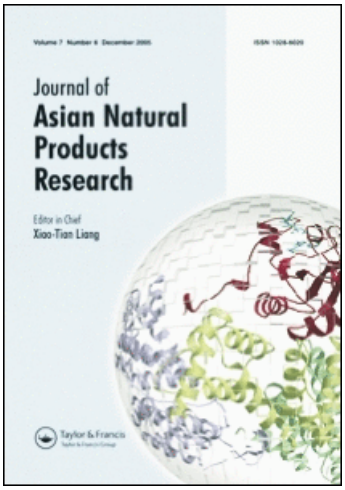

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Schisanlactone H and sphenanthin A, new metabolites from *Schisandra sphenanthera*

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From the fruits of *Schisandra sphenanthera* (Schisandraceae), a new 3,4-*seco*-lanostane triterpenoid, schisanlactone H (**1**), and a new monocyclofarnesane sesquiterpenoid, sphenanthin A (**2**), were isolated. Their structures were elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques.

Keywords: Schisandraceae; *Schisandra sphenanthera*; schisanlactone H; sphenanthin A

1. Introduction

Schisandra sphenanthera Rehd. et Wils (Schisandraceae) has long been used in traditional Chinese medicine and widely distributed in the south-west region of China [1]. The fruits of *S. sphenanthera* are used as an antitussive, tonic, and sedative agent under the name of wuweizi in traditional Chinese medicine, together with the fruits of *S. chinensis* Baill [2]. This plant has been reported to contain dibenzocyclooctadiene lignans, which were found to have some important pharmacological effects that include anti-hepatitis, antitumor, and anti-HIV activities [3,4]. Previously, we isolated a new phenolic glycoside from the stems of *S. sphenanthera* [5]. In our continuing phytochemical reinvestigation of the fruits of this plant, two minor terpenoids, schisanlactone H (**1**) and sphenanthin A (**2**), were isolated (Figure 1). This paper deals with the isolation and structural elucidation of compounds **1** and **2**.

2. Results and discussion

Schisanlactone H (**1**) was isolated as a white powder. The molecular formula was determined as $C_{31}H_{48}O_5$ by HR-ESI-MS at *m/z* 523.3398 $[M+Na]^+$, in combination with ^1H and ^{13}C NMR spectral data, indicating eight degrees of unsaturation. The ^1H NMR spectrum exhibited signals of one secondary methyl at δ 0.98 (d, $J = 6.4$ Hz), six tertiary methyls at δ 0.69, 0.72, 1.29, 1.26, 1.21, and 1.92, two olefinic proton signals at δ 5.41 and 6.61, and one methoxyl group at δ 3.70. Analysis of the ^{13}C NMR, DEPT, and HSQC spectral data revealed that **1** contained one carboxymethyl group, one α,β-unsaturated carbonyl, six quaternary carbons (including an oxygenated one and two olefinic ones), seven methines (including two unsaturated ones), eight methylenes, and seven methyls. Apart from two double bonds and two carbonyl groups, the remaining elements of unsaturation of **1** were assumed to be a tetracycline skeleton.

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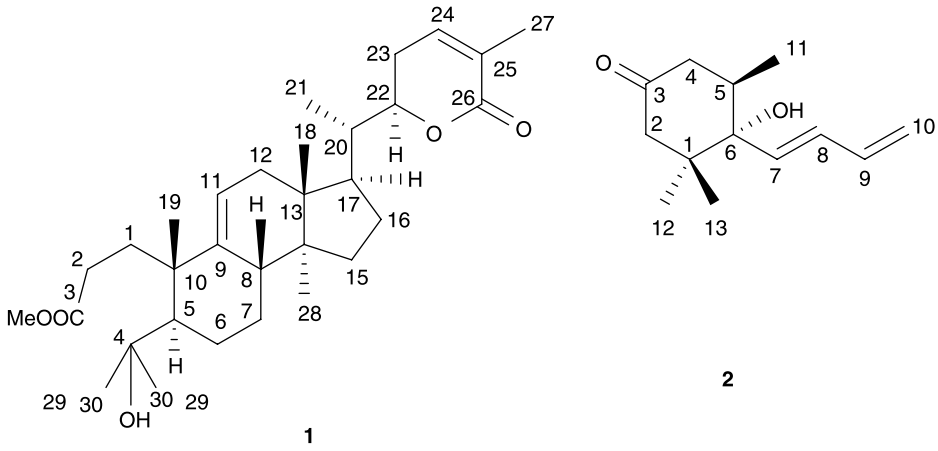

Figure 1. The structures of compounds **1** and **2**.

A careful comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with those of schisanlactone F (**3**) [6] indicated that two compounds possessed the identical constituting rings D and E, and ring A of both compounds has suffered an oxidative cleavage between C-3 and C-4. The main differences between those compounds were the olefinic carbons at C-4 and C-29, and a carboxyl group in **3** being replaced by an oxygenated quaternary carbon, a methyl group, and a carboxymethyl group in **1**, respectively. This assignment was consistent with the analysis of $^{1}H-^{1}H$ COSY, HSQC, and HMBC spectral data for **1**. Therefore, the structure of **1** was proposed as a 3,4-*seco*-lanostane derivative.

In the HMBC spectrum, cross-peaks (Figure 2) observed between Me-27 (δ 1.92, s) and C-24, C-25, and C-26, together with the fragment ion at *m/z* 111 [$C_6H_7O_2^+$] in the positive FAB-MS, indicated the presence of a six-membered α-methyl-α,β-unsaturated-δ-lactone. HMBC correlations from Me-30 (δ 1.26 or 1.29, s) to C-4, C-5, and C-29, and from Me-29 (δ 1.29 or 1.26, s) to C-4, C-5, and C-30, required that Me-29 and Me-30 be attached to the same oxygenated quaternary carbon (δ 75.5, s, C-4), but Me-29 and Me-30 cannot be distinguished. HMBC cross-peaks of OMe (δ 3.70, s) and H-2 (δ 2.44, m, H-2a; 2.35, m, H-2b) with C-3 indicated that the carboxymethyl group was connected with C-2. This was further confirmed by the fragment ion at *m/z* 440 [$M-HCOOCH_3$] in the positive FAB-MS. The proton signal at δ 5.41 (d, $J = 5.8$ Hz, H-11) showed HMBC correlations with C-9, C-10, C-12, and C-13, suggesting that the double bond was located between C-9 and C-11, which was further established by the HMBC cross-peaks observed from Me-19, H-7, and H-12 to C-9.

As is known, the configurations of Me-18, 19, 28 in lanostane triterpenes are β, β, α, respectively, and the absolute configuration of C-20 is *R*, namely Me-21 possessed an α-orientation. The ROESY

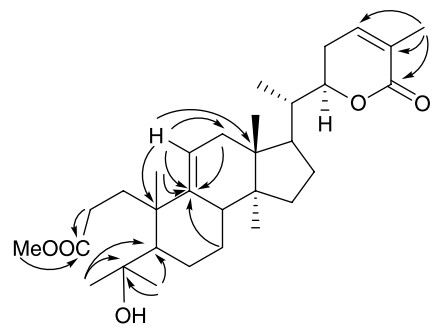

Figure 2. Key HMBC correlations of compound **1**.

spectrum of **1** showed a correlation between Me-18 and H-8, which suggested that H-8 possessed a β-configuration. In addition, we did not observe the NOE correlations between Me-19 and H-5, Me-18 and H-17, which indicated that H-5 and H-17 were located at the α-position. Moreover, the α-configuration of H-22 was deduced from the ROESY correlation between H-17 and H-22. Thus, the structure of **1** was determined as 3-*O*-methyl-4-hydroxy-$\Delta^{9,11}$ schisanlactone F and named schisanlactone H.

Sphenanthin A (**2**), a white powder, had the molecular formula of $C_{13}H_{20}O_2$ as determined by the positive FAB-MS, ^1H and ^{13}C NMR spectral data, which was verified by HR-ESI-MS at *m/z* 231.1356 $[M+Na]^+$, possessing four degrees of unsaturation. Analysis of the ^1H and ^{13}C NMR spectral data of **2** showed the presence of two tertiary methyls, one secondary methyl, three methylenes (including one terminal double bond), four methines (including three unsaturated ones), and three quaternary carbons (including an oxygenated one and a carbonyl carbon). Apart from two double bonds and a carbonyl group, there should be a ring in the molecule in accordance with the degrees of unsaturation.

Extensive analysis of the ^1H and ^{13}C NMR spectral data suggested that compound **2** possessed the same skeleton with *S* (+)-dehydrovomifoliol [7], a monocyclofarnesane sesquiterpenoid with 13 carbons. The significant differences included the obvious presence of a carbonyl (δ 211.4, s) and a conjugated double bond (δ 136.3, d, C-7; 131.4, d, C-8; 136.0, d, C-9; 117.3, t, C-10), and the lack of an α,β-unsaturated carbonyl in **2**. Moreover, the coupling constant between H-7 and H-8 ($J = 15.0$ Hz) suggested that the two olefinic protons were located at *trans*-orientation [8].

In the HMBC spectrum, Me-12 (δ 0.94, s) and Me-13 (δ 0.97, s) showed correlations with C-1, C-2, and C-6, Me-11 correlated with C-4, C-5, and C-6, which suggested that C-2, C-6, Me-12, and Me-13 were all attached to C-1, and another tertiary carbon (C-5) bearing the methyl group was situated between C-4 and C-6. HMBC cross-peaks from H-2 and H-4 to C-3, and from Me-11, Me-12, and Me-13 to C-3 give rise to the connectivity of C-2, C-3, and C-4. Correlations of H-10 with C-8 and C-9, H-9 with C-7 and C-8, H-8 with C-7 and C-10, and H-7 with C-8, C-9, and C-10 confirmed the existence of the conjugated double bond. In addition, the HMBC correlations between H-7 and C-5 and C-6 suggested that the conjugated double bond was directly attached to C-6 (Figure 3). As required by its molecular formula and the chemical shift of C-6, a hydroxyl group (δ 1.62, s) should be located at C-6. The ion peak at *m/z* 191 $[M-OH]^+$ in the positive FAB-MS also proved the existence of the hydroxyl group, which was consistent with the IR spectrum (3423 cm^{-1}). The HMBC correlations from 6-OH to C-1, C-5, and C-6 further confirmed this deduction.

The relative stereochemistry of **2** was determined by a combination of coupling constant analyses and NOESY spectrum. Me-11 and Me-13 were biogenetically β, and Me-12 was an α [7,9]. The lack of the ROESY mutual correlation between OH-6 and Me-11 suggested that OH-6 possessed an α-orientation, which was fully confirmed by the similar chemical shift of C-6 (δ 77.2) in compound **2** to that in *S* (+)-dehydrovomifoliol (δ 78.9). This indicated that the olefine moiety attached to C-6 is in the equatorial position, which is the most

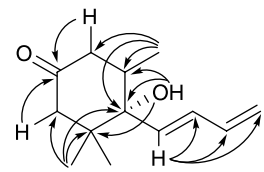

Figure 3. Key HMBC correlations of compound **2**.

stable conformation of **1** since the bulky groups are in the equatorial positions.

Thus, the structure of **2** was established as shown in Figure 1, and named as sphenanthin A.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Jasco DIP-370 digital polarimeter. UV spectra were run on a UV 210A spectrophotometer. IR spectra were recorded on a Bio-Rad FtS-135 spectrophotometer with KBr pellets. 1D and 2D NMR spectra were recorded using Bruker AM-400 and DRX-500 instruments with tetramethylsilane as an internal standard. FAB-MS were measured on a VG Auto Spec-3000 spectrometer; and HR-ESI-MS were taken on an API Qstar Pulsar instrument. Column chromatography was carried out on silica gel (200–300 mesh; Qingdao Marine Chemical Factory, Qingdao, China), Lichroprep RP-18 (43–63 μm; Merck, Darmstadt, Germany), Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden), and MCI (MCI-gel CHP-20P, 75–150 μm; Mitsubishi Chemical Corporation, Tokyo, Japan). TLC was performed on TLC plates (Si gel GF_{254}; Qingdao Marine Chemical Factory), and detected by spraying with 5% H_2SO_4–EtOH, followed by heating on a hot plate.

3.2 Plant material

The fruits of *S. sphenanthera* were purchased from the Herb Material Market of Juhuacun, Kunming, Yunnan Province, China, in July 2005, and were identified by Mrs Xiao-Lei Li, Kunming University of Science and Technology. A voucher specimen (KMUST 2005071101) has been deposited at the Laboratory of Phytochemistry, the College of Life Science and Technology, Kunming University of Science and Technology.

3.3 Extraction and isolation

The air-dried and powdered fruits of *S. sphenanthera* (8 kg) were extracted three times with 70% aq. acetone at room temperature, and the solvent was evaporated *in vacuo*, then the residue was extracted with EtOAc. The EtOAc fraction (145.9 g) was subjected to the silica gel column eluting with $CHCl_3$–acetone (gradient 1:0, 20:1, 9:1, 8:2, 7:3, 6:4, 5:5, 0:1, MeOH) to afford fractions A–H. Fraction B (22.937 g; $CHCl_3$–acetone, 20:1) was repeatedly chromatographed over silica gel, using petroleum–acetone (30:1), and $CHCl_3$–acetone (50:1) as the eluent, to give **1** (18 mg). Fraction D (13.57 g; $CHCl_3$–acetone, 8:2) was separated by repeated silica gel column (eluting with $CHCl_3$–isopropanol gradient 1:0, 100:1, 80:1, 60:1, 50:1, 40:1, 30:1), Sephadex LH-20 (MeOH), RP-18 (gradient 40–100% MeOH–H_2O), and then purified on semipreparative HPLC (Agilent-1200 HPLC system, Zorbax SB-C-18 (Agilent), 9.4 mm × 25 cm, 3 ml/min, 230 nm, 12.95 min, 48:52 MeOH–H_2O) to give **2** (3 mg).

3.3.1 Schisanlactone H (*1*)

A white powder. $[\alpha]_D^{27}$ + 90.3 (c = 0.25, CH_3COCH_3); UV λ_{max} (MeOH) nm (log ε): 204.2 (4.42); IR (KBr) ν_{max}: 3430, 3073, 2939, 2869, 1721, 1637, 1466, 1434, 1376, 1357, 1281, 1244, 1225, 1197, 1166, 1120, 1032, 851 cm^{-1}; ^1H and ^{13}C NMR spectral data: see Table 1. FAB-MS (pos.) m/z (%): 501 $[M+H]^+$(5), 483 $[M-OH]^+$(100), 452 (10), 440 (9), 401 (10), 355 (11), 233 (11), 145 (16), 111 ($[C_6H_7O_2]^+$, 22). Positive HR-ESI-MS m/z: 523.3398 $[M+Na]^+$ (calcd for $C_{31}H_{48}O_5Na$, 523.3399).

3.3.2 Sphenanthin A (*2*)

A white powder. $[\alpha]_D^{26}$ −10.0 (c = 0.15, $CDCl_3$); UV λ_{max} (MeOH) nm (log ε): 204.5 (4.21), 221.0 (4.25), 258.0 (sh), 330.0 (sh); IR (KBr) ν_{max}: 3423, 2956,

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** (ppm, in $CDCl_3$).

Position	δ_H (mult., *J*, Hz)	δ_C	Position	δ_H (mult., *J*, Hz)	δ_C
1	2.80 m	32.1 t	16	(a) 1.66 m	26.6 t
2	(a) 2.44 m	29.8 t		(b) 1.40 m	
	(b) 2.35 m		17	1.62 overlap	46.8 d
3		176.1 s	18	0.69 s	14.3 q
4		75.5 s	19	1.21 s	27.1 q
5	1.42 overlap	49.6 d	20	2.08 m	39.1 d
6	(a) 1.68 m	27.1 t	21	0.98 d (6.4)	13.1 q
	(b) 1.40 m		22	4.47 dt (3.5, 13.2)	80.6 d
7	(a) 1.71 m	26.8 t	23	(a) 2.10 m	23.5 t
	(b) 1.52 m			(b) 2.40 m	
8	2.13 overlap	43.4 d	24	6.61 d (6.3)	139.4 d
9		144.3 s	25		128.3 s
10		45.0 s	26		166.6 s
11	5.41 d (5.8)	117.5 d	27	1.92 s	17.0 q
12	(a) 2.14 d (18.4)	37.7 t	28	0.72 s	18.3 q
	(b) 1.95 dd (5.8, 18.4)		29[a]	1.29 s	27.6 q
13		44.1 s	30[a]	1.26 s	34.2 q
14		46.9 s	OMe	3.70 s	51.7 q
15	1.39 m	33.8 t			

Note: [a]Assignments may be interchanged.

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **2** (ppm, in $CDCl_3$).

Position	δ_H (mult., *J*, Hz)	δ_C	HMBC ($^{1}H \rightarrow {}^{13}C$)
1		42.8 s	
2	(a) 1.94 d (13.6)	51.4 t	1, 3, 6, 12
	(b) 2.84 d (13.6)		1, 3, 6, 12
3		211.4 s	
4	(a) 2.23 dd (13.7, 2.3)	45.1 t	5, 6
	(b) 2.42 t (13.7)		3, 5, 6, 11
5	2.27 m	36.7 d	4, 6, 11
6		77.2 s	
7	5.73 d (15.0)	136.3 d	5, 6, 8, 9, 10
8	6.34 dd (4.4, 15.0)	131.4 d	6, 7, 10
9	6.41 m	136.0 d	7, 8
10	(a) 5.13 dd (8.7, 1.8)	117.3 t	9
	(b) 5.27 dd (16.0, 1.8)		8, 9
11	0.89 d (6.6)	16.0 q	3, 4, 5, 6
12	0.94 s	24.5 q	1, 2, 3, 6, 13
13	0.97 s	24.5 q	1, 2, 3, 6, 12
6-OH	1.62 s		1, 5, 6

2925, 2854, 1710, 1604, 1462, 1283, 1010, 746 cm^{-1}; ^{1}H and ^{13}C NMR spectral data: see Table 2. FAB-MS (pos.) *m/z* (%): 209 $[M+H]^+$(69), 191 (57), 110 (20), 97 (14), 83 (100). Positive HR-ESI-MS *m/z*: 231.1356 $[M+Na]^+$ (calcd for $C_{13}H_{20}O_2Na$, 231.1360).

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