Kadsuracoccinic Acids A−C, Ring-A *seco*-Lanostane Triterpenes from *Kadsura coccinea* and Their Effects on Embryonic Cell Division of *Xenopus laevis*

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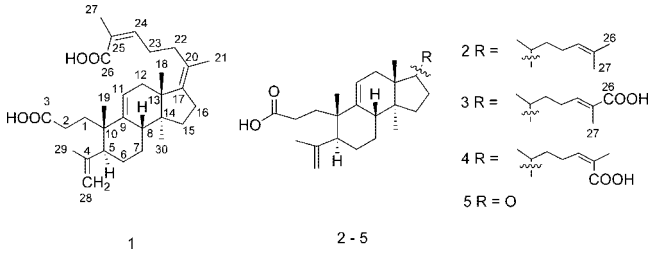
Three new 3,4-*seco*-lanostanes, kadsuracoccinic acids A−C (**1**−**3**), were isolated from the medicinal plant *Kadsura coccinea,* in addition to the known compounds kadsuric acid (**4**) and micranoic acid A (**5**). The structures of **1**−**3** were elucidated by analysis of their 2D-NMR spectroscopic data. Furthermore, the relative conformation of **1** was confirmed by an X-ray crystallographic study. This is the first report of a 3,4-*seco*-lanostane-type triterpene with a 17(20)-ene functional group. Treatment of cultured individual *Xenopus laevis* cells with **1** at the blastular stage arrested cleavage of these cells with an IC_{50} of 0.32 μg/mL.

Kadsura coccinea (Lem.) A. C. Smith (Schisandraceae) is widely distributed throughout southwest mainland China. Extracts of this plant have been used in Chinese folk medicine for the treatment of cancer and dermatosis and as an anodyne to relieve pain.[1] Previous chemical investigations of *K. coccinea* have yielded lignans and triterpenoids.[2−11] In continuation of our search for new bioactive agents from plants,[12,13] we have studied the $CHCl_3$ extract of the rhizomes of *K. coccinea*. In the present work, we report the isolation and structure elucidation of three new *seco*-lanostane triterpenes, kadsuracoccinic acids A−C (**1**–**3**), of which compound **1** possesses a 3,4-*seco*-lanostane skeleton with a 17(20)-ene functional group. Compounds **1**–**3** were accompanied by two known compounds (**4** and **5**), and, with the exception of **3**, these isolates were evaluated for their effects on the cell division of *Xenopus laevis*.



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Kadsuracoccinic acid A (**1**) crystallized as colorless prisms and gave a molecular formula of $C_{30}H_{44}O_4$ by HREIMS (found *m*/*z* 468.3233, calcd 468.3239). Inspection of the ^{13}C NMR spectrum revealed the presence of two carboxyl signals at δ_C 181.7 and 174.1. The ^1H NMR spectrum indicated three olefinic methyls at δ_H 1.58, 1.76, and 1.92, one isopropenyl (exomethylene protons at δ_H 4.87 and 4.70, H-28a and H-28b; δ_H 1.76, C*H*$_3$-29), and three tertiary methyls at δ_H 0.70, 0.81, and 1.06, respectively. Furthermore, two pairs of nonequivalent methylene protons at δ_H 2.50, 2.40 (H-1) and at δ_H 2.09, 1.78 (H-2) showed mutual correlations in the COSY spectrum (Figure 1), and both showed long-range correlations with resonance δ_C 181.7 (C-3) in the HMBC spectrum (Figure 1).

The COSY spectrum of **1** also showed cross-peaks between resonaces at δ_H 1.92 (C*H*$_3$-27) and 6.08 (t, J = 7.2 Hz, H-24) and between H-24 and the nonequivalent methylene signals at δ_H 2.23, 2.69 (H-23). The HMBC spectrum of **1** showed long-rang correlations between C*H*$_3$-27 and carbons at δ_C 174.1 (C-26), 126.3 (C-25), and 146.4 (C-24) and between H-24 and C-26 and C-25, and signals at δ_C 20.4 (C-27), 29.7 (C-23), and 33.7 (C-22). The above-mentioned data indicated the presence of the following side chain: HOOC−C(CH$_3$)=CH−CH$_2$−CH$_2$−. Further irradiation of C*H*$_3$-27 resulted in a NOE on H-24 and indicated that the double bond at C-24 has a *Z*-configuration.

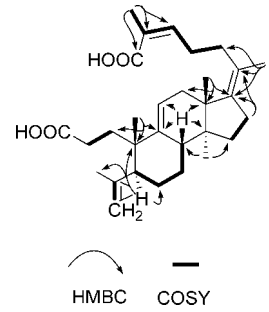

Figure 1. HMBC and COSY correlations of compound **1**.

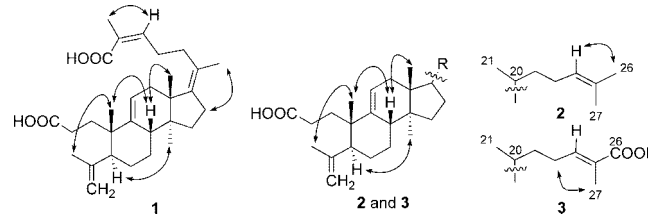

Figure 2. Key NOE correlations of compounds **1**–**3**.

These data were closely related to those previously reported for kadsuric acid (**4**),[14] but clearly differed in the signals corresponding to C-17, CH$_3$-21, and C-22. Both the signals of the C*H*-17 and C*H*-20 methine protons were absent. Moreover the C*H*$_3$-21 three-proton doublet was shifted downfield to δ_H 1.58 and appeared as a singlet signal. These observations indicated the presence of a double bond between C-17 and C-20.

The orientation of H-8 for **1** was determined on the basis of correlations of C*H*$_3$-19/H-8 and C*H*$_3$-18/H-8, respectively, in the NOESY spectrum (Figure 2). A correlation of C*H*$_3$-30 and H-5 suggested that H-5 has an α-orientation.[17] In addition, the signal at δ_H 2.21 (H-16) showed cross-peaks with C*H*$_3$-21 and the resonace at δ_H 0.81 (C*H*$_3$-18) showed cross-peaks with a nonequivalent methylene at δ_H 1.89, 2.42 (C*H*$_2$-22), indicating the double bond in C-17 has a *Z*-configuration. Thus, the structure of **1** was assigned as 3,4-*seco*-lanosta-4(28),9(11),17(20),17(*Z*),24(*Z*)-tetraene-3,26-dioic acid.

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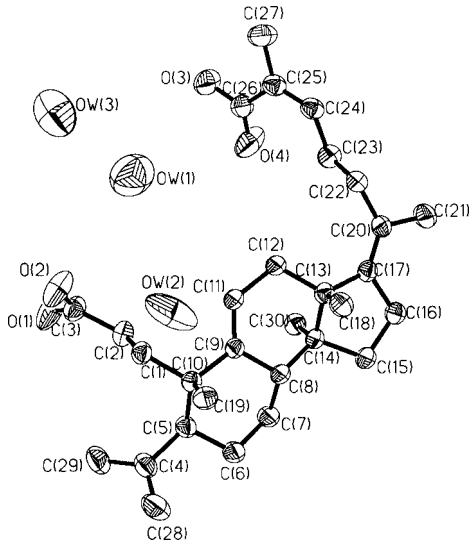

Figure 3. X-ray structure of compound **1** showing relative configuration.

To confirm the structure of **1**, this compound was subjected to a single-crystal X-ray diffraction analysis. A view of the solid-state conformation is provided in Figure 3, and it confirmed that **1** has a double bond between C-17 and C-20.

The IR and NMR spectra of kadsuracoccinic acid B (**2**) and kadsuracoccinic acid C (**3**) revealed that, like **4**, these compounds also have a *seco*-lanostane skeleton. The NMR spectra of **2** were closely related to analogous data reported for kadsuric acid (**4**), but clearly differed in the signals corresponding to the side chain at C-17. Indeed, the detailed analysis of **2** using the COSY and HMBC spectra showed a typical set of resonances associated with a 2-methylhept-2-ene system at δ_C 35.9 (C-20), 18.3 (C-21), 36.1 (C-22), 25.0 (C-23), 125.2 (C-24), 130.9 (C-25), 17.7 (C-26), and 25.7 (C-27), similar to analogous signals in the ^{13}C NMR spectrum of coccinetane A.[15,16] In turn, COSY and HMBC spectra of **3** disclosed a side chain with correlated protons, namely, COOH−C(CH_3)=CH−CH_2−CH_2−, as in compounds **1** and **4**. However, the irradiation of the CH_3-27 signal showed a NOE with CH_2-23, indicating an *E*-configuration of the double bond between C-24 and C-25 in **3**.

The relative configurations of **2** and **3** were deduced from the NOESY spectra (Figure 2). Of particular interest were NOESY cross-peaks observed between H-8 and the signals of CH_3-19 and CH_3-18, on one hand, and CH_3-30 and the signals of H-17 and H-5, on the other hand. These were in good agreement with the relative stereochemistry of poricoic acid G, a 3,4-*seco*-lanosane-type triterpene, at C-5, C-8, C-10, C-13, C-14, and C-17.[17]

Compounds **4** and **5** are known compounds, whose structures were confirmed by comparison with literature reports.[18]

The most intriguing feature of **1** is the unusual 17(20)-ene structure and the *Z*-configuration of this double bond. From a biogenetic point of view, compound **1** is achieved via dehydration after **4** was oxidized. Chart S1 (Supporting Information) shows the psossible biogenetic relationship existing between compounds **1**−**5**. Although an alternative *E*-configuration in 17(20)-ene is possible, the hydrogen bonding between the two carbonyl groups makes the dehydration easier, and the main product of dehydration should possess a *Z*-configuration (Chart S1). Compound **5** is a highly degraded triterpene. A possible biogenic fission of the C-17/C-20 bond that might be achieved via oxidation has been reported in the literature.[18]

Compounds **1**, **2**, **4**, and **5** were tested on the division of isolated cells from early *X. laevis* embryos. The amount of **3** was so small that it could not be tested. Single cells from midblastula stage embryos were able to divide in a non-nutritive medium. Under the standard conditions of the present study, most cells divided between 4 and 10 times.[19] The effects of **1**, **2**, **4**, and **5** on the division of these cells were studied at four different concentrations: 1, 10, 25,

Table 1. NMR Spectroscopic Data for **1**, **2** (600 MHz, $CDCl_3$), and **3** (600 MHz, MeOH-d_4)

	1		**2**		**3**	
no.	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	29.1	2.50, m, 2.40, m	34.1	1.58, m, 1.71, m	35.4	1.90, m, 1.72, m
2	31.8	2.09, m, 1.78, m	29.2	2.30, m	30.5	2.24, m, 1.99, m
3	181.7		180.9		178.9	
4	147.6		149.8		151.4	
5	49.4	2.09, m	45.3	2.06, d (7.2)	46.6	2.15, d (7.2)
6	27.7	1.55, m, 1.79	28.8	1.63, m	30.8	1.57, m, 1.70, m
7	26.6	1.26, m, 1.64, m	28.3	1.26, m 1.64, m	29.4	1.06, m, 1.32, m
8	41.5	2.10, m	38.7	2.57, m	40.3	2.62, m
9	142.0		146.7		148.1	
10	42.6		51.5		52.8	
11	118.9	5.36, dd (5.2)	117.7	5.31, dd (3.4)	119.3	5.35, dd (3.5)
12	36.0	2.15, m, 2.40, m	33.9	1.50, m	35.3	1.58, m
13	47.2		43.6		45.0	
14	46.2		36.3		37.4	
15	33.0	1.42, m	29.7	2.00, m	30.3	1.97, m
16	28.8	2.21, m	18.6	1.55, m, 1.65, m	19.8	1.55, m, 1.69, m
17	143.1		53.0	1.48, m	54.4	1.58, s
18	21.1	0.81, s	21.7	0.75, s	22.4	0.80, s
19	26.8	1.06, s	27.5	1.02, s	28.1	1.07, s
20	124.6		35.9	1.46, m	37.3	1.42, m
21	19.8	1.58, s	18.3	0.88, d (6.5)	18.8	0.95, d (6.5)
22	33.7	1.89, m, 2.42, m	36.1	1.04, m, 1.09, m	36.0	1.60, m, 1.18, m
23	29.7	2.23, m, 2.69, m	25.0	1.85, m, 2.02, m	26.7	2.15, m, 2.25, m
24	146.4	6.08, t (7.2)	125.2	5.10, m	144.4	6.72, t (7.2)
25	126.3		130.9		128.7	
26	174.1		25.7	1.68, s	171.9	
27	20.4	1.92, s	17.7	1.61, s	12.4	1.81, s
28	113.8	4.70, br s	111.9	4.82, br s	112.4	4.84, br s
		4.87, br s		4.87, br s		4.90, br s
29	23.1	1.76, s	26.3	1.80, s	26.3	1.81, s
30	19.1	0.70, s	24.1	0.85, s	26.7	0.87, s

and 50 μg/mL. Among the compounds tested, compound **1** induced significant cleavage arrest in the cells at a concentration 1 μg/mL [resulting in 85% cleavage arrest; $IC_{50} = 0.32$ μg/mL (0.68 μM)]. About 47% of the cell growth was inhibited by an anticancer drug, 5-fluorourasil (5-FU), at a concentration of 10 μg/mL [$IC_{50} = 6.6$ μg/mL (26.8 μM)].

As the early embryonic cell cycle in *X. laevis* consists of only S and M phases and does not include the G_1 and G_2 phases,[20,21] arrest of the cell cycle by compound **1** is unrelated to the inhibition of reactions at the G_1 to S phase transition. Arrest of the cell cycle of *X. laevis* embryos by **1** may be related to the preservation of the progression of the M phase.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a JASCO DIP-360 polarimeter. The UV spectra were obtained in MeOH on a Shimadzu UV-160 spectrophotometer, and the IR spectra were recorded on a JASCO IR A-2 spectrophotometer. The NMR spectra were recorded on a JEOL-ECA600 MHz spectrometer, with TMS as an internal standard. The MS data were obtained on a JEOL GC Mate spectrometer. Column chromatography was carried out with a CHP-20P column (Mitsubishi Chemical Corporation.). Thin-layer chromatography (TLC) was performed on Merck TLC plates (0.25 mm thickness), with compounds visualized by spraying with 5% H_2SO_4 in ethanol solution and then heating on a hot plate. HPLC was performed on a JASCO PU-2089 instrument equipped with a JASCO UV-2075 detector. Cosmosil (Cholester Waters 10 $\Phi \times$ 250 mm), FluoFix 120 N, FluoFix 120E (10 $\Phi \times$ 250 mm), and Shisheido SIL (10 $\Phi \times$ 250 mm) columns were used for preparative purposes.

Plant Material. The dried rhizomes of *K. coccinea* were collected in Guangxi Province, People's Republic of China, in April 2004 and identified by Dr. Bao-Lin Guo, Peking Union Medical College, Beijing, People's Republic of China. Voucher specimens (NK04012) are deposited in the Department of Pharmacognosy, College of Pharmacy, Nihon University.

Extraction and Isolation. The dried rhizomes of *K. coccinea* (1.75 kg) were extracted three times with 80% acetone. Evaporation of the solvent under reduced pressure from the combined extract gave an acetone extract (82.5 g). This extract was dissolved and suspended in water (2.0 L) and partitioned in turn with $CHCl_3$ (3 $\times$ 2 L), EtOAc (3 $\times$ 2 L), and *n*-butanol (3 $\times$ 2 L). The amounts extracted were 44.7, 4.0, and 14.2 g, respectively, and the residual aqueous extract yielded 20.6 g.

The $CHCl_3$ fraction was subjected to silica gel column chromatography (13 $\Phi \times$ 65 cm, eluted with $CHCl_3$ and MeOH, 95:5 $\rightarrow$ 70:30). The column chromatographic fractions (500 mL each) were combined into 11 portions according to TLC (toluene−EtOAc−HOAc, 70:33:3). Portion four was further purified by HPLC (Fluofix 120N, 10 $\Phi \times$ 250 mm, CH_3CN-H_2O, 70:30) to give **1** (11 mg), **4** (16 mg), and **5** (20 mg). Portion five was further purified by HPLC (Fluofix 120N, 10 $\Phi \times$ 250 mm, CH_3CN-H_2O, 80:20) to give **2** (10 mg) and **3** (4 mg).

Kadsuracoccinic Acid A (1): colorless prisms (acetone); mp 150–151 °C; $[\alpha]_D$ +31 (*c* 0.25, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 204 (3.96); IR (KBr) ν_{max} 3500–3210, 2939, 1695, 1637, 1455, 1372, 1295, 1081, 894, 757 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 468 $[M]^+$ (78), 453 (18), 395 (30), 369 (100), 327 (30), 261 (50); HREIMS *m/z* 468.3233 (calcd for $C_{30}H_{44}O_4$, 468.3239).

X-ray Crystallographic Data for 1. The colorless crystal was grown from *n*-hexane−EtOAc. Crystal data: $C_{30}H_{44}O_4 \cdot 2.3H_2O$, $M = 468.68$, orthorhombic system, space group $P2_12_12_1$, $a = 21.108(1)$ Å, $b = 20.366(1)$ Å, $c = 7.182(1)$ Å, $V = 3087.4(5)$ Å^3, $Z = 4$, $d = 1.087$ g/cm^3. A crystal of dimensions 0.40 $\times$ 0.40 $\times$ 0.80 mm was used for measurement on a MAC DIP-2030K diffractometer with a graphite monochromator (ω-2θ scans, $2\theta_{max} = 50°$), Mo Kα radiation. The total number of independent reflections measured was 3830, of which 3602 were observed ($|F|^2 \geq 2\sigma|F|^2$). The structure was solved and refined using the programs SHELXS-97. The program XSHELL was used as an interface to the SHELX programs and to prepare the figures. Final indices: $R_1 = 0.0762$, $wR_2 = 0.2366$ ($w = 1/\sigma|F|^2$). This material has also been deposited with the Cambridge Crystallographic Data Center (CCDC 658659). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44-(0)1223-336033 or e-mail:deposit@ccdc.cam.ac.uk].

Kadsuracoccinic Acid B (2): colorless, amorphous powder; $[\alpha]^{25}_D$ +44 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3500–3200 (OH), 2944, 2960, 1719, 1650, 1279 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 440 $[M]^+$ (100), 425 (18), 367 (40), 69 (65); HREIMS *m/z* 440.3651 (calcd for $C_{30}H_{48}O_2$, 440.3654) .

Kadsuracoccinic Acid C (3): colorless, amorphous powder; $[\alpha]^{25}_D$ +65 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3500–3200 (OH), 2950, 2671, 1701, 1639, 1540, 1430 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 470 $[M]^+$ (100), 455 (18), 397 (60); HREIMS *m/z* 470.3393 (calcd for $C_{30}H_{46}O_4$, 470.3396).

Bioassay. *X. laevis* eggs were obtained by natural amplexus of males and females after injection with human chorionic gonadotropin (200 IU) and dejellying with 2.5% cysteine. The embryos were then allowed to develop to stage 8 at room temperature.

Embryonic cells of *X. laevis* were dissected from stage 8 *X. laevis* blastulae.[12,13] Single cells from the inner surface of the caps were separated off by directing a gentle stream of calcium- and magnesium-free medium (50 mM phosphate buffer, 35 mM NaCl, 1 mM KCl, pH 7.0) as described by Godsave and Slack.[22] Two or three cells were transferred into a well of a Terasaki plate filled with 10 mL of 2 mg/mL γ-globulin in a simple salt solution (NAM/2) and cultured for 20 h at 25 °C.[12,13]

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Supporting Information Available: Biogenetic proposal for compounds **1**−**3**, 1D- and 2D-NMR spectra of **1**−**3**, and the X-ray data of **1** are available free of charge via the Internet at http://pubs.acs.org.

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