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Tetrahedron
Tetrahedron 64 (2008) 4260–4267
www.elsevier.com/locate/tet

Isolation and characterization of miscellaneous terpenoids of *Schisandra chinensis*

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Received 22 November 2007; received in revised form 22 February 2008; accepted 26 February 2008
Available online 29 February 2008

Abstract

Two new bisnortriterpenoids with 18-norschiartane skeleton, wuweizidilactones G (**1**) and H (**2**), four new highly oxygenated nortriterpenoids based on a schisanartane skeleton, schindilactones D–G (**3**–**6**), a pre-schisanartane skeleton, pre-schisanartanin B (**7**), and a novel 3,4-*seco*-21,26-olide-artane triterpenoid wuweizilactone acid (**8**), along with 24 known terpenoids with different carbon frameworks, have been isolated from the acetone extract of the stems and leaves of *Schisandra chinensis*. The terpenoids produced by this plant have chemical diversity. The structures of new compounds **1**–**8** have been characterized by spectroscopic data interpretation. The cytotoxicity and anti-HIV-1 activity of all the *Schisandra* nortriterpenoids were evaluated.

Keywords: *Schisandra*; *Schisandra* nortriterpenoids; Wuweizidilactone; Schindilactone; Wuweizilactone acid

1. Introduction

Schisandra nortriterpenoids are a structurally intriguing group of highly oxygenated, polycyclic, fused heterocyclic natural products isolated from the plants of the genus *Schisandra*.[1] A series of these compounds exhibiting different carbon frameworks and oxygenated pattern, such as schiartane,[2] 18-norschiartane,[3] 18(13→14)-*abeo*-schiartane,[4] schisanartane,[5] pre-schisanartane,[6] and wuweiziartane[7] skeletons, have been so far reported from different species of geographically distinct regions. Recently, we have initiated a program to discover structurally unique and bioactive natural products (especially the triterpenoids with novel carbon skeleton) from different *Schisandra* species. In this study, chemical constituents of a Traditional Chinese Medicine (TCM), the stems and leaves of *Schisandra chinensis*, have been extensively investigated. Our previous studies on the same species collected from the same region have led to the identification of several structurally interesting *Schisandra* nortriterpenoids, displaying novel carbon skeletons.[4,6,7] Further studies led to the isolation of a series of terpenoids with miscellaneous carbon skeletons, including 18-norschiartane (**1**, **2**, **9**, and **10**),[4] schisanartane (**3**–**6** and **11**–**17**),[6] pre-schisanartane (**7** and **18**),[6] 3,4-*seco*-21,26-olide-artane (**8**), schiartane (**19**),[2] 18(13→14)-*abeo*-schiartane (**20**–**23**),[4] artane (**24**),[8] 3,4-*seco*-lanostane (**25** and **26**),[9,10] ursane (**27** and **28**),[11] abietane (**29**),[12] clovane (**30**),[13] and menthane (**31** and **32**) skeletons,[14] which were detected in the acetone extract of this plant. Thus, the terpenoids produced by this plant showed obvious chemical diversity. The structures of new triterpenoids **1**–**8**, exhibiting four different carbon frameworks, were determined by spectroscopic data and analogous analysis with

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0040-4020/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.02.085

related known molecules. All the isolates were evaluated for their cytotoxicity and anti-HIV-1 activity.

2. Results and discussion

Wuweizidilactone G (**1**) was isolated as a white solid. Its molecular formula was determined to be $C_{35}H_{44}O_{13}$ (14 degrees of unsaturation) by means of analyzing ^{1}H, ^{13}C, and DEPT NMR spectroscopic data, and was also verified by high-resolution electrospray ionization mass spectrometry (HRESIMS) data (calcd 695.2664 $[M+Na]^{+}$, found 695.2679). DEPT NMR results indicated that there were 43 protons bound to carbon atoms and one exchangeable proton. The ^{1}H and ^{13}C NMR spectra of **1** showed the coexistence of five singlet and two doublet methyls, five methylenes, four aliphatic sp^3 methine carbon atoms, six oxygenated sp^3 methine carbons, six sp^3 quaternary carbon atoms, four ester groups, and one trisubstituted double bond (Tables 1 and 2). This observation suggested that **1** was likely to be an 18-norschiartane-type bisnortriterpenoid that was substituted by an angeloyl and an acetyl groups.[4] This assumption was subsequently confirmed by conducting a set of 2D NMR spectroscopic experiments ($^{1}H-^{1}H$ COSY, HSQC, HMBC, and ROESY spectra). The complete structure of **1** was elucidated by analyzing the 2D NMR data obtained and by comparing these results with the NMR data obtained for wuweizidilactone B (**10**).[4] The close similarities of the NMR data for rings A–F with those of a known compound **10** suggested that **1** has a similar substructure to that of **10**, but with the differences in the chemical shifts at C-23 and C-27 of G ring. These changes, along with the completely different carbon and proton chemical shifts of C-24 (δ_C 62.6, d) and C-25 (δ_C 56.3, s), indicated that the double bond in **10** was replaced by the epoxy ring in **1**. The substructure of G ring was further revealed by the $^{1}H-^{1}H$ COSY correlations of H-23 with H-24 and HMBC correlations of H_3-27 with C-24, C-25, and C-26. Furthermore, in the ROESY spectrum, the correlations of H_3-27 with H-23 suggested the α-orientation of epoxy ring between C-24 and C-25. Thus, the complete structure of **1** was established as shown in Figure 1.

Wuweizidilactone H (**2**) was obtained as a white solid and had the molecular formula of $C_{28}H_{36}O_{10}$ as determined by analysis of ^{1}H, ^{13}C, and DEPT NMR spectroscopic data, which were verified by HRESIMS (found 525.2197 $[M+Na]^{+}$, calcd 525.2206), requiring 11 degrees of unsaturation. The A–D rings' ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) were analogous to those of **1**. One of the main differences observed in the ^{13}C NMR spectrum was that the signals corresponding to angeloyl and acetyl substituents at C-7 and C-12 in **1** were absent in **2**. These observations coupled with the analysis of 2D NMR data revealed the A–D rings structure (Fig. 2).

The characteristic NMR signals of C-14 (δ_C 70.5, s) and C-15 (δ_H 3.73, br s; δ_C 54.1, d) suggested the presence of

Table 1
^{13}C NMR data of compounds**1**–**8** in pyridine-d_5, δ in parts per million[a]

No.	1	2	3	4	5	6	7	8
1	82.0 (d)	82.0 (d)	108.7 (s)	108.8 (s)	108.1 (s)	108.7 (s)	79.4 (d)	29.9 (t)
2	35.7 (t)	35.7 (t)	43.7 (t)	43.1 (t)	43.5 (t)	43.2 (t)	35.4 (t)	32.6 (t)
3	174.9 (s)	175.1 (s)	173.5 (s)	173.4 (s)	172.6 (s)	173.1 (s)	175. 5 (s)	177.0 (s)
4	84.2 (s)	84.3 (s)	84.2 (s)	84.7 (s)	85.2 (s)	84.3 (s)	84.2 (s)	149.9 (s)
5	53.8 (d)	52.8 (d)	58.9 (d)	58.3 (d)	58.0 (d)	54.6 (d)	62.4 (d)	45.8 (d)
6	31.2 (t)	33.5 (t)	36.3 (t)	36.6 (t)	22.3 (t)	28.0 (t)	23.8 (t)	27.7 (t)
7	70.1 (d)	68.8 (d)	68.4 (d)	68.8 (d)	19.9 (t)	64.2 (d)	27.1 (t)	25.2 (t)
8	46.5 (d)	45.7 (d)	60.2 (d)	60.5 (d)	48.6 (d)	61.2 (s)	56.6 (d)	46.5 (d)
9	75.1 (s)	78.6 (s)	81.6 (s)	81.8 (s)	81.4 (s)	79.9 (s)	82.6 (s)	20.9 (s)
10	98.1 (s)	98.5 (s)	97.1 (s)	97.4 (s)	98.7 (s)	97.1 (s)	98.4 (s)	27.7 (s)
11	41.9 (t)	41.8 (t)	42.5 (t)	41.5 (t)	35.9 (t)	36.6 (t)	38.4 (t)	26.9 (s)
12	70.0 (d)	68.8 (d)	31.2 (t)	30.5 (t)	31.4 (t)	32.0 (t)	25.0 (t)	32.2 (t)
13	91.2 (s)	84.2 (s)	50.3 (s)	49.9 (s)	49.9 (s)	50.7 (s)	25.9 (s)	46.1 (s)
14	69.8 (s)	70.5 (s)	209.9 (s)	209.5 (s)	209.6 (s)	208.2 (s)	216.0 (s)	48.6 (s)
15	55.4 (d)	54.1 (d)	99.0 (s)	98.1 (s)	98.9 (s)	98.2 (s)	99.1 (s)	46.7 (t)
16	26.8 (t)	24.9 (t)	45.2 (d)	45.8 (d)	44.3 (d)	46.5 (d)	31.3 (d)	71.7 (d)
17	43.2 (d)	33.9 (d)	220.4 (s)	221.6 (s)	220.0 (s)	220.0 (s)	34.5 (d)	152.5 (s)
18			26.1 (q)	26.0 (q)	25.8 (q)	26.6 (q)	28.5 (q)	25.1 (q)
19	46.4 (t)	46.6 (t)	40.7 (t)	40.8 (t)	43.2 (t)	37.6 (t)	70.7 (d)	30.1 (t)
20	35.7 (d)	22.3 (d)	44.9 (d)	33.3 (d)	44.7 (d)	44.9 (d)	31.3 (d)	131.2 (s)
21	10.6 (q)	18.9 (q)	15.0 (q)	12.4 (q)	15.0 (q)	14.7 (q)	17.8 (q)	69.9 (t)
22	85.3 (d)	34.4 (t)	40.4 (d)	41.3 (d)	40.1 (d)	40.3 (d)	79.9 (d)	23.7 (t)
23	76.2 (d)	105.8 (s)	75.6 (d)	75.0 (d)	75.3 (d)	75.1 (d)	76.8 (d)	30.3 (t)
24	62.6 (d)	148.8 (d)	69.4 (d)	71.7 (d)	69.1 (d)	68.1 (d)	33.2 (t)	133.6 (d)
25	56.3 (s)	131.9 (s)	42.2 (d)	42.2 (d)	42.1 (d)	42.5 (d)	34.4 (d)	126.2 (s)
26	173.2 (s)	171.8 (s)	178.0 (s)	177.8 (s)	177.8 (s)	178.1 (s)	180.3 (s)	173.4 (s)
27	11.4 (q)	10.4 (q)	8.0 (q)	8.0 (q)	8.3 (q)	8.4 (q)	16.6 (q)	22.2 (q)
28								21.2 (q)
29	22.1 (q)	22.1 (q)	23.0 (q)	25.4 (q)	25.3 (q)	24.9 (q)	21.9 (q)	112.0 (t)
30	28.4 (q)	28.3 (q)	29.6 (q)	29.9 (q)	29.9 (q)	29.5 (q)	28.6 (q)	20.0 (q)

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

Table 2
^{1}H NMR data of **1**–**7** in pyridine-d_5, δ in parts per million (J in hertz)[a]

Proton	**1**[b]	**2**[b]	**3**	**4**	**5**	**6**	**7**
1	4.36 (d, 5.1)	4.17 (d, 5.0)					5.02 (d, 5.2)
2α	2.79 (d, 18.1)	2.74 (d, 18.0)	3.06 (ABd, 18.0)	3.10 (ABd, 18.0)	3.22 (ABd, 18.0)	3.18 (ABd, 18.0)	2.72 (d, 18.1)
2β	3.12 (dd, 18.0, 5.1)	3.02 (dd, 18.0, 5.0)	3.15 (ABd, 18.0)	3.15 (ABd, 18.0)	3.22 (ABd, 18.0)	3.18 (ABd, 18.0)	3.32 (dd, 18.1, 5.2)
5	2.62 (overlapped)	3.15 (dd, 13.2, 3.5)	2.60–2.67 (m)	2.62 (overlapped)	2.14–2.18 (m)	2.57 (dd, 14.4, 2.6)	2.18 (overlapped)
6α	2.10–2.14 (m)	1.94–2.02 (m)	2.26 (overlapped)	2.26 (overlapped)	1.18–1.22 (m)	2.20–2.28 (m)	2.17 (overlapped)
6β	1.65–1.73 (m)	1.51–1.57 (m)	2.26 (overlapped)	2.26 (overlapped)	1.18–2.22 (m)	1.58–1.65 (m)	1.37 (overlapped)
7α			4.50–4.60 (m)	4.50–4.61 (m)	1.57–1.62 (m)	3.91 (t-like, 6.8)	2.22–2.32 (m)
7β	5.66 (br d, 7.9)	4.40 (d, 5.5)			1.88–1.95 (m)		2.00–2.09 (m)
8	2.90 (br s)	2.74 (overlapped)	2.91 (d, 9.8)	2.85 (d, 8.0)	3.66 (dd, 12.0, 5.0)		2.85 (dd, 7.0, 4.5)
11α	2.10 (overlapped)	2.01 (dd, 14.8, 9.9)	1.69–1.76 (m)	1.64–1.72 (m)	1.64 (overlapped)	1.88–1.95 (m)	1.42–1.52 (m)
11β	2.41 (br d, 16.3)	2.27 (br d, 14.8)	2.00–2.10 (m)	2.18–2.26 (m)	2.09–2.17 (m)	2.00–2.08 (m)	2.68–2.75 (m)
12α			1.55–1.59 (m)	1.58–1.64 (m)	1.32–1.42 (m)	1.59–1.68 (m)	1.38 (overlapped)
12β	5.40 (br s)	4.85 (d, 9.9)	1.83–1.89 (m)	1.74–1.80 (m)	1.80–1.86 (m)	1.97–2.04 (m)	2.45–2.52 (m)
15	3.84 (br s)	3.73 (br s)					
16α	2.00 (overlapped)	1.85–1.93 (m)					
16β	1.68 (overlapped)	1.64 (overlapped)	2.83 (overlapped)	2.82 (d, 8.3)	2.77 (d, 6.5)	2.72 (d, 6.5)	1.41 (d, 7.0)
17	2.50–2.59 (m)	2.84–2.92 (m)					0.90 (t-like, 7.0)
18			0.92 (s)	0.93 (s)	0.92 (s)	0.92 (s)	0.96 (s)
19α	2.24 (ABd, 16.1)	2.06 (ABd, 15.6)	2.62 (ABd, 16.1)	2.45 (ABd, 16.0)	2.42 (ABd, 16.3)	2.48 (ABd, 16.4)	4.20 (d, 7.3)
19β	2.36 (ABd, 16.1)	2.18 (ABd, 15.6)	2.79 (ABd, 16.1)	2.90 (ABd, 16.0)	2.57 (ABd, 16.3)	2.62 (ABd, 16.4)	
20	2.74 (overlapped)	2.26–2.40 (m)	2.65–2.76 (m)	3.30–3.43 (m)	2.58–2.69 (m)	2.65–2.76 (m)	3.50–3.61 (m)
21	0.84 (d, 6.8)	0.76 (d, 6.6)	1.12 (d, 6.9)	1.14 (d, 7.2)	1.27 (d, 7.0)	1.19 (d, 7.0)	1.70 (d, 6.4)
22α		1.38 (dd, 13.0, 3.0)	2.83 (overlapped)	2.76–2.84 (m)	2.78–2.85 (m)	2.83–2.90 (m)	5.01 (overlapped)
22β	4.05 (d, 10.4)	1.65 (overlapped)					
23	4.78 (br s)		4.65 (br s)	4.80 (br s)	4.49 (br s)	4.61 (br s)	4.95 (overlapped)
24	4.36 (br s)	7.01 (br s)	5.31 (br s)	5.11 (br s)	4.62 (br s)	5.13 (br s)	1.92–2.18 (m)
25			3.17–3.21 (m)	3.10–3.16 (m)	3.03–3.10 (m)	3.20–3.26 (m)	2.72–2.81 (m)
27	1.62 (br s)	1.77 (s)	1.25 (d, 7.2)	1.52 (d, 8.0)	1.49 (d, 7.5)	1.68 (d, 7.2)	1.18 (overlapped)
29	1.07 (s)	0.99 (s)	1.39 (s)	1.41 (s)	1.29 (s)	1.31 (s)	1.32 (s)
30	1.22 (s)	1.21 (s)	1.30 (s)	1.30 (s)	1.36 (s)	1.27 (s)	1.17 (s)

[a] The assignments were based on DEPT, ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.
[b] See the Section 4 for the data of angeloyl, acetyl, and hydroxyl groups.

epoxy ring between C-14 and C-15. Analysis of the COSY spectrum starting from the proton at δ_H 3.73 (H-15) revealed the presence of spin system $-CHCH_2CHCH(CH_3)CH_2-$ (C15/C16/C17/C20/C21 and C22). In addition, the key HMBC correlations from H_2-22 and H-24 to a ketal quaternary carbon (C-23, δ_C 105.8, s), from H_3-21 to C-17, C-20, and C-22, and from H-17 to C-13 indicated the presence of a six membered oxygen ring (F ring) and that C-22 was connected with a quaternary carbon C-23 (Fig. 2). Moreover, the ^{13}C NMR signal for C-13 shifted upfield (δ_C 84.2) in **2** compared with **1** (δ_C 91.2), which further corroborated the structural feature that the oxygenated quaternary carbon C-13 was located in a six membered ring rather than a rigid five membered ring. Considering the molecular formula, a spirocyclic moiety (rings F and G) must be located at C-23. The strong HMBC correlations of H_3-27 with C-24, C-25, and C-26 indicated the double bond at C-24 and C-25 as well as the structure of ring G. Thus, the gross structure of **2** was determined.

The relative stereochemistry of compound **2** was mainly established using information from ROESY spectrum and by comparison of its spectroscopic data to those of **1**. The same relative stereochemistry of A–E rings in compound **2** as in **1** was deduced from the similar carbon and proton chemical shifts and ROESY correlations found in **2** (Tables and Fig. 3). The strong ROESY correlations of H-15 with H-7 showed the α-orientation of the epoxy ring between C-14 and C-15. The α-orientation of the OH-12 and the β-orientation of CH_3-21 were deduced from the key correlation between H-12 and H-20 as shown in computer-generated 3D drawing (Fig. 4), which was minimized using the MM2 force field. The last question to be settled was the relative configuration of spiro carbon C-23. The weak ROE interactions of H-22β with H_3-21 and H-24 suggested that C-24 might be on the β-orientation of ring G. The most solid evidence that confirmed the configuration of C-23 was the abnormal chemical shift of C-20 observed in ^{13}C NMR spectrum. The large upfield shift of C-20 (δ_C 22.3, d) in **2** compared with that of **1** (δ_C 37.8, d) was attributed to the strong γ-*gauche* steric compression between oxygen atoms at C-23 (ring G) and H-20 (Fig. 3). Thus, the relative stereochemistry of C-23 was undoubtedly determined as *S** configuration.

Schindilactone D (**3**), a white solid, had the molecular formula $C_{29}H_{36}O_{11}$ as established on the basis of HRESIMS at *m*/*z* 583.2149 $[M+Na]^+$ (calcd 583.2155). The IR spectrum showed the presence of hydroxyl (3441 cm^{-1}) and carbonyl (1777 and 1738 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table 2) had five resonances at δ_H 0.92 (3H, s), 1.30 (3H, s), 1.39 (3H, s), 1.12 (3H, d, J=6.9 Hz), and 1.25 (3H, d, J=7.2 Hz), typical of three angular and two secondary methyls; two doublet proton resonances at δ_H 2.48 (1H, ABd, J=16.4 Hz) and 2.62 (1H, ABd, J=16.4 Hz) indicating an AB spin system (H_2-19); proton signals at δ_H 3.18 (2H, s) for H_2-2. Above information in conjunction with the ^{13}C and

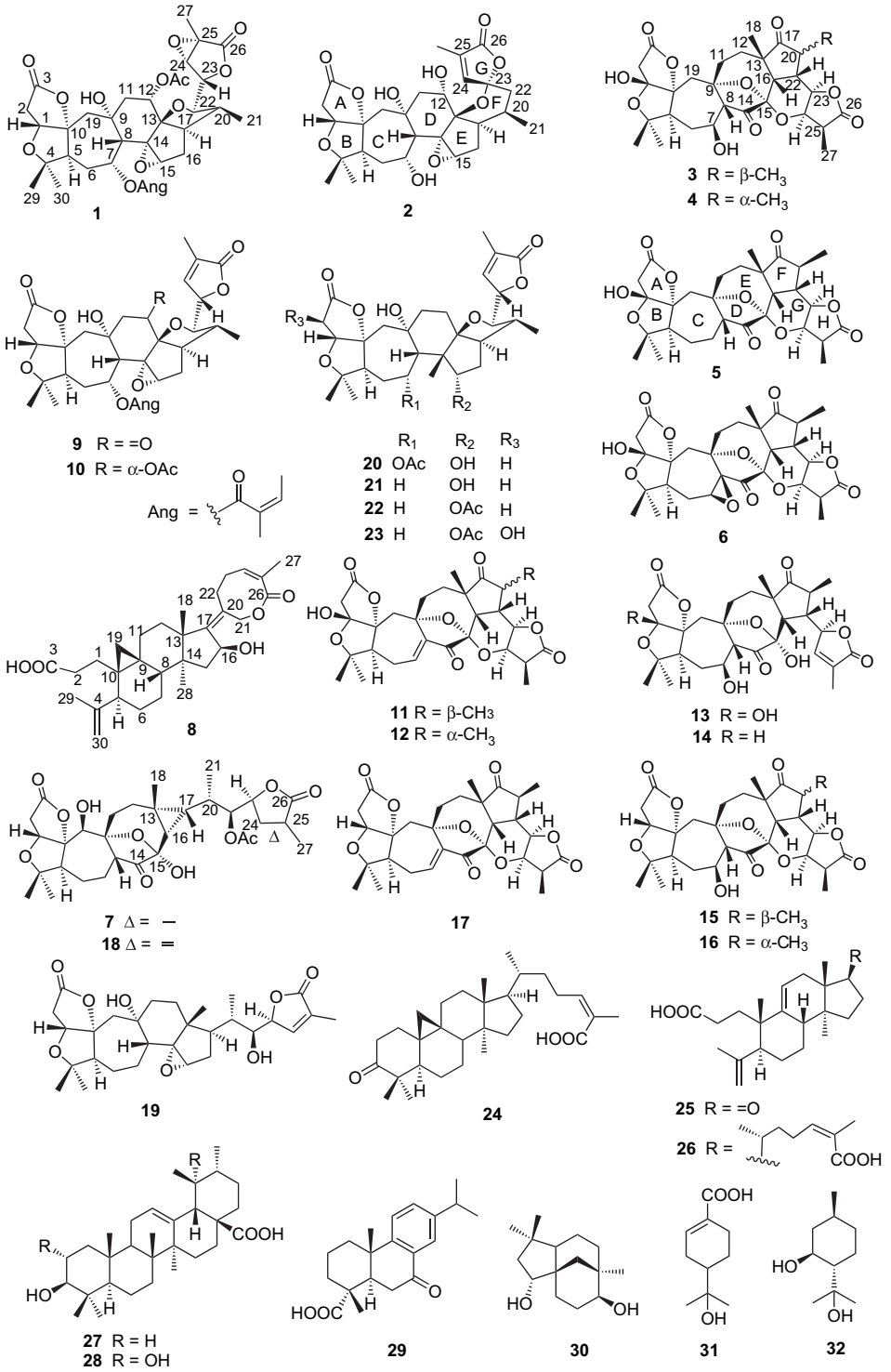


Figure 1. The structures of compounds **1**–**32**.

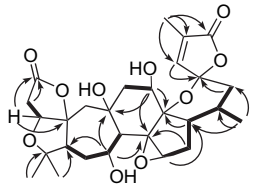


Figure 2. Key correlations of HMBC and $^{1}H-^{1}H$ COSY for **2**.

DEPT NMR spectra indicated that compound **3** was a highly oxygenated nortriterpenoid with a schisanartane core, the same as that of schindilactone A (**11**).[6] The NMR and MS data of **3** similar with those of schindilactone A indicated that **3** was a dehydration derivative of schindilactone A. This was confirmed by the absence of double bond signals in **3** and by the presence of two methines at δ_C 68.4 and 60.2, in which the carbon signal at δ_C 68.4 was attributed to an OH bearing

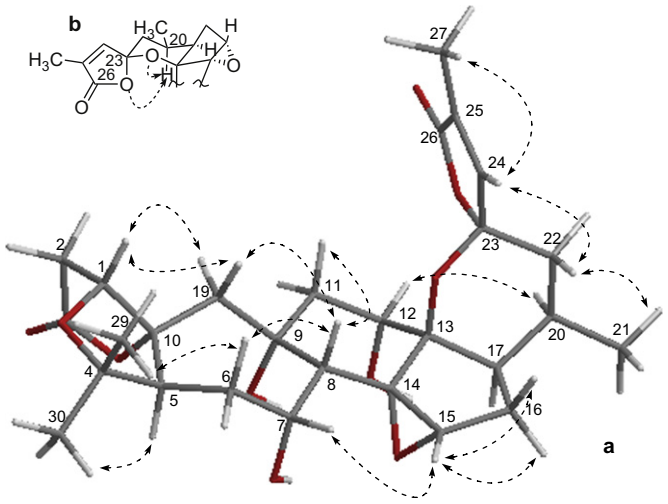


Figure 3. (a) Selected ROESY correlations and relative stereochemistries for **2**; (b) key γ-steric compression effect between oxygen atoms and H-20 in **2**.

carbon C-7. The hydroxyl group was connected to C-7, determined by the HMBC correlation between H-7 and C-5, 6, 8, 9, and 14. Because of the coupling constant (J=9.8 Hz) between H-7 and H-8 and NOESY correlation of H-7 with H-5, the relative configuration of OH-7 was assigned to be β-orientation. The ^{13}C NMR chemical shift of C-20 was about 45, so the orientation of Me-21 was determined to be β.[6] The relative stereochemistry of the schisanartane core in **3** was further confirmed by the NOESY spectrum. Therefore, compound **3** was identified as shown in Figure 1, named schindilactone D.

Compound **4** was isolated as an amorphous powder and had the same molecular formula of $C_{29}H_{36}O_{11}$ as that of **3**, which was determined by analysis of ^{13}C and DEPT NMR as well as HRESIMS data. Careful analysis of their ^{1}H and ^{13}C NMR data indicated that **3** and **4** might be C-20 epimers. This deduction was fully confirmed by the abnormal upfield chemical shift of C-20 from δ_C 44.9 in **3** to δ_C 33.3 in **4** due to the γ-steric compression effect between oxygen atom at C-23 and H-20 in **3** (Table 1). The large shift difference in ^{13}C NMR of C-20 in **3** and **4** was also found for compounds **11** and **12**.

The molecular formula of schindilactone F (**5**) was determined to be $C_{29}H_{36}O_{10}$ by analysis of HRESIMS data. The ^{13}C NMR spectrum indicated the presence of two ester carbonyls (δ_C 177.8 and 172.6), two ketone carbonyls (δ_C 220.0 and 209.6), and two oxygenated quaternary carbons at δ_C 98.7 and 98.9, which were characteristic signals for schisanartane nucleus. The comparison of the spectroscopic data of **5** with those of **3** (Table 1) showed similarities except that a hydroxyl bearing carbon (C-7) in **3** was replaced by a methylene group (δ_C 19.9, t) in **5**. The structure was shown in Figure 1.

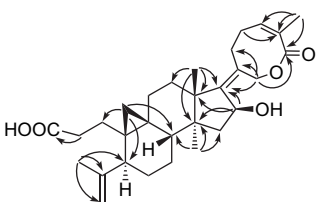


Figure 4. Key HMBC correlations of **8**.

Schindilactone F (**6**) was assigned the molecular formula $C_{29}H_{34}O_{11}$, as deduced from the positive HRESIMS (m/z 581.1989 $[M+Na]^+$). Comparison of the spectroscopic data of **6** with those of **3** revealed that they were quite similar except for the presence of an additional epoxy ring. The oxymethine and quaternary carbon (δ_C 64.2, d and 61.2, s) in the ^{13}C NMR spectrum of **6** and HMBC correlations of H-7 with C-5, C-8, and C-9 showed the presence of epoxy ring between C-7 and C-8. Moreover, the cross-peaks from H-7 to H-5 in the ROESY spectrum of **6** indicated that the epoxy ring had the β-orientation.[15] Thus, the structure of compound **6** was determined.

Pre-schisanartanin B (**7**) was obtained as an amorphous solid, and the molecular formula was $C_{31}H_{42}O_{11}$ based on its HRESIMS data (m/z 613.2480 $[M+Na]^+$). Its ^{13}C NMR spectrum showed 31 carbon signals. Analysis of the ^{1}H and ^{13}C NMR spectra of **7** showed the presence of a ketone (δ_C 216.0), two lactone carbonyl carbons (δ_C 180.3 and 175.5), and an acetyl group (δ_C 170.7 and 21.5; δ_H 2.12, s). Comparison of the ^{1}H NMR data of **7** with those of **18** suggested the similarity, except for the absence of olefinic signals attributable to H-24 and H-25. The signals corresponding to this double bond were also absent in the ^{13}C NMR spectrum of **7**, where three signals at δ_C 33.2 (t), 34.4 (d), and 180.3 (s) suggested that this compound has a 24,25-dihydropre-schisanartanin A moiety. This assignment was supported by the HMBC correlations of H_3-27 with C-24, C-25, and C-26 as well as the ^{1}H–^{1}H COSY spin system H-23/H-24/H-25/H_3-27. Due to the lack of useful correlation in ROESY spectrum, the stereochemistry of C-25 was not determined.

A molecular formula of $C_{30}H_{42}O_5$ and 10 degrees of unsaturation were determined for **8** based on accurate mass measurement and NMR data. The IR spectrum showed absorption bands due to carboxyl group (3582–3240 and 1703 cm^{-1}) and C=C double bonds (1640 cm^{-1}). The ^{1}H NMR spectrum (see Table 2) showed one isopropenyl group [δ_H 4.79 and 4.95 (each 1H, br s, H-29a and H-29b), 1.67 (3H, s, H-30)], three tertiary methyl groups [δ_H 0.86 (3H, s), 1.34 (3H, s), 1.91 (3H, s)], and typical cycloartane methylene group [δ_H 0.33 and 0.65 (each 1H, d, J=4.0 Hz)]. Analysis of the ^{13}C and DEPT NMR spectra for **8** indicated the presence of 4 methyl groups, 12 methylenes, 3 aliphatic methines, 1 olefinic methine, and 10 quaternary carbons with 2 chemical shift typical of carboxyl carbons (δ_C 177.0 and 173.4) as well as with 4 obvious olefinic carbons (δ_C 152.5, 149.9, 131.2, and 126.2). Comparison of the spectroscopic data of **8** with those of nigranoic acid[16] revealed the A–C rings' ^{1}H and ^{13}C NMR spectroscopic data were analogous with each other. Therefore, the structure of **8** was assigned as a 3,4-*seco*-cycloartane derivative.

The HMBC correlations from angular methyl resonance at δ_H 1.34 (H_3-18) to C-12 (δ_C 32.2, t), C-13 (δ_C 46.1, s), C-14 (δ_C 48.6, s), and C-17 (152.5, s) as well as from H_3-28 (δ_H 0.86, s) to C-8 (δ_C 46.5, d), C-13, C-14, and C-15 (δ_C 46.7, t) indicated that the C-17 was an olefinic carbon (Fig. 4). Although the H-16 (δ_H 5.04, overlapped) proton signal was overlapped by the H_2O signal, the assignment for this proton

resonance could be achieved by the HSQC and $^{1}H-^{1}H$ COSY NMR spectra because of the clear correlation of H-15 with H-16. In addition, the HMBC correlations of H-16 with C-13, C-14, and C-17 helped to establish the structure of D ring, accounting for the presence of double bond between C-17 and C-20. The remaining seven carbon signals in the ^{13}C NMR spectrum of **8** comprised a novel eight-membered lactone ring [a methyl (δ_C 22.2, C-27), an oxymethylene (δ_C 69.9, C-21), two methylenes (δ_C 23.7 and 30.3, C-22 and C-23), an olefinic methine (δ_C 133.6, C-24), a quaternary olefinic carbon (δ_C 126.2, C-25), and a carbonyl (δ_C 173.4, C-26)] as shown in Figure 1, based on the observations of HMBC correlations of H_3-27 with C-24, C-25, and C-26, of H_2-22 (δ_H 2.42–2.51, m) with C-17 and C-20, and of H_2-21 (5.38, ABq, J=13.6 Hz) with C-17, C-20, and C-26, as well as the $^{1}H-^{1}H$ COSY cross-peaks observed of H-22 with H-23 and of H-23 with H-24 (5.75, br s). The geometry of the double bond between C-17 and C-20 was deduced from ROESY correlation of H-16 with H_2-21 (Fig. 5).

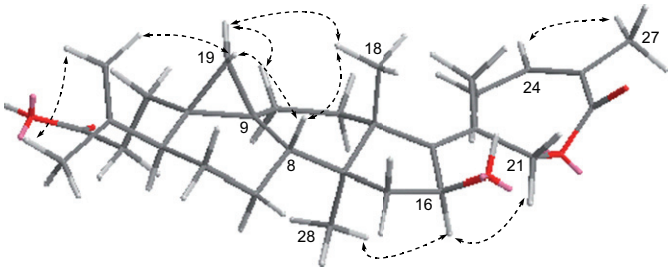


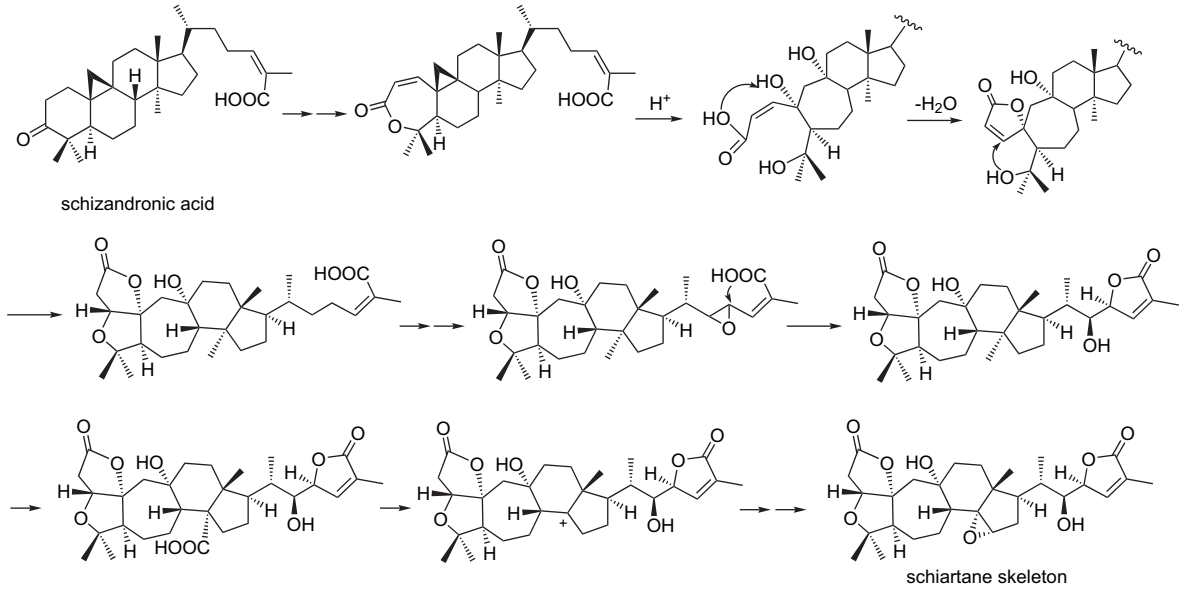
Figure 5. Key ROESY correlations for **8**.

The relative stereochemistry of compound **8** was established using information from ROESY spectrum and by comparison of its spectroscopic data to those of nigranoic acid.[16] The same relative stereochemistry of A–C rings in compound **8** as in nigranoic acid was deduced from the similar carbon and proton chemical shifts and ROESY correlations found in **1** (Fig. 5). The β-orientation for H-8 was suggested from the strong ROESY correlations of H-8 with H-19β and H_3-18 as shown in computer-generated 3D drawing. The relative configuration of OH-16 was inferred to be β, judging from ROESY cross-peaks of H-16 with H_3-28. Thus, the structure of **8**, named as wuweizilactone acid, was unambiguously determined.

3. Conclusion

The genus *Schisandra* of the family Schisandraceae has been known to be rich in bioactive lignans (mainly dibenzocyclooctadienlignans), with more than 150 structures identified from different species.[17,18] In earlier studies, we obtained a series of highly oxygenated nortriterpenoids with unprecedented carbon skeletons. While the previous publications have proposed biosynthetic pathways of schisanartane, preschisanartane, 18-norschiartane, 18(13→14)-*abeo*-schiartane, and wuweiziartane,[4,6,7] we now propose a biogenetic pathway to explain the formation of schiartane skeleton (Scheme 1). In addition, we found that the chemical constitutes of *S. chinensis* showed obvious chemical diversity and contained all the triterpenoids skeletons characterized previously from the genus *Schisandra*. Thus, the biosynthetic study of *Schisandra* nortriterpenoids from this plant may be an interesting topic and challenge for further investigation.

The anti-HIV-1 activities of **1–23** were tested by a microtiter syncytium formation infectivity assay, using the method previously described, with AZT as a positive control.[19,20] All the compounds demonstrated weak anti-HIV-1 activity with EC_{50} values ranging from 17.9 to 100 μg mL^{-1}, (AZT: EC_{50}=0.0043 μg mL^{-1}). The cytotoxicity of all the triterpenoids was also evaluated against K562 cell lines, but they were completely inactive with IC_{50} values of >100 μg mL^{-1}. Although, the compounds did not display significant bioactivity in the cytoxicity and anti-HIV assays, they are structurally



Scheme 1. Proposed mechanism for the formation of the schiartane type.

intriguing and may play a role in the pharmacological properties of these well known Chinese medicinal plants.

4. Experimental

4.1. General

Optical rotations were carried out on a Perkin–Elmer model 241 polarimeter. UV spectra were obtained in a UV 210A spectrometer. IR spectra were measured in a Bio-Rad FTS-135 spectrometer as KBr pellet. 1D and 2D NMR spectra were taken on a Bruker AM-400 or a Bruker DRX-500 NMR spectrometer with TMS as an internal standard. Mass spectra were recorded on a VG Auto spec-3000 spectrometer or on a Finnigan MAT 90 instrument. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm×25 cm column. Column chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (Pharmacia).

4.2. Extraction and isolation

The aerial parts of *S. chinensis* were collected in Tonghua prefecture, Jilin Province, People's Republic of China, in September 2005. The sample was identified by Prof. Jun-Lin Yu, and a voucher specimen (KIB 05092106) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences. Air-dried and powdered aerial parts of *S. chinensis* (12 kg) were extracted with 80% aq acetone (25 L×4, each 2 days) at room temperature. After removal of the solvents in vacuo at 45 °C, a residue (450 g) was obtained. This residue was dissolved in H_2O (3.0 L) and then extracted successively with petroleum ether (60–90 °C, 1 L×2) and EtOAc (2 L×4). The EtOAc extract (210 g) was subjected to column chromatography over MCI-gel CHP 20P (90% MeOH–H_2O, 100% MeOH).

The 90% CH_3OH fraction (170 g) was purified by flash column chromatography on silica gel (200–300 mesh, 1.5 kg), eluted in a step gradient manner with $CHCl_3$–acetone (1:0 to 0:1) to afford fractions I–VIII. Fraction II was subjected to repeated chromatography on silica gel (petroleum ether–acetone, from 30:1 to 10:1; cyclohexane–2-propanol, 60:1) and RP-18, followed by semipreparative and preparative HPLC to yield compounds **2** (1.1 g), **3** (7 mg), **4** (11 mg), **5** (8 mg), and **13** (5 mg). Fraction III was first subjected to chromatography over RP-18 (CH_3OH–H_2O, from 0:1 to 1:0) and silica gel ($CHCl_3$–acetone, from 40:1 to 20:1), followed by semipreparative HPLC to yield compounds **6** (13 mg), **16** (25 mg), **30** (4 mg), **31** (24 mg), and **32** (46 mg). In the same way, fraction IV yielded compounds **11** (16 mg), **14** (1.21 g), **15** (0.42 g), **26** (7 mg), **27** (6 mg), **28** (305 mg), and **29** (3 mg). Compound **17** (12.3 mg) was obtained from fraction V by recrystallization from CH_3OH. The remnant of fraction V was separated by silica gel chromatography and semipreparative HPLC to afford compounds **7** (12 mg), **18** (13 mg), **19** (13 mg), **20** (6 mg), **21** (7 mg), **23** (562 mg), and **25** (11 mg). Compounds **8** and **9** (45 mg), **10** (26 mg), **12** (7 mg), **22** (3 mg), and **24** (23 mg) were obtained from fraction VI. Compound **1** (9 mg) was obtained from fraction VII by repeated silica gel chromatography and semipreparative HPLC.

4.2.1. Wuweizidilactone G (*1*)

Amorphous powder; $[\alpha]_D^{19}$ +16.8 (*c* 0.26, CH_3OH); ^1H NMR data, see Table 2; angeloyl: δ_H 5.82 (q, *J*=6.2 Hz), 2.01 (br s), 2.04 (br d, *J*=6.2 Hz); acetyl: δ_H 2.07 (s); ^{13}C NMR data, see Table 1; angeloyl: δ_C 166.4 (s), 127.9 (s), 139.6 (d), 21.1 (q), 15.9 (q), acetyl: δ_C 170.7 (s), 21.4 (q); IR (KBr) ν_{max} 3439, 2973, 2934, 1782, 1730, 1704, 1642, 1454, 1375, 1249, 1232, 1153, 1121 cm^{-1}; FABMS *m/z* 673 $[M+H]^+$; HRESIMS *m/z* 695.2664 $[M+Na]^+$, calcd for $C_{35}H_{44}O_{13}Na$ *m/z* 695.2679.

4.2.2. Wuweizidilactone H (*2*)

Amorphous powder; $[\alpha]_D^{19}$ +4.8 (*c* 0.35, CH_3OH); ^1H and ^{13}C NMR data, see Tables 1 and 2; OH-7: δ_H 5.95 (br s), OH-9: δ_H 5.73 (br s), OH-12: δ_H 5.26 (br s); IR (KBr) ν_{max} 3467, 2963, 2927, 1767, 1631, 1448, 1384, 1320, 1243, 1211, 972 cm^{-1}; FABMS *m/z* 533 $[M+H]^+$; HRESIMS *m/z* 555.2197 $[M+Na]^+$, calcd for $C_{28}H_{36}O_{10}Na$ *m/z* 555.2206.

4.2.3. Schindilactone D (*3*)

White solid; $[\alpha]_D^{19}$ +16.0 (*c* 0.10, CH_3OH); IR (KBr) ν_{max} 3441, 2932, 1777, 1738, 1456, 1383, 1112, 1090, 1011 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 561 $[M+H]^+$; HRESIMS *m/z* 583.2149 $[M+Na]^+$, calcd for $C_{29}H_{36}O_{11}Na$ *m/z* 583.2155.

4.2.4. Schindilactone E (*4*)

White solid; $[\alpha]_D^{19}$ +27.5 (*c* 0.25, CH_3OH); IR (KBr) ν_{max} 3424, 2975, 2931, 1778, 1717, 1450, 1377, 1253, 1016 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 561 $[M+H]^+$; HRESIMS *m/z* 583.2162 $[M+Na]^+$, calcd for $C_{29}H_{36}O_{11}Na$ *m/z* 583.2155.

4.2.5. Schindilactone F (*5*)

White solid; $[\alpha]_D^{19}$ +68.6 (*c* 0.35, CH_3OH); IR (KBr) ν_{max} 3422, 2973, 2934, 1780, 1716, 1452, 1383, 1263, 1203, 1014, 940 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 544 $[M+H]^+$; HRESIMS *m/z* 567.2220 $[M+Na]^+$, calcd for $C_{29}H_{36}O_{10}Na$ *m/z* 567.2206.

4.2.6. Schindilactone G (*6*)

White solid; $[\alpha]_D^{19}$ +21.4 (*c* 0.56, CH_3OH); IR (KBr) ν_{max} 3414, 2979, 2933, 1791, 1779, 1716, 1452, 1380, 1218, 1006 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 559 $[M+H]^+$; HRESIMS *m/z* 581.1989 $[M+Na]^+$, calcd for $C_{29}H_{34}O_{11}Na$ *m/z* 581.1998.

4.2.7. Pre-schinsanartanin B (*7*)

Colorless crystals; $[\alpha]_D^{19}$ +80.1 (*c* 0.50 in CH_3OH); IR (KBr) ν_{max} 3441, 2934, 2871, 1764, 1739, 1638, 1456, 1373, 1238,

1069, 1035, 924 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 613 $[M+Na]^+$; HRESIMS *m/z* 613.2480 $[M+Na]^+$, calcd for $C_{31}H_{42}O_{11}Na$ *m/z* 613.2488.

*4.2.8. Wuweizilactone acid (**8**)*

White solid; $[\alpha]_D^{19}$ +91.4 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3434, 2928, 2875, 1712, 1640, 1455, 1376, 1269, 1169, 1109, 1032 cm^{-1}; ^1H NMR data (400 MHz, measured in C_5D_5N): δ_H 2.31–2.35 (1H, m, H-1a), 1.67 (1H, overlapped, H-1b), 2.53 (1H, overlapped, H-2a), 2.79–2.82 (1H, m, H-2b), 2.52 (1H, overlapped, H-5), 1.40–1.44 (1H, m, H-6α), 1.01 (1H, overlapped, H-6β), 1.21 (1H, overlapped, H-7α), 1.00 (1H, overlapped, H-7β), 1.52–1.55 (1H, m, H-8), 2.22 (1H, overlapped, H-11α), 1.29 (1H, overlapped, H-11β), 1.78 (2H, overlapped, H_2-12), 2.05 (1H, dd, *J*=13.4, 7.8 Hz, H-15α), 1.74 (1H, overlapped, H-15β), 5.04 (1H, overlapped, H-16), 1.34 (3H, s, H_3-18), 0.33 (1H, d, *J*=4.0 Hz, H-19α), 0.65 (1H, d, *J*=4.0 Hz, H-19β), 5.38 (2H, q, *J*=13.6 Hz, H_2-21), 2.42 (1H, overlapped, H-22a), 2.51 (1H, overlapped, H-22b), 2.19 (1H, overlapped, H-23a), 2.30 (1H, overlapped, H-23b), 5.75 (H, br s, H-24), 1.91 (3H, s, H_3-27), 1.86 (3H, s, H_3-28), 4.79 (H, br s, H-29a), 4.95 (1H, br s, H-29b), 1.67 (3H, s, H_3-30); ^{13}C NMR data (100 MHz, measured in C_5D_5N), see Table 1; ESIMS *m/z* $[M+Na]^+$ 505 (100), 521 $[M+K]^+$, 987 $[2M+Na]^+$; HRESIMS *m/z* $[M+Na]^+$ 505.2915, calcd for $C_{30}H_{42}O_5Na$ 505.2929.

Acknowledgements

Financial support for this research was provided by the National Natural Science Foundation of China (no. 20402016), the Natural Science Foundation of Yunnan Province (no. 2005XY04), and the XiBuZhiGuang project of the Chinese Academy of Sciences to Dr. Wei-Lie Xiao.

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