Three New Compounds from *Kadsura longipedunculata*

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Two new tetrahydrofuran lignans, kadlongirins A and B (1, 2), a new cadinane-type sesquiterpenoid, 2,7-dihydroxy-11,12-dehydrocalamenene (3), together with seven known lignans, grandisin, fragransin B_1, vladirol F, kadsuralignan C, otobaphenol, isoanwulignan, and 4-[4-(3,4-dimethoxyphenyl)-2,3-dimethylbutyl]-2-methoxyphenol, were isolated from the leaves and stems of *Kadsura longipedunculata*. The structures of these new compounds were elucidated by spectroscopic methods. Compound 2 exhibited weak anti-human immunodeficiency virus-1 activity with an EC_{50} value of 16.0 μg/ml, and therapeutic index (TI) value of 6.7.

Key words *Kadsura longipedunculata*; lignan; sesquiterpenoid; anti-human immunodeficiency virus activity

Kadsura longipedunculata Finet *et* Gagnep is a climbing plant widely distributed in the southern part of China. It has been used in folk medicine for the treatment of rheumatoid arthritis as well as gastric and duodenal ulcers.[1,2] This species has been reported to contain dibenzocyclooctadienlignans, lanostane triterpenoid acids, and lactones, which have been found to possess some beneficial pharmacological effects, including antihepatitis, antitumor, and anti-human immunodeficiency virus (HIV) activities.[3—10] Recently, we reported the isolation and structure elucidation of two novel series of triterpene dilactones with unprecedented rearranged skeletons, named as kadlongilactones A—F and longipedlactones A—I, respectively, from the leaves and stems of *K. longipedunculata* collected in the Erlang mountain region of Sichuang Province, China.[11—13] To search more novel and biologically potent active compounds, and to compare the chemical constituents' differences of the same *Kadsura* species belonging to different geographical distribution and climatic conditions, we investigated the leaves and stems of *K. longipedunculata* collected in the Yibin region of Sichuang Province, China. This paper deals with the isolation and structure elucidation of three new compounds, named as kadlongirins A and B (**1**, **2**) and 2,7-dihydroxy-11,12-dehydrocalamenene (**3**), together with seven known compounds, grandisin (**4**),[14] fragransin B_1 (**5**),[15] vladirol F (**6**),[16] kadsuralignan C (**7**),[17] otobaphenol (**8**),[18] isoanwulignan (**9**),[19] and 4-[4-(3,4-dimethoxyphenyl)-2,3-dimethylbutyl]-2-methoxy-phenol (**10**).[20] The anti-HIV-1 activities of the three new compounds were evaluated. Compound **2** showed weak anti-HIV-1 activity with an EC_{50} value of 16.0 μg/ml and therapeutic index (TI) value of 6.7.

Results and Discussion

Compound **1** was obtained as yellowish oil and its molecular formula of $C_{24}H_{32}O_8$ was established from HR-ESI-MS ($[M+Na]^+$, *m/z* 471.1994) and ^{13}C-NMR spectroscopic data, indicating 9 degrees of unsaturation. The IR spectrum showed the presence of a hydroxyl group (3426 cm^{-1}) and aromatic moieties (1593, 1512, 1463 cm^{-1}), and a very intense absorption band at 1126 cm^{-1} suggested C–O–C functionalities. Its ^{1}H-NMR spectrum (Table 1) suggested **1** as an asymmetric tetrahydrofuran lignan,[21,22] since signals corresponding to an oxybenzyl methine (δ_H 4.80, d, 10.1 Hz) and two methyl protons (δ_H 1.31, s; 0.94, d, 6.8 Hz) could be observed. The chemical shifts observed for aromatic protons at δ_H 6.74 (s, 2H), 6.97 (d, 8.3 Hz, 1H), 7.08 (d, 8.3 Hz, 1H), and 7.12 (s, 1H), associated with the presence of intense singlets corresponding to five methoxyl protons (δ_H 3.76, 3H; 3.84, 3H; 3.85, 3H; 3.86, 6H), indicated the substitution pattern as 3,4-dimethoxyphenyl and 3′,4′,5′-trimethoxyphenyl for the two aromatic rings. The ^{13}C-NMR spectrum (Table 1) corroborated the assignments made for the structural determination of both aromatic rings. As expected, the symmetric 3′,4′,5′-trimethoxyphenyl ring displayed only four different

Table 1. ^{1}H- and ^{13}C-NMR Assignments of **1** and **2**

Position	**1**		**2**	
	δ_C (mult.)	δ_H (mult., *J*, Hz)	δ_C (mult.)	δ_H (mult., *J*, Hz)
1	130.8 s		130.4 s	
2	113.4 d	7.12 (s)	113.2 d	7.06 (s)
3	149.9 s		150.8 s	
4	150.4 s		150.0 s	
5	112.2 d	6.97 (d, 8.3)	112.3 d	6.98 (d, 8.4)
6	122.2 d	7.08 (d, 8.3)	121.7d	7.07 (dd, 2.0, 8.4)
7	113.7 s		109.4 s	
8	83.5 s		73.5 s	
9	19.4 q	1.31 (s)	10.8 q	1.44 (s)
1′	138.6 s		136.2 s	
2′	105.7 d	6.74 (s)	106.9 d	6.91 (s)
3′	154.5 s		154.7 s	
4′	139.2 s		140.2 s	
5′	154.5 s		154.7 s	
6′	105.7 d	6.74 (s)	106.9 d	6.91 (s)
7′	89.0 d	4.80 (d, 10.1)	87.7 d	5.12 (s)
8′	50.4 d	2.44 (m)	71.3 s	
9′	8.9 q	0.94 (d, 6.8)	12.8 q	1.15 (s)
3-OMe	56.4 q	3.84 (s)	56.4 q	3.85 (s)
4-OMe	56.4 q	3.85 (s)	56.5 q	3.85 (s)
7-OMe	50.6 q	3.21 (s)	50.7 q	3.13 (s)
3′-OMe	56.6 q	3.86 (s)	56.5 q	3.87 (s)
4′-OMe	61.1 q	3.76 (s)	61.1 q	3.74 (s)
5′-OMe	56.6 q	3.86 (s)	56.5 q	3.87 (s)

All spectra were recorded in CD_3OD at 400 MHz. δ in ppm, *J* in Hz.

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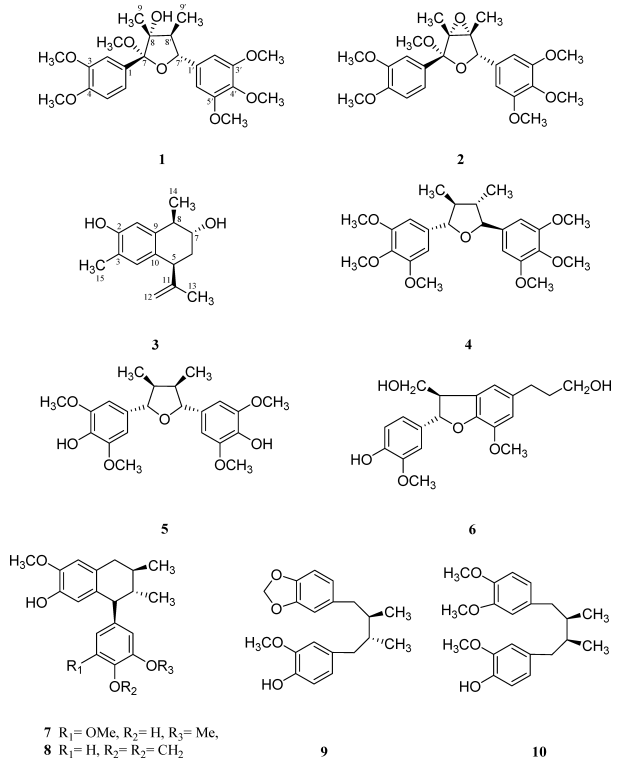

Fig. 1. The Structures of Compounds **1**—**10**

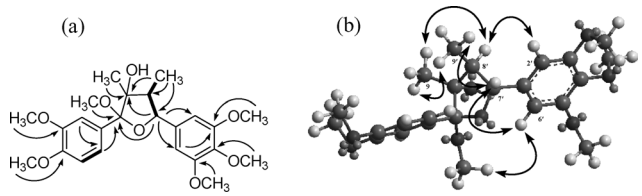

Fig. 2. (a) ^{1}H, ^{1}H COSY (—) and Key HMBC (H→C) Correlations of **1**; (b) Key ROESY (↔) Correlations of **1**

chemical shifts for aromatic carbons (δ_C 105.7, 138.6, 139.2, 154.5), while for the second aromatic ring bearing two methoxyl groups, the corresponding signals could be assigned (δ_C 112.2, 113.4, 122.2, 130.8, 149.9, 150.4). Extensive analysis of HSQC, ^{1}H–^{1}H COSY, and HMBC spectral data led to the establishment of the functional groups (Fig. 2). The HMBC correlations from H-7′ to C-2′ and C-6′, and from H-2, H-6 to C-7, confirmed the location of each oxygenated aromatic ring to the tetrahydrofuran ring. The HMBC correlations from methoxyl protons to C-3, C-4, C-3′, C-4′, and C-5′ indicated that the five methoxyl groups are attached at C-3, C-4, C-3′, C-4′, and C-5′, respectively. Except for the presence of the above five methoxyl groups, an especially upfield signal of methoxyl group (δ_H 3.21, δ_C 50.6) was observed in ^{1}H- and ^{13}C-NMR spectrum, which was attached at C-7. This was established by HMBC correlation observed from methoxyl protons to C-7 and the relative downfield shift of C-7 (δ_C 113.7, s). In addition, the HMBC correlations from H_3-9, H_3-9′, H-7′ to the oxygenated quaternary carbon C-8 (δ_C 83.5, s), along with the analysis of its molecular formula, indicated that C-8 of **1** was substituted by a hydroxyl group. The relative configuration of **1** was shown

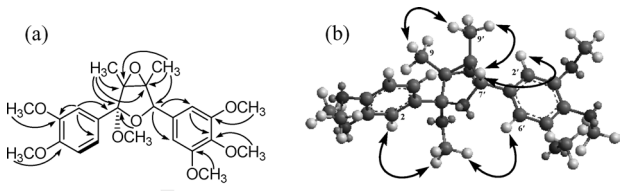

Fig. 3. (a) ^{1}H, ^{1}H COSY (—) and Key HMBC (H→C) Correlations of **2**; (b) Key ROESY (↔) Correlations of **2**

to be as depicted in Fig. 2b by the correlations observed in a ROESY experiment. ROESY correlations were observed between H-7′ and H-6′ (H-2′), H_3-9 and H_3-9′ ; H-7′ also gave a ROESY correlation to H_3-9′, which established that H-7′, H_3-9′, and H_3-9 were in β-orientation, and the ROESY correlations of H-8′ with H-2′ (H-6′), and CH_3O-7 with H-6′ (H-2′) indicated H-8′ and CH_3O-7 were in α-orientation. Thus the structure of **1** was determined, and named as kadlongirin A. Note that compound **1** is the first example of a tetrahydrofuran lignan substituted at C-7 with a methoxyl group in *Kadsura* species.

Compound **2**, obtained as yellowish oil, had the molecular formula $C_{24}H_{30}O_8$, as derived from HR-ESI-MS at *m/z* 469.1850 ($[M+Na]^+$, Calcd 469.1838). The UV, IR, and NMR spectra established that **2** possessed tetrahydrofuran lignan skeleton,[21,22] differing from **1** by the loss of two hydrogens. Detailed comparison of ^{1}H- and ^{13}C-NMR data of **2** with those of **1** showed close analogy. The major differences included the appearance of a tetrasubstituted epoxide (δ_C 71.3, s; 73.5, s) in **2** and disappearance of a methine (δ_C 50.4, d) in **1**. The HMBC (Fig. 3a) correlations from H_3-9 and H_3-9′ to C-8 and C-8′, respectively, further verified that the epoxide group was positioned between C-8 and C-8′. In the ROESY (Fig. 3b) experiment, H_3-9′ showed strong correlations with H_3-9 and H-7′, and H-7′ with H-2′ (H-6′), indicating an α-orientation of the epoxide group. CH_3O-7 (δ_H 3.13, s) showed correlation with H-2 and H-6′ (H-2′), indicating its α-orientation. Thus all of these data confirmed the structure for **2** with an epoxide of α-orientation located between C-8, and C-8′, named as kadlongirin B.

Compound **3** was obtained as yellow amorphous powder. Its molecular formula, $C_{15}H_{20}O_2$, was determined by the pseudo-molecular ion peak $[M+Na]^+$ in the positive HR-ESI-MS at *m/z* 255.1361 (Calcd 255.1360), indicating 6 degrees of unsaturation. The IR spectra revealed the presence of hydroxyl group (3423 cm^{-1}) and phenolic group (1449, 1643 cm^{-1}).[23] The ^{1}H-NMR spectrum of **1** (Table 1) showed two aromatic protons (δ_H 6.55, s; 6.73, s), suggesting a tetrasubstituted aromatic spin-system with a *para* configuration. A series of complex signals at δ_H 4.64, 4.88 (2H, each br s), δ_H 3.79 (1H, ddd, 2.4, 4.8, 6.8 Hz), δ_H 3.58 (1H, dd, 7.6, 8.4 Hz), and 2.65 (1H, m) was indicative of a characteristic olefinic methylene and carbinolic and two benzyl hydrogens. Further signals at δ_H 1.25 (3H, d, 7.1 Hz), δ_H 1.65 (3H, s), and 2.08 (3H, s) revealed the presence of two aliphatic groups and an aromatic methyl group in the molecule. The ^{13}C-NMR data (Table 2) and DEPT experiments clearly indicated the basic C_{15}-sesquiterpene skeleton of the cadinane skeleton.[24,25] The structure of **3** was elucidated from HSQC, ^{1}H–^{1}H COSY, and HMBC studies (Fig. 4a). The HMBC correlations of H_3-15 to C-2, C-3, and C-4, and H-4 to C-2, con-

Table 2. ^{1}H- and ^{13}C-NMR Assignments of **3**

Position	**3**	
	δ_C (mult.)	δ_H (mult., *J*, Hz)
1	115.3 d	6.55 (s)
2	154.9 s	
3	123.4 s	
4	131.7 d	6.73 (s)
5	44.7 d	3.58 (dd, 7.6, 8.4)
6α	33.1 t	1.78 (ddd, 6.8, 7.6, 12.2)
6β		2.03 (ddd, 2.4, 8.4, 12.2)
7	71.7 d	3.79 (ddd, 2.4, 4.8, 6.8)
8	41.9 d	2.65 (m)
9	139.8 s	
10	128.3 s	
11	150.3 s	
12a	113.9 t	4.88 (s)
12b		4.64 (s)
13	19.7 q	1.65 (s)
14	21.8 q	1.25 (d, 7.1)
15	15.9 q	2.08 (s)

All spectra were recorded in CD_3OD at 400 MHz. δ in ppm, *J* in Hz.

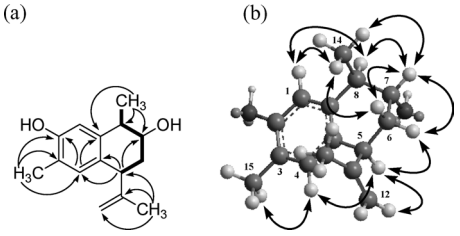

Fig. 4. (a) ^{1}H, ^{1}H COSY (—) and Key HMBC (H→C) Correlations of **3**; (b) Key ROESY (↔) Correlations of **3**

Table 3. Anti-HIV-1 Activities of the New Compounds

Compound	CC_{50} (μg/ml)[a]	EC_{50} (μg/ml)[b]	TI (CC_{50}/EC_{50})[c]
1	96.1	51.8	1.9
2	106.7	16.0	6.7
3	61.5	12.8	4.8
Positive control AZT	1146.1	2.03 ng/ml	564571.4

a) CC_{50}=concentration causing 50% cellular cytotoxicity. *b*) EC_{50}=50% effective concentration. *c*) TI=therapeutic index.

firmed the location of the phenolics function was located at C-2. In addition, the following HMBC correlations: H_3-14 to C-7, C-8, and C-9; H-5 to C-7, and C-10; and H_3-13 to C-5, C-11, and C-12, as well as the ^{1}H–^{1}H COSY spin system H-5/H_2-6/H-7/H-8/H_3-14, suggested that a hydroxyl group and an isopropenyl group were located at C-7 and C-5, respectively. The linkage of the *p*-menthane moiety with the aromatic moiety was determined through the correlations of H_3-14 to C-9, and H-5 to C-10. The ROESY spectrum (Fig. 4b) further determined the stereochemistry of **3**: correlations of H_3-14 with H-1, H-7, and H-6β, and H-7 with H_2-6 and H-8, suggested that H_3-14 and H-7 were in β-orientation; correlations of H-5 with H-4, and H-6α showed that H-5 should be positioned on the α-orientation. Therefore 2,7-dihydroxy-11,12-dehydrocalamenene was assigned as showed in Fig. 1.

The anti-HIV activity was indicated as potencies of the new compounds **1**—**3** in preventing the cytopathic effects of HIV-1 in C8166 cells, with cytotoxicity measured in parallel with the determination of antiviral activity using AZT as a positive control (EC_{50}=2.03 ng/ml and CC_{50}=1146.08 μg/ml). Compound **2** exhibited weak anti-HIV-1 activity with an EC_{50} value of 16.0 μg/ml and TI value of 6.7 (Table 3).

Experimental

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were obtained on a Bio-Rad FtS-135 spectrophotometer with KBr pellets, whereas UV data were obtained using a UV-210A spectrometer. MS were recorded on a VG Auto Spec-3000 spectrometer. 1D-NMR spectra were obtained on a Bruker DRX-400 instrument with TMS as an internal standard, and 2D-NMR spectra were obtained on a Bruker DRX-500 instrument with TMS as an internal standard. Column chromatography (CC) and TLC: Si gel (200—300 mesh) from Qingdao Marine Chemical Factory, Quingdao, People's Republic of China.

Plant Material The leaves and stems of *K. longipedunculata* were collected in Yibin region of Sichuan Province, China, in August 2004, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The air-dried and powdered leaves and stems (10 kg) of *K. longipedunculata* were extracted with 70% aqueous Me_2CO (3×30 l) at room temperature to yield an extract, which was successively extracted with petroleum ether and EtOAc. The EtOAc extract was evaporated to dryness under reduced pressure to give an extract (400 g) that was separated by Si gel CC (2.0 kg, 200—300 mesh) and eluted with a $CHCl_3$/Me_2CO gradient system (9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5) to give fractions 1—5. Fractions 1 (180 g) and 2 (108 g) were subjected to CC with Petroleum ether/$(CH_3)_2CO$ gradient system (40 : 1, 20 : 1, 10 : 1, 5 : 1, 2 : 1) to afford 5 fractions, which were further purified by semipreparative HPLC (Agilent 1100 HPLC system, U.S.A.; Zorbax SB-C-18, Agilent, 9.4 mm×25 cm, U.S.A., MeOH–H_2O) to give compounds **1** (2 mg), **3** (6 mg), **4** (8 mg), **5** (15 mg), **9** (152 mg), **8** (12 mg), and **10** (14 mg). Fraction 3 (30 g) was subjected to CC with $CHCl_3$/CH_3OH (20 : 1) to afford 6 fractions that were further purified by Sephadex LH-20 (CH_3OH) to afford another 8 fractions, which were purified by semipreparative HPLC (MeOH–H_2O) to give compounds **2** (8 mg), **6** (16 mg), and **7** (71 mg).

Kadlongirin A (**1**): Yellowish oil; $[\alpha]_D^{27.8}$ +46 (*c*=0.73, MeOH); UV (MeOH) λ_{max} (log ε): 206 (5.07), 276 (3.77), 314 (2.87), 360 (2.91) nm; IR (KBr) ν_{max}: 3426, 2994, 2961, 2936, 2834, 1593, 1512, 1463, 1423, 1410, 1372, 1329, 1267, 1235, 1163, 1126, 1099, 1082, 1026, 1009, 985, 935, 870, 810, 764 cm^{-1}; ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz): see Table 1; positive ESI-MS: *m/z* 935 $[2M+K]^+$ (3), 919 $[2M+Na]^+$ (44), 487 $[M+K]^+$ (21), 471 $[M+Na]^+$ (45), 417 (100), 399 (4), 317 (8), 209 (31); positive HR-ESI-MS: *m/z* 471.1996 $[M+Na]^+$, (Calcd for $C_{24}H_{32}O_8Na$, 471.1994).

Kadlongirin B (**2**): Yellowish oil; $[\alpha]_D^{27.5}$ +85 (*c*=0.50, MeOH); UV (MeOH) λ_{max} (log ε): 206 (5.16), 275 (3.93), 363 (2.94), 374 (2.80) nm; IR (KBr) ν_{max}: 3440, 2937, 2838, 1630, 1513, 1463, 1425, 1330, 1263, 1229, 1126, 1102 cm^{-1}; ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz): see Table 1; positive ESI-MS: *m/z* 915 $[2M+Na]^+$ (70), 485 $[M+K]^+$ (7), 469 $[M+Na]^+$ (100), 441 (65); positive HR-ESI-MS: *m/z* 469.1850 $[M+Na]^+$, (Calcd for $C_{24}H_{30}O_8Na$, 469.1838).

2,7-Dihydroxy-11,12-dehydrocalamenene (**3**): Yellow amorphous powder; $[\alpha]_D^{21.5}$ −85 (*c*=0.59, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.80), 282 (3.65), 334 (2.37), 354 (2.32), 365 (2.44) nm; IR (KBr) ν_{max}: 3423, 2964, 2931, 2875, 1643, 1621, 1504, 1449, 1413, 1373, 1260, 1189, 1131, 1052, 1031, 1005, 893 cm^{-1}; ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz): see Table 2; positive ESI-MS: *m/z* 487 $[2M+Na]^+$ (52), 255 $[M+Na]^+$ (51), 252 (100), 173 (16); positive HR-ESI-MS: *m/z* 255.1361 $[M+Na]^+$, (Calcd for $C_{15}H_{20}O_2Na$, 255.1360).

Anti-HIV-1 Assay The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[26]

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