Structure and anti-HIV activity of micrandilactones B and C, new nortriterpenoids possessing a unique skeleton from *Schisandra micrantha*†

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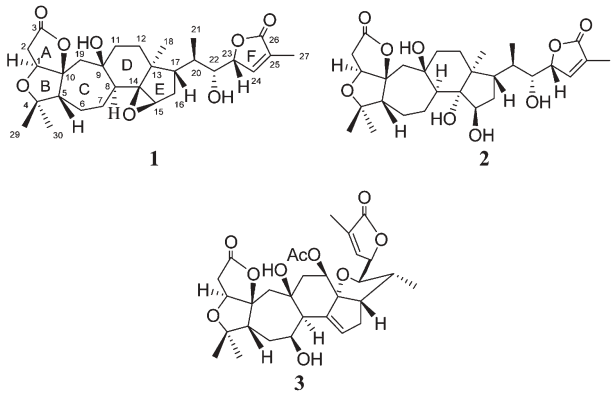
Two new highly oxygenated nortriterpenoids with a unique norcycloartane skeleton, micrandilactones B and C (1–2), were isolated from *Schisandra micrantha*; micrandilactone C (2) exhibited an EC_{50} value of 7.71 μg/mL (SI > 25.94) against HIV-1 replication with minimal cytotoxicity, and the potent anti-HIV-1 activity and unique structural features of 2 make it a promising lead for therapeutic development of a new generation of anti-HIV drug.

The current interest of our group in the phytochemical study of traditional Chinese medicine is aimed at finding new natural compounds with interesting biological activities and also investigating the occurrence of natural terpenoids, which could be used as natural sources of intermediates for the synthesis of high added value compounds. In this connection, we have focused on investigations of plants of the genus *Schisandra*,[1–7] which belongs to the economically and medicinally important family Schisandraceae. Previous studies of *Schisandra* species have reported lignans with various biological activities as typical of the genus.[8–10] We have recently reported the isolation of several novel highly oxygenated nortriterpenoids, such as micrandilactone A,[2] henridilactones A–D,[3] lancifodilactones B–E,[4] which belong to an unprecedented new nortriterpenoid skeleton with a biosynthetically modified eight-membered ring, and lancifodilactone A (**3**)[5] with an unusual new bisnortriterpenoid skeleton, from the leaves and stems of *Schisandra micrantha* A. C. Smith, *S. henryi* var. *yunnanensis* A. C. Smith, and *S. lancifolia* (Rehd. et Wils) A. C. Smith. Continued screening of the leaves and stems of *S. micrantha* led to the discovery of micrandilactones B and C (**1–2**), two novel nortriterpenoids with a unique highly oxygenated norcycloartane skeleton. Micrandilactone C (**2**) exhibited an EC_{50} value of 7.71 μg/mL (SI > 25.94) against HIV-1 replication with minimal cytotoxicity (> 200 μg/mL). Described herein are stereostructure elucidation and biological evaluation of two compounds.

Micrandilactone B (**1**) was isolated as colorless prisms, whose molecular formula of $C_{29}H_{40}O_8$ was established on the basis of HRESIMS analysis ($[M + Na]^+$, *m*/*z* 539.2634) and its ^{13}C NMR spectrum, indicating 10 degrees of unsaturation. Its EIMS displayed a small M^+ ion at *m*/*z* 516, and two fragment ions at *m*/*z* 498 and 480, corresponding to the successive loss of two molecules of water. The ^{13}C NMR spectrum of **1** (Table 1) revealed signals for 29 carbons: five methyls, seven methylenes, eight methines (including three oxygen-bearing carbons), one tri-substituted olefin, five quaternary carbons (four oxygenated) and two ester carbonyl groups. The data suggested that **1** was a highly oxygenated nortriterpene and contained seven rings. The ^1H NMR spectrum of **1** displayed signals for four tertiary methyls and one secondary methyl. In addition, the characteristic three resonances appearing as an ABX spin system at δ_H 4.28 (d, J = 4.8 Hz), 2.98 (dd, J = 4.8, 17.7 Hz) and 2.74 (d, J = 17.7 Hz) were assigned to H-1, H-2α and H-2β, respectively.[2–5] Comparison of the ^1H and ^{13}C NMR data for **1** with those of lancifodilactone A (**3**)[5] strongly suggested a similar structure for rings A–C of both compounds.



1 **2** **3**

By contrast, the data for the remaining portion of the structure of **1** were quite distinctive from those of **3**. Interpretation of the 2D NMR data, including the ^1H–^1H COSY and HMQC spectra, allowed us to construct the four partial structures (**a–d**) (Fig. 1). The HMBC spectrum was used to confirm the above proton spin-system assignments and establish the connectivities among the fragments described above. HMBC correlations of H-11α with C-8 and C-9, of H-7 with C-9, and of H_2-19 with C-9 and C-11, gave rise to the connectivity of partial structures **b** and **c** through C-9. The tertiary methyl at δ_H 0.94 (Me-18) showed HMBC correlations with C-12, C-13, C-14, and C-17, requiring the attachment of fragments **c** and **d** through C-13. This was further confirmed by the observation of HMBC correlations between H-11β and H-16β and C-13. Other correlations were noted from Me-27 to C-24, C-25 and C-26. This, along with the critical MS fragment at *m*/*z* 420 $[M - C_5H_4O_2]^+$ and a base peak at *m*/*z* 97 $[C_5H_5O_2]^+$, indicated the presence of a five-membered

† Electronic supplementary information (ESI) available: experimental procedures, copies of 1D and 2D NMR spectra, UV, IR and ESIMS data, X-ray crystallographic data for **1** and **2**. See http://www.rsc.org/suppdata/cc/b5/b501932j/

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Table 1 ^{1}H and ^{13}C NMR data for compounds **1** and **2** in pyridine-d_5[a]

	1		**2**	
No.	δ_H (mult; *J*, Hz)	δ_C	δ_H (mult; *J*, Hz)	δ_C
1	4.28 (d, 4.8)	82.1 d	4.16 (d, 4.5)	82.8 d
2α	2.98 (dd, 4.8, 17.7)	36.5 t	2.73 (dd, 4.5, 17.9)	37.2 t
2β	2.74 (d, 17.7)		2.35 (d, 17.9)	
3		174.9 s		177.6 s
4		84.8 s		86.2
5β	2.52 (dd, 3.7, 13.3)	59.2 d	2.29 (dd, 3.8, 15.1)	60.2 d
6α	1.33 (m)	27.0 t	1.28 (overlap)	29.3 t
6β	1.59 (m)		1.74 (overlap)	
7α	1.37 (m)	24.0 t	2.14 (m)	24.7 t
7β	1.95 (m)		1.60 (m)	
8	2.19 (overlap)	44.7 d	1.60 (overlap)	56.8 d
9		73.8 s		73.3 s
10		99.3 s		101.3 s
11α	1.87 (m)	38.5 t	1.58 (m)	38.5 t
11β	1.70 (m)		1.28 (m)	
12α	1.74 (m)	31.4 t	1.41 (m)	39.6 t
12β	2.26 (m)		1.98 (overlap)	
13		41.7 s		46.3 s
14		72.7 s		87.6 s
15	3.36 (br s)	54.7 d	3.73 (d, 3.5)	77.2 d
16α	1.48 (m)	31.9 t	1.88 (m)	36.0 t
16β	2.19 (m)		1.58 (overlap)	
17	1.78 (m)	45.9 d	1.94 (m)	54.7 d
18	0.94 (s)	15.3 q	1.00 (s)	18.2 q
19α	2.08 (s)	46.3 t	2.03 (ABd, 15.9)	47.0 t
19β	2.08 (s)		1.75 (ABd, 15.9)	
20	2.11 (m)	39.9 d	1.98 (m)	38.3 d
21	1.26 (d, 4.8)	14.6 q	0.98 (d, 4.5)	18.2 q
22	4.00 (br s)	72.8 d	3.63 (dd, 2.0, 6.8)	73.8 d
23	5.14 (br s)	82.0 d	5.04 (d, 1.8)	83.6 d
24	7.21 (br s)	148.8 d	7.08 (d, 1.8)	149.9 d
25		130.2 s		131.3 s
26		175.4 s		177.0 s
27	1.81 (s)	10.6 q	1.78 (s)	10.6 q
29	1.25 (s)	29.4 q	1.19 (s)	30.0 q
30	1.12 (s)	23.0 q	1.04 (s)	23.5 q
22-OH	6.62 (d, 6.1)			

[a] Data were recorded on a Bruker AM-400 MHz spectrometer (^{1}H, ^{13}C); chemical shifts (δ) are in ppm.

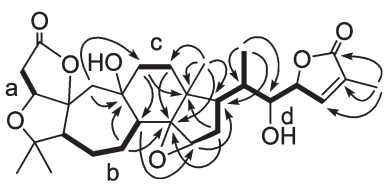

Fig. 1 Selected 2D NMR correlations for micrandilactone B (**1**). Bond in bold indicates ^{1}H–^{1}H COSY, arrow indicates HMBC.

α-methyl-α,β-unsaturated-γ-lactone ring (ring F).[5] The epoxide group was positioned between C-14 and C-15 on the basis of HMBC correlations from H-7β, H-12α and Me-18 to C-14, and from H-8 to C-15. The additional HMBC correlations from H-15 to C-16, and C-17 and from H-16β to C-14, supported this assignment and also connected the partial structures **b** and **d** through the trisubstituted epoxide.

Taking the above data into account, the planar structure of **1** was completely assembled. The relative configurations of all of the chiral centers of **1**, as well as the conformation of each ring were elucidated through the analysis of its ROESY data, coupling constants, and analogy with **3**. All the dipolar couplings observed

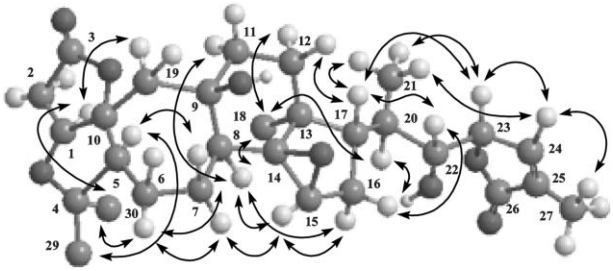

Fig. 2 Key ROESY correlations and relative configurations assigned for **1**.

for the C-1–C-10 fragment were in complete agreement with the stereochemistry reported for the same region in **3**. As depicted in the Chemdraw 3D molecular model (Fig. 2), ROESY correlation between H-15 and H-7α suggested that H-15 had α-orientation and thus the epoxide was placed in the β-position with respect to the half-chair form of ring E. The β-orientation of H-17 was supported by the observation of the ROESY cross-peak of H-17/H-12β. We could not, however, define the relative stereochemistry of C-20, C-22 and C-23, on the basis of the NMR spectra alone, since the σ-bond between C-17 and C-20 has free rotation. At this stage, a single-crystal X-ray diffraction analysis was performed, and a computer-generated, perspective drawing of the final X-ray model, less the hydrogen atom of **1**, is shown in Fig. 3.[11] Assuming the X-ray conformation for the side chain at H-17, Me-21, H-22 and H-23 were configured in *cis*-orientations, namely, all of the protons having the β-orientations.

Side by side comparison of ^{13}C NMR data between **2** and **1** (Table 1) indicated that the A/B/C and F rings were identical with those of **1**. The major difference in the ^{13}C NMR spectrum of **2** compared to **1** was the lack of a trisubstituted epoxide (δ_C 54.7, d; 72.7, s), and it being replaced by two new low field signals for an oxymethine at δ_C 77.2 (d) and an oxygenated quaternary carbon at δ_C 87.6 (s). These observations, in combination with the molecular formula of $C_{29}H_{42}O_9$ determined by HRESIMS (*m/z* 557.2739), suggested the presence of two more hydroxyls in the molecule of **2** with respect to **1**. The observed HMBC correlations between H-15 and C-13, C-14 and C-17, and between H-7, H-12, H-16 and Me-18 and C-14, along with a COSY cross-peak from H-15 to H-17, allowed the assignments of the two hydroxyls as being located at C-14 and C-15. The ROESY data for **2** were consistent with assignments for the same relative stereochemistry as in **1**. The only configuration that could not be independently assigned in **2** was that of C-14, due to a lack of relevant correlation. The remaining question was finally solved by a crystal structure analysis (Fig. 4).[12]

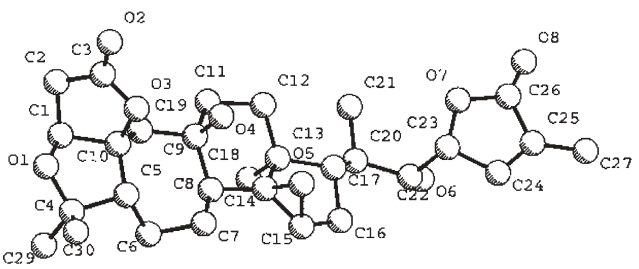

Fig. 3 X-Ray structure of micrandilactone B (**1**).

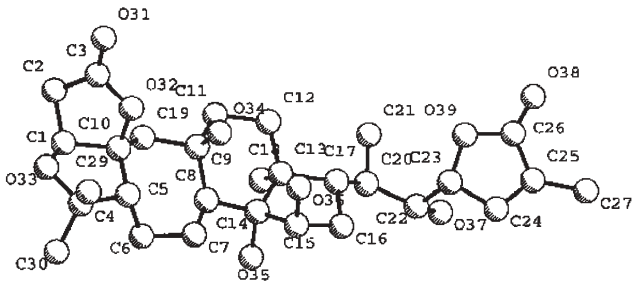

Fig. 4 X-ray structure of micrandilactone C (**2**).

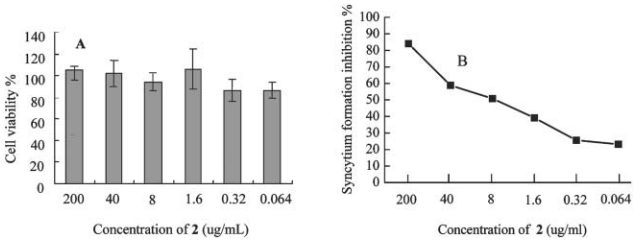

Fig. 5 Cytotoxicity and anti-HIV-1 activities of **2** on C8166 cells. Cytotoxicity (A), HIV-1_{IIIB} induced syncytium formation inhibition (B).

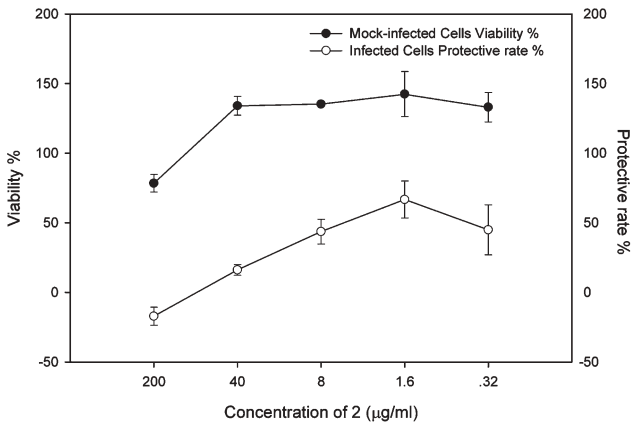

Fig. 6 Protective activities of **2** towards HIV-1_{IIIB} infected MT-4 cells.

The anti-HIV activities and cytotoxicities of **1** and **2** were tested (see supporting information: Table 2) by microtiter syncytium formation infectivity assay, using the method previously described, with AZT as a positive control.[13,14] The assays include 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 dying. Compound **2** possessed minimal cytotoxicity ($CC_{50} > 200$ μg/mL) on tested human T cell leukemia cell line C8166 at the assayed doses (Fig. 5A). The inhibitory activity of **2** on HIV-1_{IIIB} induced syncytium formation is summarized in Fig. 5B, and the EC_{50} was 7.71 μg/mL. The selectivity index (SI) was > 25.94. As shown in Fig. 6, **2** exerted its potent activity in protecting HIV-1_{IIIB} infected MT-4 host cells from dying with a selectivity index of > 425.5 at the concentration of 0.47 μg/mL. Compound **1** showed weak anti-HIV-1 activity.

This result is encouraging and warrants further structural modification to both decrease cytotoxicity and increase antiviral inhibitory activity. Micrandilactone C (**2**), which is a unique highly oxygenated nortriterpenoid with significant activity against HIV-1 replication, might be a promising lead compound for the preparation of anti-HIV drugs. Further biological evaluation is in progress to better define the anti-HIV potency of **2**. Such a study would provide valuable information on the therapeutic development of a new generation of anti-HIV drug.

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11 X-ray crystallographic analysis of micrandilactone B (**1**). $C_{29}H_{40}O_8$, M = 516.63, monoclinic, space group $P2_12_12_1$, a = 6.501(1), b = 12.672(1), c = 32.201(1) Å, V = 2652.7(2) Å^3, Z = 4, D_{calc} = 1.294 g cm^{-3}, crystal dimensions 0.10 × 0.20 × 0.40 mm. The total number of independent reflections measured was 3420, of which 2659 were observed ($|F|^2 \geqslant 8\sigma|F|^2$). The final indices were R_f = 0.069, R_w = 0.071 ($w = 1/\sigma|F|^2$).
12 X-ray data of micrandilactone C (**2**). Dimensions: 0.10 × 0.30 × 0.50 mm. $C_{29}H_{42}O_9$, M = 534.65, triclinic, space group $P1$, a = 7.878(1), b = 10.483(1), c = 18.484(1) Å, α = 84.67(1), β = 80.14(1), γ = 89.66(1)°, V = 1497.4(3) Å^3, Z = 1, d = 1.331 g cm^{-3}. The total number of independent reflections measured was 4589, of which 3735 were observed ($|F|^2 \geqslant 3\sigma|F|^2$). R_f = 0.053, R_w = 0.051 ($w = 1/\sigma|F|^2$). Crystallographic data for **1** and **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition numbers: CCDC 236725, 257495). See http://www.rsc.org/suppdata/cc/b5/b501932j/ for crystallographic data in CIF or other electronic format.
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