Three new nortriterpenoids from *Schisandra wilsoniana* and their anti-HIV-1 activities

Guang-Yu Yang, a,b Yin-Ke Li, c Xing-Jie Zhang, d Xiao-Nian Li, a Liu-Meng Yang, d Yi-Ming Shi, a Wei-Lie Xiao, a,* Yong-Tang Zheng, d Han-Dong Sun a,b*

a State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China
b Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, Yunnan, China
c School of Chemistry and Biotechnology, Yunnan Nationalities University, Kunming 650031, Yunnan, China
d Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, Yunnan, China

Received 5 July 2011; Accepted 21 July 2011
© The Author(s) 2011. This article is published with open access at Springerlink.com

**Abstract:** Three new highly oxygenated nortriterpenoids, Wilsonianadilactones D–F (1–3), were isolated from the leaves and stems of *Schisandra wilsoniana*. Their structures were established by means of spectroscopic analysis. Compounds 1–3 showed weak anti-HIV-1 activity with the therapeutic index (TI) values (CC50/EC50) greater than 8.16, 14.7, and 17.5, respectively.

**Keywords:** nortriterpenoid, Wilsonianadilactone, *Schisandra wilsoniana*

**Introduction**

*Schisandra wilsoniana* A. C. Smith (Schisandraceae) is a climbing plant mainly distributed in Heqing, Lijiang, Dali, and Yulong prefectures of Yunnan Province in the mainland of China. In previous work, a number of chemical constituents, such as nortriterpenoids,2 carotane sesquiterpenoids,3,4 neo-lignans,5 and dibenzocyclooctadiene lignans,6 were isolated from this plant. Some of these compounds showed in vitro anti-HIV-1 and anti-HBV activities.7,8 Motivated by the discovery of new and bioactive metabolites from this plant, our group has reinvestigated the chemical constituents of the leaves and stems of *S. wilsoniana*, which led to the isolation and characterization of three new highly oxygenated nortriterpenoids, Wilsonianadilactones D–F (1–3). Their structures were established by means of MS and extensive NMR spectra. This paper deals with the isolation, structural characterization, and anti-HIV-1 activity of these compounds.

**Results and Discussion**

A 70% aqueous acetone extract prepared from the leaves and stems of *S. wilsoniana* was partitioned between EtOAc and H2O. The EtOAc layer was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18, and semi-preparative RP-HPLC to afford compounds 1–3. Their structures were shown in Figure 1, and 1H and 13C NMR spectroscopic data were listed in Tables 1 and 2.

**Figure 1** The structures of compounds 1–3.

Wilsonianadilactone D (1) was obtained as optically active white crystals. Its molecular formula, C22H20O13, was established on the basis of HRESIMS analysis ([M + Na]+, m/z 615.2057, calc 615.2054) and its 1H and 13C NMR spectra. Analysis of the 1H and 13C NMR (Tables 1 and 2), HSQC and HMBC (Figure 2) spectra of 1 revealed the presence of 29 carbons, including two ester carbonyl groups, two ketos, four methyls, six methylenes (including an oxygenated one), eight methines (including four oxygenated ones), and seven quaternary carbons (including six oxygenated ones), which suggested a highly oxygenated triterpene skeleton. Comparison of the 1D and 2D NMR data of 1 with those of micrandilactone A9 revealed that compound 1 was structurally similar to micrandilactone A.10 The differences were resulted from the ap-
Pearance of an oxygenated methylene signal (δ 68.8 t; δ 3.59, 3.68, Abδ, J = 12.0 Hz), and the lack of a methyl signal (δ 27.7 s; δ 1.24 s in micrandilactone A) in 1. This indicated that a methyl group (C-29) in micrandilactone A was replaced by an oxygenated methylene group in 1 (Figure 1). This was also supported by the HMBC correlations of H-29 (δ1 3.59, 3.68) with C-4 (δ 87.9), C-30 (δ 16.4) and C-5 (δ 55.5); and of H-5 (δ1 3.02) and H-30 (δ 1.44) with C-29 (δ 68.8) (Figure 2). The other chiral centers were deduced to be the same as those of micrandilactone A by analysis of ROESY spectrum of 1 (Figure 3) and comparison of chemical shifts with those of micrandilactone A. Thus, the structure of 1 was established and given the trivial name of wilsonianadilactone D.

The molecular formula of C13H20O4 from its HRESIMS ([M + Na]+, m/z 581.2362, calcd 581.2363, [M + Na]+) analysis of compound 3 demonstrated that it had the same molecular formula of C13H20O4 as that of lancifodium lactone A.11 The 1H and 13C NMR spectra data of 3 were very similar to those of lancifodium lactone A. Analysis of HSQC, and HMBC (Figure 2) spectra of 3 showed that obvious differences were resulted from the location of acetoxyl group. The HMBC correlation of H-7 (δ1 4.82) with the acetyl carbon (δ 170.1) revealed that the acetoxyl group should be located at C-7 in 3 other than at C-12 in lancifodium lactone A. The ROESY correlations (Figure 3) observed in 3 and comparison of chemical shifts and coupling constants of 3 with those of lancifodium lactone A11 suggested that the two compounds have the same relative configurations. Therefore, the structure of compound 3 was determined as shown, and given the trivial name of wilsonianadilactones F (Figure 1).

Since some of nortriterpenoids from Schisandra spp exhibited modest or strong anti-HIV activities,2,14 compounds 1–3 were tested for their potencies in preventing the cytopathic effects of HIV-1 in C8166 cells and cytotoxicity was measured in parallel with the determination of antiviral activity, using AZT as a positive control (EC50 = 0.0045 μg/mL and CC50 > 200 μg/mL).19 Compounds 1–3 showed weak anti-HIV-1 activities with EC50 values of 24.5, 13.6 and 11.4 μg/mL, respectively, and all exerted minimal cytotoxicity against C8166 cells (CC50 > 200 μg/mL). The therapeutic index (TI) values (CC50/EC50) of 1–3 were greater than 8.16, 14.7, and 17.5, respectively.

**Table 1.** The 13C NMR (125 MHz, Pyridine-d$_5$, δ s in ppm) data of 1–3.

| Pos. | 1     | 2     | 3     |
|-----|-------|-------|-------|
| 1   | 81.8  | CH    | 81.8  | CH    | 83.4  | CH    |
| 2   | 35.1  | CH$_2$| 35.1  | CH$_2$| 35.9  | CH$_2$|
| 3   | 175.2 | C     | 175.2 | C     | 175.3 | C     |
| 4   | 87.9  | C     | 85.8  | C     | 84.4  | C     |
| 5   | 55.5  | C     | 56.2  | C     | 54.8  | C     |
| 6   | 38.0  | CH$_2$| 37.2  | CH$_2$| 34.8  | CH$_2$|
| 7   | 67.7  | CH    | 67.5  | CH    | 66.1  | CH    |
| 8   | 59.7  | CH    | 58.4  | CH    | 51.5  | CH    |
| 9   | 83.0  | C     | 83.2  | C     | 78.1  | C     |
| 10  | 93.9  | C     | 94.2  | C     | 98.7  | C     |
| 11  | 43.2  | CH$_2$| 42.2  | CH$_2$| 42.4  | CH$_2$|
| 12  | 32.5  | CH$_2$| 32.2  | CH$_2$| 74.8  | CH    |
| 13  | 49.3  | C     | 48.8  | C     | 98.1  | C     |
| 14  | 54.1  | CH    | 54.6  | CH    | 140.8 | CH    |
| 15  | 99.8  | C     | 99.2  | C     | 130.0 | C     |
| 16  | 209.9 | C     | 209.2 | C     | 31.9  | CH$_2$|
| 17  | 220.7 | C     | 219.9 | C     | 45.2  | CH    |
| 18  | 30.9  | CH$_2$| 30.8  | CH$_2$|
| 19  | 41.1  | CH$_2$| 41.2  | CH$_2$| 46.5  | CH$_2$|
| 20  | 80.2  | C     | 79.6  | C     | 37.9  | C     |
| 21  | 18.9  | CH$_2$| 18.5  | CH$_2$| 12.8  | CH$_2$|
| 22  | 75.5  | C     | 75.2  | C     | 82.2  | C     |
| 23  | 75.2  | CH    | 74.6  | CH    | 81.4  | CH    |
| 24  | 76.7  | CH    | 75.9  | CH    | 147.1 | CH    |
| 25  | 42.5  | CH$_2$| 42.2  | CH$_2$| 130.7 | C     |
| 26  | 177.6 | C     | 178.1 | C     | 174.3 | C     |
| 27  | 8.1   | CH$_2$| 8.3   | CH$_2$| 10.4  | CH$_2$|
| 28  | 68.8  | CH$_2$| 70.1  | CH$_2$| 28.5  | CH$_2$|
| 29  | 16.4  | CH$_2$| 17.2  | CH$_2$| 22.3  | CH$_2$|
| OAc | 170.3 | C     | 170.1 | C     |
|     | 21.2  | CH$_2$| 21.3  | CH$_2$|

**Figure 2** Selected HMBC and 1H-1H COSY correlations of 1 and 3.

**Figure 3** Selected ROESY correlations of 1 and 3.
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 DRX-500 spectrometers. Column chromatography was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 column (9.4 mm × 25 cm). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H2SO4 in EtOH.

Table 2. The 1H NMR (500 MHz, Pyridine-d5, δ in ppm, J in Hz) data of 1–3.

|     | 1                      | 2                      | 3                      | 1                      | 2                      | 3                      |
|-----|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Pos | 1                      | 2                      | 3                      | 1                      | 2                      | 3                      |
| 1   | 4.17, d (6.2)          | 4.27, d (5.9)          | 4.18, d (4.6)          | 17                     | 1.57, s                | 1.58, s                |
| 2n  | 2.94, dd (6.4, 18.5)   | 2.89, dd (6.2, 18.0)   | 2.99, dd (4.5, 18.2)   | 18                     | 1.5, d                  | 1.58, s                |
| 2f  | 2.75, d (18.5)         | 2.71, d (18.5)         | 2.65, d (17.4)         | 19a                    | 2.24, ABd (15.5)        | 2.50, ABd (15.3)        |
| 5   | 3.02, dd (4.5,13.5)    | 3.99, dd (4.5, 13.1)   | 3.29, dd (4.6, 13.0)   | 19β                    | 2.52, ABd (15.5)        | 2.21, ABd (15.3)        |
| 6a  | 1.95–1.98, overlap     | 1.93–1.97, overlap     | 2.02–2.07, overlap     | 20                     | 2.38, m                |                        |
| 6f  | 2.22–2.47, m           | 2.23–2.48, m           | 1.54–1.59, m           | 21                     | 1.60, s                | 1.58, s                |
| 7   | 4.40, dd (9.2, 10.4)   | 4.38, dd (9.2, 10.2)   | 4.82, br s             | 22                     | 3.68, dd (4.3, 9.6)     |                        |
| 8   | 2.99, d (10.4)         | 2.96, d (10.2)         | 2.60, br s             | 23                     | 4.96, d (1.8)           | 4.93, d (2.1)           |
| 11a | 1.95–1.98, overlap     | 1.93–1.97, overlap     | 2.28, dd (2.6, 15.4)   | 24                     | 5.40, dd (1.8, 2.2)     | 5.2, br s              |
| 11β | 1.74–1.79, m           | 1.72–1.76, m           | 2.16, dd (2.6, 15.4)   | 25                     | 3.23–3.27, m            | 3.20–3.25, m           |
| 12a | 1.95–1.98, overlap     | 1.93–1.97, overlap     | 5.26, br s             | 27                     | 1.20, d (7.1)           | 1.22, d (7.2)           |
| 12β | 1.58–1.62, m           | 1.55–1.59, m           | 2.9a                   | 29a                    | 3.59, ABd (12.0)        | 3.66, ABd (12.1)        |
| 14  | 3.23, s                | 3.20, s                | 29β                    | 3.68, ABd (12.0)       | 3.84, ABd (12.1)        |                        |
| 15  | 6.27, br s             | 14.4, s                | 1.46, s                | 30                     | 0.96, s                |                        |
| 16  | 2.35, m                | OAc                    |                         | 32                     | 2.12, s                | 2.03, s                |

Experimental Section

General Experimental Procedures. 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 DRX-500 spectrometers. Unless otherwise specified, chemical shift (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were performed on a VG Autospec-3000 spectrometer. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China). Preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 column (9.4 mm × 25 cm). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H2SO4 in EtOH.

Plant Materials. The leaves and stems of S. wilsoniana were collected in Heqing prefecture of Yunnan Province, China, in August 2006. The specimen was identified by Prof. Xi-Wen Li and a voucher specimen (No. KIB 2008-08-12) 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. The dried leaves and stems of S. wilsoniana (5.0 kg) were powdered and extracted with 70% aqueous MeCO (8 L × 4) for 24 hours at room temperature and filtrated. The filtrate was concentrated and partitioned with EtOAc. The EtOAc part (83.6 g) was chromatographed on silica gel column eluting with CHCl3–MeOH (1:0, 9:1, 8:2, 2:1, 1:1, and 0:1) to afford fractions I-VI. Fraction II (9:1, 14.4 g) was repeatedly chromatographed on silica gel (200–300 mesh) and Sephadex LH-20, then purified by preparative HPLC (MeOH:H2O:NaOEt:CH3CN:H2O, 40:55 and 40:60, and MEOH:CH3CN:H2O, 10:40:50 and 15:35:50) to yield compounds 1 (11.5 mg), 2 (8.3 mg) and 3 (14.7 mg).

Wilsonianadilactone D (1): colorless prisms; mp 180–181 °C; [α]D20 + 46.8 (c 0.25, MeOH); UV: end absorption; IR (KBr) νmax 3442, 2978, 2936, 1778, 1738, 1636, 1452, 1374, 1228, 1211, 1121, 1062, 1040, 1002, 915, 865 cm−1; 1H and 13C NMR, data see Tables 1 and 2; positive ion ESIMS m/z 615 [M + Na]+; HRESIMS m/z 615.2057 (calcd for C36H34NaO13[M + Na]+, 615.2054).

Wilsonianadilactone E (2): colorless crystals; mp 184–185 °C; [α]D20 + 43.2 (c 0.25, MeOH); UV: end absorption; IR (KBr) νmax 3448, 2962, 2925, 1792, 1743, 1735, 1654, 1460, 1431, 1346, 1116, 1012 cm−1; 1H and 13C NMR data see Tables 1 and 2; positive ion ESIMS m/z 657 [M + Na]+; HRESIMS m/z 657.2166 (calcd for C36H34NaO14[M + Na]+, 657.2159).

Wilsonianadilactone F (3): colorless crystals; mp 195–196 °C; [α]D20 + 25.8 (c 0.24, MeOH); UV: end absorption; IR (KBr) νmax 3443, 2972, 2935, 1762, 1729, 1628, 1436, 1376, 1248, 1186, 1067, 1028, 1008 cm−1; 1H and 13C NMR data see Tables 1 and 2; positive ion ESIMS m/z 581 [M + Na]+; HRESIMS m/z 581.2362 (calcd for C36H34NaO10[M + Na]+, 581.2363).

Cytotoxicity Assay. The cytotoxicity assay against C8166 cells (CC50) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytotoxic effects of HIV-1 (EC50).

Acknowledgments

This project was supported financially by the NSFC (No. 20802082 and 30830115), the projects from the Chinese Academy of Sciences (KSCX2-EW-Q-10, KSCX1-YW-R-24 and KSCX2-YW-R-185), the Major State Basic Research Development Program of China (No. 2009CB522303 and 2009CB940900), the Yong Academic and Technical Leader Rising Foundation of Yunnan Province (2006PY01-47), and the Natural Science Foundation of Yunnan Province (2005XY04 and 2006BB042Q).

Open Access This article is distributed under the terms of the Crea...
References

[1] Flora Yunnanica; Science Press: Beijing, 2000; Vol. 11, p 16.
[2] Yang, G. Y.; Xiao, W. L.; Chang, Y.; Wang, R. R.; Pu, J. X.; Gao, X. M.; Lei, C.; Sun, H. D. Helv. Chim. Acta. 2008, 91, 1871–1878.
[3] Ma, W. H.; Huang, H.; Zhou, P.; Chen, D. F. J. Nat. Prod. 2009, 72, 676–678.
[4] Yang, Y. E.; Li, X. Q.; Tang, C. P. Chin. J. Nat. Med. 2011, 9, 7–16.
[5] Zhang, X. J.; Yang, G. Y.; Wang, R. R.; Pu, J. X.; Sun, H. D.; Xiao, W. L.; Zheng, Y. T. Chem. Biodiv. 2010, 7, 2692–2701.
[6] Yang, G. Y.; Li, Y. K.; Wang, R. R.; Xiao, W. L.; Yang, L. M.; Pu, J. X.; Zheng, Y. T.; Sun, H. D. J. Asian Nat. Prod. Res. 2010, 12, 470–476.
[7] Yang, G. Y.; Li, Y. K.; Wang, R. R.; Li, X. N.; Xiao, W. L.; Yang, L. M.; Pu, J. X.; Zheng, Y. T.; Sun, H. D. J. Nat. Prod. 2010, 73, 915–919.
[8] Ma, W. H.; Lu, Y.; Huang, H.; Zhou, P.; Chen, D. F. Bioorg. Med. Chem. Lett. 2009, 19, 4958–4962.
[9] Yang, G. Y.; Fan, P.; Wang, R. R.; Cao, J. L.; Xiao, W. L.; Yang, L. M.; Pu, J. X.; Zheng, Y. T.; Sun, H. D. Chem. Pharm. Bull. 2010, 58, 734–737.
[10] Li, R. T.; Zhao, Q. S.; Li, S. H.; Han, Q. B.; Sun, H. D.; Lu, Y.; Zhang, L. L.; Zheng, Q. T. Org. Lett. 2003, 5, 1023–1026.
[11] Li, R. T.; Li, S. H.; Zhao, Q. S.; Lin, Z. W.; Sun, H. D.; Lu, Y.; Wang, C.; Zheng, Q. T. Tetrahedron Lett. 2003, 44, 3531–3534.
[12] Xiao, W. L.; Li, R. T.; Li, S. H.; Li, X. L.; Sun, H. D.; Zheng, Y. T.; Wang, R. R.; Lu, Y.; Wang, C.; Zheng, Q. T. Tetrahedron Lett. 2005, 46, 1263–1266.
[13] Xiao, W. L.; Zhu, H. J.; Shen, Y. H.; Li, R. T.; Li, S. H.; Sun, H. D.; Zheng, Y. T.; Wang, R. R.; Lu, Y.; Wang, C.; Zheng, Q. T. Org. Lett. 2005, 7, 2145–2148.
[14] Xiao, W. L.; Li, R. T.; Huang, S. X.; Pu, J. X.; Sun, H. D. Nat. Prod. Rep. 2008, 25, 871–891.
[15] Luo, X.; Chang, Y.; Zhang, X. J.; Pu, J. X.; Gao, X. M.; Wu, Y. L.; Wang, R. R.; Sun, H. D. Tetrahedron Lett. 2009, 50, 5962–5964.
[16] Lei, C.; Xiao, W. L.; Huang, S. X.; Chen, J. J.; Pu, J. X.; Sun, H. D. Tetrahedron Lett. 2010, 66, 2306–2310.
[17] Lei, C.; Huang, S. X.; Xiao, W. L.; Li, X. N.; Pu, J. X.; Sun, H. D. J. Nat. Prod. 2010, 73, 1337–1343.
[18] He, F.; Pu, J. X.; Huang, S. X.; Wang, Y. Y.; Xiao, W. L.; Li, L. M.; Liu, J. P.; Sun, H. D. Org. Lett. 2010, 12, 1208–1211.
[19] Wang, R. R.; Yang, L. M.; Wang, Y. H.; Pang, W.; Tam, S. C.; Tien, P.; Zheng, Y. T. Biochem. Biophys. Res. Commun. 2009, 382, 540–544.