Three New Lignans, Longipedunins A—C, from *Kadsura longipedunculata* and Their Inhibitory Activity against HIV-1 Protease

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Three new lignans, longipedunins A (1), B (2) and C (3), together with three known compounds, benzoyl-binankadsurin A (4), acetyl-binankadsurin A (5) and schisanlactone A (6), were isolated from *Kadsura longipedunculata*. Their structures and stereochemistry were determined by spectral and single-crystal X-ray analyses. Compounds 1 and 6 showed appreciable inhibitory activity against HIV-1 protease with IC_{50} values of 50 and 20 μM, respectively.

Key words longipedunin; *Kadsura longipedunculata*; HIV-1 protease; lignan; X-ray crystal structure

As a major limitation of current chemotherapy against the AIDS virus (HIV-1) is the rapid emergence of resistant mutants, the identification of new anti-HIV agents is still urgently needed. One of our approaches is to find new inhibitory compounds against HIV-1 protease (PR), which is essential for the maturation of the virus and is regarded as one of the most promising targets. We have isolated some triterpenes,[1—3] saponins,[4] tannins,[1,3] and amides[5] as HIV-1 PR inhibitory substances from several folk medicines. To find other different types of HIV-1 PR inhibitory compounds to compare their activity and to select the most promising ones for further development, we have worked on the EtOH extract of the roots and stems of *Kadsura longipedunculata* FINET. et GAGNEP. (Schizandraceae). *K. longipedunculata* is an evergreen shrub, widely indigenous to the southern part of China. As a folk medicine, its roots and stems are commonly used for the treatment of gastric and duodenal ulcers, rheumatoid arthritis, menstrual irregularities and other feminine disorders.[6] From this folk medicine, three new lignans, longipedunins A (**1**), B (**2**) and C (**3**), together with benzoyl-binankadsurin A (**4**),[7] acetyl-binankadsurin A (**5**)[8] and schisanlactone A (**6**)[9] (Fig. 1) were isolated. The isolation, structural elucidation and HIV-1 protease inhibitory activity of the isolated compounds are reported herein.

Results and Discussion

Longipedunin A (**1**), obtained as colorless prisms (mp 176—178 °C), has the molecular formula $C_{31}H_{32}O_8$ as revealed by its high-resolution electron impact mass spectrum (HR-EI-MS) (*m*/*z* 532.2073). The nature of the structure was deduced from ^{1}H- and ^{13}C-NMR spectra, the UV spectrum with absorption maxima at 222 (log ε 4.19), 258 (log ε 3.91), and 278 (log ε 3.91) nm, and the IR spectrum with bands at 3470 (OH), 1690 (ester), 1620 and 1590 (aromatic) cm^{-1}, indicating that **1** could be a dibenzocyclooctadiene lignan with hydroxyl and ester groups. The ^{1}H-NMR spectrum of **1** showed signals due to two secondary methyl groups (δ 1.01, 1.13, 3H each, d, J=7.0 Hz), assigned to CH_3-7 and CH_3-8 groups, respectively; one multiplet signal at δ 2.12 (2H, m) which exhibited a ^{1}H–^{1}H correlation with two secondary methyls was assigned to H-7 and H-8; another multiplet signal at δ 2.69 (2H, m), which exhibited a ^{1}H–^{1}H correlation with δ_H 2.12 was assigned to H_2-6. Other partial structures could be assembled by the HMBC experiment (Fig. 2), where three methoxy groups (δ 3.55, 3.84, 3.93, each 3H, s) were assigned to C-2, C-14 and C-3, respectively; one singlet at δ 5.49 (1H, s) was assigned to C1-OH; another singlet at δ 5.72 (1H, s) was assigned to H-9 as a benzylic methine carrying an oxygen-bearing group; two signals at δ 5.95 and 5.99 (1H each, d, J=1.0 Hz) were characteristic for a methylenedioxy group on C-12 and C-13; and two aromatic protons (δ 6.46, 6.56, 1H each, s) , assigned to C-4 and C-11, respectively. The ^{1}H-NMR spectrum also showed the presence of a *trans*-cinnamic acid ester with proton signals at δ_H 5.86, 7.07

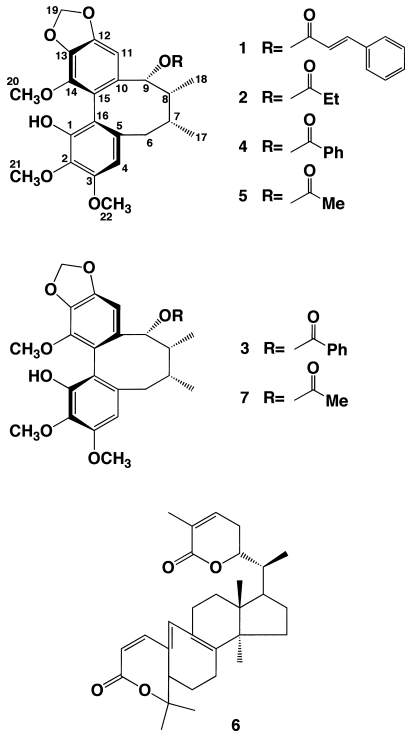

Fig. 1. Structures of Compounds **1**—**7**

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(1H each, d, J=16.0 Hz) and aromatic proton signals at δ 7.34 (5H, m). Carbon signals at δ_C 117.8, 144.2 (C-24, 25), 128.0 (C-27, 31), 128.7 (C-28, 30), 130.0 (C-29), as well as a signal at δ_C 134.4 (C-26), and a carbonyl carbon at δ_C 166.0 (C-23) supported this deduction. Furthermore, the mass spectrum of **1** presented a molecular ion at m/z 532 and a characteristic peak at m/z 385 [$M^+ - C_6H_5C_2H_2COO$], which confirmed the presence of a cinnamic acid ester. These findings were further supported by the long-range correlation (HMBC) spectrum of **1** (Fig. 2). The nuclear Oberhauser effect (NOE) spectrum showed an enhancement between H-11 and H-9, suggesting that a cinnamic acid ester group was located at the 9α position. It showed correlated peaks between CH_3-7 and H-4, CH_3-8 and H-9, indicating that both CH_3-7 and CH_3-8 were α-oriented; an additional peak between H-4 and H-6α led to a twist-boat-chair conformation of the cyclooctadiene ring[10] (Fig. 2). To confirm its complete structure, single-crystal X-ray analyses were carried out. As shown in Fig. 3, the structure of **1** was unambiguously confirmed as deduced by spectral analysis. A relatively highfield signal at δ 3.55 (C2-OCH_3) of the methoxyl might be explained by shielding effects of the *trans*-cinnamic acid ester group in structure **1**. The biphenyl group in **1** was determined to have an *S*-biphenyl configuration by a CD spectrum with a negative Cotton effect around 312 nm.[11] On the basis of the above results, the absolute structure of longipedunin A was thus determined as **1**.

Longipedunin B (**2**), obtained as an amorphous powder, has the molecular formula $C_{25}H_{30}O_8$ as revealed by its HR-EI-MS (m/z 458.1894), and the UV, IR, CD, and NMR spectral features are similar to those of **1**. The structural difference between **2** and **1** is only in the acyl moiety of C-9α: a propionyl acid ester group in **2** instead of a cinnamic acid ester group in **1**. The mass spectrum of **2** showed a molecular ion at m/z 458 and a characteristic peak at m/z 385 [$M^+ - C_2H_5COOH$], which confirmed the presence of the above substitution. Thus, the structure of **2** was determined as shown in Fig. 1.

Longipedunin C (**3**), obtained as an amorphous powder, had the molecular formula $C_{29}H_{30}O_8$ as revealed by its HR-EI-MS (m/z 506.1941). Comparison of the UV, IR and ^{1}H-NMR spectra of **3** with those of benzoyl-binankadsurin A (**4**), showed that they possessed the same substitutions. Thus the plane structure of **3** was the same as that of **4**, but the stereo-structure between them was quite different. The CD

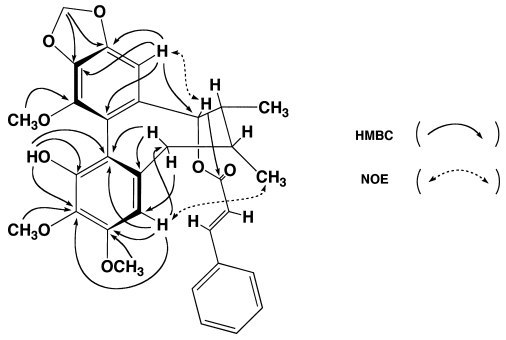

Fig. 2. Key HMBC Correlations and NOE Interactions of **1**

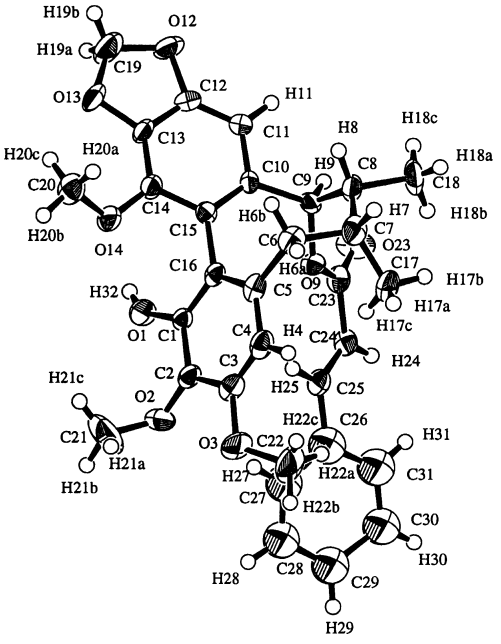

Fig. 3. The X-Ray Crystallographic Structure (ORTEP Drawing) of **1**

Table 1. ^{1}H-NMR Data for Compounds **1**—**3** ($CDCl_3$)

Position	**1**[a]	**2**[b]	**3**[b]
4	6.46 (s)	6.39 (s)	6.37 (s)
6	2.69 (2H, m)	2.63 (2H, m)	2.05, 2.27 (m)
7	2.12 (m)	2.05 (m)	2.16 (m)
8	2.12 (m)	2.05 (m)	2.16 (m)
9	5.72 (br s)	5.56 (br s)	5.69 (d, 1.2 Hz)
11	6.56 (s)	6.50 (s)	6.89 (s)
17	1.01 (3H, d, 7.0 Hz)	0.91 (3H, d, 7.0 Hz)	0.84 (3H, d, 7.1 Hz)
18	1.13 (3H, d, 7.0 Hz)	1.08 (3H, d, 7.0 Hz)	1.05 (3H, d, 7.1 Hz)
19	5.95 (d, 1.0 Hz)	5.94 (d, 1.4 Hz)	5.95 (d, 1.5 Hz)
	5.99 (d, 1.0 Hz)	5.98 (d, 1.4 Hz)	6.01 (d, 1.5 Hz)
20	3.84 (3H, s)	3.84 (3H, s)	3.91 (3H, s)
21	3.55 (3H, s)	3.90 (3H, s)	3.91 (3H, s)
22	3.93 (3H, s)	3.90 (3H, s)	3.92 (3H, s)
1-OH	5.49 (s)	—	5.69 (s)
R	5.86 (1H, d, 16.5 Hz)	1.74, 1.86 (each 1H)	8.04 (2H, dd, 8.1, 1.4 Hz)
	7.07 (1H, d, 16.5 Hz)	0.85 (3H, t, 7.6 Hz)	7.42 (2H, t, 8.1 Hz)
	7.34 (5H)		7.54 (1H, m)

a) 500 MHz. *b*) 400 MHz.

Table 2. ^{13}C-NMR Data for Compounds **1**—**3** ($CDCl_3$)

Position	**1**[a)]	**2**[b)]	**3**[b)]
1	146.8 s	146.6 s	146.9 s
2	133.3 s	133.4 s	133.5 s
3	150.2 s	150.3 s	152.3 s
4	106.9 d	107.2 d	104.0 d
5	133.4 s	133.7 s	139.4 s
6	38.6 t	38.6 t	34.9 t
7	34.8 d	35.0 d	40.7 d
8	41.7 d	41.6 d	38.9 d
9	82.8 d	82.7 d	76.7 d
10	135.5 s	135.9 s	131.9 s
11	102.7 d	102.8 d	102.4 d
12	148.9 s	148.9 s	148.5 s
13	136.0 s	136.1 s	135.7 s
14	141.2 s	141.3 s	140.9 s
15	119.2 s	119.0 s	120.2 s
16	117.1 s	117.0 s	114.0 s
17	15.1 q	14.8 q	8.7 q
18	19.7 q	19.8 q	21.9 q
19	101.2 t	101.2 t	101.1 t
20	59.8 q	59.8 q	59.8 q
21	60.5 q	60.8 q	61.1 q
22	55.7 q	55.8 q	55.7 q
23	166.0 s	173.6 s	165.2 s
24	117.8 d	27.0 t	130.7 s
25	144.2 d	8.6 q	129.6 d
26	134.4 s		128.3 d
27	128.0 d		132.8 d
28	128.7 d		128.3 d
29	130.0 d		129.6 d
30	128.7 d		
31	128.0 d		

a) 125 MHz. *b*) 100 MHz.

Table 3. Inhibitory Activity of Compounds **1**—**6** against HIV-1 Protease

Compound	% Inhibition (100 μg/ml)	IC_{50} (μg/ml)
1	77.8±3.3	50
2	41.0±12.6	>100
3	47.3±1.4	>100
4	21.6±3.4	>100
5	12.8±4.1	>100
6	94.8±0.1	20
Acetyl pepstatin	100	0.15

spectrum of **3** exhibited a positive Cotton effect around 254 nm, indicating an *R*-biphenyl configuration.[11] Direct comparisons of the CD and ^{13}C-NMR spectra of **3** with those of schisantherin O (**7**)[12] showed that they were almost identical, their structural difference being only in the acyl moiety of C-9α. Therefore, longipedunin C was assigned as **3**, which was a diastereomer of benzoyl-binankadsurin A (**4**).

Longipedunin A (**1**), longipedunin B (**2**), longipedunin C (**3**), benzoyl-binankadsurin A (**4**), acetyl-binankadsurin A (**5**) and schisanlactone A (**6**) were tested for HIV-1 protease inhibitory effects. As shown in Table 3, schisanlactone A (**6**) showed potent HIV-1 protease inhibitory activity with IC_{50} values of 20 μM. It is interesting to note that unlike common HIV-1 protease inhibitors, schisanlactone A (**6**) has neither hydroxyl nor carboxyl group in its structure, but showed inhibitory activity against HIV-1 PR with a potency comparable to some triterpene acids.[3] Among dibenzocyclooctadiene lignans (**1**—**5**) with the same skeleton, longipedunin A (**1**) demonstrated higher activity (IC_{50} 50 μM) than the others, indicating that a *trans*-cinnamic acid ester group might be an important functional group.

Experimental

General Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured in MeOH solution using a JASCO DIP-360 automatic polarimeter at 28 °C. CD spectra were taken on J-805 in MeOH solution. IR spectra were measured with a JASCO FT-IR-230 infrared spectrophotometer. UV spectra were taken on a Shimadzu UV-2200 UV-VIS spectrophotometer in MeOH solution. ^{1}H- and ^{13}C-NMR spectra were taken on a Varian Unity Plus 500 (^{1}H, 500 MHz; ^{13}C 125 MHz) or a JEOL JNA-LA 400 WB-FT (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer with chemical shifts being represented in parts per million (ppm) and tetramethylsilane (TMS) as an internal standard. EI-MS and HR-EI-MS were measured on a JEOL JMS-AX 505 HAD mass spectrometer at an ionization voltage of 70 eV.

Chromatography BW-820MH (Fuji Silysia) was used for column chromatography. TLC was performed on Merck Kieselgel 60 F_{254} plates (Merck Co., Darmstadt, Germany), and spots on the plate were observed under a UV light and visualized by spraying with 10% H_2SO_4 reagent followed by heating. Preparative TLC was carried out on pre-coated silica gel 60 F_{254} plates (0.5 mm, Merck Co.). Medium pressure liquid chromatography (MPLC) was carried out on a LiChroprep Si60 (size A, Merck Co.).

Extraction and Isolation The air-dried roots and stems of *kadsura longipedunculata* (8.0 kg) were pulverized and extracted 6 times with 95% EtOH (each 5 l) at room temperature. The EtOH extract was evaporated *in vacuo* to yield an oily residue (1240 g), which was suspended in water (2000 ml), followed by extraction with ether (2000 ml×5). The ether solution was concentrated to yield a darkly brown residue (365 g), which was applied to a silica gel column and eluted with petroleum ether containing increasing amounts of EtOAc. The fractions eluted with petroleum ether–EtOAc (70 : 30) were subjected to repeated column chromatography with hexane–EtOAc (85 : 15) to yield longipedunin A (**1**, 18 mg). Those obtained from petroleum ether–EtOAc (60 : 40) elution were combined and subjected to MPLC (benzene–EtOAc 80 : 20) to yield longipedunin B (**2**, 4 mg); and those obtained from petroleum ether–EtOAc (50 : 50) elution were combined and subjected to preparative TLC to yield longipedunin C (**3**, 6 mg).

Longipedunin A (**1**): Colorless prisms (MeOH); mp 176—178 °C; $[\alpha]_D$ +52.2° (c=0.2, MeOH); CD (c=2×10^{-4} g/ml, MeOH): −3700 (312 nm); UV λ_{max} (MeOH) nm (log ε): 222 (4.19), 258 (3.91), 278 (3.91); IR (KBr) ν_{max} cm^{-1}: 3470, 1690, 1620, 1590; EI-MS *m/z* (%): 532 (100), 506 (54), 401 (20), 385 (100), 369 (100), 353 (100), 341 (89), 147 (100), 105 (100); HR-EI-MS *m/z* 532.2073 (Calcd for $C_{31}H_{32}O_8$, 532.2098 M$^+$); ^{1}H- and ^{13}C-NMR see Tables 1 and 2.

Single-crystal analysis was made on a Rigaku AFC7R diffractometer with graphite monochromated Mo$K\alpha$ radiation (50 kV, 120 mA) and a rotating anode generator. Crystal data: $C_{31}H_{32}O_8$, formula weight=532.59, crystal size (w×d×h mm^3)=0.22×0.26×0.43, colorless prismatic, monoclinic, space group $P2_1$(#4), a=11.320 (2) Å, b=10.665 (3) Å, c=11.680 (2) Å, β=107.74 (1), V=1343.0 (5) Å^3, Z=2, D_{calcd}=1.317 g/cm^3, F_{000}=564.00, μ(Mo$K\alpha$)=0.95 cm^{-1}. Number of reflections observed (I>2.0σ(I))=2317, R1=0.0395, R_w=0.1380. Further details of the X-ray structure data are available on request from the Cambridge Crystallographic Data Centre (deposition number CCDC 286065).

Longipedunin B (**2**): Amorphous powder; $[\alpha]_D$ +8.3° (c=0.1, MeOH); CD (c=1×10^{-4} g/ml, MeOH) −14060 (312 nm); UV λ_{max} (MeOH) nm (log ε): 218 (3.80), 254 (3.18), 280 (2.81) ; IR (KBr) ν_{max} cm^{-1}: 3400, 1720, 1460, 1360, 1100; EI-MS *m/z* (%): 458 (8), 457 (28), 384 (35), 383 (100), 368 (19), 340 (17), 83 (10); HR-EI-MS *m/z* 458.1894 (Calcd for $C_{25}H_{30}O_8$, 458.1941 M$^+$); ^{1}H- and ^{13}C- NMR see Tables 1 and 2.

Longipedunin C (**3**): Amorphous powder; $[\alpha]_D$ +25.4° (c=0.2, MeOH); CD (c=2×10^{-4} g/ml, MeOH); UV λ_{max} (MeOH) nm (log ε): 225 (4.15), 254 (3.48), 280 (3.00); IR (KBr) ν_{max} cm^{-1}: 3400, 1700, 1460, 1260, 1080; EI-MS *m/z* (%): 506 (46), 466 (38), 438 (33), 385 (54), 384 (100), 355 (57), 105 (100); HR-EI-MS *m/z* 506.1941 (Calcd for $C_{29}H_{30}O_8$, 506.1941 M$^+$); ^{1}H- and ^{13}C-NMR see Tables 1 and 2.

Benzoyl-binankadsurin A (**4**), acetyl-binankadsurin A (**5**) and schisanlactone A (**6**) were identified on the basis of comparison of their physical and spectral properties with those reported in the literature.[7—9]

HIV Protease Assay An HIV protease assay kit (Bachem Feinchemikalien AG, Bubendorf, Switzerland; lot no. PR D-00070) was used.

The HIV protease (HIV PR) inhibition assay was performed, and inhibitory activity was calculated as described previously[1]. Acetyl pepstatin from the same assay kit was used as a positive control, with its IC_{50} being 0.24 μM.

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