Novel Anti-HIV Lancilactone C and Related Triterpenes from *Kadsura lancilimba*

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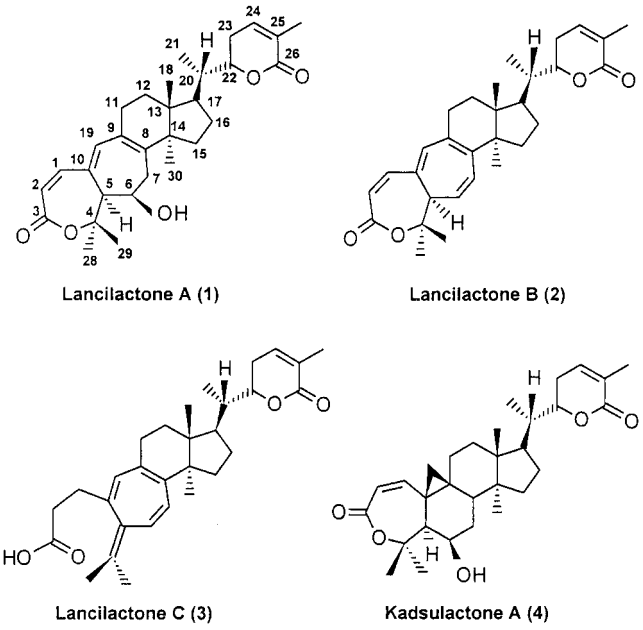
Three new triterpene lactones, lancilactones A (**1**), B (**2**), and C (**3**), together with the known kadsulactone A (**4**), were isolated from the stems and roots of *Kadsura lancilimba*. Their structures and stereochemistries were determined primarily from mass and NMR spectral data. Compound **3** inhibited HIV replication with an EC_{50} value of 1.4 μg/mL and a therapeutic index of greater than 71.4.

The stems and roots of *Kadsura lancilimba* How. (Schizandraceae), a plant indigenous to southern China, have been used to treat stomach-ache and enterogastritis in Chinese folk medicine. The ethanolic extract of this plant was found to exhibit significant in vitro anti-lipid peroxidative effects.[1] In previous papers, we reported that several new dibenzocyclooctadiene lignans from *Kadsura interior* showed significant inhibitory activity in vitro against HIV replication in H9 lymphocytes.[2,3] Recently, we also screened other *Kadsura* species for potential anti-AIDS agents. The Et_2O extract of *K. lancilimba* showed significant anti-HIV activity. Bioassay-directed fractionation of this active extract has now led to the isolation and characterization of three new triterpene lactones, lancilactones A (**1**), B (**2**), and C (**3**), along with the known kadsulactone A (**4**). Lancilactone C (**3**) was identified as an anti-HIV principle. We report herein on the structure elucidation and anti-HIV activity of **1**, **2**, and **3**.



Results and Discussion

Lancilactone A (**1**), obtained as colorless needles, had the molecular formula $C_{30}H_{40}O_5$ as revealed by HRMS. The IR spectrum showed the presence of a hydroxyl group (3508 cm^{-1}) and two lactone groups (1699, 1678 cm^{-1}). The ^1H NMR spectrum (Table 1) showed signals for one secondary (δ 1.03) and five tertiary (δ 0.80, 1.02, 1.57, 1.59, and 1.93) methyls. The ^{13}C NMR (Table 2), DEPT, and HETCOR spectra indicated that **1** contains 30 carbon atoms and 39 carbon-bonded hydrogen atoms. Carbon atom multiplicities were determined by a DEPT experiment. Thirteen low-field signals corresponded to two carbonyl (δ 166.4 and 166.3), eight olefinic (δ 117.6, 128.5, 130.5, 139.1, 137.9, 144.2, 144.6, and 148.7), and three oxygenated carbons (δ 80.3, 80.5, and 84.2); the high-field region showed six methyl, six methylene, five methine, and three quaternary carbons. These data were consistent with the HRMS empirical formula and suggested that **1** was a triterpene.

A six-membered α,β-unsaturated lactone ring was assigned to the side chain of **1** due to the presence of a mass spectral fragment at *m/z* 111 (Scheme 1) and the results of $^1H-^1H$ COSY and HECTOR spectra. In the former spectrum, the following correlations were found: H-23 with H-22 and H-24, CH_3-27 with H-24, CH_3-21 with H-20, and H-20 with H-22. In the long-range HETCOR, the CH_3-27 protons were correlated with C-24 and C-26, and the CH_3-21 protons were correlated with C-17 and C-20. Thus, as shown, the side chain of **1** contains an α-methyl-α,β-unsaturated δ-lactone substituted at the δ-position.

The seven-membered α,β-unsaturated lactone ring of **1** was assigned as ring A based on biogenetic considerations and the analytical NMR results. A long-range HETCOR experiment showed that H-1 was correlated with a carbonyl, two methyls and the low-field (δ 80.5) C-4, and H-5 was correlated with C-1, C-10, and C-19. From the ^1H and ^{13}C NMR spectra, **1** has only six methyl groups and no cyclopropane ring. Furthermore, the UV spectrum [λ_{max} (log ϵ) 208 (4.33), 265 (4.04), 327 (4.35) nm] indicated that three double bonds are conjugated to suggest an A,B-*seco*-9,19-cyclolanostene skeleton.[7]

A hydroxyl group was located at C-6 because the carbon and attached proton both were shifted to low-field (δ 84.2,

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Table 1. ^{1}H NMR Data of Compounds **1**−**3** (δ in $CDCl_3$)

proton	1	2	3
1	6.79, 1 H, m	6.58, 1 H, m	2.84, 2 H, m
2	5.82, 1 H, m	5.79, 2 H, m	2.58, 2 H, m
5	2.45, 1 H, m	1.71, 1 H, m	
6	5.02, 1 H, m	5.82, 1 H, m	6.25, 1 H, s
7	1.93, 2 H, m	6.16, 1 H, m	6.76, 1 H, s
11	1.42, 2 H, m	2.61, 2 H, m	2.90, 2 H, m
12	1.53, 2 H, m	1.88, 1.65, 2 H, AB	1.94, 2 H, m
15	2.13, 1 H, 2.41, 1 H, AB	1.88, 1.65, 2 H, AB	1.76, 1.92, 2 H, AB
16	1.83, 2 H, m	1.93, 2 H, m	1.58, 1.97, 2 H, AB
17	1.58, 1H, m	1.57, 1 H, m	1.69, 1 H, m
18	0.80, 3 H, s	0.91, 3 H, s	0.66, 3 H, s
19	6.42, 1 H, s	6.16, 1 H, m	6.90, 1 H, s
20	2.08, 1 H, m	2.10, 1 H, m	2.12, 1 H, m
21	1.03, 3 H, d, J = 6.5 Hz	1.07, 3 H, d, J = 6.5 Hz	1.08, 3 H, d, J = 6.5 Hz
22	4.48, 1 H, d−t	4.52, 1 H, d−t	4.54, 1 H, m
23	2.13, 2 H, m	2.13, 2.40, 2 H, AB	2.19, 2.43, 2 H, AB
24	6.61, 1 H, m	6.62, 1 H, m	6.64, 1 H, m
27	1.93, 3 H, s	1.93, 3 H, s	1.94, 3 H, s
28	1.57, 3 H, s	1.51, 3 H, s	1.90, 3 H, s
29	1.59, 3 H, s	1.62, 3 H, s	1.69, 3 H, s
30	1.02, 3 H, s	0.91, 3 H, s	1.07, 3 H, s
3-COOH			11.42, 1 H, br s

Table 2. ^{13}C NMR Data of Compounds **1**−**3** (δ in $CDCl_3$)

carbon	1	2	3
1	144.6 d	141.6 d	28.2 t
2	117.6 d	118.7 d	34.7 t
3	166.4 s	166.7 s	178.7 s
4	80.5 s	78.7 s	135.4 s
5	53.5 d	51.4 d	133.6 s
6	84.2 d	122.9 d	123.5 d
7	37.8 t	125.0 d	126.5 s
8	128.5 s	149.5 s	144.4 s
9	148.7 s	133.1 s	135.1 s
10	137.9 s	127.2 s	134.6 s
11	31.0 t	28.5 t	25.5 t
12	26.8 t	30.9 t	30.7 t
13	51.4 s	51.1[a] s	45.3 s
14	44.9 s	44.3[a] s	49.5 s
15	26.7 t	26.7 t	31.8 t
16	30.1 t	30.6 t	27.3 t
17	46.2 d	46.3 d	46.7 d
18	15.8 q	15.9 q	16.1 q
19	144.2 d	135.9 d	128.3 d
20	39.4 d	39.5 d	39.4 d
21	13.7 q	13.7 q	13.7 q
22	80.3 d	80.3 d	80.5 d
23	23.6 t	23.6 t	23.5 t
24	139.1 d	139.2 d	139.4 d
25	130.5 s	128.4 s	128.3 s
26	166.3 s	166.4 s	166.5 s
27	17.0 q	17.0 q	17.0 q
28	27.3 q	29.2 q	26.1 q
29	29.2 q	25.2 q	19.3 q
30	27.2 q	26.5 q	27.5 q

[a] These data are interchangeable.

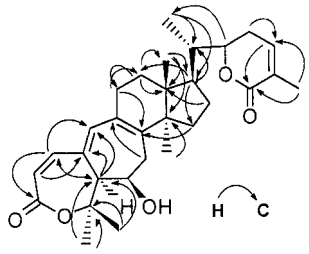
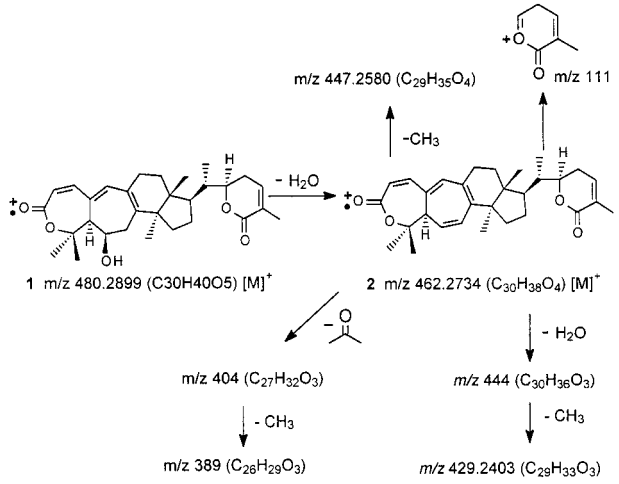


Figure 1. The long-range HETCOR responses of 1.

Scheme 1. Mass spectral fragmentation of compounds 1 and 2

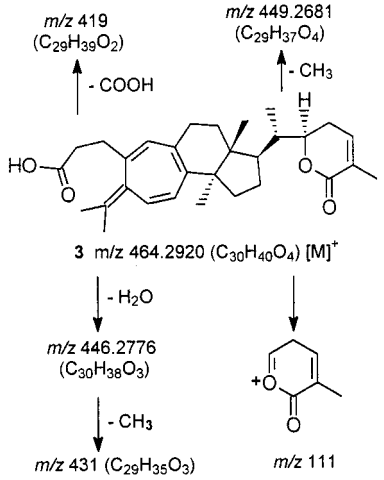


δ 5.03) in the respective NMR spectra. The C_6−H also correlated with H-5 and H-7 in the ^{1}H−^{1}H COSY. The ^{1}H−^{1}H COSY and HETCOR spectra were used for assignment of all proton resonances as listed in Table 1, and a long-range HETCOR was used for assignment of all carbon resonance as listed in Table 2. Figure 1 summarizes the observed long-range HETCOR correlations. Structure **1** is compatible with all ^{1}H and ^{13}C NMR data.

The stereochemistry of **1** was determined from the CD spectrum and 2D ^{1}H NOE (NOESY). Lancilactone A (**1**) shows a strong negative Cotton effect at 235 nm (−77 568); thus, C-22 was assigned the (*S*)-configuration.[4,8] In the NOESY spectrum of **1**, CH_3-18 showed cross peaks with H-20, as well as with CH_3-29, indicating that CH_3-18 and H-20 should be in syn- and β-positions. The CH_3-30 had a cross peak with H-17, indicating that this methyl has α-configuration; H-5 showed a cross peak with CH_3-28, indicating that H-5 also has α-configuration. Irradiation of H-6 resulted in NOE enhancement of H-5, confirming an α-configuration for H-6. Thus, the structure of **1** was assigned as shown.

Lancilactone B (**2**), obtained as colorless needles, had the molecular formula $C_{30}H_{38}O_4$ as revealed by its HRMS. Similarities in all spectral data indicated that **2** was related in structure to compound **1**. Comparison of the ^{1}H and ^{13}C NMR spectra suggested that **2** also had an A,B-*seco*-9,19-cyclolanostene skeleton and two lactone groups. The most prominent differences in the ^{1}H NMR spectra of **1** and **2** were the disappearance of the oxygenated proton (δ 5.02) in the former compound and the appearance of two olefinic

Scheme 2. Mass spectral fragmentation of compound 3



protons (δ 5.82 and 6.16) in the latter compound. Also, H-5 shifted upfield from δ 2.45 in **1** to δ 1.71 in **2**. The DEPT spectra of **2** showed two additional olefinic carbons (δ 122.9, 125.0) and one less oxygenated carbon than found in the spectra of **1**. Furthermore, in the UV spectrum, the absorbency wavelength was shifted from 327 nm in **1** to 334 nm in **2**. These data suggested loss of water and formation of a double bond between C-6 and C-7, consistent with the absence of a hydroxyl absorption in the IR spectrum of **2**. Thus, the structure of **2** was assigned as shown.

Lancilactone C (**3**), obtained as colorless granules, had the molecular formula $C_{30}H_{40}O_4$ as revealed by its HRMS. The ^{1}H NMR spectrum (Table 1) showed signals for one secondary (δ 1.08) and five tertiary (δ 0.66, 1.07, 1.69, 1.90, 1.94) methyls. The ^{13}C NMR (Table 2), DEPT, HETCOR spectra indicated that **2** contains 30 carbon atoms and 39 carbon-bonded hydrogen atoms. The multiplicity of the carbon atoms was determined by the DEPT experiment. The ^{13}C NMR spectrum showed two carbonyl carbons at δ 166.5 and 178.7; 10 olefinic carbons at δ 123.5, 126.5, 128.3 $\times$ 2, 133.6, 134.6, 135.1, 135.4, 139.4, and 144.4; and an oxygenated carbon at δ 80.5; the high-field region showed six methyl, seven methylene, three methine, and two quaternary carbons. These data are consistent with the HRMS empirical formula and again suggested that **3** was a triterpene. Compounds **2** and **3** have the same side-chain moiety as shown by similarities in the ^{1}H and ^{13}C NMR spectra. The MS fragment at *m*/*z* 111 supports this conclusion (Scheme 2). However, in the ^{13}C NMR spectrum, the carbonyl carbon at δ 166.7 in **2** shifted downfield to δ 178.7 in **3**, and a carboxylic acid proton was present at δ 11.42 in the ^{1}H NMR spectrum of **3**. Because lancilactones B and C were isolated from the same source, we postulated that the carboxylic acid might arise from a similarly located seven-membered lactone ring. Cleavage of the ring A lactone between C-3 and C-4 would form a carboxylic acid at C-3 and a double bond between C-4 and C-5. ^{1}H and ^{13}C NMR results support this conclusion. The proton/carbon signals of C-1 and C-2 shifted upfield from δ 6.58, 5.29/141.6, and 118.7 in **2** to δ 2.84, 2.58/28.2, and 34.7 in **3**, respectively. In the long-range HETCOR, H-1 correlated with C-2, C-5, and C-10, and in the NOESY spectrum, showed cross peaks with H-2, H-19, and H-6. Thus, the structure of **3** was assigned as shown.

Lancilactones A (**1**), B (**2**) and C (**3**), and kadsulactone A (**4**) were tested for in vitro inhibitory effects against HIV replication in H9 lymphocytes. The data are listed in Table 3. Among these compounds, lancilactone C (**3**) demonstrated potent anti-HIV activity with an EC_{50} value of 1.4 μg/mL and a therapeutic index (TI) of greater than 71.4. The other compounds showed no suppression. The structural difference between active compound **3** and inactive compounds **1**, **2**, and **4** is the opened ring A lactone in **3**. Therefore, the carboxylic acid may play a role in the anti-HIV activity of **3**. Additional derivative synthesis and SAR studies are in progress.

Table 3. Anti-HIV Activities of Compounds **1**−**4**

compounds	EC_{50} (μg/mL)	IC_{50} (μg/mL)	TI
1	no suppression	14.3	
2	no suppression	7.1	
3	1.4	>100	>71.4
4	no suppression	8.6	
AZT	0.045	1875	41 667

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler micromelting point apparatus and are uncorrected. The IR spectra were recorded as KBr pellets on a Nicolet Magna FTIR 750 spectrophotometer. The UV spectra were measured on a Shimadzu UV-250 spectrophotometer in absolute MeOH. MS were determined on a Varian MAT-711 mass spectrometer for EIMS and HP 5989A mass spectrometer for HRMS. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AC-300 spectrometer with TMS as internal standard and $CDCl_3$ as solvent. Optical rotations were measured with a JASCO J-500A spectropolarimeter equipped with a JASCO DP-500N data processor. Analytical TLC was performed on HSG F_{254} plates (Yantai Institute of Chemical Technology) with petroleum ether: EtOAc (3:2). Si gel H (200−300 mesh, Qing Dao) was used for column chromatography. Spots on the plate were observed under UV light and visualized by spraying with vanillin−H_2SO_4 followed by heating.

Plant Material. The stems and roots of *K. lancilimba* How. were collected in Long-Sheng County, Guang-Xi autonomous region, People's Republic of China in November 1992. A voucher specimen (KL-9211GX-1) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Shanghai Medical University, Shanghai, People's Republic of China.

Extraction and Isolation. The stems and roots of *K. lancilimba* were air-dried, ground, and extracted with 95% EtOH. The EtOH extract was evaporated in vacuo to yield a semisolid (882.5 g); H_2O (2000 mL) was added to the residue, and this solution was extracted with Et_2O 16 times. This Et_2O solution was concentrated to yield 210 g of residue. The residue was chromatographed on Si gel (1700 g), employing petroleum ether containing increasing amounts of EtOAc. The fractions eluted with petroleum ether−EtOAc (80:20) gave lancilactone C (**3**, 132 mg). The fractions eluted with petroleum ether−EtOAc (50:50) gave lancilactone A (**1**, 68 mg), lancilactone B (**2**, 7.63 g), and kadsulactone A (**4**, 290 mg).

Lancilactone A (**1**): obtained as colorless needles (EtOAc−petroleum ether); mp 247−249 °C; $[\alpha]_D$ +378.46° (*c* 3.28, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (4.33), 265 (4.04), 327 (4.35) nm; CD (*c* 0.10, MeOH) $[\theta]^{27}$ (nm) 54 720 (218, tr), −77 568 (235, pk), −33 408 (278, tr), −88 896 (325, pk), 0 (400); IR (KBr) ν_{max} 3508 (OH), 2966, 1699, 1678, 1558, 1371 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; EIMS (70 eV) *m*/*z* 480 $[M]^+$ (6), 462 (17), 447 (12), 444 (19), 429 (12), 404 (49), 377 (6.9), 363 (3.9), 233 (27), 209 (32), 195 (48), 171 (100), 128 (50), 111 (58), and 95 (58); HRMS *m*/*z* 480.2899 (calcd for $C_{30}H_{40}O_5$, 480.2876).

Lancilactone B (**2**): obtained as colorless needles ($CHCl_3$−EtOAc); mp 245−247 °C; $[\alpha]_D$ −306.92° (*c* 6.88, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (4.32), 243 (4.33), 334 (4.16) nm; CD (*c* 0.225, MeOH) $[\theta]^{27}$ (nm) 48 993 (203), −2382 (221), −45 666 (246), −5092 (272), −16 345 (305); IR (KBr) ν_{max} 2974, 1734, 1697, 1599, 1504 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2;

EIMS (70 eV) *m*/*z* 462 $[M]^+$ (1.0), 447 (1.0), 429 (1.0), 404 (16), 389 (61.3), 223 (2.6), 195 (4.3), 171 (9.5), 128 (1.7), 111 (1.9), and 88 (39); HRMS *m*/*z* 462.2734 (calcd for $C_{30}H_{38}O_4$, 462.2770).

Lancilactone C (**3**): obtained as colorless granules ($CHCl_3$−petroleum ether); mp 217−219 °C; $[\alpha]_D$ +43.37° (*c* 3.60, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 212 (4.56), 238 (4.07), 278 (3.18) nm; CD (*c* 0.0925, MeOH) $[\theta]^{27}$ (nm) +105 140 (197), −54 175 (240), −33 308 (265, tr); IR (KBr) ν_{max} 2968, 1713, 1701, 1450, 1375 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; EIMS (70 eV) *m*/*z* 464 $[M]^+$ (20), 449 (9), 446 (24), 431 (20), 404 (13), 361 (8.2), 337 (8.2), 263 (32), 223 (37), 209 (38), 179 (72), 165 (100), 111 (42), and 95 (60); HRMS *m*/*z* 464.2920 (calcd for $C_{30}H_{40}O_4$, 464.2927).

Compound **4** was identified as kadsulactone A[4] based on its UV, CD, and ^1H and ^{13}C NMR spectral data.

HIV Growth Inhibition Assay. The H9 T cell line was maintained in continuous culture with complete medium (RPMI 1640 and 10% fetal calf serum) at 5% CO_2 and 37 °C and was used in experiments only when in log phase of growth. The cells were incubated with HIV-1 (IIIB isolate, $TCID_{50}$ 10^4 IU/mL, at a multiplicity of infection of 0.1−0.01 IU/cell) for 1 h at 37 °C and 5% CO_2. The cells then were washed thoroughly to remove unabsorbed virions and resuspended at 4×10^5 cells/mL in complete medium. Aliquots (1 mL) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in the culture medium). After a 4-day incubation at 37 °C, cell density of uninfected cultures was determined by counting cells in a Coulter counter to assess toxicity of the test compound. A p24 antigen ELISA assay was used to determine the level of virus released in the medium of the HIV-infected cultures. The p24-antigen assay uses an HIV-1 anti-p24 specific monoclonal antibody as the capture antibody coated on 96-well plates. Following a sample incubation period, rabbit serum containing antibodies for HIV-1 p24 is used to tag any p24 "captured" onto the microtiter well surface. Peroxidase-conjugated goat anti-rabbit serum is then used to tag HIV-1 p24 specific rabbit antibodies that have complexed with captured p24. The presence of p24 in test samples is then revealed by addition of substrate. The cutoff for the p24 ELISA assay is 12.5 pg/mL; p24 in the culture medium was quantitated against a standard curve containing known amounts of p24. The effective (EC_{50}) and inhibitory (IC_{50}) concentrations (for anti-HIV activity and cytotoxicity, respectively) were determined.

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