Isolation and Structure Elucidation of Nortriterpenoids from *Schisandra rubriflora*

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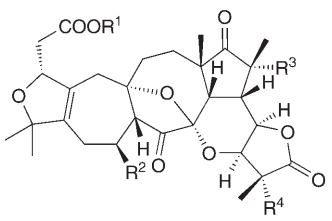
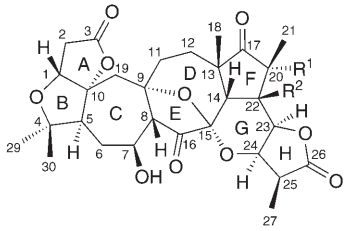
Seven new highly oxygenated nortriterpenoids, rubriflorins D–J (**1**–**7**), were isolated from the leaves and stems of *Schisandra rubriflora*, and their structures were elucidated on the basis of extensive analysis of spectroscopic data. These new compounds feature the opening of ring A compared with related known nortriterpenoids isolated from the genus *Schisandra* and showed weak activity against HIV-1.

Introduction. – The species of the genus *Schisandra* are known to be rich sources of bioactive lignans possessing various beneficial pharmacological effects such as antihepatitis, antitumor, and anti-HIV-1 activity [1–6], and many species are widely used in traditional Chinese medicine. Recently, some species of this genus have not only been used as traditional Chinese medicine but also as an important material for food and drink industries, which was developed into a series of products such as fruit juice, jelly, wine, food for health maintenance, *etc.* [7–12]. In recent years, considerable efforts of our group have been devoted to the discovery of anti-HIV-bioactive and novel triterpenoids from the genus *Schisandra*, and this led to the isolation of a series of nortriterpenoids with an important diversity of highly oxygenated structures [13–18], some of which showed anti-HIV activities [16–18].

Schisandra rubriflora (FRANCH.) REHD. et WILS, a climbing plant native to the province Yunnan, has been used as sedative and tonic agents in traditional Chinese medicine for a long time, and its fruits are eaten locally. Our phytochemical studies of this species led to the isolation of two novel nortriterpenoids, rubriflordilactones A and B [19], and the current reinvestigation of this plant led to the discovery of seven new nortriterpenoids, rubriflorins D–J (**1**–**7**), together with two known compounds, micrandilactone A (**8**) [13] and lancifodilactones C (**9**) [14]. These new compounds featured the opening of lactone ring A as compared with related known nortriterpenoids isolated from the genus *Schisandra* [13–19]. In addition, considering that some nortriterpenoids isolated from the *Schisandra* genus showed modest or strong anti-HIV activities [16–18], all new compounds, except for compound **2**, were tested for their



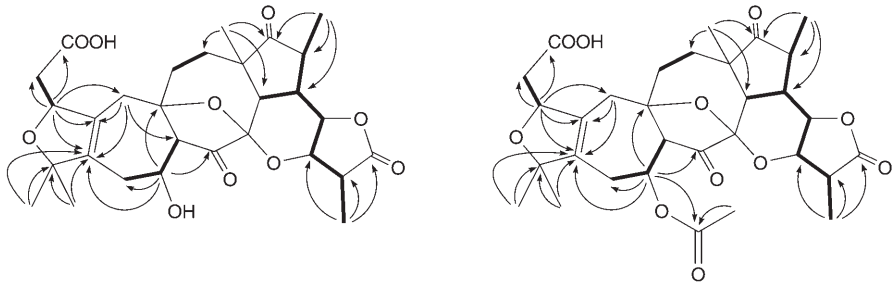
1 $R^1 = R^3 = R^4 = H$, $R^2 = OH$
2 $R^1 = R^3 = R^4 = H$, $R^2 = AcO$
3 $R^1 = R^3 = H$, $R^2 = AcO$, $R^4 = OH$
4 $R^1 = Et$, $R^2 = OH$, $R^3 = R^4 = H$
5 $R^1 = Et$, $R^2 = R^4 = OH$, $R^3 = H$
6 $R^1 = Et$, $R^2 = R^3 = R^4 = OH$
7 $R^1 = Me$, $R^2 = OH$, $R^3 = R^4 = H$



8 $R^1 = R^2 = OH$
9 $R^1 = R^2 = H$

anti-HIV-1 activities. In this paper, we report the isolation, structural elucidation, and biological evaluation of the new compounds.

Results and Discussion. – Rubriflorin D (**1**) was obtained as an amorphous powder. Its empirical formula $C_{29}H_{36}O_{10}$ was established from its HR-ESI-MS ($[M+Na]^+$ at 567.2194) and ^{13}C-NMR data, indicating 12 degrees of unsaturation. The IR spectrum of **1** showed absorption bands for an OH group (3443 cm^{-1}) and a carboxylic acid (1710 and 3045 – 2882 cm^{-1}). Detailed analysis of the ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HSQC, HMBC, ^{1}H,^{1}H-COSY (*Fig. 1*), and ROESY data (*Fig. 2*) and comparison with data of lancifodilactone (**9**) [14] confirmed the proposed structure of rubriflorin D (**1**).



1 **2**

Fig. 1. *Selected 2D-NMR correlations of compounds* **1** *and* **2**. Bonds in bold indicate ^{1}H,^{1}H-COSY, arrows indicate HMBC.

The ^{1}H-NMR spectrum of **1** exhibited three tertiary and two secondary Me groups. The ^{1}H- and ^{13}C-NMR and HSQC data revealed that **1** contained 29 C-atoms, including one ester, one carboxylic acid, two carbonyl, five Me, five CH_2, and nine CH groups (including four oxygenated ones), and six quaternary C-atoms (including two olefinic and three oxygenated ones). These suggested that compound **1** was a highly oxygenated nortriterpenoids and required the presence of seven rings to satisfy the observed degrees of unsaturation. The NMR spectra of **1** were similar to those of lancifodilactone C (**9**) [14], and the obvious differences were the appearance of a C=C bond and the lack of a CH group and a

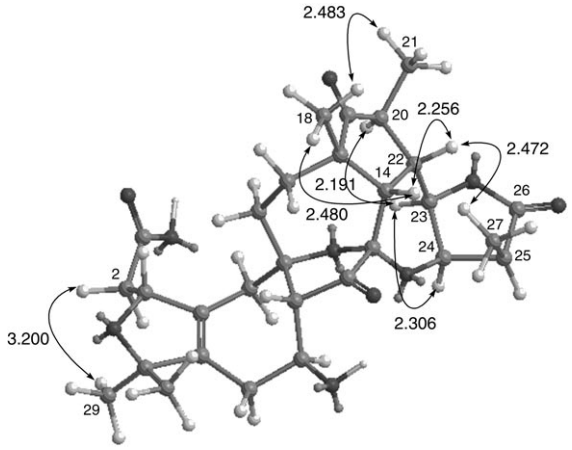


Fig. 2. *Computer-generated molecular model showing key ROESY correlations and corresponding interatomic distances* [Å] *of compound* **1**

oxygenated quaternary C-atom in **1**. Combinative analysis of 1D and 2D NMR data revealed that **1** possessed the same rings D–H as **9**. In the HMBC plot, two geminal Me groups, Me(29) and Me(30), showed strong correlations to C(4) and an olefinic C-atom (δ(C)136.0), and of H–C(1) (δ(H) 5.46) and CH_2(2) (δ(H) 2.73 and 3.01) exhibited strong correlations to another olefinic C-atom (δ(C) 128.8), which suggested a C=C bond between C(5) and C(10)[1]). This established that ring A of **9** was opened in **1** and C(3) changed from an ester group in **9** to a carboxylic acid group in **1**. Moreover, an oxymethine signal at δ(H) 4.84 was assigned to H–C(7) based on the HMBC correlations from this proton signal to C(5), C(6), C(9), and C(16), and the correlation with H–C(8) in the ^{1}H,^{1}H-COSY experiment (*Fig. 1*). The planar structure of **1** was thus established as shown. Its relative configuration was determined by a ROESY experiment, together with 1D NMR data comparison with those of **9** [14]. The strong ROESY correlation CH_2(2)/Me(29) determined the α-orientation of the CH_2(2) group. Accordingly, H–C(1) was β-orientated. H–C(7) was deduced to be α-orientated from the large coupling constant with H–C(8) (J = 9.3 Hz), which was similar to that of **9** (J = 9.6 Hz). According to ^{1}H,^{1}H-coupling constants and the pivotal ROESY correlations H–C(14)/Me(18), Me(18)/Me(21), H–C(14)/H–C(22), H–C(20)/H–C(23), H–C(23)/H–C(24), and H–C(22)/Me(27) (*Fig. 2*), all of the other chiral centers of **1** were identical with those of **9**. In addition, a computer-generated 3D structure was obtained by CHEM 3D ULTRA V 8.0, with MM2 force-field calculations for energy minimization (*Fig. 2*). The calculated interatomic distances between H–C(2)/Me(29) (3.200 Å), H–C(14)/Me(18) (2.480 Å), Me(18)/Me(21)(2.483 Å), H–C(14)/H–C(22) (2.256 Å), H–C(20)/H–C(23) (2.191 Å), H–C(23)/H–C(24) (2.306 Å), and H–C(22)/CH_3(27) (2.472 Å) are all less than 4.00 Å (*Fig. 2*); this further supported the well-defined ROESY correlations observed for each of these proton pairs.

The molecular formula of **2** was deduced as $C_{31}H_{38}O_{11}$ from its HR-ESI-MS and ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum (*Table 2*) was very similar to that of **1**, except for the signal of an additional acetyl group (*s* at δ(H) 2.07), which was supported by the presence of additional ^{13}C-NMR signals (δ(C) 169.7 and 20.9; *Table 1*). The strong HMBC correlation of H–C(7) (δ(H) 5.82) with the C=C of the acetyl group indicated that the latter was located at O–C(7) (*Fig. 1*). The downfield shift of the H–C(7) signal of **2** (δ(H) 5.82) as compared to **1** (δ(H) 4.84) supported this

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Table 1. *^{13}C-NMR Data* (C_5D_5N) *of Rubriflorins D–J* (**1**–**7**[1]). δ in ppm.

	1	**2**	**3**	**4**	**5**	**6**	**7**
C(1)	83.6 (*d*)	83.5 (*d*)	83.3 (*d*)	83.2 (*d*)	83.5 (*d*)	83.3 (*d*)	83.2 (*d*)
C(2)	42.3 (*t*)	42.7 (*t*)	42.4 (*t*)	42.3 (*t*)	42.6 (*t*)	42.1 (*t*)	42.3 (*t*)
C(3)	174.3 (*s*)	173.7 (*s*)	173.7 (*s*)	171.6 (*s*)	172.2 (*s*)	171.5 (*s*)	172.0 (*s*)
C(4)	89.1 (*s*)	88.8 (*s*)	88.7 (*s*)	89.2 (*s*)	89.8 (*s*)	89.3 (*s*)	89.3 (*s*)
C(5)	136.0 (*s*)	135.5 (*s*)	135.5 (*s*)	136.3 (*s*)	136.5 (*s*)	136.3 (*s*)	136.4 (*s*)
C(6)	33.1 (*t*)	30.4 (*t*)	30.5 (*t*)	33.0 (*t*)	32.5 (*t*)	32.7 (*t*)	32.9 (*t*)
C(7)	69.3 (*d*)	69.7 (*d*)	69.9 (*d*)	69.3 (*d*)	69.7 (*d*)	69.5 (*d*)	69.3 (*d*)
C(8)	58.7 (*d*)	55.2 (*d*)	55.3 (*d*)	58.7 (*d*)	58.9 (*d*)	58.8 (*d*)	58.7 (*d*)
C(9)	85.2 (*s*)	85.2 (*s*)	85.3 (*s*)	85.2 (*s*)	85.7 (*s*)	85.8 (*s*)	85.2 (*s*)
C(10)	128.8 (*s*)	129.3 (*s*)	129.5 (*s*)	128.4 (*s*)	128.7 (*s*)	128.1 (*s*)	128.3 (*s*)
C(11)	40.9 (*t*)	40.0 (*t*)	41.1 (*t*)	41.0 (*t*)	41.2 (*t*)	40.8 (*t*)	41.0 (*t*)
C(12)	30.7 (*t*)	30.6 (*t*)	30.7 (*t*)	30.7 (*t*)	31.0 (*t*)	30.3 (*t*)	30.7 (*t*)
C(13)	50.5 (*s*)	50.4 (*s*)	50.4 (*s*)	50.5 (*s*)	50.9 (*s*)	49.8 (*s*)	50.5 (*s*)
C(14)	45.3 (*d*)	45.2 (*d*)	45.2 (*d*)	45.3 (*d*)	45.6 (*d*)	45.3 (*d*)	45.3 (*d*)
C(15)	99.7 (*s*)	99.8 (*s*)	100.0 (*s*)	99.7 (*s*)	100.3 (*s*)	99.4 (*s*)	99.7 (*s*)
C(16)	211.1 (*s*)	209.8 (*s*)	209.4 (*s*)	211.1 (*s*)	211.6 (*s*)	210.8 (*s*)	211.1 (*s*)
C(17)	220.3 (*s*)	220.1 (*s*)	219.7 (*s*)	220.2 (*s*)	221.2 (*s*)	219.9 (*s*)	220.2 (*s*)
C(18)	26.9 (*q*)	26.8 (*q*)	26.4 (*q*)	26.8 (*q*)	27.1 (*q*)	27.3 (*q*)	26.8 (*q*)
C(19)	34.1 (*t*)	34.2 (*t*)	34.3 (*t*)	34.2 (*t*)	34.5 (*t*)	34.1 (*t*)	34.1 (*t*)
C(20)	44.9 (*d*)	44.9 (*d*)	44.8 (*d*)	44.9 (*d*)	45.2 (*d*)	74.6 (*s*)	44.9 (*d*)
C(21)	14.6 (*q*)	14.6 (*q*)	14.6 (*q*)	14.4 (*q*)	15.0 (*q*)	24.6 (*q*)	14.7 (*q*)
C(22)	40.3 (*d*)	40.3 (*d*)	40.5 (*d*)	40.4 (*d*)	40.7 (*d*)	42.1 (*d*)	40.4 (*d*)
C(23)	75.1 (*d*)	75.0 (*d*)	73.4 (*d*)	75.0 (*d*)	73.4 (*d*)	73.2 (*d*)	75.0 (*d*)
C(24)	69.0 (*d*)	69.2 (*d*)	74.5 (*d*)	69.0 (*d*)	75.1 (*d*)	76.4 (*d*)	69.0 (*d*)
C(25)	43.1 (*d*)	42.2 (*d*)	76.8 (*s*)	43.2 (*d*)	77.2 (*s*)	76.6 (*s*)	42.2 (*d*)
C(26)	177.7 (*s*)	177.7 (*s*)	176.9 (*s*)	177.7 (*s*)	177.5 (*s*)	176.9 (*s*)	177.6 (*s*)
C(27)	8.3 (*q*)	8.3 (*q*)	18.2 (*q*)	8.3 (*q*)	18.3 (*q*)	18.1 (*q*)	8.3 (*q*)
C(29)	29.3 (*q*)	26.3 (*q*)	26.7 (*q*)	29.3 (*q*)	26.7 (*q*)	26.8 (*q*)	29.3 (*q*)
C(30)	26.4 (*q*)	28.7 (*q*)	28.6 (*q*)	26.4 (*q*)	29.6 (*q*)	29.3 (*q*)	26.4 (*q*)
MeO							51.5 (*q*)
EtO				60.5 (*t*)	61.1 (*t*)	60.4 (*t*)	
				14.7 (*q*)	14.7 (*q*)	14.3 (*q*)	
AcO		20.9 (*q*)	20.9 (*q*)				
		169.7 (*s*)	169.6 (*s*)				

deduction. Moreover, H–C(7) was deduced to be α-orientated from the similarity of the ^{1}H,^{1}H-coupling constants with those of **1** (*Table 2*). Thus, the acetyl group was established to be β-orientated.

The HR-ESI-MS analysis of compound **3** demonstrated that it had the molecular formula $C_{31}H_{38}O_{12}$. The NMR data of **3** were very similar to those of **2**. Analysis of the 1D and 2D NMR spectra revealed that the difference was the presence of a CH group (H–C(25)) in **2** which was oxygenated by an OH group to a quaternary C-atom (C(25)) in **3**. In addition, the ROESY correlation of Me(27) with H–C(22) suggested that the OH group was α-orientated.

The molecular formula of **4** was deduced as $C_{31}H_{40}O_{10}$ from its HR-ESI-MS and ^{13}C-NMR data (*Table 1*). Its ^{1}H- (*Table 3*) and ^{13}C-NMR spectra were very similar to that of **1**, except for signals of an additional Et group. The obvious HMBC correlation

Table 2. *^{1}H-NMR Data* (C_5D_5N) *of Rubriflorins D – F* (**1** – **3**)[1]). δ in ppm, J in Hz.

	1	**2**	**3**
H–C(1)	5.46 (*dd*, *J* = 3.2, 7.3)	5.35 (*dd*, *J* = 3.5, 7.5)	5.39 (*dd*, *J* = 3.5, 7.5)
CH_2(2)	2.73 (*dd*, *J* = 7.3, 12.5),	2.69 (*dd*, *J* = 7.5, 13.2),	2.69 (*dd*, *J* = 7.5, 13.2),
	3.01 (*dd*, *J* = 3.2 12.5)	2.95 (*dd*, *J* = 3.5, 13.2)	2.96 (*dd*, *J* = 3.5, 13.2)
CH_2(6)	2.64 – 1.68 (H_α)[a]),	2.48 – 2.52[a])	2.49 – 2.54[a])
	2.46 (*dd*, *J* = 8.7, 12.3, H_β)		
H–C(7)	4.84 (*dd*, *J* = 8.7, 9.3)	5.82 (*dd*, *J* = 9.0, 9.5)	5.79 (*dd*, *J* = 8.9, 9.5)
H–C(8)	3.05 (*d*, *J* = 9.3)	3.04 (*d*, *J* = 9.5)	3.05 (*d*, *J* = 9.5)
CH_2(11)	1.82 – 1.86 (*m*, H_α),	1.92 – 1.96 (*m*, H_α),	1.91 – 1.96 (*m*, H_α),
	1.74 – 1.78 (*m*, H_β)	1.71 – 1.76 (*m*, H_β)	1.71 – 1.76 (*m*, H_β)
CH_2(12)	1.86 – 1.91 (*m*, H_α),	1.90 – 1.95 (*m*, H_α),	1.90 – 1.95 (*m*, H_α),
	1.47 – 1.52 (*m*, H_β)	1.48 – 153 (*m*, H_β)	1.47 – 1.52 (*m*, H_β)
H–C(14)	2.66 (*d*, *J* = 6.9)	2.72 – 2.76[a])	2.71 – 2.75[a])
Me(18)	0.93 (*s*)	0.98 (*s*)	0.92 (*s*)
CH_2(19)	2.51 (*AB*, *J* = 15.8, H_α),	2.73 (*AB*, *J* = 15.0, H_α),	2.72 (*AB*, *J* = 14.7, H_α),
	3.13 (*AB*, *J* = 15.8, H_β)	2.73 (*AB*, *J* = 15.0, H_β)	2.72 (*AB*, *J* = 14.7, H_β)
H–C(20)	2.83 – 2.88[a])	2.78 – 2.83 (*m*)	2.76 – 2.80 (*m*)
Me(21)	1.24 (*d*, *J* = 6.9)	1.20 (*d*, *J* = 6.4)	1.24 (*d*, *J* = 6.3)
H–C(22)	2.82 – 2.86[a])	2.79 – 2.84[a])	2.79 – 2.84[a])
H–C(23)	4.69 (br. *s*)	4.71 (br. *s*)	4.64 (br. *s*)
H–C(24)	4.75 (br. *d*, *J* = 1.7)	5.26 (br. *s*)	4.77 (br. *s*)
H–C(25)	3.15 – 3.20 (*m*)		3.08 – 3.13 (*m*)
Me(27)	1.31 (*d*, *J* = 6.8)	1.86 (*s*)	1.30 (*d*, *J* = 6.7)
Me(29)	1.34 (*s*)	1.29 (*s*)	1.29 (*s*)
Me(30)	1.28 (*s*)	1.35 (*s*)	1.36 (*s*)
AcO		2.07 (*s*)	2.04 (*s*)

[a]) Overlapped.

of the Me*C*H_2O signal (δ(H) 4.12 – 4.16) with the C=O signal (δ(C) 171.6) assigned to C(3) indicated that the EtO group should be located at C(3).

The structures of rubriflorins H – J (**5** – **7**) were determined to be as shown on the basis of their spectral data (*Tables 1* and *3*) and comparison with those of **4** in an analogous manner as used for the structure elucidation of **3**.

The extraction and isolation of compounds **1** – **7** were carried out under neutral conditions at low temperature, which suggested that the opening of the lactone ring A did not occur during the isolation process. In addition, all new compounds, except for **2**, were tested for their potencies in preventing the cytopathic effects of HIV-1 in C8166 and for cytotoxicity measured in parallel with the determination of antiviral activity, by using AZT (= 3′-azido-3′-deoxythymidine) as a positive control (EC_{50} = 0.0043 μg/ml and CC_{50} > 200 μg/ml). All compounds showed weak anti-HIV-1 activities with EC_{50} in the range 15.5 – 95.5 μg/ml, and compounds **3** and **4** exerted minimal cytotoxicity against C8166 cells (CC_{50} > 200 μg/ml) (*Table 4*). Compound **2** was not tested for its bioactivity since only a limited amount of material was available.

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Table 3. *^{1}H-NMR Data* (C_5H_5N) *of Rubriflorins G–J* (**4**–**7**)[1]). δ in ppm, J in Hz.

	4	**5**	**6**	**7**
H–C(1)	5.28 (*d*, *J* = 3.0, 7.5)	5.28 (*d*, *J* = 7.6)	5.24 (*d*, *J* = 7.7)	5.27 (*d*, *J* = 7.4)
CH_2(2)	2.55 (*dd*, *J* = 7.5, 12.4), 2.86 (*dd*, *J* = 3.0, 12.4)	2.54–2.58[a]), 2.87–2.92[a])	2.49–2.54[a]), 2.77–2.82[a])	2.54 (*dd*, *J* = 7.4, 12.3), 2.85 (br. *d*, *J* = 12.3)
CH_2(6)	2.64–2.68 (H_α)[a]), 2.45 (*dd*, *J* = 6.9, 11.5, H_β)	2.75 (*d*, *J* = 11.3, H_α), 2.57 (*dd*, *J* = 8.7, 11.3, H_β)	2.71 (*d*, *J* = 11.8, H_α), 2.48–2.53 (H_β)[a])	2.63–2.67 (H_α)[a]), 2.45 (*dd*, *J* = 8.7, 11.9, H_β)
H–C(7)	4.82 (*dd*, *J* = 9.1, 9.1)	4.90 (*dd*, *J* = 8.7, 9.4)	4.87 (*dd*, *J* = 8.8, 9.2)	4.83 (*dd*, *J* = 8.7, 9.3)
H–C(8)	3.06 (*d*, *J* = 9.1)	3.18 (*d*, *J* = 9.4)	3.21 (*d*, *J* = 9.2)	3.07 (*d*, *J* = 9.3)
CH_2(11)	1.82–1.87 (*m*, H_α), 1.73–1.77 (*m*, H_β)	1.87–1.92 (H_α)[a]), 1.76–1.80 (*m*, H_β)	1.98–2.02 (H_α)[a]), 1.75–1.80 (*m*, H_β)	1.81–1.85 (*m*, H_α), 1.73–1.77 (*m*, H_β)
CH_2(12)	1.85–1.90 (*m*, H_α), 1.47–1.52 (*m*, H_β)	1.88–1.93 (H_α)[a]), 1.49–1.53 (*m*, H_β)	1.97–2.01 (H_α)[a]), 1.53–1.57 (*m*, H_β)	1.84–1.89 (*m*, H_α), 1.46–1.50 (*m*, H_β)
H–C(14)	2.67 (*d*, *J* = 6.9)	2.83 (*d*, *J* = 8.3)	2.82 (*d*, *J* = 8.2)	2.67 (*d*, *J* = 6.9)
Me(18)	0.92 (*s*)	0.98 (*s*)	0.98 (*s*)	0.93 (*s*)
CH_2(19)	2.35 (*AB*, *J* = 15.5, H_α), 3.07 (*AB*, *J* = 15.5, H_β)	2.41 (*AB*, *J* = 15.4, H_α), 3.12 (*AB*, *J* = 15.4, H_β)	2.38 (*AB*, *J* = 15.5, H_α), 3.17–3.22 (H_β)[a])	2.36 (*AB*, *J* = 15.4, H_α), 3.12 (*AB*, *J* = 15.4, H_β)
H–C(20)	2.83–2.88[a])	2.78–2.83 (*m*)		2.71–176 (*m*)
Me(21)	1.23 (*d*, *J* = 6.9)	1.23 (*d*, *J* = 6.6)	1.56 (*s*)	1.23 (*d*, *J* = 6.9)
H–C(22)	2.82–2.86[a])	2.83–2.87[a])	3.16–3.20[a])	2.82–2.86[a])
H–C(23)	4.68 (br. *s*)	4.73 (br. *s*)	4.78 (br. *s*)	4.70 (br. *s*)
H–C(24)	4.73 (br. *d*, *J* = 1.7)	5.33 (br. *s*)	5.63 (br. *s*)	4.74 (*d*, *J* = 1.7)
H–C(25)	3.17–3.22 (*m*)			3.17–3.22 (*m*)
Me(27)	1.31 (*d*, *J* = 6.8)	1.84 (*s*)	1.79 (*s*)	1.31 (*d*, *J* = 6.8)
Me(29)	1.33 (*s*)	1.37 (*s*)	1.27 (*s*)	1.34 (*s*)
Me(30)	1.29 (*s*)	1.40 (*s*)	1.34 (*s*)	1.30 (*s*)
EtO	4.12–4.16 (*m*), 1.12 (*t*, *J* = 7.1)	4.16–4.20 (*m*), 1.17 (*t*, *J* = 7.1)	4.06–4.10 (*m*), 1.06 (*t*, *J* = 7.1)	
MeO				3.63 (*s*)

[a]) Overlapped.

Table 4. *Summary of Cytotoxicities and Anti-HIV-1 Activities of the New Compounds*

Compounds	Cytotoxicity (CC_{50}) [μg/ml][a])	Anti-HIV-1_{IIIB} activity (EC_{50}) [μg/ml]	Selectivity index (CC_{50}/EC_{50})
1	102.6	19.1	5.37
3	> 200	87.1	> 2.30
4	> 200	95.5	> 2.09
5	89.5	15.5	5.77
6	106.5	15.8	6.74
7	92.9	19.1	4.86
AZT	> 200	0.0043	> 46511.63

[a]) Minimal cytotoxicity against C8166 cells when $CC_{50} > 200$ μg/ml.

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Experimental Part

General. Column chromatography (CC): silica gel (200-300 mesh; *Qing-dao Marine Chemical, Inc.*, Qingdao, China). Prep. HPLC: *Agilent 1100* liquid chromatograph; *Zorbax SB-C$_{18}$* column (9.4 mm × 25 cm); TLC: silica gel plates; visualization by heating the plates sprayed with 10% H_2SO_4 in EtOH. M.p.: *XRC-1* micro melting point apparatus; uncorrected. Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrophotometer; λ_{max}(log ε) in nm. IR Spectra: *Tenor 27* spectrophotometer; KBr pellets; in cm^{-1}. 1D and 2D NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; unless otherwise specified, chemical shifts δ in ppm with reference to the solvent signals. Mass spectra: *VG Autospec-3000* spectrometer at 70 eV; in *m*/*z*.

Plant Material. The leaves and stems of *S. rubriflora* were collected in Dali Prefecture of Yunnan Province, China, in August 2003. The specimen was identified by Prof. *Xi-Wen Li*, and a voucher specimen (No. KIB 2003-08-02) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The plant material (2.5 kg) was powdered and extracted with 70% aq. Me_2CO (4 × 5 l) for 24 h at r.t. The filtrate was concentrated to 1 l and partitioned with AcOEt, to give the AcOEt part and the H_2O-soluble portion. The AcOEt part (57.0 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 1:0, 9:1, 8:2, 2:1, 1:1, and 0:1): *Fractions I–V. Fr. II* (10.4 g) was repeatedly subjected to CC (silica gel (200–300 mesh), *Sephadex LH-20*) and finally purified by prep. HPLC (MeOH/H_2O 45:55 and MeOH/MeCN/H_2O 10:40:50): **1** (7 mg), **4** (20 mg), and **7** (15 mg). *Fr. III* (12.9 g) was further subjected to CC (silica gel, $CHCl_3$/Me_2CO 10:1, 5:1, 2:1, and 1:1): *Fr. III.A–III.F. Fr. III.B* (1.8 g) was purified by recrystallization and repeated CC (silica gel, *Rp-18*, *Sephadex LH-20* (MeOH), followed by prep. HPLC (MeOH/H_2O 35:65 and MeOH/MeCN/H_2O 15:30:55): **2** (2 mg), **3** (7 mg), and **9** (18 mg). Similarly, *Fr. III.C* (2.1 g) was purified by all chromatography methods mentioned above: **5** (10 mg), **6** (7 mg), and **8** (17 mg).

Rubriflorin D (= *(1*S*,3a*R*,3b*S*,4*S*,5a*S*,7a*S*,9*S*,13*S*,13a*R*,14a*S*,14b*S*,15a*R*)-1,3a,3b,4,5,5a,6,7,9,11,12,13,13a,14,14b,15a-Hexadecahydro-13-hydroxy-1,4,5a,11,11-pentamethyl-2,5,14-trioxo-2*H,*8*H-*7a,14a-epoxy-3,10,15-trioxaazuleno[6′,5′:5,6]cyclooct[1,2,3-*cd*]-*as-*indacene-9-carboxylic Acid*[2]); **1**): Amorphous white powder. $[\alpha]_D^{26} = +87.9$ ($c = 0.44$, MeOH). UV (MeOH): 206 (3.79). IR (KBr): 3443, 3045, 2958, 2926, 2882, 1773, 1736, 1632, 1459, 1379, 1162, 1074, 1015, 884. NMR: *Tables 1* and *2*. ESI-MS (pos.): 567 ($[M+Na]^+$). HR-ESI-MS: 567.2194 ($[M+Na]^+$), $C_{29}H_{36}NaO_{10}^+$; calc. 567.2206).

Rubriflorin E (= *(1*S*,3a*R*,3b*S*,4*S*,5a*S*,7a*S*,9*S*,13*S*,13a*R*,14a*S*,14b*S*,15a*R*)-13-(Acetyloxy)-1,3a,3b,4,5,5a,6,7,9,11,12,13,13a,14,14b,15a-hexadecahydro-1,4,5a,11,11-pentamethyl-2,5,14-trioxo-2*H,*8*H-*7a,14a-epoxy-3,10,15-trioxaazuleno[6′,5′:5,6]cyclooct[1,2,3-*cd*]-*as-*indacene-9-carboxylic Acid*[2]); **2**): White crystals. M.p. 184–185°. $[\alpha]_D^{25} = +162.1$ ($c = 0.12$, MeOH). UV (MeOH): 205 (3.44). IR (KBr): 3037, 2972, 2935, 2879, 1788, 1741, 1632, 1379, 1238, 1121, 1035, 1018, 599. NMR: *Tables 1* and *2*. ESI-MS (pos.): 609 ($[M+Na]^+$). HR-ESI-MS: 609.2308 ($[M+Na]^+$), $C_{31}H_{38}NaO_{11}^+$; calc. 609.2311).

Rubriflorin F (= *(1*R*,3a*R*,3b*S*,4*S*,5a*S*,7a*S*,9*S*,13*S*,13a*R*,14a*S*,14b*S*,15a*S*)-13-(Acetyloxy)-1,3a,3b,4,5,5a,6,7,9,11,12,13,13a,14,14b,15a-hexadecahydro-1-hydroxy-1,4,5a,11,11-pentamethyl-2,5,14-trioxo-2*H,*8*H-*7a,14a-epoxy-3,10,15-trioxaazuleno[6′,5′:5,6]cyclooct[1,2,3-*cd*]-*as-*indacene-9-carboxylic Acid*[2]); **3**): White crystals. M.p. 180–181°. $[\alpha]_D^{25} = +159.1$ ($c = 0.22$, MeOH). UV (MeOH): 205 (3.84). IR (KBr): 3442, 3036, 2970, 2932, 2886, 1786, 1737, 1632, 1380, 1236, 1117, 1036, 1015, 594. NMR: *Tables 1* and *2*. ESI-MS (pos.): 625 ($[M+Na]^+$). HR-ESI-MS: 625.2248 ($[M+Na]^+$, $C_{31}H_{38}NaO_{12}^+$; calc. 625.2260).

Rubriflorin G (= *(1*S*,3a*R*,3b*S*,4*S*,5a*S*,7a*S*,9*S*,13*S*,13a*R*,14a*S*,14b*S*,15a*R*)-1,3a,3b,4,5,5a,6,7,9,11,12,13,13a,14,14b,15a-Hexadecahydro-13-hydroxy-1,4,5a,11,11-pentamethyl-2,5,14-trioxo-2*H,*8*H-*7a,14a-ep-*

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[2]) The given stereodescriptors are arbitrary since only the relative configurations were determined.

oxy-3,10,15-trioxaazuleno[6′,5′:5,6]cyclooct[1,2,3-cd*]*-as-*indacene-9-carboxylic Acid Ethyl Ester*; **4**): White crystals. M.p. 199–200°. $[\alpha]_D^{27} = +107.2$ ($c = 0.38$, MeOH). UV (MeOH): 205 (3.68). IR (KBr): 3446, 2971, 2931, 1782, 1736, 1629, 1458, 1377, 1161, 1073, 1015, 833. NMR: *Tables 1* and *3*. ESI-MS (pos.): 595 ($[M+Na]^+$). HR-ESI-MS: 595.2513 ($[M+Na]^+$, $C_{31}H_{40}NaO_{10}^+$; calc. 595.2519).

Rubriflorin H (=*(1*R,*3a*R,*3b*S,*4*S,*5a*S,*7a*S,*9*S,*13*S,*13a*R,*14a*S,*14b*S,*15a*S*)-1,3a,3b,4,5,5a,6,7,9,11,12,13,13a,14,14b,15a-Hexadecahydro-1,13-dihydroxy-1,4,5a,11,11-pentamethyl-2,5,14-trioxo-2*H,*8*H-*7a,14a-epoxy-3,10,15-trioxaazuleno[6′,5′:5,6]cyclooct[1,2,3*-cd*]*-as-*indene-9-carboxylic Acid Ethyl Ester*[2]); **5**): White crystals. M.p. 218–219°. $[\alpha]_D^{25} = +108.1$ ($c = 0.13$, MeOH). UV (MeOH): 205 (3.77). IR (KBr): 3443, 2974, 2926, 1788, 1747, 1629, 1459, 1379, 1291, 1112, 1013, 884. NMR: *Tables 1* and *3*. ESI-MS (pos.): 611 ($[M+Na]^+$). HR-ESI-MS: 611.2129 ($[M+Na]^+$, $C_{31}H_{40}NaO_{11}^+$; calc. 627.2125).

Rubriflorin I (=*(1*R,*3a*R,*3b*S,*4*R,*5a*S,*7a*S,*9*S,*13*S,*13a*R,*14a*S,*14b*S,*15a*S*)-1,3a,3b,4,5,5a,6,7,9,11,12,13,13a,14,14b,15a-Hexadecahydro-1,4,13-trihydroxy-1,4,5a,11,11-pentamethyl-2,5,14-trioxo-2*H,*8*H-*7a,14a-epoxy-3,10,15-trioxaazuleno[6′,5′:5,6]cyclooct[1,2,3*-cd*]*-as-*indene-9-carboxylic Acid Ethyl Ester*[2]); **6**: White crystals. M.p. 211–212°. $[\alpha]_D^{25} = +118.3$ ($c = 0.28$, MeOH). UV (MeOH): 205 (3.74). IR (KBr): 3441, 2973, 2931, 1786, 1746, 1630, 1457, 1377, 1288, 1113, 1011, 880, 620. NMR: *Tables 1* and *3*. ESI-MS (pos.): 627 ($[M+Na]^+$). HR-ESI-MS: 627.2429 ($[M+Na]^+$, $C_{31}H_{40}NaO_{12}^+$; calc. 627.2417).

Rubriflorin J (=*(1*S,*3a*R,*3b*S,*4*S,*5a*S,*7a*S,*9*S,*13*S,*13a*R,*14a*S,*14b*S,*15a*R*)-1,3a,3b,4,5,5a,6,7,9,11,12,13,13a,14,14b,15a-Hexadecahydro-13-hydroxy-1,4,5a,11,11-pentamethyl-2,5,14-trioxo-2*H,*8*H-*7a,14a-epoxy-3,10,15-trioxaazuleno[6′,5′:5,6]cyclooct[1,2,3*-cd*]*-as-*indacene-9-carboxylic Acid Methyl Ester*[2]); **7**): White crystals. M.p. 197–198°. $[\alpha]_D^{25} = +116.1$ ($c = 0.14$, MeOH). UV (MeOH): 205 (3.85). IR (KBr): 3443, 2968, 2928, 1776, 1737, 1629, 1457, 1440, 1379, 1285, 1162, 1073, 1015, 884. NMR: *Tables 1* and *3*. ESI-MS (pos.): 581 ($[M+Na]^+$). HR-ESI-MS: 581.2119 ($[M+Na]^+$, $C_{30}H_{38}NaO_{10}^+$; calc. 581.2123).

Anti-HIV-1 Assay. The cytotoxicity assay against C8166 cells (CC_{50}) was assessed by using the MTT method, and the anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) [20].

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