Notes

Two New Lignans, Interiotherins A and B, as Anti-HIV Principles from *Kadsura interior*

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Two new lignans, interiotherins A (**1**) and B (**2**), along with two known lignans, angeloylgomisin R (**3**) and schisantherin D (**4**), were isolated from *Kadsura interior*. Their structures and stereochemistries were determined from spectral data. Compounds **1** and **4** inhibit HIV replication with EC_{50} values of 3.1 and 0.5 μg/mL, respectively.

The stems of *Kadsura interior* A. C. Smith (Schizandraceae), a plant indigenous to southern China, are known as "Ji-Xue-Teng" in traditional Chinese medicine and have been used for the treatment of menstrual irregularities, blood deficiencies, and other feminine disorders.[1] Many dibenzocyclooctadiene lignans including kadsurin and interiorin have been isolated from *K. interior*.[2,3] Kadsurin and interiorin have been found to exhibit significant in vitro and in vivo antilipid peroxidative effects.[4,5] In the course of our continuing search for natural products as anti-AIDS agents, the EtOH extract of the stems of *K. interior* was found to show significant inhibitory activity in vitro against HIV replication in H9 lymphocytes. Bioactivity-directed fractionation of this extract has led to the isolation and identification of two new dibenzocyclooctadiene lignans, interiotherins A (**1**) and B (**2**), along with angeloylgomisin R (**3**)[6] and schisantherin D (**4**) (Figure 1).[7] The isolation, structural elucidation, and anti-HIV activity of interiotherins A and B are reported herein.

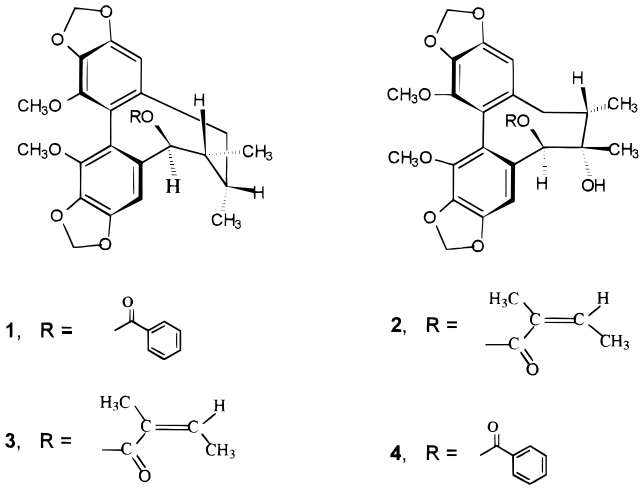

Figure 1.

Interiotherin A (**1**), obtained as colorless prisms (mp 153−154 °C), has the molecular formula $C_{29}H_{28}O_8$ as revealed by its HRMS (*m/z* 504.1785). The UV spectrum of **1** showed maximum absorption at 222 (log ϵ, 4.84), 255 (4.10), 278 (3.79) nm, indicating that **1** is a dibenzocyclooctadiene lignan.[8] The ^{1}H-NMR spectrum of **1** was similar to that of **3**, showing signals due to two secondary methyl groups (δ 0.86, δ 1.02, 3H each, d, J = 7.0 Hz), assignable to CH_3-7 and CH_3-8 groups, respectively; two methylenedioxy moieties (δ 6.0, δ 5.88, 2H each, AB); two methoxy groups (δ 3.80, δ 3.58, 3H each, s) on two aromatic rings; and a benzoyl group (δ 7.64, 2H, m, δ 7.49, 1H, m, δ 7.31, 2H, m). A multiplet signal at δ 2.06 (2H, m), which exhibited ^{1}H−^{1}H correlation with the two secondary methyls was assigned to H-7 and H-8. The signal at δ 5.88 (1H, m), which also correlated with the multiplet signal at δ 2.06, was assigned to a benzylic methine carrying an oxygen-bearing group. Two aromatic protons appeared at high field at δ 6.70 and δ 6.50, indicating that the methylenedioxy moieties should be linked adjacent to these aromatic protons.[9] The NOESY spectrum of **1** showed correlated peaks between CH_3-8 and H-4, CH_3-8 and H-6, indicating that **1** possesses a boat conformation of the cyclooctadiene ring. A correlated peak between H-4 and H-6 indicates that the benzoyl group was located in a β-position.[10] The configuration of the biphenyl group in **1** was determined by comparison of its CD spectrum with that of compound **3**. Both compounds showed a positive Cotton effect around 235 nm and a negative one around 251 nm, indicating that both compounds possess an *S*-biphenyl configuration.[8] The absolute structure of interiotherin A was thus elucidated as **1**.

Interiotherin B (**2**), obtained as colorless prisms (mp 96−98.5 °C), has the molecular formula $C_{27}H_{30}O_9$ as revealed by its HRMS (*m/z* 498.1926). The UV spectrum of **2** showed maximum absorption at 221 (log ϵ,

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

compound	IC_{50} (μg/mL)	EC_{50} (μg/mL)	TI
1	41	3.1	13.2
2	41	no	suppression
3	34	no	suppression
4	25.3	0.5	50.6
AZT	500	0.002	250 000

4.82), 256 (4.07), and 278 (3.63) nm, indicating that **2** is also a dibenzocyclooctadiene lignan.[8] The ^{1}H-NMR spectrum of **2** was similar to that of **4**, showing signals due to a secondary methyl group (δ 1.13, 3H, d, J = 7.0 Hz), assignable to the CH_3-8 group; a tertiary methyl group (δ 1.32, 3H, s), assignable to the CH_3-7 group; two methylenedioxy moieties (δ 6.01, δ 5.96, 2H each, AB); two methoxy groups (δ 3.84, δ 3.77, 3H each s) on two aromatic rings; and an angeloyl group (δ 1.41, 3H, q, δ 1.86, 3H, m, δ 6.01, 1H, m). The multiplet signal at δ 1.93, which exhibited ^{1}H−^{1}H correlation with the secondary methyl, was assigned to H-8. The signal at δ 5.50 (1H, s) was assigned to H-6, a benzylic methine carrying an oxygen-bearing group. Two aromatic protons appeared highfield at δ 6.71 and δ 6.46, indicating that the methylenedioxy moieties should be linked adjacent to these aromatic protons.[9] The NOESY spectrum of **2** showed a correlated peak between 6-H and 4-H, indicating that the angeloyl group was located in a β-position. Correlated peaks between 7-Me and 8-Me, 9β-H and 8-Me, 7-Me and 6α-H, and 7-Me and 8-H indicate that **2** has a twist-boat-chair conformation of the cyclooctadiene ring. The biphenyl group in **2** was determined to have a *S*-biphenyl configuration from its characteristic CD spectrum.[8] The absolute structure of interiotherin B was thus elucidated as **2**.

Interiotherin A (**1**), interiotherin B (**2**), angeloylgomisin R (**3**), and schisantherin D (**4**) were tested for in vitro inhibitory effects against HIV replication in H9 lymphocytes. The data are listed in Table 1. Among these compounds, schisantherin D (**4**) demonstrated potent anti-HIV activity with an EC_{50} value of 0.5 μg/mL and a therapeutic index (TI) of 50.6. The EC_{50} and TI values of interiotherin A (**1**) were 3.1 μg/mL and 13.2, respectively. Interiotherin B (**2**) and angeloylgomisin R (**4**) showed weak anti-HIV activity.

Experimental Section

General Experimental Procedures. Mps were determined on a Kofler micromelting point apparatus and are uncorrected. The IR spectra were recorded as KBr pellets on a Perkin-Elmer 783 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 an 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. Optical rotations were measured with a JASCO J-500A spectropolarimeter equipped with a JASCO DP-500N data processor. Analytical TLC was performed on Si gel plates (Yantai Institute of Chemical Technology) with petroleum ether−EtOAc (4:1). 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 10% H_2SO_4 followed by heating.

Plant Material. The stem bark of *Kadsura interior* A. C. Smith was collected in Feng-Qing County, Yunnan Province, People's Republic of China in August 1992. A voucher specimen 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 stem bark (6 kg) of *K. interior* was air-dried, ground, and extracted three times with 95% EtOH at room temperature. The EtOH extract was evaporated *in vacuo* to yield a semisolid (1034 g). H_2O (2500 mL) was added to the residue, and the resulting solution was extracted five times with Et_2O. This Et_2O solution was concentrated to yield 215 g of residue. The residue was chromatographed on Si gel (1250 g), employing petroleum ether containing increasing amounts of EtOAc. The fractions eluted with petroleum ether−EtOAc (90:10) gave interiotherin A (**1**, 219 mg) and angeloylgomisin R (**3**, 141 mg). The fractions eluted with petroleum ether−EtOAc (80:20) were subjected to repeated column chromatography with the same solvent to yield interiotherin B (**2**, 686 mg). Similarly, the same fractions were further purified by chromatography with petroleum ether−EtOAc (70:30) as eluent to afford schisantherin D (**4**, 142 mg).

Interiotherin A (1): prisms (MeOH); mp 153−154 °C; $[\alpha]_D$ −53.4° (*c* 2.23, $CHCl_3$); CD (*c* 0.035, MeOH) $[\theta]^{25}$ (nm) +56 113 (235), −70 989 (251); UV (MeOH) λ_{max} (log ϵ) 222 (4.84), 255 (4.10), 278 (3.79) nm; IR ν_{max} 1705, 1620, 1500, 1480 cm^{-1}; EIMS (70 eV) *m*/*z* [M]$^+$ 504 (100), 382 (80.9), 122 (17.4), 105 (35.3), 77 (16.9); HRMS *m*/*z* calcd for $C_{29}H_{28}O_8$ 504.1784, found *m*/*z* 504.1785; ^{1}H NMR 0.86 (3H, d, J = 7.0 Hz, H-18), 1.02 (3H, d, J = 7.0 Hz, H-17), 2.03−2.08 (2H, m, H-7, H-8), 2.26 (2H, d, J = 6.5 Hz, H-9), 3.58 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 5.88 (3H, m, OCH_2O, H-6), 6.0 (2H, m, OCH_2O), 6.7 (1H, s, H-4), 7.31 (2H, m, H-3′, 5′), 7.49 (1H, m, H-4′), 7.64 (2H, m, H-2′, 6′); ^{13}C-NMR, see Table 2.

Interiotherin B (2): prisms (MeOH); mp 96−98.5 °C; $[\alpha]_D$ +50.8° (*c* 4.25, $CHCl_3$); CD (*c* 0.030, MeOH) $[\theta]^{24}$ (nm) +92 954 (236), −101 254 (256); UV (MeOH) λ_{max} (log ϵ) 221 (4.82), 256 (4.07), 278 (3.63) nm; IR ν_{max} 3520, 1715, 1620, 1500, 1475 cm^{-1}; EIMS (70eV) *m*/*z* [M]$^+$ 498 (16.4), 398 (100), 355 (77.2), 326 (93.1), 83 (40.5), 55 (63.4); HRMS *m*/*z* calcd for $C_{27}H_{30}O_9$ 498.1890, found *m*/*z* 498.1926; ^{1}H NMR 1.13 (3H, d, J = 7.0 Hz, H-17), 1.32 (3H, d, J = 7.0 Hz, H-18), 1.41 (3H, s, α-CH_3), 1.86 (3H, m, β-CH_3), 1.93 (1H, m, H-8), 2.12−2.40 (2H, m, H-9), 3.77 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 5.50 (1H, s, H-6), 5.96 (2H, m, OCH_2O), 6.01 (3H, m, OCH_2O, H-β), 6.71 (1H, s, H-4); ^{13}C NMR, see Table 2.

Angeloylgomisin R (**3**) and schisantherin (**4**) were identified on the basis of comparison of their physical and spectral properties with those reported in the literature.[6,7]

HIV Growth Inhibition Assay. Test samples are first dissolved in DMSO. The following are the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μg/mL.

As the test samples are being prepared, an aliquot of the T cell line, HS1, is infected with HIV-1 (IIIB isolate), while another aliquot is mock-infected with culture medium (RPMI-1640 with 10% fetal calf serum supplemented with L-glutamine). The mock-infected aliquot is used for toxicity determinations. The stock virus used

Table 2. ^{13}C-NMR Data of Compounds **1-4** ($CDCl_3$)

carbon	1	2	3	4
1	141.8	141.7	141.6	141.6
2	137.0	137.0	136.9	136.9
3	148.1	148.3	147.9	148.1
4	106.5	103.0	106.7	102.4
5	122.7	121.0	123.0	120.6
6	81.7	84.7	80.7	84.9
7	36.8[a]	72.4	37.3[b]	72.2
8	36.9[a]	42.7	37.0[b]	42.7
9	37.9	36.6	37.7	36.4
10	121.5	121.7	121.2	121.4
11	102.7	106.0	102.3	105.9
12	149.0	149.0	148.7	148.8
13	134.4	134.2	134.2	133.9
14	141.4	140.7	141.3	140.2
15	136.0	135.6	135.3	135.3
16	131.3	129.7	131.7	129.1
17	14.4	19.1	15.9	18.8
18	19.5	28.5	17.6	28.3
Bz, 1′	132.8			129.1
2′	129.9			132.8
3′	128.1			127.8
4′	130.6			129.5
5′	128.1			127.8
6′	129.9			132.8
C=O	165.6			164.6
angl				
α-CH_3		20.0	20.0	
β-CH_3		15.9	15.5	
α-C		127.4	128.0	
β-C		139.9	137.9	
C=O		165.9	166.8	

[a],[b]Assignments within the same vertical column may be reversed.

for these studies typically has a $TCID_{50}$ value of 10^4 Infectious Units/mL. The appropriate amount of virus for a multiplicity of infection between 0.1 and 0.01 Infectious Units/cell is added to the first aliquot of H9 cells. The other aliquot of H9 cells receives only culture medium and is then incubated under conditions identical to those for the HIV-infected H9 cells. After a 4-h incubation at 37 °C and 5% CO_2, both cell populations are washed three times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or culture medium (positive infected control/negative drug control). In addition, AZT is also assayed during each experiment as a positive drug control. The plates are incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants are collected on day 4 for use in our in-house p24 antigen ELISA assay. p24 Antigen is a core protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity is determined by performing cell counts on the mock-infected H9 cells, which received either culture medium (no toxicity), test sample, or AZT.

If a test sample has suppressive capability and is not toxic, its effects are reported in the following terms: IC_{50}, the concentration of test sample that is toxic to 50% of the mock-infected H9 cells; EC_{50}, the concentration of the test sample that is able to suppress HIV replication by 50%; and therapeutic index (TI), the ratio of IC_{50} to EC_{50}. The biological data of compounds **1**−**4** are given in Table 1.

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