Triterpenoids from *Schisandra rubriflora*

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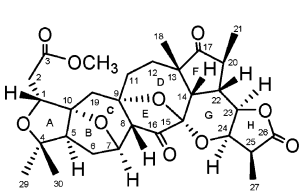
Investigation of an organic extract of *Schisandra rubriflora* led to the isolation of three new highly oxygenated nortriterpenoids, named rubriflorins A−C (**1**−**3**), together with six related known compounds (**4**−**9**). Their structures were elucidated by spectroscopic evidence. Compounds **1**−**3** feature the opening of ring A compared with related known nortriterpenoids isolated from the genus *Schisandra* and showed anti-HIV-1 activities with EC_{50} values of 10.0, 16.2, and 81.3 μg/mL, respectively.

Phytochemical studies on species the genus *Schisandra* have revealed that they are rich sources of lignans possessing various beneficial pharmacological effects, and over 19 species are widely used as sedative and tonic agents in traditional Chinese medicine.[1−7] In a survey of biologically active natural products from the genus *Schisandra*, we earlier reported a series of highly oxygenated nortriterpene derivatives with unprecedented carbon skeletons.[8−17] As part of our ongoing phytochemical investigation on the species of the genus, *Schisandra rubriflora* (Franch.) Rehd. et Wils (Schisandraceae), a species native to Yunnan Province, was chosen for chemical constituent studies, and two novel nortriterpenoids, rubriflordilactones A and B, were reported recently.[17] Our continuing study of this plant has led to the isolation of three new nortriterpenoids, rubriflorins A−C (**1**−**3**), along with six related known compounds identified as micrandilactone A (**4**),[8] lancifodilactones C and D (**5**, **6**),[9] henridilactones A and B (**7**, **8**),[10] and micrandilactone G (**9**).[11] Compounds **1**−**3** were tested for their anti-HIV-1 activities. This paper describes the isolation, structure elucidation, and biological activities of the new compounds.

Powdered dried leaves and stems of *S. rubriflora* were extracted in 70% aqueous acetone. The filtrate was concentrated and partitioned with EtOAc. The EtOAc fraction was submitted to successive chromatographic fractionation and purification to yield compounds **1**−**9**.

The positive ion ESIMS of **1** exhibited a pseudomolecular ion at *m*/*z* 581 for $[M + Na]^+$. The molecular formula was established as $C_{30}H_{38}O_{10}$ by HRESIMS (*m*/*z* 581.2362 $[M + Na]^+$), suggesting 12 degrees of unsaturation. Examination of the ^1H and ^{13}C NMR data of **1** showed the presence of three tertiary methyls and two secondary methyls, five methylenes, 10 methines (four oxygenated), five quaternary carbons (four oxygenated), two ester groups, two carbonyl groups, and one methoxy group. The above data accounted for all of the protons, indicating the absence of free hydroxyl groups. In addition, the lack of any olefinic moieties required the presence of eight rings to satisfy the degrees of unsaturation.

Considering that a series of oxygenated nortriterpenoids had been isolated from the genus *Schisandra*,[8−17] it was reasonable to presume that **1** was also a nortriterpenoid derivative. Comparison of ^1H and ^{13}C NMR data of **1** with those of micrandilactone G (**9**),[11] along with a detailed analysis of HSQC, $^1H-^1H$ COSY, and HMBC spectra, revealed that **1** possessed the same rings (D−H) as **9**. However, the data for the remaining structure of **1** were quite



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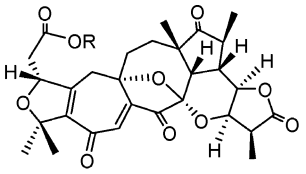
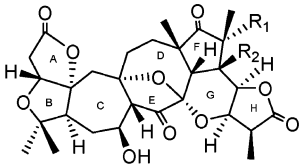
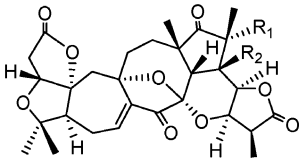


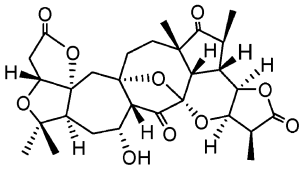
2 R = CH_3
3 R = C_2H_5



	R_1	R_2
4	OH	OH
5	H	H



	R_1	R_2
6	H	H
7	OH	H
8	OH	OH



9

distinctive from those of **9**. Further analysis of the HMBC spectrum of **1** (Figure 1) showed the following correlations: both CH_3-29 and CH_3-30 with C-4 and C-5; H-1 with C-2, C-3, C-4, C-5, C-10, and C-19; the proton signal of a methoxy group at δ_H 3.61 with an ester group (C-3); and H-7 with C-5, C-8, C-9, C-10, and C-16. This evidence, together with two obvious spin systems deduced from $^1H-^1H$ COSY spectra of **1**, H-1/H-2 and H-5/H6/H-7/H-8, established the planar structure of **1** as shown.

The relative stereochemistry of **1** was determined from analysis of molecular models, energy minimized using the MM2 force field in CS Chem 3D V 8.0 overlaid with key correlations observed in the ROESY NMR spectrum (Figure 2). The relative configurations of C-13, C-14, C-20, C-22, C-23, C-24, and C-25 were determined to be the same as those of **9** by strong ROESY correlations of H-14/CH_3-18, H-14/H-21, H-20/H-23, H-23/H-24, H-14/H-22, and H-22/CH_3-27. These correlations were all supported by calculated interatomic distances of less than 4.00 Å, although most of them were approximately 2.40 Å. Biogenetically, the relative orientation

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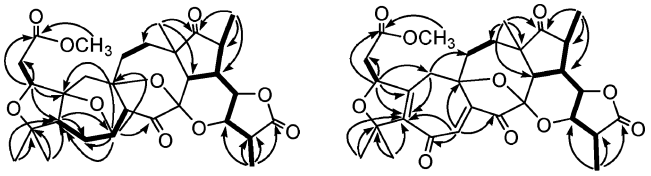

1 2

Figure 1. Selected HMBC (→) and $^1H-^1H$ COSY (—) correlations of **1** and **2**.

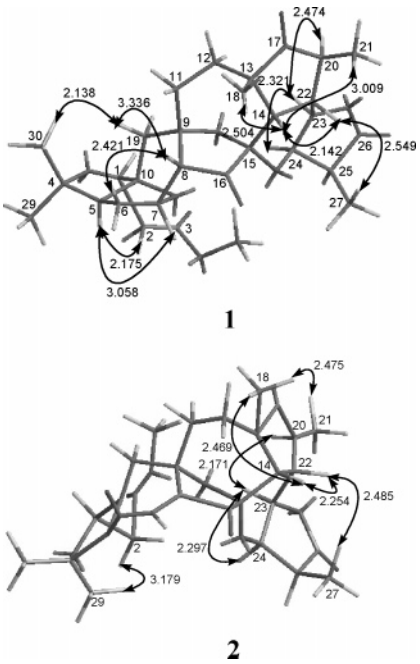

Figure 2. Computer-generated molecular model showing key ROESY correlations and corresponding interatomic distance (Å) of compounds **1** and **2**.

of H-5 was α and CH_3-30 was *β*. The ROESY cross-peak of H-5 with H_2-2 suggested that C-2 was α- and that H-1 was *β*-oriented. The ROESY correlation observed from H-5 to H-7 suggested that H-7 was α-oriented, which was supported by a calculated interatomic distance of 3.06 Å. This, aided by a Dreiding molecular model analysis, established the oxygen bridge between C-7 and C-10 to have α-orientation for the rigid ring system. Compared with related nortriterpenoids,[8−17] compound **1** features an open lactone ring and has an oxygen bridge between C-7 and C-10.

Rubriflorin B (**2**) was obtained as pale yellow crystals. Its molecular formula was established to be $C_{30}H_{34}O_{10}$ by the HRESIMS data (*m*/*z* 577.2051 $[M + Na]^+$, calcd 577.2049). The ^{13}C NMR spectrum showed 30 carbon signals including three tertiary methyls, two secondary methyls, four methylenes, eight methines (one olefinic carbon), seven quaternary carbons (three olefinic), two ester groups, three carbonyl groups, and one methoxy group. This suggested that **2** also was a nortriterpenoid derivative. Comparison of ^1H and ^{13}C NMR data of **2** with those of **1** suggested that **2** was structurally related to **1**. The differences between **1** and **2** can be explained by replacement of an oxygenated quaternary carbon, three methine carbons (one oxygenated), and a methylene carbon in **1** by four olefinic carbons and a conjugated carbonyl group (δ_C 185.5) in **2**. Analysis of 2D NMR spectra showed that **2** had the same rings (D−H) as **1**. The signals of C-1, C-2, C-3, C-4, C-19, C-29, and C-30 were assigned by analysis of HSQC, HMBC, and $^1H-^1H$ COSY spectra. The chemical shift of the ketocarbonyl group (δ_C 200.8, C-16) suggested that it was α,*β*-unsaturated, and an olefinic methine was consequently assigned to the *β*-position of the α,*β*-unsaturated ketone moiety on the basis of HMBC correlations observed from the olefinic proton signal at δ_H 6.79 to C-9 and C-16, which showed a double bond between C-7 (δ_C 130.4) and C-8 (δ_C 142.5). This deduction was supported by HMBC correlations from H-19 to C-8 and C-9. In addition, cross-peaks of H-1, H_2-7, H-19, CH_3-29, and CH_3-30 with the olefinic carbon (δ_C 140.9), and H-1, H_2-2 with the olefinic carbon (δ_C 150.2), enabled us to assign the two carbons to C-5 and C-10, respectively. The conjugated carbonyl group (δ_C 185.5) was assigned to C-6. This assignment was supported by the upfield shift of the carbonyl group of the conjugated system of the dienone moiety. According to the observed ROESY correlations of H_2-2/H-29, H-14/CH_3-18, H-14/H-21, H-20/H-23, H-23/H-24, H-14/H-22, and H-22/CH_3-27, and comparison of ^1H and ^{13}C data with those of **1**, all of the chiral centers of **2** were established to be identical with those of **1** (Table 1 and Figure 2).

HRESIMS analysis of rubriflorin C (**3**) demonstrated that it had the molecular formula $C_{31}H_{36}O_{10}$ (*m*/*z* 569.2395 $[M + H]^+$), which is more than that of **2** by a methylene group. The ^1H and ^{13}C NMR data were similar to those of **2**. The only difference observed in the ^1H and ^{13}C NMR spectra was that the methoxy group attached to C-3 in **2** was replaced by an ethoxy group in **3**. This conclusion was supported by the presence of ethoxy group signals at δ_H 4.14 (m) and 1.13 (t, $J = 7.1$) and δ_C 61.4 and 14.7 in the NMR spectra of **3**. The above deduction was confirmed by HMBC correlations observed from the methylene proton signal at δ_H 4.14, assigned to the ethyl group, to C-3 (δ_C 170.9). A possible biosynthetic pathway to compounds **1**−**3** was proposed (Figure S1, Supporting Information), starting from micrandilactone G (**9**) as the biogenetic precursor.

Since some nortriterpenoids isolated from *Schisandra* genus are reported to have modest or strong anti-HIV activities,[12,17] the new compounds were tested for their ability to prevent the cytopathic effects of HIV-1 in C8166, and their cytotoxicity was measured in parallel with the determination of antiviral activity using AZT as a positive control ($EC_{50} = 0.0043$ μg/mL and $CC_{50} > 200$ μg/mL). Compounds **1**−**3** showed anti-HIV-1 activities with EC_{50} values of 10.0, 16.2, and 81.3 μg/mL, respectively. Compound **3** showed minimal cytotoxicity against C8166 cells ($CC_{50} > 200$ μg /mL), and compounds **1** and **2** showed cytotoxicity against C8166 cells with CC_{50} values of 89.1 and 100.3 μg /mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were obtained on a XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. Unless otherwise specified, chemical shifts (*δ*) were expressed in ppm with reference to the solvent signals. Mass spectra were performed on a VG Autospec-3000 spectrometer under 70 eV. Column chromatography was performed with silica gel (200−300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm, column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The leaves and stems of *S. rubriflora* were collected in August 2003 from Dali Prefecture of Yunnan Province, China. The specimen was identified by Prof. Xi-Wen Li. A voucher specimen, No. KIB 2003-08-02, has been 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. Air-dried and powdered stems and leaves (3.1 kg) were extracted with 70% aqueous Me_2CO (4 × 5 L) at room temperature and concentrated *in vacuo* to give a crude extract (110 g), which was partitioned between H_2O and EtOAc. The EtOAc part (77.0 g) was chromatographed on a silica gel column eluting with $CHCl_3$−

Table 1. ^{1}H and ^{13}C NMR Data for Rubriflorins A−C (**1**−**3**)[a]

	1		**2**		**3**	
no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	80.0 d	5.28 (dd, 4.8, 9.4)	84.6 d	5.43 (dd, 3.2, 7.2)	84.7 d	5.44 (dd, 3.2, 7.3)
2	36.8 t	2.75 (br d, 9.4)	39.9 t	2.91 (dd, 7.2, 13.1)	40.1 t	2.91 (dd, 7.3, 13.2)
		2.80 (br d, 4.8)		3.03 (dd, 3.2, 13.1)		3.03 (dd, 3.2, 13.2)
3	171.7 s		170.8 s		170.9 s	
4	81.2 s		90.6 s		90.7 s	
5	56.3 d	2.85 (m)	140.9 s		141.1 s	
6 α	29.4 t	2.50 (m)	185.5 s		185.6 s	
6 *β*		1.80 (overlapped)				
7	79.5 d	4.98 (t, 7.2)	130.4 d	6.79 (s)	130.3 d	6.80 (s)
8	51.9 d	2.92 (d, 7.2)	142.5 s		142.5 s	
9	80.0 s		83.3 s		83.2 s	
10	93.7 s		150.2 s		150.1 s	
11 α	40.8 t	1.95 (m)	36.1 t	1.88 (m)	36.0 t	1.89 (m)
11 *β*		1.40 (m)		1.76 (m)		1.77 (m)
12 α	30.3 t	1.82 (overlapped)	31.9 t	1.86 (m)	31.9 t	1.87 (m)
12 *β*		1.42 (m)		1.48 (m)		1.48 (m)
13	50.5 s		51.3 s		51.2 s	
14	45.6 d	2.63 (d, 6.9)	46.1 d	2.66 (d, 7.0)	46.1 d	2.67 (d, 7.0)
15	99.8 s		101.1 s		101.0 s	
16	210.0 s		200.8 s		200.7 s	
17	219.8 s		220.2 s		220.0 s	
18	26.8 q	0.92 (s)	27.1 q	0.95 (s)	27.0 q	0.95 (s)
19 α	26.4 t	1.83 (AB d, 14.4)	37.5 t	2.79 (AB d, 16.7)	37.5 t	2.82 (AB d, 16.7)
19 *β*		2.05 (AB d, 14.4)		3.24 (AB d, 16.7)		3.25 (AB d, 16.7)
20	45.0 d	2.69 (m)	45.4 d	2.72 (m)	45.3 d	2.73 (m)
21	14.5 q	1.29 (d, 6.9)	14.9 q	1.29 (d, 7.0)	14.9 q	1.29 (d, 7.0)
22	40.3 d	2.81 (m)	40.5 d	2.84 (m)	40.5 d	2.85 (m)
23	74.9 d	4.67 (br s)	75.1 d	4.79 (br s)	75.2 d	4.78 (br s)
24	69.5 d	4.73 (br d, 1.9)	69.4 d	4.87 (br s)	69.4 d	4.86 (br s)
25	42.1 d	3.23 (m)	42.8 d	3.23 (m)	42.9 d	3.23 (m)
26	177.3 s		180.0 s		177.9 s	
27	8.5 q	1.33 (d, 7.1)	8.8 q	1.41 (d, 7.2)	8.7 q	1.40 (d, 7.2)
29	30.0 q	1.31 (s)	29.3 q	1.67 (s)	29.2 q	1.67 (s)
30	23.3 q	1.26 (s)	27.7 q	1.50 (s)	27.7 q	1.50 (s)
OCH_3	51.6 q	3.61 (s)	51.5 q	3.64 (s)		
OC_2H_5					61.4 t	4.14 (m)
					14.7 q	1.13 (t, 7.1)

[a] Data were recorded in C_5D_5N on a Bruker AM-125 (^{13}C NMR) and an AM-500 (^{1}H NMR) MHz; chemical shifts (δ) are expressed in ppm with reference to the most downfield signal of C_5D_5N (δ 8.71 ppm) for ^{1}H and to the center peak of the most downfield signal of C_5D_5N (δ 149.9 ppm) for ^{13}C, respectively.

CH_3OH (1:0, 9:1, 8:2, 2:1, 1:1, and 0:1) to afford fractions I−V. Fraction II (10.4 g) was repeatedly chromatographed on silica gel and Sephadex LH-20 and then by semipreparative HPLC (CH_3OH-H_2O, 45:55, and $CH_3OH-CH_3CN-H_2O$, 10:40:50) to yield compounds **1** (7 mg), **4** (8 mg), and **9** (19 mg). Fraction III (12.9 g) was further chromatographed on a silica gel column eluting with $CHCl_3-Me_2CO$ (10:1, 5:1, 2:1, 1:1) to afford subfractions A−F. Subfraction B (2.1 g) was purified by crystallization and repeated chromatography over silica gel, RP-18, and Sephadex LH-20 (CH_3OH), followed by semipreparative and preparative HPLC (CH_3CN-H_2O, 35:65, CH_3OH-H_2O, 37:63, and $CH_3OH-CH_3CN-H_2O$, 10:33:57) to yield compounds **2** (5 mg), **3** (7 mg), **5** (10 mg), **6** (13 mg), **7** (6 mg), and **8** (15 mg).

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}).[18]

Rubriflorin A (1): white crystals; mp 188−189 °C; $[\alpha]_D^{25}$ +121.1 (*c* 0.26, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 208 (3.55) nm; IR (KBr) ν_{max} 2975, 2945, 1779, 1728, 1644, 1455, 1450, 1377, 1284, 1167, 1083, 1005, 874 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; ESIMS *m*/*z* 581 $[M + Na]^+$; HRESIMS *m*/*z* 581.2362 $[M + Na]^+$ (calcd 581.2362 for $C_{30}H_{38}O_{10}Na$).

Rubriflorin B (2): pale yellow crystals; mp 175−176 °C; $[\alpha]_D^{25}$ +93.5 (*c* 0.11, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 207 (3.66) nm; IR (KBr) ν_{max} 2920, 1779, 1740, 1634, 1460, 1384, 1181, 1017, 579 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; ESIMS *m*/*z* = 577 $[M + Na]^+$; HRESIMS *m*/*z* 577.2051 $[M + Na]^+$ (calcd 577.2049 for $C_{30}H_{34}O_{10}Na$).

Rubriflorin C (3): pale yellow crystals; mp 177−178 °C; $[\alpha]_D^{25}$ +98.5 (*c* 0.13, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 207 (3.85) nm; IR (KBr) ν_{max} 2929, 1777, 1738, 1631, 1457, 1381, 1179, 1015, 580 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; ESIMS *m*/*z* 569 $[M + H]^+$; HRESIMS *m*/*z* 569.2395 $[M + H]^+$ (calcd 569.2386 for $C_{31}H_{37}O_{10}$).

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Supporting Information Available: Detailed anti-HIV-1 activity testing method, possible biosynthetic pathway, and NMR spectra of rubriflorins A−C (**1**−**3**). These materials are available free of charge via the Internet at http://pubs.acs.org.

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