Rubrisandrins A and B, Lignans and Related Anti-HIV Compounds from *Schisandra rubriflora*

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Received May 30, 2006

Bioactivity-directed fractionation of an ethanolic extract of the fruits of *Schisandra rubriflora* led to the isolation and identification of dibenzocyclooctadiene lignans including the new lignans rubrisandrins A (**1a** + **1b**) and B (**2**) and the known lignans gomisin J (**3**), (±)-gomisin M_1 (**4**), (+)-gomisin M_2 (**5**), schisanhenol (**6**), deoxyschisandrin, schisantherin B, schisandrin, tigloylgomisin P, gomisin O, angeloylgomisin P, and epigomisin O. Their structure and stereochemistry were determined by spectroscopic methods, including 2D-NMR techniques. Compounds **1** and **3**−**6** were active as anti-HIV agents. (±)-Gomisin M_1 (**4**) exhibited the most potent anti-HIV activity, with EC_{50} and therapeutic index (TI) values of <0.65 μM and >68, respectively.

The fruits of *Schisandra* plants are commonly used in China as tonic, sedative, and astringent agents.[1] Lignans are bioactive constituents of *Schisandra* medicinal plants, which show various beneficial activities including antihepatitis, antitumor, and antilipid peroxidation effects.[2−5] In our previous studies, dibenzocyclooctadiene lignans with anti-HIV activity were isolated from *Kadsura interior* of the same family.[6,7] In our continuing efforts to discover natural anti-AIDS agents, an ethanolic extract of the fruits of *Schisandra rubriflora* (Schisandraceae), which is indigenous to southern China, inhibited HIV replication.[8] Bioactivity-directed fractionation of this extract led to the isolation and identification of new dibenzocyclooctadiene lignans, named rubrisandrins A (**1** = **1a** + **1b**) and B (**2**), together with 11 known lignans, gomisin J (**3**),[9] (±)-gomisin M_1 (**4**),[10] (+)-gomisin M_2 (**5**),[10] schisanhenol (**6**),[11] deoxyschisandrin,[12] schisantherin B,[13] schisandrin,[13] tigloylgomisin P,[14] gomisin O,[15] angeloylgomisin P,[14] and epigomisin O.[15] Five of these compounds were active in an HIV growth inhibition assay with a therapeutic index (TI) greater than 5. In particular, **4** exhibited the most potent anti-HIV activity, with an EC_{50} less than 0.65 μM and a TI greater than 68. This paper deals with the isolation and characterization of the new compounds and the results of preliminary anti-HIV screening.

Results and Discussion

Repeated column chromatography of the Et_2O extract of the fruits of *S. rubriflora* yielded the new dibenzocyclooctadiene lignans rubrisandrins A (**1a** + **1b**), as an inseparable mixture in a 2:1 ratio, and B (**2**), together with 11 known lignans. Their structure and sterochemistry were elucidated by spectroscopic methods. Rubrisandrin A was obtained as an inseparable mixture (**1** = **1a** + **1b**) of two regioisomers, which could be distinguished by ^{1}H, ^{13}C, and 2D-NMR data. In the ^{13}C NMR spectrum, two groups of carbon signals in a ratio of ca. 2:1 were found for **1a** and **1b**. Accordingly, the corresponding proton signals were assigned on the basis of HMQC, and the structures of **1a** and **1b** could be further elucidated by HMBC and NOESY spectra.

Compound **1**, obtained as an amorphous solid, has the molecular formula $C_{22}H_{28}O_6$ from HREIMS (*m*/*z* 388.1868). With maximum absorptions at 221 and 250 nm, the UV spectrum of **1**, along with ^{1}H NMR and ^{13}C NMR spectra as discussed below, indicated that **1a** and **1b** are dibenzocyclooctadiene lignans.[16]

The ^{1}H NMR spectrum of **1a** (see Table 1) showed signals for two secondary methyl groups (δ_H 0.74, 1.00, each 3H, d, $J = 7.1$ Hz), assignable to the *cis*-oriented CH_3-9 and CH_3-9′, respectively.[17] The presence of four benzylic methylene signals (δ_H 2.45, 1H, dd, $J = 13.6$, 1.8 Hz; 2.55, 1H, dd, $J = 13.5$, 7.4 Hz; 2.24, 1H, dd, $J = 13.2$, 9.5 Hz; 2.04, 1H, d, $J = 13.0$ Hz) indicated that, like the known gomisin J, **1a** has no substitution at C-7 and C-7′.[9] On the basis of the HMQC spectrum, the protons at δ_H 2.45 and 2.55 were attached to the same carbon (δ_C 38.8) as were the protons at δ_H 2.24 and 2.04 (δ_C 35.3). Furthermore, HMBC correlations of the proton at δ_H 2.04 with the carbons at δ_C 21.8 (CH_3-9) and 40.9 (C-8) and of the proton at δ_H 2.55 with the carbons at δ_C 12.5 (CH_3-9′) and 33.8 (C-8′) indicated that the δ_H 2.04 and 2.24 resonances corresponded with H_2-7 and the δ_H 2.45 and 2.55 resonances with H_2-7′ (see Figure 2).

The ^{1}H NMR spectrum of **1a** also showed signals due to two aromatic protons (δ_H 6.66, 6.38, each 1H, s) and four *O*-methyl groups (δ_H 3.60, 3.93, 3.90, and 3.91, each 3H, s) on two aromatic rings. HMBC correlations of the protons at δ_H 6.38 and 6.66 with the carbons at δ_C 35.5 (C-7) and 38.8 (C-7′), respectively, suggested that these two proton resonances corresponded with H-6 and H-6′, respectively. Their corresponding carbon signals were assigned as δ_C 103.9 and 113.7, respectively, by HMQC techniques. On the basis of HMBC correlations of H-6 with the carbons at δ_C 151.8, 133.5, and 146.6 and of H-6′ with the aromatic carbons at δ_C 147.8, 137.7, and 150.2, these six carbons were assigned to C-5, C-4, C-3, C-5′, C-4′, and C-3′, respectively. The positions of the four *O*-methyl substituents were elucidated from the HMBC cross-peaks of the *O*-methyl resonances at δ_H 3.60, 3.93, 3.90, and 3.91 with the carbons at δ_C 150.2 (C-3′), 137.7 (C-4′), 151.8 (C-5), and 133.5 (C-4), respectively (see Figure 2).

The presence of two phenolic hydroxyls was deduced from the following spectral data: an IR band at 3419 cm^{-1}, no carbon signal typical for a methylenedioxy at δ_C 100−102 in the ^{13}C NMR spectrum (see Table 1), and two proton signals at δ_H 5.71, 5.73 (br s) with no HMQC correlations.[18] Since no *O*-methyl resonance (ca. δ_C 55) correlated with C-5′ and the proton at δ_H 6.66 (H-6′) correlated with the carbon at δ_C 147.8 (C-5′) in the HMBC spectrum, one hydroxyl was located at C-5′.[18] The other hydroxyl was located at C-3, which was confirmed by the HMBC correlations of the proton at δ_H 5.73 (OH-3) with the carbons at δ_C 146.6 (C-3) and 115.8 (C-2).

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10.1021/np060239e CCC: $33.50
Published on Web 11/15/2006

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data for **1a** and **1b** (in $CDCl_3$)

	1a		**1b**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		139.8		140.6
2		115.8		120.4
3		146.6		150.1
4		133.5		137.4
5		151.8		149.0
6	6.38 (1H, s)	103.9	6.66 (1H, s)	110.6
7	2.24 (1H, dd, 13.2, 9.5)	35.5	2.03 (1H, d, 12.9)	35.4
	2.04 (1H, d, 13.0)		2.22 (1H, dd, 13.3, 9.5)	
8	1.84 (1H, m)	40.9	1.84 (1H, m)	40.9
9	1.00 (3H, d, 7.1)	21.8	0.98 (3H, d, 7.4)	21.6
1′		135.3		134.4
2′		121.5		116.7
3′		150.2		146.8
4′		137.7		133.8
5′		147.8		150.5
6′	6.66 (1H, s)	113.7	6.38 (1H, s)	107.3
7′	2.45 (1H, dd, 13.6, 1.8)	38.8	2.55 (1H, dd, 13.5, 2.0)	39.2
	2.55 (1H, dd, 13.5, 7.4)		2.58 (1H, dd, 13.5, 7.4)	
8′	1.89 (1H, m)	33.8	1.89 (1H, m)	33.7
9′	0.74 (3H, d, 7.1)	12.5	0.75 (3H, d, 7.0)	12.8
3-OMe			3.60 (3H, s)	60.4
4-OMe	3.91 (3H, s)	61.1	3.92 (3H, s)	61.1
5-OMe	3.90 (3H, s)	55.7		
3′-OMe	3.60 (3H, s)	60.4		
4′-OMe	3.93 (3H, s)	60.9	3.92 (3H, s)	61.1
5′-OMe			3.89 (3H, s)	55.7
OH	5.71, 5.73 (br s)		5.71, 5.73 (br s)	

The CD spectrum of **1** had a negative Cotton effect at 248 nm and a positive Cotton effect at 210 nm, indicating that **1** has an *S*-biphenyl configuration.[19] The NOESY correlations between H-6′/CH_3-9′ and H-6/H-7 in **1a** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring[20] (see Figure 3). The substituent positions and stereochemical assignments in the cyclooctadiene ring of **1a** were supported by the NOESY correlations between the protons H-6/CH_3O-5, H-6′/H-7′α, H-6′/CH_3-9′, H-8′/H-8, CH_3-9′/CH_3-9, H-6/H-7β, H-8/H-7′β, and H-7α/CH_3-9. Thus, the structure of rubrisandrin A **1a** was determined as shown in Figure 1.

The ^{1}H, ^{13}C, and 2D-NMR data of **1b** were similar to those of **1a**. The ^{1}H NMR spectrum of **1b** indicated the presence of two secondary methyl groups (δ_H 0.75, 3H, d, $J = 7.0$ Hz; 0.98, each 3H, d, $J = 7.4$ Hz), which could be assigned to CH_3-9′ and CH_3-9, respectively. The ^{1}H NMR spectrum also showed the presence of two benzylic methylene (δ_H 2.58, 1H, dd, $J = 13.5$, 7.4 Hz; 2.55, 1H, dd, $J = 13.5$, 2.0 Hz; δ_H 2.22, 1H, dd, $J = 13.3$, 9.5 Hz; 2.03, 1H, d, $J = 12.9$ Hz), four *O*-methyl (δ_H 3.92, 3.92, 3.89, and 3.60, each 3H, s), two hydroxy (δ_H 5.71 and 5.73, each 1H, br s), and two aromatic protons (δ_H 6.66 and 6.38, each 1H, s).

The HMBC spectrum showed that the aromatic proton at δ_H 6.38 correlated with the carbon at δ_C 39.2 (CH_2-7′), the benzylic methylene protons at δ_H 2.55 and 2.58 (CH_2-7′) correlated with the carbon at δ_C 33.7 (C-8′), and the methyl proton at δ_H 0.75 (CH_3-9′) correlated with the carbons at δ_C 33.7 (C-8′) and 39.2 (C-7′), indicating that the δ_H 6.38 resonance could be assigned as H-6′. Also, the resonance at δ_H 6.66 was assigned as H-6 on the basis of the following HMBC correlations between protons and carbons at δ_H 6.66/δ_C 35.4 (C-7), δ_H 2.03 and 2.22 (CH_2-7)/δ_C 40.9 (C-8), and δ_H 0.98 (CH_3-9)/δ_C 40.9 and 35.4 (C-7) (see Figure 4).

Cross-peaks of the proton at δ_H 6.66 (H-6) with the carbons at δ_C 149.0, 137.4, and 150.1 and of the proton at δ_H 6.38 (H-6′) with the carbons at δ_C 150.5 and 133.8 indicated that these carbon resonances corresponded with C-5, C-4, C-3, C-5′, and C-4′, respectively. The HMBC correlations of four *O*-methyls (δ_H 3.92, 3.89, 3.92, 3.60) with carbons at δ_C 133.8, 150.5, 137.4, and 150.1, respectively, revealed that these four substitutions were located at C-4′, C-5′, C-4, and C-3, respectively. Thus, the two hydroxyls on the aromatic rings were located at C-3′ and C-5 (see Figure 4), which was confirmed by the HMBC correlations of the proton at δ_H 2.03 with the carbon at δ_C 149.0 and of the hydroxyl proton at δ_H 5.71 with the carbons at δ_C 116.7 (C-2′) and 133.8 (C-4′). The last aromatic ring substitutions were confirmed from NOESY correlations of the hydroxyl proton at δ_H 5.73 with the carbon at δ_C 3.92 (CH_3O-4) and of the *O*-methyl protons (CH_3O-4) with the carbon at δ_C 3.60 (CH_3O-3).

NOESY correlations between H-6′/CH_3-9′ and H-7β/H-6 indicated a twist-boat-chair (TBC) conformation for the cyclooctadiene ring of **1b**.[20] The substituent positions and stereochemical assignments in this ring were elucidated from the NOESY correlations of the protons at H-6′/H-7′α, H-6/H-8, H-6/H-7β, HO-3′/CH_3O-4′, CH_3O-3/CH_3O-4, CH_3-9′/CH_3-9, and H-8/H-7′β. Thus, the structure of **1b** was determined as that in Figure 1.

Rubrisandrin B (**2**), obtained as an amorphous solid, has the molecular formula $C_{22}H_{28}O_6$ as determined by HREIMS (*m*/*z* 388.1854). The UV spectrum of **2** showed maximum absorptions at 217 and 268 nm and, together with the ^{1}H NMR data, indicated that **2** was a dibenzocyclooctadiene lignan.[13] The ^{1}H NMR spectrum showed the presence of two methyls, two benzylic methylenes, four *O*-methyls, and two aromatic protons. The appearance of two methyl doublets (δ_H 0.75, 1.01, each 3H, d, $J = 7.1$ Hz) indicated the presence of two secondary methyl groups, which could be assigned as CH_3-9′ and CH_3-9, respectively. Four benzylic methylene protons (δ_H 2.60, 1H, dd, $J = 13.7$, 7.6 Hz; 2.50, 1H, d, $J = 13.6$, 1.7 Hz; δ_H 2.05, 1H, d, $J = 13.4$ Hz and 2.29, 1H, dd, $J = 13.3$, 9.6 Hz) indicated that there was no substitution at C-7 and C-7′ in the cyclooctadiene ring.

The absence of a typical methylenedioxy carbon signal at δ_C 100−102 in the ^{13}C NMR spectrum[18] and the presence of two proton signals at δ_H 5.75 and 5.76 (each 1H, br s) in the ^{1}H NMR spectrum suggested the presence of two hydroxyls on the aromatic rings, which was confirmed by an IR band at 3424 cm^{-1}. The positions of the two aromatic protons, two hydroxyls, and four *O*-methyl groups were elucidated by ^{1}H NMR and ^{13}C NMR spectral analysis. As in **1a** and **1b**, the two aromatic proton signals at δ_H 6.41 and 6.42 (each 1H, s) were assigned as H-6 and H-6′. These signals were shifted upfield from those (δ_H 6.63, 2H) in gomisin J,[21] and the corresponding protonated aromatic carbon signals at δ_C 107.8 and 104.3 in **2** were also shifted upfield about 3 ppm compared with those in deoxyschisandrin. These findings suggested that the hydroxy groups in **2** were located at the *para*-positions (C-3 and C-3′) relative to the aromatic protons.[17] These assignments were supported by the ^{1}H NMR chemical shifts of the four *O*-methyl groups (δ_H 3.93, 3.92, 3.91, and 3.89, each 3H, s), indicating that no *O*-methyl group was located at C-3 or C-3′.[8] Two *O*-methyl groups were located at C-5 and C-5′ (δ_C 55.8 × 2), adjacent to the H-6 and H-6′ aromatic protons,[17] and the remaining *O*-methyl groups were assigned at C-4 and C-4′ (δ_C 61.1 × 2).

The observation of cross-peaks between the protons at δ_H 2.60/2.50, δ_H 2.60/1.91, and δ_H 1.91/0.75 in the ^{1}H−^{1}H COSY spectrum and correlation of the protons at δ_H 2.60/6.42 in the NOESY spectrum supported the assignment of the resonances at δ_H 6.42 to H-6′, δ_H 2.60 and 2.50 to H_2-7′, and δ_H 1.91 to H-8′. In the same way, ^{1}H−^{1}H COSY correlations of the protons at δ_H 2.05/2.29, 2.29/1.81, and 1.81/1.01 and NOESY correlation of the protons at δ_H 6.41/2.05 indicated assignment of the resonances at δ_H 6.41 with H-6, δ_H 2.29 and 2.05 with H_2-7, and δ_H 1.81 with H-8.

Compound **2** has an *S*-biphenyl configuration on the basis of a negative Cotton effect at 249 nm and a positive Cotton effect at 212 nm in the CD spectrum. The NOESY correlations between

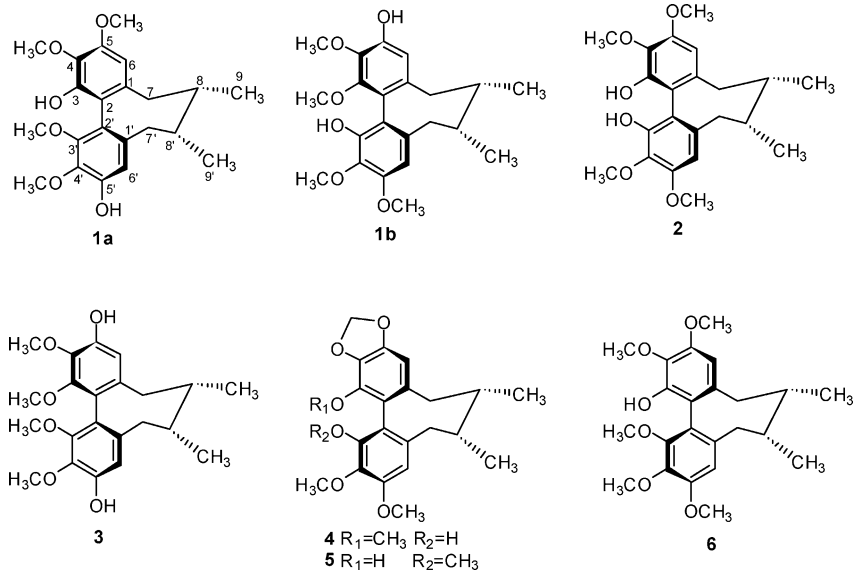

Figure 1. Structures of compounds **1**−**6**.

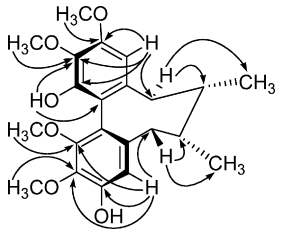

Figure 2. HMBC correlations of **1a**.

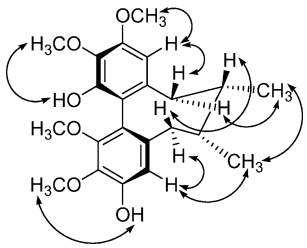

Figure 3. NOESY correlations of **1a**.

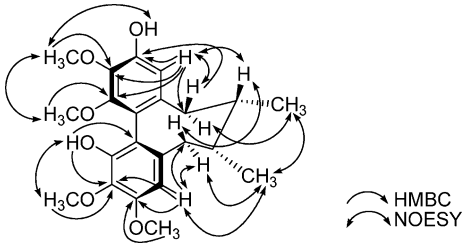

Figure 4. HMBC and NOESY correlations of **1b**.

H-6′/H-7′α, CH_3-9/H-7α, and H-7α/H-6 indicated a TBC conformation for the cyclooctadiene ring. The stereochemical assignments in the cyclooctadiene ring were strengthened by the correlations of H-6′/CH_3O-5′, H-6′/H-7′α, H-6′/CH_3-9′, H-7α/H-6, H-6/CH_3O-5, OH-3′/CH_3O-4′, and OH-3/CH_3O-4 in the NOESY spectrum (see Figure 5). Thus, the structure of **2** was determined as shown in Figure 1.

Except for **2**,[22] the compounds isolated from *S. rubriflora* were tested for in vitro inhibitory effects against HIV replication in H9 lymphocytes.[23] The results are shown in Table 2. (±)-Gomisin M_1 (**4**) was the most potent compound, with an EC_{50} less than 0.65 μM and a TI greater than 68. Compounds **1** and **3**−**6**, which all

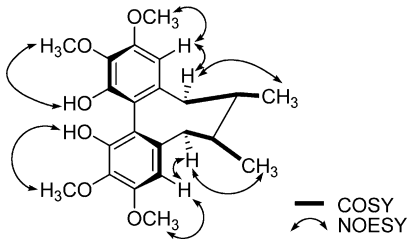

Figure 5. $^1H-^1H$ COSY and NOESY correlations of **2**.

Table 2. Anti-HIV Activities of the Lignans from *S. rubriflora*

compound	EC_{50} (μM)	IC_{50} (μM)	TI (IC_{50}/EC_{50})
1[a]	11.3	>64	5.7
3	3.9	23.2	6.0
4	<0.65	44.5	>68
5	2.4	47.1	19.4
6	5.7	42.0	7.4
deoxyschisandrin	no suppression	>60	no suppression
schisantherin B	40	>49	1.2
schisandrin	no suppression	>58	no suppression
tigloylgomisin P	37	>49	1.3
gomisin O	36	>60	1.7
angeloylgomosin P	no suppression	>49	no suppression
epigomisin O	42	>60	1.4
AZT	0.023	1870	80900

[a] Inseparable mixture of **1a** and **1b** (2:1)

contain aromatic hydroxyl(s), showed anti-HIV activity, while the remaining lignans, which are without an aromatic hydroxyl, were inactive. This observation suggested that the aromatic hydroxyl groups are important to the anti-HIV activity of these lignans. Comparison of the anti-HIV potencies and the structures of **4** and **5** suggested that the position of the hydroxyl could enhance the anti-HIV activity. Compounds **4** and **5** are structurally similar except for the substituents on C-3 and C-3′. Compound **4**, with a 3′-OH, was more potent (TI > 68) than **5** (TI = 19.4), with a 3-OH.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 spectropolarimeter. UV spectra were measured on a Shimadzu UV-260 spectrophotometer in absolute MeOH. CD spectra were measured with a JASCO J-715 spectropolarimeter. IR spectra were recorded as KBr pellets on the Avatar 360E.S.P spectrophotometer (Themo Nicolet Co.). ^1H NMR and ^{13}C NMR spectra were measured on a Bruker AV500 or DRX400 spectrometer. Mass spectra were determined on a HP5989A mass spectrometer for EIMS

and a Kratos Concept 1H series mass spectrometer for HREIMS. Analytical and preparative TLC were performed on silica gel plates (Yan-tai Institute of Chemical Technology) with petroleum ether−EtOAc (3:1). Silica gel (100−200 mesh, 200−300 mesh, and 300−400 mesh, Qingdao Marine Chemical Factory) was used for column chromatography. Spots were observed under UV light and visualized by spraying with 10% H_2SO_4, followed by heating. Semipreparative HPLC was conducted on an Agilent 1100 instrument (Agilent, Santa Clara, CA) equipped with a UV detector, and the data were captured by HP ChemStation software. The HPLC separation was performed on a RP-18 column (Waters Xterra, 5 μm, 4.6 × 250 mm). The column temperature was controlled at 30 °C, and the detection wavelength was set at 254 nm. The flow rate was 2.0 mL/min.

Plant Material. The fruits of *S. rubriflora* were collected in Li-Jiang County, Yunnan Province, People's Republic of China, in October of 2002, and identified by one of the authors (D.-F.C.). A voucher specimen (DFC-WWZ-LJ0201) has been deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.

Extraction and Isolation. The fruits (23 kg) of *S. rubriflora* were air-dried, ground, and extracted exhaustively with 95% EtOH at room temperature. The alcoholic extract was evaporated in vacuo to yield a semisolid (5.5 kg), which was suspended in water (8 L) and extracted with Et_2O (×7). This Et_2O solution was concentrated to yield 1.3 kg of residue. The residue (200 g) was chromatographed on silica gel (4 kg), eluting with petroleum ether containing increasing amounts of acetone. Fraction 3 [petroleum ether−acetone (95:5)] gave deoxyschisandrin (6.3 g). Schisanhenol (**6**, 4.5 g) was recrystallized from fraction 4 [petroleum ether−acetone (9:1)]. Fraction 4 (18.3 g) was also subjected to repeated silica gel column chromatography with petroleum ether−acetone (50:1−10:1). Preparative TLC with benzene−EtOAc (4:1) of fraction 4-3 yielded epigomisin O (5 mg). Fraction 4-4 was subjected to repeated column chromatography with petroleum ether−EtOAc (25:1) to yield (+)-gomisin M_2 (**5**, 1.14 g). Preparative TLC with petroleum ether−EtOAc (5:1) of fraction 4-5 gave gomisin O (3 mg). Fraction 4-6 was subjected to repeated column chromatography with petroleum ether−EtOAc (20:1) to yield angeloylgomosin P (16 mg) and (±)-gomisin M_1 (**4**, 211 mg). Fraction 5 [petroleum ether−acetone (8:2)] was subjected to repeated column chromatography with petroleum ether−EtOAc (9:1−3:1). Repeated column chromatography of fraction 5-2 with petroleum ether−EtOAc (4:1) gave tigloylgomisin P (45 mg) and with petroleum ether−EtOAc (3:1) yielded schisandrin (655 mg) and schisantherin B (3.9 g). Preparative TLC with benzene−EtOAc (3:1) of fraction 5-3 yielded **3** (7 mg). Fraction 6 [petroleum ether−acetone (7:3)] was subjected to repeated column chromatography with $CHCl_3$−EtOAc (4:1). The residue was purified with semipreparative HPLC with MeOH−H_2O (75:25) to yield **1** (14 mg) and **2** (1 mg).

Rubrisandrin A (1a + 1b): amorphous solid, $[\alpha]_D^{22}$ +18.5 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.75), 250 (4.03) nm; CD (*c* 0.08, MeOH), $[\theta]^{15}$ (nm) −10 786 (248), +13 234 (210); IR (KBr) ν_{max} 3419, 2937, 2871, 1584, 1489, 1123, 1004, 751 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 388 [M^+] (100), 341(7), 331(10), 221(7), 167-(7), 83(16), 85(11); HREIMS *m*/*z* 388.1868 (calcd for $C_{22}H_{28}O_6$, 388.1886).

Rubrisandrin B (2): amorphous solid, $[\alpha]_D^{22}$ +33.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.93), 268 (4.06) nm; CD (*c* 0.12, MeOH), $[\theta]^{15}$ (nm) −57 007 (249), +81 653 (212); IR (KBr) ν_{max} 3424, 2933, 1584, 1489, 1456, 1402, 1341, 1093, 1004, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.42 (1H, s, H-6′), 6.41 (1H, s, H-6), 2.60 (1H, dd, J = 13.7, 7.6 Hz, H-7′α), 2.50 (1H, d, J = 13.6, 1.7 Hz, H-7′β), 1.91 (1H, m, H-8′), 1.81 (1H, m, H-8), 2.29 (1H, dd, J = 13.3, 9.6 Hz, H-7β) 2.05 (1H, d, J = 13.4 Hz, H-7α), 0.75 (3H, d, J = 7.1 Hz, CH_3-9′), 1.01 (3H, d, J = 7.1 Hz, CH_3-9), 3.93, 3.92 (each 3H, s, CH_3O-4′, -4), 3.91 (3H, s, CH_3O-5), 3.89 (3H, s, CH_3O-5′), 5.75, 7.76 (each 1H, s, HO-3′, -3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.4, 150.1, 146.1 × 2, 139.7 × 2, 134.5, 133.2 (C, C-3′−5′, C-5−3, C-1′, and C-1), 114.4, 115.3 (C, C-2 and C-2′), 107.8, 104.3 (CH, C-6′ and C-6), 61.1 × 2, 55.8 × 2 (CH_3, CH_3O-4′, -5′, -5, -4), 40.9, 39.3, 35.7, 33.8 (CH or CH_2, C-7′−C-7), 21.8, 12.7 (CH_3, CH_3-9, -9′); HREIMS *m*/*z* 388.1854 (calcd for $C_{22}H_{28}O_6$, 388.1886).

Known compounds were identified by comparison of their physical and spectroscopic data with literature values.[9−15]

HIV Growth Inhibition Assay. The anti-HIV inhibitory assay was performed at Panacos Pharmaceuticals according to the following reported procedure.[23] 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 5% CO_2 and 37 °C. The cells were then washed thoroughly to remove unabsorbed virions and resuspended at 4 × 10^5 cells/mL. Aliquots (1 mL) were placed in 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 is 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.

Acknowledgment. This investigation was supported by grants from the National Natural Science Foundation of China (30271586), the Foundation for Doctoral Programs (2000026519), Trans-Century Training Program Foundation for the Talents, and the Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions (1999-71) from the Ministry of Education, People's Republic of China (to D.-F.C.) and by Grant AI-33066 from the National Institute of Allergy and Infectious Diseases (NIAID) (to K.-H.L.). The authors are grateful to Dr. S. L. Morris-Natschke at University of North Carolina for manuscript reading.

Supporting Information Available: NMR spectra of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP060239E