as that of kadlongilactone A (**9**). The kadlongilactone-type triterpenoids are rarely naturally occurred and just only reported from the plants of *Kadsura longipedunculata* up to now.[1,2] Our present research expanded considerably the library for this class of natural products and also illuminated the variety of the metabolites of the *K. coccinea* species. Particularly, cytotoxicity assay showed that some of these triterpenoids showed significant biological activity against K562, Bel-7402, and A549 cell lines in vitro.

4. Experimental

4.1. General

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 DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in parts per million with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight spectrometer. Column chromatography was performed with silica gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} (9.4 mm×25 cm) column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column (34 mm×15 cm). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

4.2. Plant material

The stems of *K. coccinea* were collected in October 2005 from Honghe Prefecture of Yunnan Province, PR China. The specimen was identified by Prof. Xi-Wen Li. A voucher specimen, No. KIB 2005-10-10, has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

Air-dried and powdered stems (13 kg) were extracted with 70% aqueous Me_2CO (4×50 L) at room temperature and concentrated in vacuo to give a crude extract, which was partitioned between H_2O and EtOAc. The EtOAc part (413 g) was chromatographed on a silica gel column eluting with $CHCl_3$–Me_2CO (1:0, 40:1, 9:1, 8:2, 2:1, 1:1, and 0:1) to afford five fractions I–V. Fraction I–III were repeatedly chromatographed on silica gel (petroleum ether/Me_2CO, 20:1 to 2:1), respectively. Fraction III-4 (813 mg) was performed on reversed-phase column (RP-18) eluting with MeOH/H_2O (30%–90%) to give 17 fractions. These 17 fractions were then performed on preparative HPLC (MeOH–H_2O, 60:40) and semipreparative HPLC (MeOH–H_2O, 60:40) to yield compounds **1** (4 mg), **2** (3 mg), **3** (4 mg), **4** (4 mg), **5** (3 mg), **7** (4 mg), **11** (5 mg), **15** (5 mg), and **16** (8 mg). Fraction II-2 was performed on semipreparative HPLC (MeOH–H_2O, 75:25) to afford compounds **6** (5 mg) and **14** (30 mg). Fraction I-2 was performed on semipreparative HPLC (MeOH–H_2O, 75:25) to give compound **13** (7 mg). Compounds **8** (42 mg), **9** (100 mg), **10** (6 mg), **12** (65 mg), **17** (92 mg), and **18** (11 mg) were crystallized from fractions III-2, III-4, III-5, I-8, II-3, and II-2, respectively.

4.3.1. Kadcoccilactone K (**1**)

Colorless crystals; $[\alpha]_D^{24.3}$ −7.37 (*c* 0.11, MeOH); UV (MeOH): λ_{max} (log ε) 208 (4.23), 287 nm (3.81); IR (KBr): ν_{max} 3441, 2926, 1703, 1631, 1612, 1579, 1433, 1384, 1286, 1197, 1113, 1087, 1004, 977 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; positive ESIMS *m*/*z* (%): 545 (10) $[M+Na]^+$, 301 (45), 159 (8), 99 (100), 91 (60), 78 (95); positive HRESIMS: 545.2527 $[M+Na]^+$ (calcd 545.2515 for $C_{31}H_{38}O_7Na$).

4.3.2. Kadcoccilactone L (**2**)

White amorphous solid; $[\alpha]_D^{24.0}$ −60.09 (*c* 0.21, MeOH); UV (MeOH): λ_{max} (log ε) 284 (3.90), 221 (4.05), 207 nm (4.17); IR (KBr): ν_{max} 3441, 2921, 1696, 1637, 1613, 1454, 1379, 1339, 1292, 1261, 1235, 1208, 1167, 1130, 1056, 994 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; positive FABMS *m*/*z* (%): 491 (25) $[M+H]^+$, 473 (100), 455 (62), 239 (95), 105 (53), 91 (45), 69 (64); positive HRFABMS: 491.2445 $[M+H]^+$ (calcd 491.2444 for $C_{30}H_{35}O_6$).

4.3.3. Kadcoccilactone M (**3**)

White amorphous solid; $[\alpha]_D^{28.4}$ −194.12 (*c* 0.08, MeOH); UV (MeOH): λ_{max} (log ε) 287 (3.81), 223 (4.22), 210 nm (4.29); IR (KBr): ν_{max} 3443, 2919, 2851, 1694, 1635, 1611, 1460, 1433, 1383, 1336, 1293, 1261, 1235, 1209, 1166, 1130, 1099, 974, 920, 897, 823 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; positive FABMS *m*/*z* (%): 506 (34) $[M]^+$, 99 (100); positive HRESIMS: 529.2213 $[M+Na]^+$ (calcd 529.2202 for $C_{30}H_{34}O_7Na$).

4.3.4. Kadcoccilactone N (**4**)

White amorphous solid; $[\alpha]_D^{22.9}$ −160.19 (*c* 0.27, MeOH); UV (MeOH): λ_{max} (log ε) 205 nm (3.78); IR (KBr): ν_{max} 3434, 2924, 1698, 1636, 1451, 1375, 1340, 1291, 1237, 1210, 1191, 1166, 1129, 1044, 1024, 997, 965, 929, 900 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; positive ESIMS *m*/*z* (%): 533 (100) $[M+Na]^+$, 522 (5), 301 (9), 275 (10), 267 (6); positive HRESIMS: 533.2521 $[M+Na]^+$ (calcd 533.2515 for $C_{30}H_{38}O_7$ Na).

4.3.5. Kadcoccilactone O (**5**)

White amorphous solid; $[\alpha]_D^{24.1}$ −59.52 (*c* 0.29, MeOH); UV (MeOH): λ_{max} (log ε) 280 (3.95), 219 (3.73), 203 nm (3.77); IR (KBr): ν_{max} 3444, 2937, 1704, 1450, 1375, 1302, 1234, 1214, 1130, 1056, 985 cm^{-1}; ^1H and ^{13}C NMR data see Tables 2 and 3; positive FABMS *m*/*z* (%): 509 (43) $[M+H]^+$, 457 (20), 315 (46), 223 (100), 145 (34), 115 (75), 71 (64); positive HRESIMS: 531.2351 $[M+Na]^+$ (calcd 531.2358 for $C_{30}H_{36}O_7Na$).

*4.3.6. Kadcoccilactone P (**6**)*

White amorphous solid; $[\alpha]_D^{26.0}$ −56.78 (*c* 0.27, MeOH); UV (MeOH): λ_{max} (log ε) 205 (4.00), 219 (3.93), 192 nm (3.72); IR (KBr): ν_{max} 3474, 2982, 2948, 2865, 1698, 1636, 1453, 1392, 1375, 1354, 1336, 1292, 1235, 1206, 1165, 1130, 1020, 979, 942, 914, 898, 845 cm^{-1}; ^1H and ^{13}C NMR data see Tables 2 and 3; negative ESIMS *m*/*z* (%): 512 (37) $[M]^-$, 511 (92) $[M-H]^-$, 489 (30), 255 (45), 181 (58), 111 (48), 62 (100); negative HRESIMS: 511.2688 $[M-H]^-$ (calcd 511.2695 for $C_{30}H_{39}O_7$).

*4.3.7. Kadcoccilactone Q (**7**)*

White amorphous solid; $[\alpha]_D^{23.1}$ +41.92 (*c* 0.17, MeOH); UV (MeOH): λ_{max} (log ε) 253 (3.68), 211 nm (3.66); IR (KBr): ν_{max} 3442, 2980, 2943, 2876, 1712, 1664, 1469, 1449, 1383, 1374, 1334, 1289, 1242, 1211, 1179, 1121, 1094, 1051, 1020, 998, 985, 950, 915 cm^{-1}; ^1H and ^{13}C NMR data see Tables 2 and 3; positive FABMS *m*/*z* (%): 499 (36) $[M+H]^+$, 481 (53), 463 (50), 325 (47), 281 (16), 231 (38), 203 (33), 155 (97), 109 (100); positive HRESIMS: 521.2877 $[M+Na]^+$ (calcd 521.2879 for $C_{30}H_{42}O_6Na$).

*4.3.8. Kadcoccilactone R (**8**)*

White amorphous solid; $[\alpha]_D^{23.0}$ +75.52 (*c* 0.19, acetone); UV (MeOH): λ_{max} (log ε) 206 (4.03), 195 (3.62), 192 nm (3.61); IR (KBr): ν_{max} 3434, 2968, 1716, 1452, 1375, 1288, 1242, 1198, 1156, 1123, 1057, 1030, 989, 953, 851 cm^{-1}; ^1H and ^{13}C NMR data see Tables 2 and 3; positive FABMS *m*/*z* (%): 486 (8) $[M]^+$, 469 (100), 451 (20), 433 (9), 355 (42), 83 (35), 61 (19); positive HRESIMS: 509.3234 $[M+Na]^+$ (calcd 509.3242 for $C_{30}H_{46}O_5Na$).

4.4. Cytotoxicity bioassays

Cytotoxicity of compounds against suspended tumor cells (K562, Bel-7402, and A549 human tumor cells) was determined by trypan blue exclusion method and against adherent cells was determined by sulforhodamine B (SRB) assay. Cells were plated in 96-well plate 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. After compound treatment, cells were counted (suspended cells) or fixed and stained with SRB (adherent cells) as previously reported.[18,19]

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Supplementary data

^1H and ^{13}C NMR spectra of compounds **1**–**8**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.10.011.

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