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# Rubriflordilactones A and B, Two Novel Bisnortriterpenoids from *Schisandra rubriflora* and Their Biological Activities

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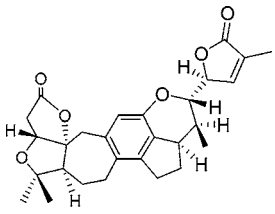
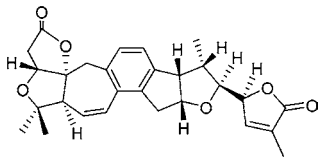
## ABSTRACT



**Rubriflordilactone A (1)**



**Rubriflordilactone B (2)**

**Rubriflordilactones A (1) and B (2), two novel highly unsaturated rearranged bisnortriterpenoids possessing a biosynthetically modified aromatic D-ring, were isolated from the leaves and stems of *Schisandra rubriflora*. Their structures were established on the basis of extensive spectroscopic methods, including two-dimensional NMR techniques, and confirmed by X-ray crystallographic analysis. Compound 1 showed weak anti-HIV-1 activity, and compound 2 exhibited an $EC_{50}$ value of 9.75 $\mu$g/mL (SI = 12.39) against HIV-1 replication with low cytotoxicity.**

Plants of the genus *Schisandra* belong to the economically and medicinally important family Schisandraceae. In China, over 19 species are known to be a rich source of lignans possessing various beneficial pharmacological effects such as antihepatitis, antitumor, and anti-HIV-1 activity.[1−4] Recently, some triterpenoids isolated from this genus showed anti-HIV-1 activity[5,6] and inhibitory activity toward cholesterol biosynthesis.[7−10]

In our previous research on this genus, we reported two bioactive compounds, nigranoic acid[6] and micrandilactone C,[11] and the isolation and characterization of a series of

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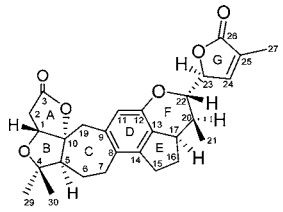
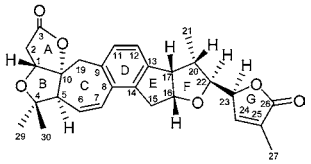
highly oxygenated nortriterpenoids with new skeleton, including micrandilactones A−G,[12−13] lancifodilactones A−G,[14−17] and henridilactones A−D.[18]

To search for more new bioactive compounds from the plants of this genus, we examined the leaves and stems of *Schisandra rubriflora* (Franch.) Rehd. et Wils, which led to the isolation of two novel highly unsaturated rearranged bisnortriterpenoids, rubriflordilactones A (**1**) and B (**2**). Both compounds possessed a bisnortripenoid backbone derived from cycloartane and featured a biosynthetically modified aromatic ring D, which first occurred in cycloartane triterpenoid. In addition, compounds **1** and **2** were tested for their cytotoxic activity against K562 cells and for anti-HIV-1 activity. Described in this paper are their structure elucidation and biological activities.



**1**



**2**

The leaves and stems of *S. rubriflora* were collected in Dali Prefecture of Yunnan Province, China, in August 2003 and identified by Prof. Su-Gong Wu. The air-dried and powdered stems and leaves (3.1 kg) were extracted with 70% aqueous $Me_2CO$ (3 × 8 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 fraction (77.0 g) was repeatedly chromatographed on silica gel and sephadex LH-20 (MeOH) to yield colorless mixed crystals of **1** and **2** (25 mg). Further purification with semipreparative HPLC (Agilent 1100 HPLC system, Germany; Zorbax SB-C-18, Agilent, 9.4 mm × 25 cm, U.S.A., MeOH/MeCN/$H_2O$ 20:18:62) led to the isolation of rubriflordilactones A (**1**, 12 mg) and B (**2**, 7 mg).

Rubriflordilactone A (**1**),[19] isolated as colorless crystals, showed a quasi-molecular ion peak at *m/z* 463 ([M − H$^-$]) in its negative ESI mass spectrum. The molecular formula of **1** was revealed as $C_{28}H_{32}O_6$ by HRESIMS data (found 463.2282, calcd 463.2277), corresponding to 13 degrees of unsaturation in the molecule. The $^1$H NMR spectrum (Table 1) exhibited three tertiary methyls and a secondary methyl.

**Table 1.** $^1$H and $^{13}$C NMR Assignments of **1** and **2**[a]

| | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ (mult, *J*, Hz) | $\delta_C$ | $\delta_H$ (mult, *J*, Hz) | $\delta_C$ |
| 1 | 4.30 (d, 6.1) | 80.0 d | 4.42 (d, 5.3) | 79.9 d |
| 2α | 2.83 (d, 18.3) | 36.1 t | 2.86 (d, 18.0) | 35.9 t |
| 2β | 3.19 (dd, 6.1, 18.3) | | 3.23 (overlapped) | |
| 3 | | 175.5 s | | 175.1 s |
| 4 | | 84.1 s | | 85.1 s |
| 5 | 2.32 (overlapped) | 60.2 d | 2.87 (overlapped) | 60.0 d |
| 6α | 1.83 (m) | 24.1 t | 5.72 (br d, 12.6) | 128.1 d |
| 6β | 1.58 (m) | | | |
| 7α | 2.99 (dd, 2.6, 16.2) | 30.8 t | 6.50 (d, 12.6) | 127.9 d |
| 7β | 2.71 (overlapped) | | | |
| 8 | | 134.5 s | | 133.2 s |
| 9 | | 126.7 s | | 130.8 s |
| 10 | | 99.1 s | | 103.4 s |
| 11 | 6.50 (s) | 116.1 d | 7.53 (d, 5.5) | 131.5 d |
| 12 | | 148.3 s | 7.89 (d, 5.5) | 129.3 d |
| 13 | | 124.8 s | | 140.3 s |
| 14 | | 145.1 s | | 144.5 s |
| 15α | 2.59 (overlapped) | 31.3 t | 3.23 (overlapped) | 30.7 t |
| 15β | 1.69 (m) | | 2.88 (overlapped) | |
| 16α | 2.67 (m) | 30.0 t | 4.86 (overlapped) | 83.5 d |
| 16β | 2.06 (m) | | | |
| 17 | 3.21 (m) | 37.8 d | 3.29 (dd, 5.3, 5.5) | 58.4 d |
| 19α | 2.84 (d, 15.6) | 40.7 t | 3.00 (d, 15.2) | 38.2 t |
| 19β | 3.54 (d, 15.6) | | 3.22 (d, 15.2) | |
| 20 | 2.30 (m) | 30.1 d | 2.31 (m) | 42.5 d |
| 21 | 0.82 (3H, d, 7.1) | 13.2 q | 1.17 (3H, d, 8.2) | 18.6 q |
| 22 | 4.05 (dd, 1.3, 7.8) | 83.5 d | 3.73 (dd, 5.2, 6.7) | 86.9 d |
| 23 | 4.96 (overlapped by $H_2O$) | 82.2 d | 4.86 (overlapped) | 81.1 d |
| 24 | 7.29 (br s) | 145.1 d | 6.88 (br s) | 146.8 d |
| 25 | | 132.0 s | | 130.8 s |
| 26 | | 171.9 s | | 173.0 s |
| 27 | 1.89 (3H, s) | 10.8 q | 1.76 (3H, s) | 10.7 q |
| 29 | 1.10 (3H, s) | 28.4 q | 1.24 (3H, s) | 28.3 q |
| 30 | 1.32 (3H, s) | 21.0 q | 1.32 (3H, s) | 21.9 q |

[a] Data were recorded in $C_5D_5N$ on Bruker AM-400 MHz ($^1$H, $^{13}$C) and Bruker DRX-500 MHz spectrometers (COSY, HMBC, NOESY). Chemical shifts ($\delta$) are expressed in ppm with reference to the most downfield signal of $C_5D_5N$ ($\delta$ 8.71 ppm) for $^1$H and to the center peak of the most downfield signal of $C_5D_5N$ ($\delta$ 149.9 ppm) for $^{13}$C.

The $^{13}$C and DEPT NMR spectra displayed 28 carbons, including two ester groups, eight quaternary carbons (two oxygenated sp$^3$ carbons and six olefinic carbons), eight methines (three oxygenated ones and two olefinic carbons), six methylenes, and four methyls. The data suggested that **1** was a highly oxygenated unsaturated bisnortriterpene and contained seven rings. In addition, the characteristic three resonances appearing as an ABX spin system at $\delta$ 4.30 (d, *J*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(19) Rubriflordilactone A (**1**): colorless crystals, mp 195−196 °C; $[\alpha]^{25.7}_D$ = −58.07 (*c* 0.114, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 215 (4.33), 260 (3.12) nm; IR (KBr) $\upsilon_{max}$ 3435, 2964, 2927, 2856, 1755, 1734, 1443, 1389, 1218, 1162, 924, 790 cm$^{-1}$; NMR can be found in Table 1; negative ESIMS *m/z* (rel int) 463 (100, [M − H]$^-$); HR-ESIMS found 463.2282, calcd for $C_{28}H_{31}O_6$ 463.2277.

= 6.1 Hz), 2.83 (d, $J$ = 18.3 Hz), and 3.19 (dd, $J$ = 6.1, 18.3) were assigned to H-1, H-2α, and H-2$\beta$, respectively.[11−14] Comparison of the $^1$H and $^{13}$C NMR data for **1** with those of micrandilactone B [11] strongly suggested a similar structure for rings A−C of both compounds, and this was confirmed by analysis of 2D NMR spectral data of **1**, which led to the establishment of partial structure **1a** (Figure 1).

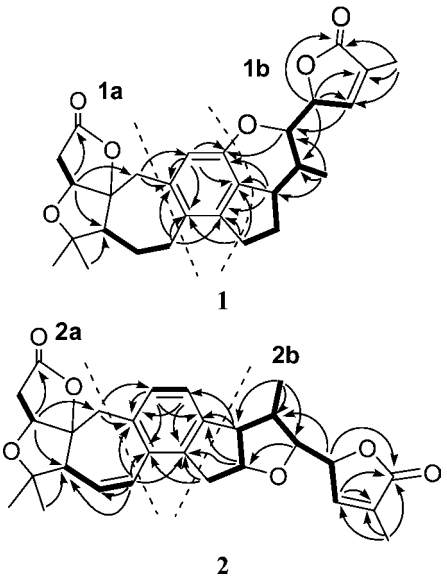


**Figure 1.** Fragments and key COSY (—), and HMBC (→) correlations of **1** and **2**.

The MS fragment at $m/z$ 97 $[C_5H_5O_2]^+$ and the HMBC correlations of both H-23 ($\delta$ 4.96) and Me-27 ($\delta$ 1.89) with C-24, C-25, and C-26 indicated the presence of a five-membered α-methyl-α,$\beta$-unsaturated-$\gamma$-lactone ring (G). In addition, HMBC also showed correlations from H-24 ($\delta$ 7.29) to C-22, H-23 to C-20, and Me-21($\delta$ 0.82, d, $J$ = 7.1 Hz) to C-17, C-20, and C-22. The above evidence, along with a proton spin system deduced from $^1$H−$^1$H COSY correlations, H-15/H-16/H-17/H-20/H-21/H-22/H-23/H-24, led to the establishment of partial structure **1b** (Figure 1). Apart from fragments **1a** and **1b**, there are still six olefinic carbons (five quaternary ones and one methine) to be assigned. Analysis of HMBC data showed the following correlations: H-7 with C-8, C-9, and C-14; H-19 with C-9 and C-11; H-15 with C-8, C-13, and C-14; H-17 with C-12, C-13, and C-14; H-22 with C-12; and H-11 with C-8, C-9, C-12, and C-13. The above evidence revealed the presence of a penta-substituted aromatic ring that connected fragments **1a** and **1b** and established the planar structure of **1**, which was finally confirmed by X-ray crystallographic analysis (Figure 2).[20]

The relative stereochemistry of **1** was also determined by X-ray analysis, together with NOESY correlations. According to the IUPAC sequence rule,[21] based on the chiral center with the lowest locant, the relative configuration of the seven chiral centers, C-1, C-5, C-10, C-17, C-20, C-22 and C-23, were deduced as $S$*, $S$*, $R$*, $R$*, $S$*, $S$*, and $S$*, respectively.

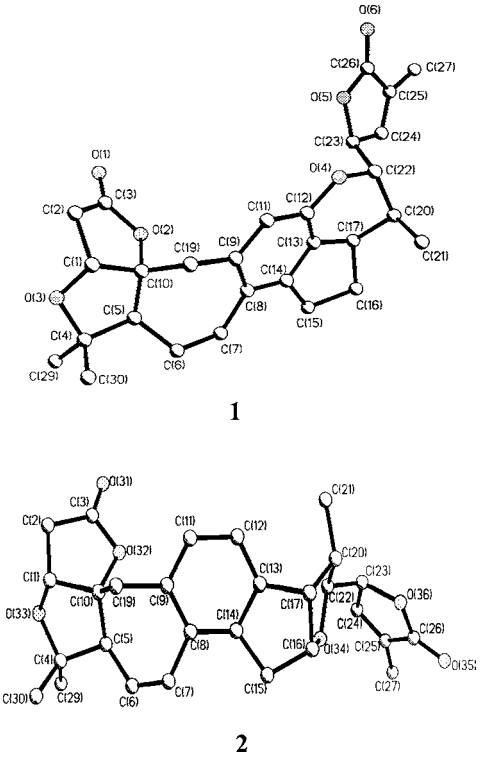


**Figure 2.** X-ray structures of **1** and **2** showing relative configuration.

Rubriflordilactone B (**2**),[22] was obtained as colorless prisms and has the molecular formula of $C_{28}H_{29}O_6$ as determined by analysis of $^1$H, $^{13}$C, and DEPT NMR spectral data, which

(20) Crystallographic data for **1**: $C_{28}H_{32}O_6$, $M$ = 464.54, orthorhombic, space group $P2_12_12$, $a$ = 32.771 (7) Å, $b$ = 13.440 (3) Å, $c$ = 5.621 (11) Å, $V$ = 2475.7 (9) Å$^3$, $Z$ = 4, $d$ = 1.246 g/cm$^3$, crystal dimensions 0.10 × 0.30 × 0.50 mm was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator ($\omega$−2$\theta$ scans, $2\theta_{max}$ = 50.0°), Mo Kα radiation. The total number of independent reflections measured was 2591, of which 2561 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: $R_1$ = 0.0917, $wR_2$ = 0.1465, $S$ = 1.125, $(\Delta/\sigma)_{max}$ = 0.003, $(\Delta\rho)_{min}$ = − 0.274e/Å$^3$, $(\Delta\rho)_{max}$= 0.471e/Å$^3$. Crystallographic data for **2**: $C_{28}H_{30}O_6$, $M$ = 462.52, orthorhombic, space group $P2_12_12_1$, $a$ = 10.299 (2) Å, $b$ = 10.771 (2) Å, $c$ = 21.683 (4) Å, $V$ = 2405.3 (8) Å$^3$, $Z$ = 4, $d$ = 1.277 g/cm$^3$, crystal dimensions 0.05 × 0.30 × 0.40 mm was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator ($\omega$−2$\theta$ scans, $2\theta_{max}$ = 50.0°), Mo Kα radiation. The total number of independent reflections measured was 2869, of which 2270 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: $R_f$ = 0.066, $R_w$ = 0.138 ($w = 1/\sigma\,|F|^2$). The crystal structures (**1** and **2**) were solved by direct methods using SHELXS-97 (Sheldrich, G. M. Univerisy of Gottingen: Gottingen, Germany, 1997) and expanded using difference Fourier techniques, refined by SHELXL-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1997) and full-matrix least-squares calculations. Crystallographic data for the structures of **1** and **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition numbers CCDC 288108 and 288109). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

(21) *IUPAC Nomenclature of Organic Chemistry*, Pergamon: New York, 1979; sections A−H. Recommendation for section A, spiro hydrocarbons.

(22) Rubriflordilactone A (**2**): colorless crystals, mp 201−202 °C; $[\alpha]^{27.9}_D$ = −119.84 ($c$ 0.514, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 216 (4.27), 255 (3.22) nm; IR (KBr) $\upsilon_{max}$ 3439, 2969, 2924, 2866, 1765, 1744, 1441, 1384, 1214, 1168, 1041, 928, 793 cm$^{-1}$; NMR can be found in Table 1; negative ESIMS $m/z$ (rel int) 461 (100, [M − H]$^-$); HR-ESIMS found 461.1955, calcd for $C_{28}H_{29}O_6$ 461.1964.

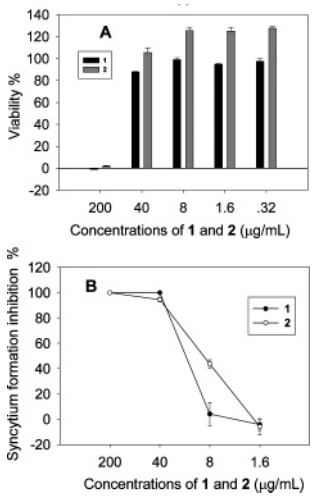


**Figure 3.** Cytotoxicity and anti-HIV-1 activities of **1** and **2** on C8166 cells: (A) cytotoxicity; (B) inhibition of HIV-$1_{IIIB}$-induced syncytium formation.

was verified by HR-ESIMS (found 461.1955, calcd 461.1964), requiring 14 degrees of unsaturation. The $^1$H NMR spectrum displayed signals due to three tertiary methyls and a secondary methyl. The $^{13}$C NMR spectrum of **1** exhibited signals for 28 carbons, including two ester groups, seven quaternary carbons (two oxygenated ones and five olefinic carbons), 12 methines (four oxygenated ones and five olefinic carbons), three methylenes, and four methyls. The above analysis suggested that **2** was also a highly oxygenated unsaturated bisnortriterpene like **1** and shared partial structural features with **1**. The presence of rings A−C (**2a**) and the five-membered α-methyl-α,$\beta$-unsaturated-$\gamma$-lactone ring G (Figure 1) was deduced by detailed comparison of $^1$H and $^{13}$C NMR spectral data with those of **1** and analysis of HMBC correlations of **2**. These, along with the critical HMBC correlation of H-22 ($\delta$ 3.73, dd, $J$ = 5.2, 6.7 Hz) with C-16 ($\delta$ 83.5) and a proton spin system deduced from $^1$H−$^1$H COSY correlations, H-15/H-16/H-17/H-20/H-21/H-22/H-23/H-24, led to the establishment of partial structure **2b** (Figure 1). The remaining six carbons were deduced to form a tetra-substituted aromatic ring by analysis of HMBC correlations as shown in Figure 1, which linked fragments **2a** and **2b** and led to the establishment of the planar structure of **2**. The relative configuration of the eight chiral centers, C-1, C-5, C-10, C-16, C-17, C-20, C-22, and C-23, were determined as *S**, *S**, *R**, *R**, *S**, *S**, *S**, and *S**, respectively, by analysis of NOESY data and confirmed by X-ray crystallographic analysis (Figure 2).[20]

Compounds **1** and **2** were tested for cytotoxicity against human tumor K562 cells by the MTT method as previously reported, and *cis*-platin was used as the positive control.[23] Both compounds showed no inhibitory activity against K562 cells with $IC_{50}$ values more than 200 $\mu$g/mL.

In addition, the anti-HIV activities and cytotoxicities of **1** and **2** were tested by microtiter syncytium formation infectivity assay, using the method previously described, with AZT as a positive control.[24,25] The assays included cytotoxicity in C8166 and MT-4 cells, inhibition of syncytium formation in HIV-$1_{IIIB}$-infected C8166 cells, and effect in protecting HIV-$1_{IIIB}$-infected MT-4 host cells from lytic effects in vitro. Compound **2** possessed low cytotoxicity ($CC_{50}$ = 120.7 $\mu$g/mL) on tested human T cell leukemia cell line C8166 at the assayed doses (Figure 3A). The inhibitory activity of **2** on HIV-$1_{IIIB}$-induced syncytium formation is summarized in Figure 3B, and the $EC_{50}$ was 9.75 $\mu$g/mL. The selectivity index (SI) was 12.39. As shown in Figure 4D, **2** exerted its obvious protection of HIV-$1_{IIIB}$ inducted

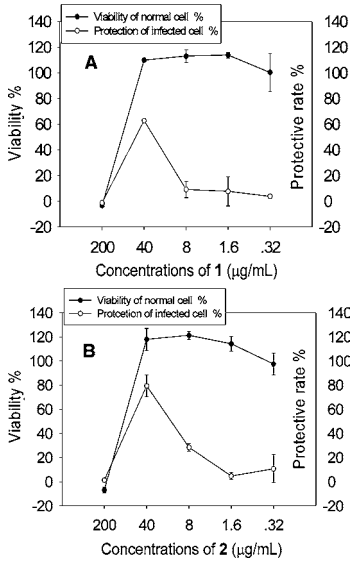


**Figure 4.** Protective activities of (A) **1** and (B) **2** toward HIV-$1_{IIIB}$-infected MT-4 cells.

MT-4 host cells lytic effects with a selectivity index of 6.09. Compound **1** showed weak anti-HIV-1 activity. Further biological evaluation is in progress to better define the anti-HIV-1 potency of **2**.

**Acknowledgment.** This project was supported by grants from the Natural Science Foundation of Yunnan Province (no. 2005XY04), the National Natural Science Foundation of China (no. 20402016), and Key Scientific and Technological projects of China (2004BA719A14) and Yunnan Province (2004NG12).

**Supporting Information Available:** 1D and 2D NMR spectra and crystallographic data of rubriflordilactones A (**1**) and B (**2**). These materials are available free of charge via the Internet at http://pubs.acs.org.

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