ORGANIC LETTERS

2007
Vol. 9, No. 11
2079−2082

Isolation and Characterization of Biogenetically Related Highly Oxygenated Nortriterpenoids from *Schisandra chinensis*

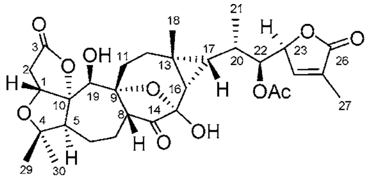
Sheng-Xiong Huang,[†] Rong-Tao Li,*,[†] Jing-Ping Liu,[†] Yang Lu,[‡] Ying Chang,[‡] Chun Lei,[†] Wei-Lie Xiao,[†] Li-Bin Yang,[†] Qi-Tai Zheng,[†] and Han-Dong Sun*,[†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, and Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, People's Republic of China

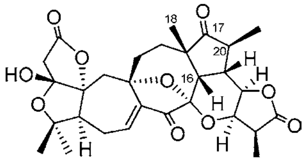
hdsun@mail.kib.ac.cn

Received February 28, 2007

ABSTRACT



pre-schisanartanin (1)



schindilactone A (2)

Pre-schisanartanin (1) and schindilactones A–C (2–4) were isolated from *Schisandra chinensis*. Among them, compound 1 had an unprecedented carbon skeleton. More importantly, the structure features of this compound provided new insight into the biosynthesis of triterpenoids with schisanartane skeleton. Their structures were determined by extensive spectroscopic analysis, single-crystal X-ray diffraction, and on the basis of the absolute structure of one related nortriterpenoid (5), which was determined for the first time by a modified Mosher method.

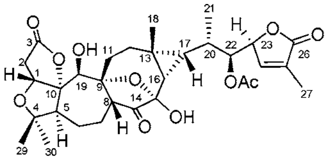
Over the past 10 years or so, considerable efforts of our group have been devoted to the discovery of bioactive and novel triterpenoids from the genus *Schisandra* species of the family Schisandraceae. About 40 highly oxygenated nortriterpenoids with three unprecedented carbon skeletons have been isolated and characterized,[1] some of which have potent anti-HIV-1 activity with low toxicity.[1a,d] The schisanartane skeleton triterpenoids are the main constituents of *Schisandra* species and are also a unique family of cycloartane derived triterpenoids because of their octacyclic backbone, which includes a 7/8/5 consecutive carbocycle and more than 12 chiral centers. This unusual ring assembly and highly oxygenated structure features are distinctive from any other naturally occurring triterpenoids. As a consequence, these structurally complex molecules have brought great interest and challenges to the chemists for total synthesis and biogenetic studies.[2]

[†] Kunming Institute of Botany.
[‡] Institute of Materia Medica.

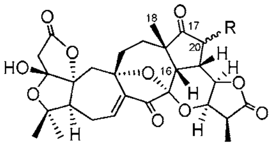
(1) (a) Sun, H. D.; Qiu, S. X.; Liu, L. Z.; Wang, Z. Y.; Lin, Z. W.; Pengsuparp, T.; Pezzuto, J. M.; Fong, H. H. S.; Cordell, G. A.; Famsworth, N. R. *J. Nat. Prod.* **1996**, *59*, 525−527. (b) Li, R. T.; Zhao, Q. S.; Li, S. H.; Han, Q. B.; Sun, H. D.; Lu, Y.; Zhang, L. L.; Zheng, Q. T. *Org. Lett.* **2003**, *5*, 1023−1026. (c) 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. (d) Li, R. T.; Han, Q. B.; Zheng, Y. T.; Wang, R. R.; Yang, L. M.; Lu, Y.; Sang, S. Q.; Zheng, Q. T.; Zhao, Q. S.; Sun, H. D. *Chem. Commun.* **2005**, 2936−2938. (e) Li, R. T.; Xiang, W.; Li, S. H.; Lin, Z. W.; Sun, H. D. *J. Nat. Prod.* **2004**, *67*, 94−97. (f) Li, R. T.; Xiao, W. L.; Shen, Y. H.; Zhao, Q. S.; Sun, H. D. *Chem. Eur. J.* **2005**, *11*, 2989−2996. (g) Li, R. T.; Shen, Y. H.; Xiang, W.; Sun, H. D. *Eur. J. Org. Chem.* **2004**, 807−811. (h) 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. *Org. Lett.* **2005**, *7*, 1263−1266. (i) 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. (j) Xiao, W. L.; Tian, R. R.; Pu, J. X.; Li, X.; Wu, L.; Lu, Y.; Li, S. H.; Li, R. T.; Zheng, Y. T.; Zheng, Q. T.; Sun, H. D. *J. Nat. Prod.* **2006**, *69*, 277−279.

10.1021/ol070510z CCC: $37.00

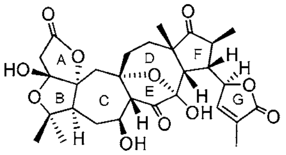
Schisandra chinensis (Turcz.) Baill. is a climbing plant widely distributed in the northeastern part of China, Korea, and Japan. It has long been used as sedative and tonic agents in traditional Chinese medicine. Investigation on this plant revealed that this plant is abundant in dibenzocyclooctadienlignans, which have been found to possess some beneficial pharmacological effects, including antihepatitis, antitumor, and anti-HIV activities.[3] In recent years, the fruits of it have been widely sold as phytomedicinal and dietary supplements in Europe, American, and Asian. With an aim to isolate structurally interesting and bioactive nortriterpenoids, as well as key intermediates to clarify the biogenetic pathway of triterpenoids with the schisanartane skeleton, purification of an extract of the aerial part of *S. chinensis* led to the isolation of micrandilactone B (**5**),[1d] lancifodilactone C (**6**),[1e] henridilactone D (**7**),[1g] and four new biogenetically related highly oxygenated nortriterpenoids that were named as pre-schisanartanin (**1**) and schindilactones A−C (**2**−**4**). Among them, compound **1** has an unprecedented carbon skeleton. And this compound provides us new insight into the biosynthesis of triterpenoids with the schisanartane skeleton. Here, we describe the structure elucidation and biological properities of these novel nortriterpenoids and propose a biosynthetic route for their generation, which can be traced back to micrandilactone B (**5**).



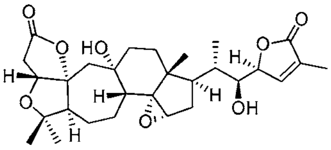
1



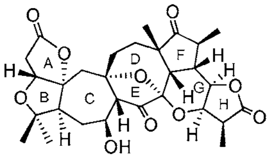
2 R = β-CH_3
3 R = α-CH_3



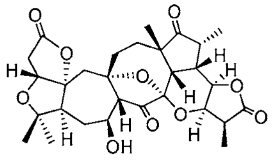
4



5



6



7

The absolute configuration of the known metabolite **5**, $[\alpha]_D^{25}$ 7.8 (*c* 0.20, CH_3OH) was determined for the first time by a modified Mosher method.[4] Comparison of the ^1H NMR chemical shifts between the (*R*)- and (*S*)-MTPA esters of **5** led to the assignment of the *S*-configuration at C-22 for micrandilactone B (**5**) (Figure 1). Therefore, the complete absolute configuration of **5** was unambiguously determined as shown in its formula.

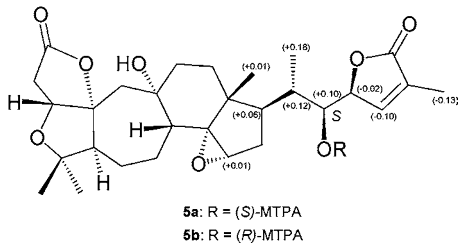


Figure 1. ^1H NMR chemical shift differences $\Delta\delta$ ($\delta_S - \delta_R$) in ppm for the MTPA esters of **5**.

Pre-schisanartanin (**1**) was obtained as colorless crystals that gave an ion peak at *m*/*z* 611.2474 in the HRESIMS, consistent with an elemental composition of $C_{31}H_{40}O_{11}Na$ (calcd 611.2468). The IR spectrum of **1** showed bands characteristic of hydroxyl and carbonyl groups (3441 and 1764 cm^{-1}). Analysis of the ^1H and ^{13}C NMR spectra of **1** showed the presence of a ketone (δ_C 216.0), two lactone carbonyl carbons (δ_C 175.5 and 174.3), and a trisubstituted double bond. Also evident was a three-membered carbon ring due to the characteristic signals in the ^1H and ^{13}C NMR spectra (δ_C 26.1, s, C-13; δ_H 0.84, t like, $J = 7.0$ Hz, H-17) (Table 1). Comprehensive analysis of 2D NMR data, including the results of COSY, HSQC, and HMBC experiments, enabled the complete planar structure of pre-schisanartanin to be assigned, as in **1**, to a highly oxygenated nortriterpene skeleton including a unique 7/8/3 consecutive carbocycle. Portions of the relative stereochemistry of **1** were readily assigned by the ROE NMR method. However, the relative stereochemistry of C-20, C-22, and C-23 could not be determined by the NMR data 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,[5] and a computer-generated, perspective drawing of the X-ray model, less the hydrogen atom of **1**, was shown in Figure 2, which confirmed the planar structure of **1** and revealed the relative stereochemistry of C-20, C-22, and C-23 as represented in **1**.

Structure determinations of schindilactones A−C (**2**−**4**) were conducted by careful analysis of their 1D and 2D NMR spectroscopic data and analogy to that of lancifodilactone C (**6**). The structure of schindilactone A (**2**) was further confirmed by the X-ray experiment (Figure 2).[5] Thus, we discuss only the elucidation of altered structural features of **3** and **4**. Compounds **2** and **3** had the same molecular

---

(2) (a) Tang, Y. F.; Zhang, Y. D.; Dai, M. J.; Luo, T. P.; Deng, L. J.; Chen, J. H.; Yang, Z. *Org. Lett.* **2005**, *7*, 885−888. (b) Zhang, Y. D.; Tang, Y. F.; Luo, T. P.; Shen, J.; Chen, J. H.; Yang, Z. *Org. Lett.* **2006**, *8*, 107−110.

(3) (a) Chang, J. B.; Reiner, J.; Xie, J. X. *Chem. Rev.* **2005**, *105*, 4581−4609. (b) Opletal, L.; Sovová, H.; Bártlová, M. *J. Chromatogr. B* **2004**, *812*, 357−371.

(4) (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519. (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

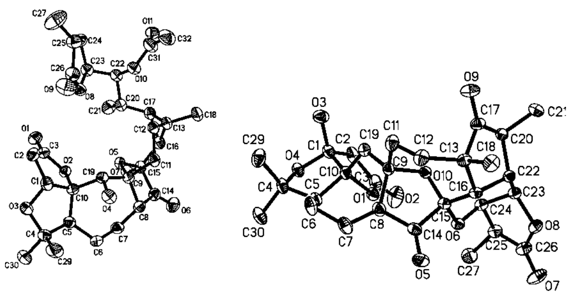
(5) Crystal data of pre-schisanartanin (**1**) and schindilactone A (**2**) can be found in the Supporting Information. CCDC-636567 and CCDC-636568 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax (+44) 1223−336-033; or deposit@ccdc.cam.ac.uk).

Table 1. ^{13}C NMR Data of Compounds **1**−**4** (pyridine-d_5, 100 MHz, δ in ppm)[a]

position	1	2	3	4
C-1	79.4	108.3	108.3	109.0
C-2	35.4	44.0	43.9	43.1
C-3	175.5	173.3	173.2	173.6
C-4	84.2	83.9	83.9	84.7
C-5	62.4	58.4	58.5	58.1
C-6	23.8	23.6	23.6	36.9
C-7	27.1	135.2	134.7	69.9
C-8	56.6	138.0	138.3	59.7
C-9	82.6	81.9	82.2	83.6
C-10	98.4	96.5	96.4	97.5
C-11	38.4	39.7	39.7	43.5
C-12	24.8	31.5	30.7	32.8
C-13	26.1	50.6	50.3	50.5
C-14	216.0	198.8	198.9	215.1
C-15	99.1	99.0	98.2	101.2
C-16	31.3	45.7	46.7	53.8
C-17	34.5	220.4	221.5	221.9
C-18	28.4	26.4	26.7	28.0
C-19	70.7	40.8	41.2	40.2
C-20	31.5	44.9	33.4	46.3
C-21	17.8	14.8	12.7	15.6
C-22	76.4	40.3	41.3	46.4
C-23	82.6	75.3	74.7	80.2
C-24	147.0	68.5	71.0	151.3
C-25	130.7	42.4	42.6	131.5
C-26	174.3	178.2	177.9	174.6
C-27	10.5	8.5	8.5	10.8
C-29	21.9	24.4	24.4	25.5
C-30	28.6	29.0	29.0	30.1

[a] The assignments were based on DEPT, $^1H-^1H$ COSY, HSQC, and HMBC experiments.

formula, $C_{29}H_{34}O_{10}$, as determined by HRESIMS. Careful analysis of their ^1H and ^{13}C NMR data indicated that **2** and **3** might be C-20 epimers. This deduction was fully confirmed by the abnormal upfield shift of C-20 from δ_C 44.2 in **2** to δ_C 33.2 in **3** due to the γ-steric compression effect between the oxygen atom at C-23 and H-20 in **3** (Table 1). The large shift difference in ^{13}C NMR of C-20 in **2** and **3** was also found for compounds **6** and **7**. Careful investigation on the ^1H and ^{13}C NMR data of **4** revealed it had structural similarity (rings A−F) to that reported for lancifodilactone C (**6**) and henridilactone D (**7**). The critical FABMS peak at m/z 97 $[C_5H_5O_2]^+$ as well as the HMBC correlations of H_3-27 (δ_H 2.32) with C-24, C-25, and C-26 indicated the presence of a five-membered α-methyl-α,β-unsaturated-γ-lactone ring. On the basis of this deduction, there must be a hydroxyl group at C-15 rather than an ether bond between C-15 and C-24 as in **6** and **7**. Thus, the structure of schindilactone C was determined as shown in **4**.



1 **2**

Figure 2. X-ray molecular structures of **1** and **2**.

It was noteworthy to mention that the absolute structures of **1**−**4**, as shown in their formulas, were confirmed after determination of the absolute structure of **5** as they had the same sign of optical rotation and on the basis of biosynthetic reasoning.

Compound **1** has a complex cyclic nortriterpenoid skeleton without precedent among known natural products. Especially, the structural feature of the 8/3 consecutive carbocycle in **1** provides us new insight into the formation of the 8/5 consecutive carbocycle in nortriterpenoids with schisanartane skeleton from the viewpoint of biosynthesis. The co-occurrence of **1** with various nortriterpenoid lactones (**2**−**7**) within the same plant raises the possibility that triterpenoids with the schisanartane skeleton result from a further modification of an existing metabolite. Herein, we propose a new possible biosynthetic route for schisanartane in order to trigger further studies and make valuable contributions to biomimetic semisynthesis or total chemical synthesis.[6]

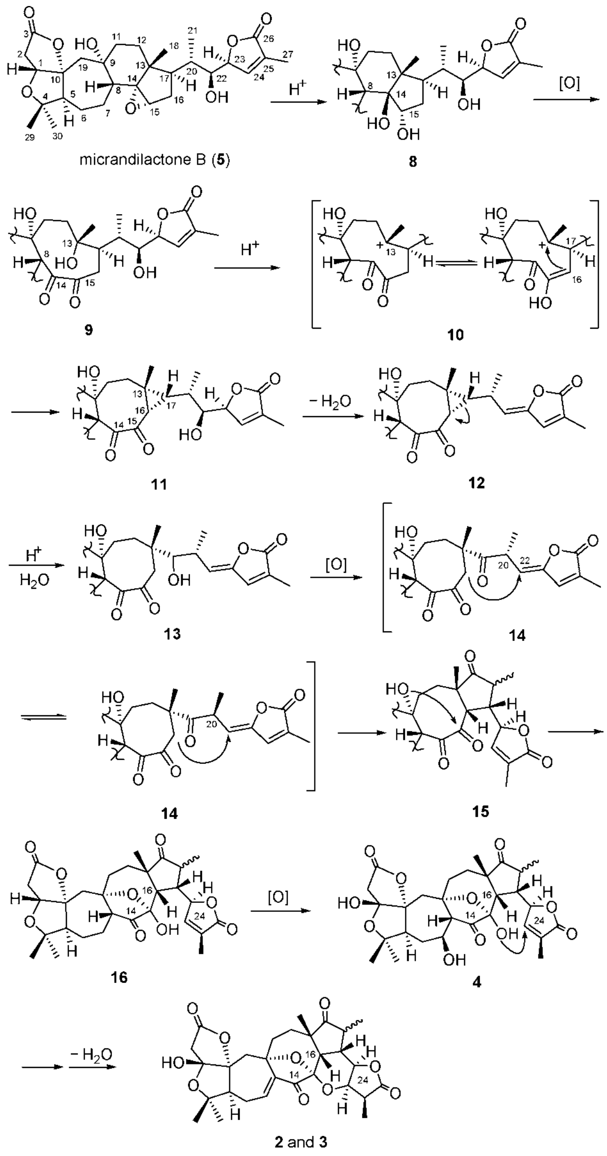
Given the core skeleton of *seco*-cycloartane is still preserved in micrandilactone B (**5**) and the key structure moiety of 28-nor-14,15-epoxy is very common in naturally occurring triterpenoids, it is concluded that **5** should occur early in Scheme 1 at the biogenetic origin of the schisanartane skeleton. The epoxy ring in **5** is suitable for the enzymatic opening that results in a 14,15-dihydroxy intermediate **8**. Afterward, **8** undergoes an oxidative cleavage of the C13−C14 carbon bond and oxidation of OH-15 to generate the 14,15-diketone intermediate **9**. This intermediate **9** can easily convert to the nine-membered-ring enolic cation intermediate **10** in acidic condition. The C-13 cation reacts with the electrophilic centers (C-16), thus giving **11**, which may be the precursor of **1**. Therefore, **11** can be converted to **1** in a few plausible steps.[7] The cleavage of the three-membered ring in **12**, then oxidation of **13** gives an intermediate **14**. Subsequent conversion of **14** into **16** may involve two consecutive ring closures between C-16 and C-22 (Michael addition), 9-OH and the carbonyl group at C-15. The oxidation of C-1 and C-7 of **16** can yield **4**. Schindilactone C (**4**) undergoes a Michael addition between 15-OH and C-24, and then dehydrogenation at C-7 and C-8 generates schindilactones A (**2**) and B (**3**). This pathway is consistent with some previously reported studies. In fact, this pathway explains why we always obtain the C-20 epimeric schisanartane type of nortriterpenoids. Moreover, the inter-

---

[6] Details about the biomimetic semisynthesis route of the schisanartane type of triterpenoids are presented in the Supporting Information.

[7] See the Supporting Information for proposed biosynthetic pathways leading to pre-schisanartanin (**1**) and lancifodilactone G.

Scheme 1. Hypothetical Biogenetic Route of the Schisanartane Type of Compounds **2**−**4**



mediate **15** can easily convert to its C-14 enol isomer, and then this enol isomer follows further ring closures and oxidation approaches to afford lancifodilactone G.[1i] This assumption reasonably accounts for the occurrence of the enol structure moiety in lancifodilactone G,[7] though the corresponding ketone isomer is more stable than the enol structure.

Compounds **1**−**7** were tested for cytotoxicity against human tumor K562, HT-29, and A549 cells by the MTT method as previously reported.[8] All compounds showed no inhibitory activity against those cells with IC_{50} values of >100 μg/mL. Additionally, the anti-HIV-1 activities of **1**−**7** were tested by microtiter syncytium formation infectivity assay, using the method previously described, with AZT as a positive control.[9] Pre-schisanartanin **1** demonstrated anti-HIV-1 activity with an EC_{50} value of 13.81 μg/mL (AZT: EC_{50} = 2.26 μg/mL). Compounds **2**−**7** showed weak anti-HIV-1 activity with EC_{50} values of >50 μg/mL.

Acknowledgment. The authors are grateful to Dr. Hao Xu, the Scripps Research Institute, University of California, for their generous support and helpful discussion on the proposed biogenetic pathway and on the gross biomimetic semisynthesis route. We also appreciate Prof. Jun-Lin Yu, Tonghua Teachers College, for collecting the plant material. Financial support of this research was provided by the National Natural Science Foundation of China (No. 20402016), the Natural Science Foundation of Yunnan Province (Nos. 2005XY04 and 2006B0042Q), and Technical Leader Raising Foundation of Yunnan Province (No. 2006PY01-47).

Supporting Information Available: Detailed description of the experimental procedures, a listing of ^1H NMR data of compounds **1**−**4**, IR and MS data for compounds **1**−**4**, and CIF files of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL070510Z

---

(8) Niu, X. M.; Li, M. L.; Zhao, Q. S.; Mei, S. X.; Na, Z.; Wang, S. J.; Lin, Z. W.; Sun, H. D. *Planta Med.* **2002**, *68*, 528−533.

(9) (a) Wang, J. H.; Tam, S. C.; Huang, H.; Ouyang, D. Y.; Wang, Y. Y.; Zheng, Y. T. *Biochem. Biophys. Res. Commun.* **2004**, *317*, 965−971. (b) Wang, Q.; Ding, Z. H.; Liu, J. K.; Zheng, Y. T. *Antiviral Res.* **2004**, *64*, 189−194.