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A New Sesquiterpene from *Schisandra sphenanthera*

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Abstract: A new sesquiterpene, schisandrathera E (**1**) and seven dibenzocyclooctadiene lignans as schisantherin D (**2**), schisantherin B (**3**), tigloylgomisin P (**4**), schisphenin E (**5**), angeloylgomisin H (**6**), (+)-deoxyschizandrin (**7**), and (+)-gomisin K_3 (**8**) were isolated from the leaves of *Schisandra sphenanthera* Rehder & E.H.Wilson. Their structures were elucidated by spectroscopic and mass spectrometric analyses, including 1D-, 2D-NMR, HR-ESI-MS and ECD spectra. Compound **1** displayed moderate cytotoxicity against both PC3 and MCF4 cell lines in MTT assay with IC_{50} values of 22.60 and 7.80 µM, respectively.

Keywords: *Schisandra sphenanthera*; schisandrathera ; sesquiterpene; dibenzocyclooctadiene.

1. Introduction

The plant *Schisandra sphenanthera* has been used as a traditional herb to treat for various types of diseases including cough, diarrhea, diabetes, insomnia [1]. The phytochemistry investigation of this plant resulted in numerous lignan compounds [2-4]. In the course of searching for new bio-active compounds from natural resources, we reported herein the isolation procedure and chemical structural elucidation of a new sesquiterpene, schisandrathera E (**1**), together with seven known dibenzocyclooctadiene lignans (**2**-**8**) including four *S*-biphenyl and three *R*-biphenyl configurations of C_{18}-dibenzocyclooctadiene skeleton from the leaves of *S. sphenanthera*. Their chemical structures were extensively elucidated by using means of 1D, 2D-NMR experiments and compared their NMR data with the published values. In addition, the stereochemistry of **1** was successfully determined by

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both theoretical and calculated CD spectra, as well as all eight isolated compounds were tested for cytotoxic activity against two cancer cell lines PC3 and MCF4.

2. Materials and Methods

2.1. General

The ESI-MS and HR-ESI-MS were taken using an Agilent 6530 Q-TOF LC/MS systems. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer with TMS as an internal Standard. Column chromatography (CC) was performed using Kieselgel 60, 70-230 mesh and 230-400 mesh (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) used pre-coated silica gel 60 F254 (Merck, Darmstadt, Germany). Preparative HPLC were carried out on an Agilent 1100 system (Agilent technologies, Santa Clara, CA, USA), using J'sphere ODS-H80 semi-preparative column (20×250 mm, YMC, Kyoto, Japan).

2.2. Plant Material

The leaves of *Schisandra sphenanthera* Rehder & E.H.Wilson were collected in Kon Tum province in February 2017, and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources. A voucher specimen (NCCT-P110) was deposited at the Institute of Marine Biochemistry.

2.3. Extraction and Isolation

The dried plant (5.0 kg) was powdered and sonicated with MeOH three times (each time 10L) at room temperature. The crude MeOH (220 g) extract was obtained by solvent evaporation under reduced pressure, which was suspended with water and partitioned with dichloromethane, ethyl acetate to give 22.3 g dichloromethane, 70.0 g ethyl acetate extracts and water layer. The ethyl acetate crude part was first chromatographed on a silica gel column using a stepwise gradient of hexane/ethyl acetate to give seven fractions ESS1-ESS7. Fraction ESS1 was subjected to a reserve phase C-18 (RP-18) and eluted with acetone/water (1.5/1 v/v) to give six smaller fractions ESS1.1- ESS1.6. Purification ESS1.2 fraction with eluent of hexane/ethyl acetate (5/1 v/v) resulted in four fractions ESS1.2.1-ESS1.2.4. Compounds **4** (4.8 mg) and **5** (4.4 mg) were isolated from ESS1.2.2 by using preparative HPLC column eluting with acetonitrile/water (4/1 v/v). Fraction ESS1.2.3 was subjected to a silica gel CC eluting with hexane/ethyl acetate (4/1 v/v) to give five fractions ESS1.2.3A-ESS1.2.3E. Compound **7** was obtained from ESS1.2.3B by using preparative HPLC column eluting with acetonitrile/water (5.5/1 v/v). Compounds **2** (6.5 mg) and **8** (5.0 mg) were isolated from ESS1.2.3D by using preparative HPLC column eluting with acetonitrile/water (4.5/1 v/v) while compound **3** (4.0 mg) was purified from ESS1.2.3E by using preparative HPLC column and eluting with acetonitrile/water column (4/1 v/v). ESS3 fraction was subjected to a RP-18 CC using acetone/water (0.8/1 v/v) as eluent to give five fractions ESS3.1-ESS3.5. Fraction ESS3.5 was loaded into a silica gel CC eluting with hexane/ethyl acetate (3.7/1 v/v) to get compound **6** (4.3 mg). Fraction ESS1.2.3 was chromatographed on a silica gel CC eluting with hexane/acetone (10/1 v/v) to get fraction ESS1.2.3A, which was further purified by preparative HPLC column eluting with acetonitrile/water (49/1 v/v) to give compound **1** (6.3 mg).

*Schisandrathera E (**1**)*: Colorless oil, $[\alpha]_D^{26}$ -8.4° (*c* = 0.1, MeOH); HR-ESI-MS: *m/z* 257.1897 $[M + Na]^+$ (calcd. for $C_{16}H_{26}ONa$: 257.1881); ^{1}H-NMR and ^{13}C-NMR, see Table 1.

*Schisantherin D (**2**)*: Colorless prisms, $[\alpha]_D^{26}$ -130° (*c* = 0.5, MeOH); ESI-MS: *m/z* 521 $[M + H]^+$; ^{1}H-NMR and ^{13}C-NMR, see Table S1, S2.

*Schisantherin B (**3**)*: Colorless prisms, $[\alpha]_D^{26}$ -30° (*c* = 0.3, MeOH); ESI-MS: *m/z* 515 $[M + H]^+$; ^{1}H-NMR and ^{13}C-NMR, see Table S1, S2.

*Tigloylgomisin P (**4**)*: Colorless prisms, $[\alpha]_D^{26}$ -42° (*c* = 0.4, MeOH); ESI-MS: *m/z* 515 $[M + H]^+$; ^{1}H-NMR and ^{13}C-NMR, see Table S1, S2.

*Schisphenin E (**5**)*: Colorless prisms, $[\alpha]_D^{26}$ -22° (*c* = 0.5, MeOH); ESI-MS: *m/z* 433 $[M + H]^+$; ^{1}H-NMR and ^{13}C-NMR, see Table S1, S2.

*Angeloylgomisin H (**6**)*: Amorphous solid, $[\alpha]_D^{26}$ +112° (*c* = 0.4, MeOH); ESI-MS: *m/z* 501 $[M + H]^+$; ^{1}H-NMR and ^{13}C-NMR, see Table S3.

*(+)-Deoxyschizandrin (**7**)*: Amorphous solid, $[\alpha]_D^{26}$ +15° (c = 0.4, MeOH); ESI-MS: m/z 417 $[M + H]^+$; ^{1}H-NMR and 13C-NMR, see Table S3.

*(+)-Gomisin K$_3$ (**8**)*: Colorless needles, $[\alpha]_D^{26}$ +65° (c = 0.4, MeOH); ESI-MS: m/z 403 $[M + H]^+$; ^{1}H-NMR and ^{13}C-NMR, see Table S3.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as colorless oil, which exhibited an ion peak at *m/z* 257.1897 $[M + Na]^+$ in HR-ESI-MS (Figure S1) confirming its molecular formula of $C_{16}H_{26}O$ (calcd. for $C_{16}H_{26}ONa$: 257.1881), indicating four degrees of unsaturation. The ^{1}H-NMR spectrum displayed two aromatic protons at δ_H 5.78 (d, *J* = 7.5 Hz) and 5.52 (dt, *J* = 7.5, 2.0), a methoxy group at δ_H 3.28 (s), three methyl groups including one upfield singlet at δ_H 0.84, and two doublets at δ_H 0.93 (d, *J* = 7.0 Hz) and 0.99 (d, *J* = 7.0 Hz) (Figure S2). The ^{13}C-NMR and HSQC spectra (Figure S3-4) of **1** exhibited sixteen carbons, which were assigned for a sesquiterpene and a methoxy group as shown in Table 1. Two >C=CH- double bonds were identified at δ_C 113.9, 122.5, 138.3, and 159.8, an oxygenated methylene group was assigned at δ_C 79.7. Compound **1** was suggested to contain two rings by analyzing of NMR data and above mass spectrum by using degrees of unsaturation as shown in Figure 1.

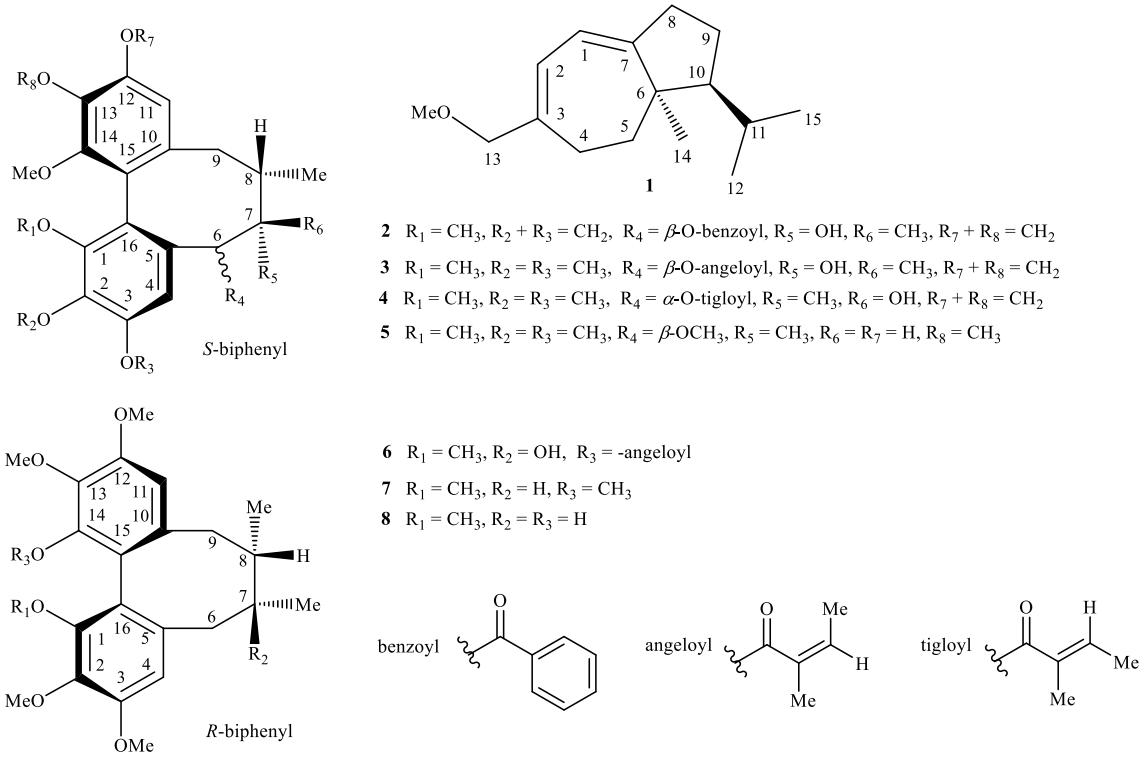

Figure 1. The chemical structures of compounds **1-8**

Furthermore, cross peaks from H-1 (δ_H 5.52) to H-2 (δ_H 5.78), from H-4 (δ_H 2.34) to H-5 (δ_H 2.00/1.39), from H-9 (δ_H 1.86/1.48) to H-8 (δ_H 2.44/2.32) and H-10 (δ_H 1.32), and from H-11 (δ_H 1.71) to H-10, H-12 (δ_H 0.99), and H-15 (δ_H 0.93) were observed in the ^{1}H-^{1}H COSY spectrum of **1** confirming its structural fragmentations (Figure S6, S8). In addition, the HMBC correlations from olefinic proton H-1 to sp^3 non-protonated carbons C-3 (δ_C 138.3) and C-6 (δ_C 47.8), from H-2 to C-7/C3/C-4, from H-5 to C-3/C-6/C-7, from H-13 to C-2/C-3/C-4, from methoxy protons (δ_H 3.85) to C-13, and from H-14 to C-5/C-6/C-7 were observed confirming two double bonds were at C-1/C-7 and C-2/C-3, methoxy group linked to C-13, and the methyl group was at C-6, as well as the presence of 1-(methoxymethyl)-6,7-disubstituted-cyclochepta-1,3-diene was established. The HMBC interactions between H-8 and C-7/C-10, between H-9 and C-6/C-7/C-10 and from H-1 to C-8 suggested that five-member ring was fused with 1-(methoxymethyl)-6,7-disubstituted-cyclochepta-1,3-diene at C-6 and C-7. Moreover, the HMBC interactions from two methyl groups together with H-11 to sp^3 methine C-10 suggested that isopropyl unit attached to C-10 of five-member carbon ring (Figure S5, S8). The overall planar structure of **1** was quite similar with that of hortonones B, except for the addition of a methoxy group and the disappearance of the ketone functional group [5].

Table 1. NMR data for compound **1**

Position	δ_C[a,b]	δ_H[a,c]
1	113.9 (CH)	5.52 (1H, dt, *J* = 7.5, 2.0 Hz)
2	122.5 (CH)	5.78 (1H, d, *J* = 7.5 Hz)
3	138.3 (C)	-
4	28.0 (CH_2)	2.34 (2H, m)
5	33.9 (CH_2)	2.00 (1H, m)
		1.39 (1H, m)
6	47.8 (C)	-
7	159.8 (C)	-
8	31.8 (CH_2)	2.44 (1H, dd, *J* = 17.5, 8.5 Hz)
		2.32 (1H, m)
9	27.0 (CH_2)	1.81 (1H, m)
		1.48 (1H, m)
10	57.6 (CH)	1.32 (1H, m)
11	28.3 (CH)	1.71 (1H, m)
12	24.1 (CH_3)	0.99 (3H, d, *J* = 7.0 Hz)
13	79.7 (CH_2)	3.85 (2H, s)
14	15.6 (CH_3)	0.84 (3H, s)
15	22.3 (CH_3)	0.93 (3H, d, *J* = 7.0 Hz)
OMe	57.2 (CH_3)	3.28 (3H, s)

Measured in [a]$CDCl_3$, [b]125 MHz, [c]500 MHz

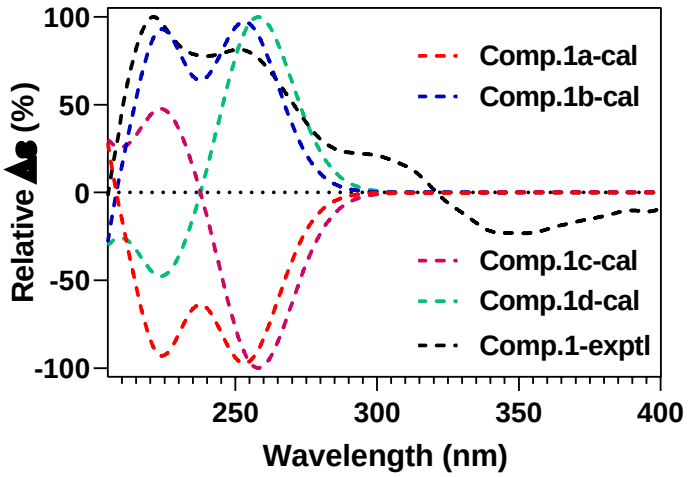

Figure 2. Theoretical calculated ECD spectra of four possible stereoisomers and experimental CD for compound **1**

Finally, the absolute configurations of two stereogenic centers at C-6 and C-10 in **1** were determined by both experimental and calculated ECD analysis. Four possible configurations **1**a-(6*S*,10*R*), **1**b-(6*R*,10*S*), **1**c-(6*R*,10*R*), and **1**d-(6*S*,10*S*) were submitted to calculate their theoretical ECD spectra and compared with experimental results (Figure 2). The experimental CD analysis displayed two positive CEs at 221 and 253 nm, which were in good agreements with **1**b (two positive CEs at 223 and 253 nm). Consequently, the complete structure of compound **1** was elucidated as shown in Figure 1, a new sesquiterpene named as schisandrathera E.

In addition, the remaining seven dibenzocyclooctadiene lignans were determined as schisantherin D (**2**) [6], schisantherin B (**3**) [4], tigloylgomisin P (**4**) [7], schisphenin E (**5**) [8], angeloylgomisin H [7], (+)-deoxyschizandrin [9], (+)-gomisin K_3 (**8**) [10] by comparing their NMR data with that in the reported literatures.

3.2 Cytotoxicity Activity

The cytotoxic behavior of these compounds **1**-**8** were evaluated against two cancer cell lines following MTT assay [11]. At concentration of 30µM, compounds **2**-**6** were inactive as less than 50% of dead cells were observed. Compound **1** displayed moderate cytotoxicity toward both PC3 and MCF4 cell lines with IC_{50} values of 22.60 and 7.80 µM, respectively, while capecitabine was used as positive control with IC_{50} values of 11.2 and 7.17 µM, respectively. The IC_{50} values of compounds **7**-**8** were calculated and tabulated in table S5.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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