Lanostane-Type Triterpenoids from the Roots of *Kadsura coccinea*

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Seven new lanostane-type triterpenoids, seco-coccinic acids A−F (**1**−**6**) and coccinilactone A (**7**), were isolated from the roots of *Kadsura coccinea*. Their structures were established on the basis of spectroscopic data analysis. The absolute configuration at C-24 of compound **5** was confirmed by the modified Mosher's method. The cell growth inhibitory effects of these compounds were determined in human leukemia HL-60 cells, and it was found that compounds **1**, **2**, **3**, and **5** exhibited antiproliferative effects with GI_{50} values ranging from 6.8 to 42.1 μM.

Lanostane-type triterpenoids have been isolated from members of the genus *Kadsura*, such as *K. japonica*,[1] *K. ananosma*,[2,3] *K. coccinea*,[4] *K. heteroclita*,[5–8] *K. longipedunculata*,[9–12] and *K. lancilimba*.[13] Several of these triterpenoids have been found to have potential anti-HIV, anticancer, and cholesterol biosynthesis inhibitory activities. For example, schisanlactone E and changnanic acid isolated from *K. longipedunculata* have been found to have an antiproliferative effect against murine leukemia P388 cells,[12] and ananosic acids B and C isolated from *K. ananosma* exhibited cytotoxicity against human CCRF-CEM cells and HeLa cells.[2]

Kadsura coccinea (Lem.) A. C. Sm. (Schizandraceae) is distributed widely in the southern part of mainland China. The dried roots of *K. coccinea*, called "Heilaohu" in Chinese,[14] are used as a folk medicine for the treatment of rheumatoid arthritis and for gastric and duodenal ulcers.[15] The isolation and structure elucidation of seven new lanostane-type triterpenoids (**1**−**7**) from the air-dried roots of *K. coccinea* are described herein, and the antiproliferative effects of these lanostane-type triterpenoids were determined against human leukemia HL-60 cells. Compounds **1**, **2**, **3**, and **5** as well as the crude chloroform extract showed antiproliferative effects.

Results and Discussion

The ethanol extract of the air-dried roots of *K. coccinea* was partitioned with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, successively. Six new lanostane-type triterpenoids (**1**−**6**) were obtained from the petroleum ether extract through a series of chromatographic separations, while a new triterpenoid (**7**) was isolated from the $CHCl_3$ extract of *K. coccinea*. The structures of **1**−**7** were elucidated on the basis of spectroscopic methods.

Compound **1** was obtained as colorless needles, and its molecular formula was determined as $C_{30}H_{48}O_3$ on the basis of HRFABMS (*m/z* 457.3674 $[M + H]^+$). The FABMS showed a characteristic fragmentation for a 3,4-seco-triterpenoid with a prominent peak at *m/z* 383 $[M - CH_2CH_2COOH]^+$.[16] The IR spectrum of compound **1** indicated the presence of a carbonyl (1710 cm^{-1}) group. The ^1H NMR spectrum showed four methyl singlets and three methyl doublets at δ 0.97 ($J = 5.8$ Hz), 0.92 ($J = 6.7$ Hz), and 0.91 ($J = 6.4$ Hz). The ^{13}C NMR spectrum exhibited 30 carbon signals that were sorted by a DEPT experiment as seven methyls, 10 methylenes, six methines, and seven quaternary carbons, including one carboxylic group at δ 176.9, one terminal double bond at δ 149.7 and 112.2, and a ketone carbonyl group at δ 210.2. On the basis of the above data, compound **1** was determined as a lanostane-type triterpenoid.[17] The ^1H and ^{13}C NMR data were assigned from the $^1H-^1H$ COSY, HMQC, and HMBC spectra (Tables 1 and 2). The presence of a seco-ring A was demonstrated by signals at δ 176.9 (C-3), 149.7 (C-4), and 112.2 (C-28).[18] The occurrence of a terminal double bond in **1** was shown by the two broad singlets at δ 4.99 and 4.98 in the ^1H NMR spectrum. In the HMBC spectrum, the correlations between C-5 (δ 45.7) and H-28 (δ 4.99 and 4.98), H-19 (δ 0.93), and H-29 (δ 1.85) implied that the terminal double bond was located between C-4 and C-28. A trisubstituted olefinic proton at δ 5.32 showed long-range correlations with C-5 (δ 45.7) and C-6 (δ 29.7), indicating that a double bond is located between C-7 and C-8. The carbonyl group signal at δ 210.2 had correlations with H-22 (δ 2.47 and 2.18) and H-24 (δ 2.31 and 2.29), indicating it to occur at C-23, which was further supported by a prominent peak at *m/z* 57 (C_4H_9) and 85 (C_5H_9O) in the EIMS. In a NOESY experiment, H-9 at δ 2.64 showed correlations with CH_3-18 at δ 0.82 and CH_3-19 at δ 0.93, which indicated that H-9 is β-oriented. On the basis of the above spectroscopic data, compound **1** (seco-coccinic acid A) was established as 23-oxo-3,4-seco-9β*H*-lanost-4(28),7-dien-3-oic acid.

Compounds **2**−**4** were all obtained as colorless needles. The ^1H NMR spectra of these three substances showed six methyl singlets and a methyl doublet, and the ^{13}C NMR spectra revealed 30 carbon signals in all cases. Except for their side chains, the proton and carbon NMR data of compounds **2**−**4** were similar to those of compound **1**, including resonances at δ 176.9 (C-3), 149.7 (C-4), and 112.2 (C-28) characteristic of a seco-ring A unit.[18] On the basis of these spectroscopic data, compounds **2**−**4** were found to be identical with **1** in their ring A−D portions.

The molecular formula of compound **2** was determined as $C_{30}H_{46}O_3$ on the basis of its HRFABMS (*m/z* 455.3539 $[M + H]^+$). The IR spectrum showed the presence of a conjugated carbonyl (1680 cm^{-1}) and a carboxylic acid (1711 cm^{-1}) group. Compound **2** exhibited differences from compound **1** in the NMR spectra with the appearance of two olefinic carbon signals at δ 123.8 and 153.9 in the ^{13}C NMR spectrum and two methyl singlets at δ 2.22 and 1.77 in the ^1H NMR spectrum, confirming the presence of a double bond at C-24. In the HMBC spectrum of compound **2**, a conjugated carbonyl group signal at δ 201.8 had correlations with H-22 (δ 2.56 and 2.18) and H-24 (δ 6.18), indicating a ketone carbonyl group at C-23. On the basis of these data, the structure of compound **2** was assessed as a triterpenoid with a 23-keto-Δ^{24} side chain. Thus, **2** (seco-coccinic acid B) was assigned as 23-oxo-3,4-seco-9β*H*-lanost-4(28),7,24-trien-3-oic acid.

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Table 1. ^{1}H NMR Data (500 MHz) of Compounds **1**−**7** (δ_H, pyridine-d_5, *J* in Hz)

H	1	2	3	4	5	6	7
1	2.03 (1H, m)	2.03 (1H, m)	2.02 (1H, m)	2.03 (1H, m)	2.03 (1H, m)	2.76 (2H, m)	1.90 (2H, m)
	1.99 (1H, m)	1.98 (1H, m)	1.97 (1H, m)	1.92 (1H, m)	1.96 (1H, m)		
2	2.57 (2H, m)	2.57 (2H, m)	2.56 (2H, m)	2.57 (2H, m)	2.57 (2H, m)	2.37 (1H, m)	2.78 (2H, m)
						2.11 (1H, m)	
5	2.26	2.26	2.25	2.26	2.26	2.19 (1H, m)	1.66 (1H, m)
	(1H, d, 7.1)	(1H, d, 7.1)	(1H, d, 7.1)	(1H, d, 6.9)	(1H, d, 7.1)		
6	2.38 (1H, m)	2.39 (1H, m)	2.38 (1H, m)	2.40 (1H, m)	2.40 (1H, m)	1.92 (1H, m)	1.54 (2H, m)
		2.09 (1H, m)	2.06 (1H, m)				
	2.07 (1H, m)			2.07 (1H, m)	2.08 (1H, m)	1.73 (1H, m)	
7	5.32	5.31	5.3	5.32	5.31	1.50 (2H, m)	1.87 (2H, m)
	(1H, d, 2.5)	(1H, d, 2.8)	(1H, d, 3.0)	(1H, d, 3.5)	(1H, d, 2.8)		
8						2.12 (1H, m)	2.14 (1H, m)
9	2.64 (1H, m)	2.64 (1H, m)	2.63 (1H, m)	2.65 (1H, m)	2.64 (1H, m)		
11	1.69 (1H, m)	1.68 (1H, m)	1.68 (1H, m)	1.53 (2H, m)	1.67 (1H, m)	5.58	5.26 (1H, d, 5.8)
	1.54 (1H, m)	1.55 (1H, m)	1.52 (1H, m)		1.55 (1H, m)	(1H, d, 5.5)	
12	1.81 (1H, m)	1.81 (1H, m)	1.80 (1H, m)	1.77 (1H, m)	1.84 (1H, m)	2.08 (1H, m)	2.09 (1H, m)
				1.62 (1H, m)			
	1.64 (1H, m)	1.64 (1H, m)	1.64 (1H, m)		1.64 (1H, m)	1.87 (1H, m)	1.89 (1H, m)
15	1.56 (1H, m)	1.54 (1H, m)	1.54 (1H, m)	1.54 (1H, m)	1.55 (1H, m)	1.36 (2H, m)	1.36 (2H, m)
	1.44 (1H, m)	1.46 (1H, m)	1.45 (1H, m)	1.46 (1H, m)	1.44 (1H, m)		
16	1.90 (1H, m)	1.93 (1H, m)	1.90 (1H, m)	1.96 (2H, m)	1.97 (1H, m)	1.52 (2H, m)	1.55 (2H, m)
	1.24 (1H, m)	1.29 (1H, m)	1.26 (1H, m)	1.28 (1H, m)	1.30 (1H, m)		
17	1.53 (1H, m)	1.55 (1H, m)	1.53 (1H, m)	1.53 (1H, m)	1.52 (1H, m)	1.63 (1H, m)	1.66 (1H, m)
18	0.82 (3H, s)	0.82 (3H, s)	0.81 (3H, s)	0.81 (3H, s)	0.82 (3H, s)	0.70 (3H, s)	0.72 (3H, s)
19	0.93 (3H, s)	0.93 (3H, s)	0.93 (3H, s)	0.94 (3H, s)	0.94 (3H, s)	1.11 (3H, s)	1.16 (3H, s)
20	2.17 (1H, m)	2.18 (1H, m)	2.19 (1H, m)	1.52 (1H, m)	1.50 (1H, m)	1.47 (1H, m)	2.20 (1H, m)
21	0.97	1.01	1.01	0.95	0.96	0.97	1.01
	(3H, d, 5.8)	(3H, d, 5.7)	(3H, d, 6.4)	(3H, d, 5.8)	(3H, d, 5.8)	(3H, d, 6.4)	(3H, d, 5.8)
22	2.47 (1H, m)	2.56 (1H, m)	2.70 (1H, m)	2.28 (1H, m)	1.40 (1H, m)	1.60 (1H, m)	2.18 (1H, m)
			2.40 (1H, m)				
	2.18 (1H, m)	2.18 (1H, m)		2.25 (1H, m)	0.97 (1H, m)	1.20 (1H, m)	2.48 (1H, m)
23				5.92 (1H, m)	1.93 (1H, m)	2.34 (1H, m)	
					1.69 (1H, m)	2.17 (1H, m)	
24	2.31 (1H, m)	6.18 (1H, s)	2.8	5.93 (1H, m)	4.37 (1H, m)	7.21 (1H, m)	2.31 (2H, m)
	2.29 (1H, m)						
			(2H, d, 7.1)				
25	2.24 (1H, m)						2.24 (1H, m)
26	0.92	1.77 (3H, s)	1.51 (3H, s)	1.55 (3H, s)	5.29 (1H, s)		0.94
	(3H, d, 6.7)				4.98 (1H, s)		(3H, d, 6.4)
27	0.91	2.22 (3H, s)	1.51 (3H, s)	1.55 (3H, s)	1.92 (3H, s)	2.13 (3H, s)	0.92
	(3H, d, 6.4)						(3H, d, 6.7)
28	4.99 (2H, s)	4.99 (2H, s)	4.97 (2H, s)	5.00 (2H, s)	5.00 (2H, s)	4.97 (2H, s)	1.39 (3H, s)
29	1.85 (3H, s)	1.84 (3H, s)	1.84 (3H, s)	1.86 (3H, s)	1.86 (3H, s)	1.83 (3H, s)	1.42 (3H, s)
30	1.07 (3H, s)	1.06 (3H, s)	1.04 (3H, s)	1.04 (3H, s)	1.05 (3H, s)	0.80 (3H, s)	0.78 (3H, s)

Compound **3** gave a $[M + Na]^+$ ion in the HRFABMS at *m*/*z* 495.3438, consistent with a molecular formula of $C_{30}H_{48}O_4$. It showed IR absorptions for hydroxyl, ketone carbonyl, and carboxylic acid groups at 3457, 1723, and 1700 cm^{-1}, respectively. Compound **3** exhibited an oxygenated carbon signal at δ 69.5 in the ^{13}C NMR spectrum, confirming the presence of a hydroxyl group in the side chain. In the HMBC spectrum, the signal appearing at δ 69.5 had long-range correlations with H-24 (δ 2.80) and the methyl protons of H-26 (δ 1.51) and H-27 (δ 1.51), which suggested a hydroxyl group was located at C-25. The carbonyl carbon signal resonating at δ 211.3 was assigned to C-23 due to the long-range correlation with the methylene protons of H-22 (δ 2.28 and 2.25) and H-24 (δ 2.80). Thus, **3** (seco-coccinic acid C) was established as 25-hydroxy-23-oxo-3,4-seco-9*βH*-lanost-4(28),7-dien-3-oic acid.

Compound **4** showed a quasimolecular ion peak $[M + H]^+$ at *m*/*z* 457.3639 ($C_{30}H_{48}O_3$) in the HRFABMS. The IR spectrum showed the presence of a hydroxyl (3428 cm^{-1}) and a carboxylic acid (1705 cm^{-1}) group. Its ^{13}C NMR spectrum displayed an oxygenated carbon at δ 69.7 and olefinic carbons at δ 124.6 and 141.7. The carbon signal at δ 69.7 was assigned to C-25 due to long-range correlations with the methyl protons of H-26 (δ 1.55) and H-27 (δ 1.55) in the HMBC spectrum. The HMBC correlations between the signal at δ 141.7 and H-22 (δ 2.28), H-26 (δ 1.55), and H-27 (δ 1.55) and between the signal at δ 124.6 and H-22 (δ 2.28) indicated that a double bond is located at C-23. Accordingly, **4** (seco-coccinic acid D) was elucidated as 25-hydroxy-3,4-seco-9*βH*-lanost-4(28),7,23-trien-3-oic acid.

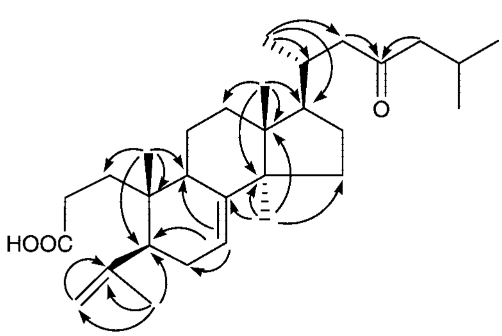
Compound **5** was obtained as colorless needles with a molecular formula of $C_{30}H_{48}O_3$, established on the basis of the HRFABMS (*m*/*z* 479.3495 $[M + Na]^+$). The ^{1}H NMR spectrum of compound **5** showed signals of five methyl singlets and a methyl doublet at δ 0.96 (J = 5.8 Hz, CH_3-21). The ^{13}C NMR spectrum revealed 30 carbon signals, which were sorted by a DEPT experiment as six methyls, 11 methylenes, six methines, and seven quaternary carbons, including one oxygenated methine, one carboxylic acid group, and two terminal double bonds. Compound **5** was found to be identical with compound **1** in the ^{1}H and ^{13}C NMR data except for the side chain. In the HMBC spectrum, the long-range correlations between the oxygenated carbon signal at δ 75.7 and H-23 (δ 1.93 and 1.69), H-26 (δ 5.29 and 4.98), and H-27 (δ 1.92) suggested a hydroxyl group located at C-24. The olefinic carbon at δ 110.1 showed HMBC correlations with proton signals at δ 4.37 (H-24) and 1.92 (H-27), indicating that another terminal double bond was located at C-26. The NOESY correlations of H-9/H-18, H-9/H-19, and H-9/H-29 indicated that H-9 is *β*-oriented. The configuration of the chiral center C-24 was determined through the NMR study of MTPA esters of the methyl ester **5a**. In the ^{1}H NMR spectrum of the (*R*)-MTPA ester **5b**, H_2-26 and H_3-27 appeared deshielded, whereas H_2-23 and H_1-20 were shielded, in comparison to the analogous data for (*S*)-MTPA ester **5c** (Figure 2). Thus, the configuration at

Table 2. ^{13}C NMR Data (125 MHz) of Compounds **1**−**7** (δ_C, pyridine-d_5)

C	1	2	3	4	5	6	7
1	30.0 t	30.0 t	30.0 t	30.0 t	30.0 t	29.9 t	34.9 t
2	29.9 t	30.0 t	30.0 t	30.0 t	30.0 t	33.4 t	32.3 t
3	176.9 s	176.9 s	177.0 s	176.9 s	176.9 s	176.8 s	174.4 s
4	149.7 s	149.7 s	149.5 s	149.5 s	150.4 s	148.2 s	85.9 s
5	45.7 d	45.7 d	45.7 d	45.7 d	45.7 d	49.4 d	52.9 d
6	29.7 t	29.7 t	29.7 t	29.7 t	29.7 t	28.2 t	25.7 t
7	118.3 d	118.3 d	118.3 d	118.2 d	118.2 d	27.0 t	28.4 t
8	146.9 s	147.0 s	146.9 s	147.1 s	147.1 s	42.7 d	42.2 d
9	39.2 d	39.2 d	39.1 d	39.2 d	39.2 d	143.0 s	147.2 s
10	36.7 s	36.7 s	36.7 s	36.7 s	36.7 s	42.9 s	42.2 s
11	18.9 t	18.9 t	18.9 t	18.9 t	18.9 t	118.8 d	117.4 d
12	34.1 t	34.1 t	34.1 t	34.1 t	34.3 t	37.9 t	37.6 t
13	44.0 s	44.0 s	44.0 s	43.9 s	44.0 s	44.2 s	44.3 s
14	51.9 s	51.9 s	51.9 s	51.8 s	51.9 s	47.5 s	47.5 s
15	34.4 t	34.4 t	34.4 t	34.5 t	34.4 t	33.9 t	34.0 t
16	28.6 t	28.6 t	28.6 t	28.5 t	28.6 t	28.2 t	27.4 t
17	53.2 d	53.5 d	53.2 d	53.0 d	53.5 d	51.2 d	51.5 d
18	21.8 q	21.8 q	21.8 q	21.9 q	21.9 q	14.9 q	14.7 q
19	24.3 q	24.3 q	24.3 q	24.3 q	24.3 q	27.2 q	23.7 q
20	33.1 d	33.7 d	33.0 d	36.9 d	36.4 d	36.3 d	33.0 d
21	19.7 q	19.7 q	19.8 q	18.6 q	18.8 q	18.4 q	19.7 q
22	50.4 t	51.7 t	52.1 t	39.3 t	32.5 t	35.5 t	50.7 t
23	210.2 s	200.7 s	211.3 s	124.6 d	32.8 t	26.0 t	210.2 s
24	52.5 t	123.8 d	56.0 t	141.7 d	75.7 d	142.5 d	52.5 t
25	24.7 d	153.9 s	69.5 s	69.7 s	149.5 s	129.0 s	24.7 t
26	22.7 q	27.3 q	30.4 q	30.9 q	110.1 t	170.7 s	22.6 q
27	22.6 q	20.6 q	30.2 q	30.9 q	18.2 q	12.9 q	22.7 q
28	112.2 t	112.2 t	112.1 t	112.2 t	112.2 t	114.1 t	25.4 q
29	26.0 q	26.0 q	26.0 q	26.0 q	26.0 q	23.6 q	33.0 q
30	27.5 q	27.6 q	27.5 q	27.5 q	27.6 q	18.5 q	18.6 q

C-24 was concluded as *R*.[19] Thus, **5** (seco-coccinic acid E) was assigned as 24*R*-hydroxy-3,4-seco-9*βH*-lanost-4(28),7,25(26)-trien-3-oic acid.

Compound **6** was obtained as colorless needles, and its molecular formula was determined as $C_{30}H_{46}O_4$ on the basis of its HRFABMS (*m/z* 471.3472 [M + H]$^+$). The ^1H NMR spectrum showed five methyl singlets and a methyl doublet at δ 0.97 (J = 6.4 Hz, CH_3-21). The ^{13}C NMR spectrum revealed 30 carbon signals, which were sorted by the DEPT experiment as six methyls, 10 methylenes, six methines, and eight quaternary carbons. The signals in the ^{13}C NMR spectrum at δ 176.8 (C-3) and 170.7 (C-26) and the absorbance band at 1698 cm^{-1} in the IR spectrum showed the presence of two carboxylic acid groups. The ^1H NMR spectrum revealed two trisubstituted olefinic protons at δ 5.58 (d, J = 5.5 Hz, H-11) and 7.21 (H-24), as well as two signals of the terminal double bond at δ 4.98 (H-28a) and 4.96 (H-28b). These data suggested that compound **6** is a 3,4-seco-triterpenoid derivative.[18] The ^1H and ^{13}C NMR data were assigned by the ^1H−^1H COSY, HMQC, and HMBC spectra (Tables 1 and 2). In the HMBC spectrum, the trisubstituted olefinic proton at δ 5.58 showed long-range correlations with C-12 (δ 37.9) and two quaternary carbons



HMBC (H→C)

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

Table 3. GI_{50} Values of Triterpenoids That Inhibit HL-60 Cell Growth[a]

compound	GI_{50} ± SE (μM)
1	6.8 ± 1.36
2	13.3 ± 0.99
3	12.1 ± 2.37
5	42.1 ± 10.2
5-FU	3.8 ± 0.48

[a] GI_{50} is the concentration that inhibited 50% of cell growth. The data shown are means ± SE of three independent experiments.

C-10 (δ 42.9) and C-13 (δ 44.2), indicating that a double bond is located between C-9 and C-11. Proton signals of H-23 (δ 2.34 and 2.17) and H-27 (δ 2.13) were correlated with an olefinic carbon at δ 142.5, which implied that another double bond was located between C-24 and C-25. A carboxylic acid group was determined at C-26 by the HMBC correlations between the signal at δ 170.7 and an olefinic proton at δ 7.21 (H-24) and a methyl proton at δ 2.13 (H-27). The relative configuration of compound **6** was determined by the NOESY spectrum, in which H-27 showed correlation with H-23 but no NOE correlation was observed between H-27 and H-24, indicating that the double-bond geometry was in the *E* configuration. The H-8 signal showed NOE correlations with H-18, H-19, and H-29, respectively. Accordingly, compound **6** (seco-coccinic acid F) was assigned as 24(*E*)-3,4-seco-8*βH*-lanost-4(28),9(11),24-triene-3,26-dioic acid.

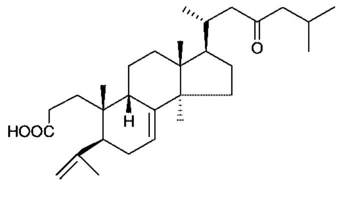
Compound **7** was obtained as colorless needles, and its molecular formula of $C_{30}H_{48}O_3$ was established by the HRFABMS (*m/z* 457.3685 [M + H]$^+$). The ^1H NMR spectrum showed five methyl singlets and three methyl doublets at δ 1.01 (J = 5.8 Hz, CH_3-21), 0.94 (J = 6.4 Hz, CH_3-26), and 0.92 (J = 6.7 Hz, CH_3-27). Altogether 30 carbon signals were revealed in the ^{13}C NMR spectrum, which were sorted by a DEPT experiment as eight methyls, nine methylenes, six methines, and seven quaternary carbons, including one oxygenated quaternary carbon at δ 85.9 and a carbonyl carbon at δ 174.4, suggesting compound **7** possesses a lactone ring unit.[17] Compound **7** was found to be identical with compound **1** in the side chain, including a ketone located at C-23. In the HMBC spectrum, H-28 (δ 1.39), H-29 (δ 1.42), and H-5 (δ 1.66) were correlated with C-4 (δ 85.9), and a ketone carbon at C-3 (δ 174.4) showed correlations with the H-1 (δ 1.90) and H-2 (δ 2.78) methylene protons. A trisubstituted olefinic proton at δ 5.26 showed long-range correlations with C-12 (δ 37.6) and C-10 (δ 42.2), indicating that a double bond is located between C-9 and C-11. The stereochemistry of compound **7** was determined by its NOESY spectrum, in which correlations of H-8/H-18, H-8/H-19, and H-8/H-28 suggested that H-8 is β-oriented. Thus, the structure of **7** was assigned as 23-oxo-8*βH*-lanost-9(11)-en-3,4-olide, and this compound was given the trivial name coccinilactone A.

The growth inhibitory effects of these compounds against HL-60 human leukemia cells were determined. Compounds **1**, **2**, **3**, and **5** showed inhibitory activity, while compound **1** was the most effective, with a GI_{50} value of 6.8 μM. The GI_{50} values of these four compounds are shown in Table 3. The crude chloroform extract showed inhibitory effect on HL-60 cell growth with a GI_{50} value of 16.1 ± 4.89 μg/mL.

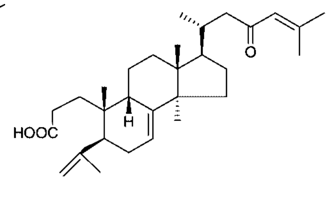
Experimental Section

General Experimental Procedures. Melting points (uncorrected) were measured on a Yanaco MP-S3 micromelting point apparatus. Optical rotations were obtained with a JASCO DIP-370 polarimeter. IR spectra were conducted on a JASCO FT/IR-300E spectrometer. 1D (^1H, ^{13}C, and DEPT) and 2D (HMBC, HMQC, ^1H−^1H COSY, and NOESY) NMR spectra were recorded on a JEOL ECP-500 NMR spectrometer. The chemical shifts are quoted relative to TMS, and the coupling constants are in Hz. FABMS and HRFABMS were taken on a JEOL JMS-700 MStation. EIMS (70 eV) were obtained on a Shimadzu GCMS-QP5050A spectrometer. The chromatographic silica

Chart 1



1



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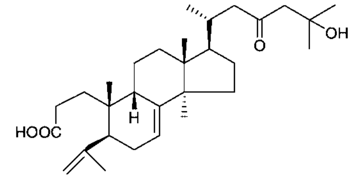

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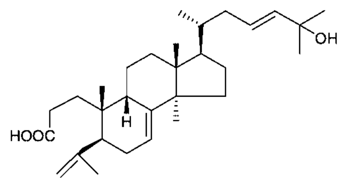

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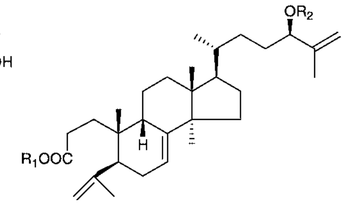
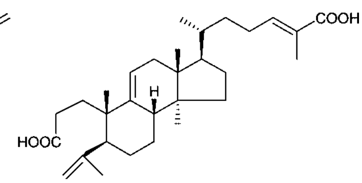


5 $R_1 = R_2 = H$

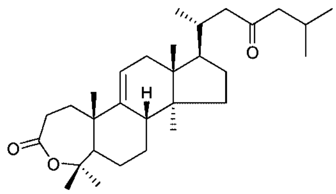
5a $R_1 = Me, R_2 = H$

5b $R_1 = Me$, $R_2 = (R)$-MTPA

5c $R_1 = Me$, $R_2 = (S)$-MTPA



6



7

gel (200−300 mesh) was produced by Qingdao Ocean Chemical Factory, and Sephadex LH-20 was obtained from GE Healthcare.

Plant Material. The roots of *Kadsura coccinea* were purchased from Guangxi Medicinal Material Corporation, Nanning, China, and identified by Associate Professor Maoxiang Lai of Guangxi Institute of Traditional Chinese Medicine. A voucher specimen (KC 0509) was deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, People's Republic of China.

Extraction and Isolation. The roots of *K. coccinea* (10 kg) were extracted with 95% EtOH to give an alcohol extract. A portion (1340 g) of the residue after removing EtOH was suspended in water and partitioned with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, successively. The petroleum ether extract (150 g) was subjected to silica gel column chromatography with a petroleum ether−EtOAc gradient solvent system (100:0 → 0:100) to provide 12 fractions. Fractions 5 [petroleum ether−EtOAc (100:7)], 6 [petroleum ether−EtOAc (100:8)], and 10 [petroleum ether−EtOAc (100:30)] were recrystallized using acetone to afford compounds **1** (110 mg), **2** (290 mg), and **3** (10 mg), respectively. Repeated chromatography of fraction 8 [petroleum ether−EtOAc (100:15)] on a column of silica gel with gradient elution using petroleum ether with increasing proportions of acetone (15:1 → 11:1) afforded compounds **4** (8 mg), **5** (7 mg), and **6** (6 mg). The $CHCl_3$ extract (50 g) was fractionated into 12 fractions by silica gel column chromatography. Fraction 8 [petroleum ether−acetone (100:15)] was subsequently chromatographed on silica gel columns, together with Sephadex LH-20 [$CHCl_3$−MeOH (1:1)], to furnish compound **7** (22 mg).

Seco-coccinic acid A (1): colorless needles (acetone); mp 148−149 °C; $[\alpha]^{20}_D$ −36.8 (*c* 0.056, $CHCl_3$); IR (KBr) ν_{max} 2949, 2874, 1710, 1633, 1462, 1370, 1275, 1198, 1109, 1063, 902, 841 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 457.3674 $[M + H]^+$ (calcd for $C_{30}H_{49}O_3$, 457.3682).

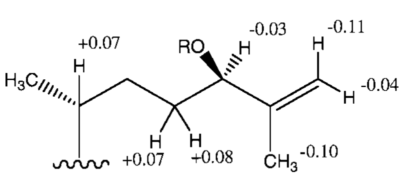


Figure 2. $\Delta\delta^{SR}$ (= $\delta^S - \delta^R$) values obtained from the ^1H NMR spectra of **5b** and **5c**.

Seco-coccinic acid B (2): colorless needles (acetone); mp 145−146 °C; $[\alpha]^{20}_D$ −25.4 (*c* 0.032, $CHCl_3$); IR (KBr) ν_{max} 2951, 2864, 1711, 1680, 1637, 1451, 1385, 1023, 957, 892 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 455.3539 $[M + H]^+$ (calcd for $C_{30}H_{47}O_3$, 455.3525).

Seco-coccinic acid C (3): colorless needles (acetone); mp 181−182 °C; $[\alpha]^{20}_D$ −28.2 (*c* 0.023, $CHCl_3$); IR (KBr) ν_{max} 3457, 2945, 2874, 1723, 1700, 1655, 1456, 1378, 1197, 1070, 976, 899 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 495.3438 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3450).

Seco-coccinic acid D (4): colorless needles (acetone); mp 183−185 °C; $[\alpha]^{20}_D$ −31.1 (*c* 0.010, $CHCl_3$); IR (KBr) ν_{max} 3428, 2923, 2846, 2375, 1705, 1627, 1462, 1376, 911, 864, 818 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 457.3639 $[M + H]^+$ (calcd for $C_{30}H_{49}O_3$, 457.3682).

Seco-coccinic acid E (5): colorless needles (acetone); mp 161−162 °C; $[\alpha]^{20}_D$ −20.9 (*c* 0.020, $CHCl_3$); IR (KBr) ν_{max} 3264, 3070, 2958, 2923, 2874, 2650, 1696, 1455, 1375, 1315, 1273, 1106, 1068, 1003, 905 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z*: 479.3495 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3501).

Preparation of Methyl Ester of 5 (5a) and (*R*)- and (*S*)-MTPA Esters of 5a (5b and 5c). After adding $TMSCHN_2$ (0.2 mL) into a solution of compound **5** (2.0 mg) in dried MeOH−THF (1:2, 1 mL), the mixture was reacted for 10 h at room temperature to give the methyl ester of compound **5** (**5a**, 2.3 mg). To each solution of compound **5a** (each 1.0 mg) in pyridine-d_5 (0.75 mL) was separately added (*R*)-(−)-MTPA Cl (10 L) and (*S*)-(+)-MTPA Cl (10 L) at room temperature, followed by stirring at room temperature for 4 h. Each reaction mixture was transferred into a 5 mm NMR tube, and the ^1H NMR data were determined.

(*R*)-MTPA Ester of 5a (5b): colorless, amorphous solid; ^1H NMR (pyridine-d_5) δ 5.67 (1H, t, J = 5.3 Hz, H-24), 5.28 (1H, d, J = 2.9 Hz, H-7), 5.22 (1H, s, H_a-26), 5.03 (1H, s, H_b-26), 4.99 (1H, s, H_a-28), 4.97 (1H, s, H_b-28), 3.81 (3H, s, OMe), 2.60 (1H, m, H-9), 1.87 (1H, m, H_a-23), 1.82 (3H, s, H-29), 1.77 (3H, s, H-27), 1.57 (1H, m, H_b-23), 1.33 (1H, m, H-20), 1.00 (3H, s, H-30), 0.85 (3H, s, H-18), 0.81 (3H, d, J = 6.2 Hz, H-21), 0.76 (3H, s, H-19).

(*S*)-MTPA Ester of 5a (5c): colorless, amorphous solid; ^1H NMR (pyridine-d_5) δ 5.64 (1H, t, J = 5.7 Hz, H-24), 5.28 (1H, brs, H-7), 5.11 (1H, s, H_a-26), 4.99 (1H, s, H_b-26), 4.99 (1H, s, H_a-28), 4.96 (1H, s, H_b-28), 3.81 (3H, s, OMe), 2.62 (1H, m, H-9), 1.94 (1H, m, H_a-23), 1.83 (3H, s, H-29), 1.67 (3H, s, H-27), 1.65 (1H, m, H_b-23), 1.40 (1H, m, H-20), 1.01 (3H, s, H-30), 0.88 (3H, d, $J = 6.2$ Hz, H-21), 0.85 (3H, s, H-18), 0.78 (3H, s, H-19).

Seco-coccinic acid F (6): colorless needles (acetone); mp 111−112 °C; $[\alpha]^{20}_{D}$ +51.8 (*c* 0.022, $CHCl_3$); IR (KBr) ν_{max} 2926, 2856, 1698, 1655, 1456, 1385, 1284, 1069, 897 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 471.3472 $[M + H]^+$ (calcd for $C_{30}H_{47}O_4$, 471.3474).

Coccinilactone A (7): colorless needles (acetone); mp 178−179 °C; $[\alpha]^{20}_{D}$ +80.2 (*c* 0.027, $CHCl_3$); IR (KBr) ν_{max} 2946, 2874, 2377, 2345, 1715, 1464, 1368, 1292, 1209, 1118, 1045, 977 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 457.3685 $[M + H]^+$ (calcd for $C_{30}H_{49}O_3$, 457.3682).

Cell Culture and Growth Inhibition Assay. Human leukemia HL-60 cells (obtained from American Type Culture Collection, Rockville, MD) were cultured in RPMI-1640 medium (Gibco, New York, NY) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mmol glutamine, and 10% heat-inactivated fetal bovine serum (Gibco). Cell growth inhibition assay was performed as reported previously.[20] Cells were seeded at a density of 1×10^5 cells/mL and incubated with various concentrations of the tested compounds for 3 days. The compounds were dissolved in dimethyl sulfoxide (DMSO), and the amount of DMSO was controlled lower than 0.1% in the final contration. The number of cells in each group was determined by hemocytometer, and the cell viability was determined using trypan blue staining. The growth inhibitory ability of these compounds was calculated and expressed as the ratio of the cell number in treated group to that of the untreated group. The concentration (GI_{50}) that inhibited half of the cell growth was calculated. 5-Fluorouracil (5-FU) was used as a positive control, and 0.1% DMSO was used as a negative control.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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