

## Intermedins A and B; New Metabolites from *Schisandra propinqua* var. *intermedia*

Hong-Mei Li<sup>1</sup>, Chun Lei<sup>2</sup>, Yong-Ming Luo<sup>1</sup>, Xiao-Nian Li<sup>2</sup>, Xiao-Lei Li<sup>1</sup>, Jian-Xin Pu<sup>2</sup>, San-Yun Zhou<sup>1</sup>, Rong-Tao Li<sup>1,2</sup>, and Han-Dong Sun<sup>2</sup>

<sup>1</sup>The College of Life Science and Technology, Kunming University of Science and Technology, Kunming 650224, Yunnan, P. R. China, and <sup>2</sup>State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P. R. China

(Received October 20, 2007; Revised February 10, 2008; Accepted April 15, 2008)

A new dibenzocyclooctadiene lignan, intermedin A (**1**), and a new natural bisabolane sesquiterpenoid, intermedin B (**2**), were isolated from the aerial parts of *Schisandra propinqua* var. *intermedia*. Their structures were elucidated on the basis of extensive spectroscopical analysis.

**Key words:** *Schisandra propinqua* var. *intermedia*, Dibenzocyclooctadiene, Sesquiterpenoid, Intermedin A, Intermedin B, Chemical constituents

### INTRODUCTION

Plants of the genus *Schisandra*, belonging to the economically and medicinally important family Schisandraceae, were commonly used in Chinese traditional medicine as tonics, sedatives, and astringent agents (Xiao *et al.*, 2005a). Previous studies reported that this genus contained dibenzocyclooctadiene lignans, lanostane triterpenoid acids, and lactones, which have been found to possess some beneficial pharmacological effects, including anti-hepatitis, antitumor, and anti-HIV activities (Pu *et al.*, 2005). In our recent research, we have found more than 40 highly oxygenated nortriterpenoids with unprecedented carbon skeletons, some of which have potent anti-HIV-1 activity with low toxicity (Li *et al.*, 2003a; Li *et al.*, 2003b; Li *et al.*, 2004a; Li *et al.*, 2004b; Li *et al.*, 2005a; Li *et al.*, 2005b; Xiao *et al.*, 2005b; Huang *et al.*, 2007). In order to discover more secondary metabolites with potential bioactivities and novel structures, and search for potential lead compounds for drug development, we investigated the aerial parts of *Schisandra propinqua* var. *intermedia* collected from Yunnan Province of China. Systematic phytochemical investigation of this plant led to the isolation of a new

lignan, intermedin A (**1**), and a new natural bisabolane sesquiterpenoid, intermedin B (**2**), which was previously reported as a synthesized product by Uehara to ascertain the absolute configuration of bisacurone A (Uehara *et al.*, 1990). This paper deals with the isolation and structural elucidation of intermedins A (**1**) and B (**2**).

### MATERIALS AND METHODS

#### General experimental procedure

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. CD spectra were measured with a JASCO J-8115 spectropolarimeter. 1D- and 2D-NMR Spectra were recorded using Bruker AM-400 and DRX-500 instruments with tetramethylsilane (TMS) as an internal standard. EI-MS were measured on a VG Auto Spec-3000 spectrometer; and HR-ESI-MS were taken on an API Qstar Pulsar instrument. HPLC separations were performed on a HP 1100 apparatus equipped with a UV detector and Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column. Column chromatography was performed with silica gel (200-300 mesh, Qing-dao Marine Chemical Inc., Qing-dao, Peoples Republic of China), silica gel H (60 μm, Qing-dao Marine Chemical Inc.). TLC was performed on TLC plates (Si gel GF<sub>254</sub>), and detected by spraying with 5% H<sub>2</sub>SO<sub>4</sub>-EtOH,

Correspondence to: Rong-Tao Li, The College of Life Science and Technology, Kunming University of Science and Technology  
Tel: 86-871-3801018, Fax: 86-871-3801191  
E-mail: rongtaoli99@yahoo.com.cn

followed by heating on a hot plate.

### Plant materials

The aerial parts of *S. propinqua* var. *intermedia* were collected in honghe region, Yunnan Province, China, in September 2005, and were identified by Prof. Xi-Wen Li, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

### Extraction and isolation

The air-dried and powdered aerial parts of *S. propinqua* var. *intermedia* (8.3 kg) were extracted with 70% aq. acetone (3×15 L, 3 d, each) at r. t. to yield the extract, which was dissolved in H<sub>2</sub>O and then extracted with EtOAc. The EtOAc extract (80 g) was subjected to silica gel column eluting with CHCl<sub>3</sub>-acetone (gradient 1: 0, 9: 1, 8: 2, 7: 3, 6: 4, 5: 5, 0: 1) to afford fractions A-E. Fr. B (9: 1 and 8: 2) was decolorized with *MCI-gel CHP-20P* column (90% MeOH-H<sub>2</sub>O) to yield Fr. B-1~B-4. Fr. B-1 (6.063 g, 90% MeOH-H<sub>2</sub>O) was purified on silica gel column (CC) eluting with CHCl<sub>3</sub>-isopropyl alcohol (100: 1) to afford four parts. Fr. B-1-4 (2.636 g) was subjected to CC, CHCl<sub>3</sub>-isopropyl alcohol (80: 1) as eluent, to obtain Fr. B-1-4-1~B-1-4-5. Fr. B-1-4-3 (1.385 g) was isolated on CC (petroleum ether-isopropyl alcohol 30:1) to get four parts. Fr. B-1-4-3-4 (55 mg) was subjected to Sephadex LH-20 and finally purified on semipreparative HPLC (MeOH/H<sub>2</sub>O 65:35) to give **1** (5 mg). B-1-4-3-1 (605 mg) was also subjected to Sephadex LH-20, then reverse chromatography (MeOH-H<sub>2</sub>O gradient 20%→100%), and finally purified on semipreparative HPLC with 65% MeOH in H<sub>2</sub>O as the mobile phase to give **2** (13 mg).

### Intermedin A (1)

Colorless oil;  $[\alpha]_D^{27}$  -60.6 (c 0.03, MeOH); UV  $\lambda_{max}$  (MeOH) nm (log  $\epsilon$ ): 191 (4.28), 204 (4.45), 221 (4.45), 250 (sh), 280 (sh); IR (KBr) cm<sup>-1</sup>: 3432, 2924, 2853, 1723, 1114; CD (c 0.06, MeOH) (mdeg): 216 (+9.07), 222 (+14.39), 239 (-20.43), 253 (-14.17); EI-MS *m/z* (rel. int. %): 637 (1, [M+H]<sup>+</sup>), 536 (25), 371 (55), 330 (27), 105 (100), 83 (18), 77 (15), 55 (14); ESI-MS (pos.) *m/z*: 659 ([M+Na]<sup>+</sup>), 675 ([M+K]<sup>+</sup>) and 1295 ([2M+Na]<sup>+</sup>); HR-ESI-MS (pos.) *m/z*: 659.2461 ([M+Na]<sup>+</sup>, C<sub>35</sub>H<sub>40</sub>O<sub>11</sub>Na<sup>+</sup>; calc. 659.2468); For <sup>1</sup>H- and <sup>13</sup>C-NMR data see Table I.

### Intermedin B (2)

Colorless oil;  $[\alpha]_D^{26}$  -11.5 (c 0.45, MeOH); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 0.90 (3H, d, *J* = 5.9 Hz, Me-14), 1.88 (3H, s, Me-12), 2.14 (3H, s, Me-13), 4.43 (1H, br s, 5-OH), 4.96 (1H, s, H-15a), 5.06 (1H, s, H-15b), 5.76 (1H, d, *J* = 10.0

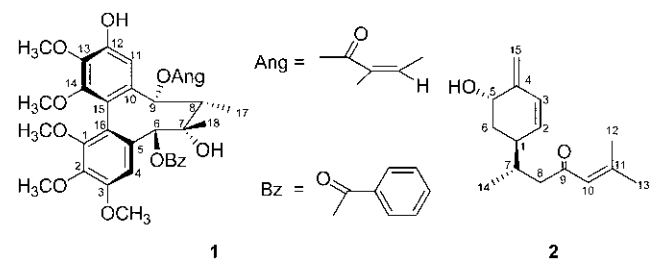
**Table I.** <sup>1</sup>H- and <sup>13</sup>C-NMR data of **1** (400 MHz for <sup>1</sup>H, and 100 MHz for <sup>13</sup>C, CDCl<sub>3</sub>,  $\delta$  ppm, *J*<sub>H<sub>z</sub>)</sub>

No.	$\delta_H$ [mult., J(Hz)]	$\delta_C$	No.	$\delta_H$ [mult., J(Hz)]	$\delta_C$
1		151.6 s	18	1.38 (3H, s)	28.8 q
2		139.0 s	-OCH <sub>3</sub>	3.14 (3H, s)	59.3 q
3		152.1 s		3.33 (3H, s)	59.7 q
4	6.87 (1H, s)	111.1 d		3.45 (3H, s)	60.3 q
5		129.2 s		3.83 (3H, s)	60.4 q
6	6.03 (1H, s)	85.0 d		3.95 (3H, s)	56.2 q
7		74.2 s	-OH	5.68 (1H, br s)	
8	2.40 (1H, m)	43.1 d	PhCO- C=O		164.9 s
9	5.90 (1H, s)	83.4 d	1'		129.3 s
10		134.8 s	2', 6'	7.41 (2H, d, 7.5)	129.6 d
11	6.82 (1H, s)	109.5 d	3', 5'	7.30 (2H, m)	128.2 d
12		149.1 s	4'	7.49 (1H, t, 7.4)	133.2 d
13		138.8 s	-Ang C=O		165.2 s
14		150.0 s	$\alpha$ -C		125.6 s
15		120.4 s	$\beta$ -C	6.00 (1H, overlapped)	142.7 d
16		122.4 s	$\alpha$ -Me	1.28 (3H, s)	19.8 q
17	1.34 (3H, d, 7.1)	17.1 q	$\beta$ -Me	1.89 (3H, d, 7.0)	15.7 q

Hz, H-3), 6.06 (1H, s, H-10), 6.15 (1H, d, *J* = 10.0 Hz, H-2); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ : 32.7 (d, C-1), 133.7 (d, C-2), 127.0 (d, C-3), 144.8 (s, C-4), 69.3 (d, C-5), 31.6 (t, C-6), 35.7 (d, C-7), 48.5 (t, C-8), 200.6 (s, C-9), 124.0 (d, C-10), 155.4 (s, C-11), 20.7 (q, C-12), 27.7 (q, C-13), 16.6 (q, C-14), 113.6 (t, C-15); EI-MS *m/z* (rel. int. %): 214 (18), 199 (100), 197 (34), 159 (18), 149 (23), 83 (34); FAB-MS *m/z* (rel. int. %): 235 [M+H]<sup>+</sup>(4), 217 (18), 119 (39), 83 (100); HR-ESI-MS (pos.) *m/z*: 235.1695 ([M+H]<sup>+</sup>, C<sub>15</sub>H<sub>23</sub>O<sub>2</sub>; calc. 235.1698).

## RESULTS AND DISCUSSION

Compound **1** gave quasi-molecular ion peaks at *m/z* 659 ([M+Na]<sup>+</sup>), 675 ([M+K]<sup>+</sup>) and 1295 ([2M+Na]<sup>+</sup>) in the positive ESI-MS, indicating its molecular weight of 636. The molecular formula was determined as C<sub>35</sub>H<sub>40</sub>O<sub>11</sub> by HR-ESI-MS (*m/z* 659.2461, [M+Na]<sup>+</sup>) with 16 unsatura-



**Fig. 1.** Structures of compounds **1** and **2**

tions. The UV spectrum of **1**, with a maximum absorption at 221 nm and two shoulders at 250 and 280 nm, along with the corresponding 1D-NMR spectra (Table I), indicated **1** was a dibenzocyclooctadiene lignan (Chen *et al.*, 2004).

The  $^1\text{H-NMR}$  spectrum of **1** showed signals of two aromatic protons ( $\delta_{\text{H}}$  6.87 and 6.82 (each 1H, s)) of a biphenyl moiety. The signals at  $\delta_{\text{H}}$  3.14, 3.33, 3.45, 3.83, 3.95 (each 3H, s), and 5.68 (1H, br s) indicated the presence of five methoxylys, and a phenolic hydroxyl, as substituents on the biphenyl rings (Shen *et al.*, 2007). One tertiary methyl at  $\delta_{\text{H}}$  1.38, one oxygenated quaternary carbon at  $\delta_{\text{C}}$  74.2, and one secondary methyl at  $\delta_{\text{H}}$  1.34 (d,  $J = 7.1$  Hz) as shown in the HMBC spectrum (Fig. 2), were also found in the cyclooctadiene ring. Moreover, the 1D-NMR spectral data of **1** indicated the presence of an angeloyl and a benzoyl moieties (Chen *et al.*, 2004; Chen *et al.*, 1996), which were further supported by the base peak at  $m/z$  105 ( $\text{Ph CO}^+$ ) and significant peaks at  $m/z$  77 ( $\text{C}_6\text{H}_5^+$ ), 83 [ $\text{Me CH} = \text{C}(\text{Me}) \text{CO}^+$ ], 55 ( $\text{Me CH} = \text{C Me}^+$ ) in the EI-MS spectrum (Zhai and Cong, 1990; Ikeya *et al.*, 1979 a; Ikeya *et al.*, 1979 b). The HMBC correlations from the five methoxy groups ( $\delta_{\text{H}}$  3.14, 3.33, 3.45, 3.83, 3.95) to C-14, C-13, C-1, C-2, C-3, respectively and from the phenolic hydroxyl at  $\delta_{\text{H}}$  5.68 to C-11, C-12, C-13, indicated that these five methoxy groups and the phenolic hydroxyl group were attached to C-1, C-2, C-3, C-13, C-14, C-12, respectively. In the HMBC spectrum of **1**, it was found that one oxymethine proton at  $\delta_{\text{H}}$  6.03 exhibited HMBC correlations with the biphenyl methine carbon ( $\delta_{\text{C}}$  111.1, C-4) and C-7; Similarly, another oxymethine proton at  $\delta_{\text{H}}$  5.90 correlated with another biphenyl methine carbon ( $\delta_{\text{C}}$  109.5, C-11) and C-8. Thus, the two oxymethine protons were assigned as H-6 and H-9, respectively. Moreover, the HMBC correlations were observed from H-6 ( $\delta_{\text{H}}$  6.03) and H-2', 6' ( $\delta_{\text{H}}$  7.41) to the carbonyl carbon ( $\delta_{\text{C}}$  164.9) of the benzoyl group, and from H-9 ( $\delta_{\text{H}}$  5.90) and  $\alpha\text{-CH}_3$  ( $\delta_{\text{H}}$  1.28) to the carbonyl carbon ( $\delta_{\text{C}}$  165.2) of the angeloyl group, established that the benzoyl and the angeloyl groups being located at C-6 and C-9 of the cyclooctadiene ring, respectively.

The stereochemistry of the cyclooctadiene moiety was elucidated by the NOE correlations observed in the ROESY spectrum (Fig. 2). The strong NOE correlations observed between H-11 and H-9, and between H-4 and H-6 suggested the  $\beta$ -orientation of H-9 and the  $\alpha$ -orientation of H-6, respectively. Therefore, the angeloyl and benzoyl groups were located on the  $\alpha$ - and  $\beta$ -face of the molecule, respectively. Moreover, the NOE interaction between H-9 and H-8, and between H-8 and Me-18 reflected the  $\alpha$ -orientation of both methyl and hydroxyl groups at C-8 and C-7, respectively. The lack of NOE response between H-4 and Me-18 further supported the

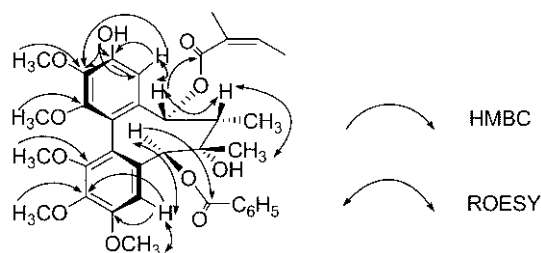


Fig. 2. Key HMBC and ROESY correlations of **1**

$\beta$ -orientation of Me-18. Further, the ROESY correlations between H-4 and H-6, Me-17 and H-9, as well as between H-9 and H-11 indicated a twist-boat-chair (TBC) conformation of the cyclooctadiene ring (Chen *et al.*, 2004; Shen *et al.*, 2007). Besides, **1** showed two negative Cotton effects around 235–255 nm and two positive ones around 215–225 nm, indicating that **1** possess an *S*-biphenyl configuration (Ikeya *et al.*, 1979a). Therefore, the complete structure of **1** was determined to be that shown in Fig. 1, named as intermedin A.

Compound **2** was composed of 15 carbons: three methyls, two methylenes, one terminal double bond, six methines (including three unsaturated ones), two unsaturated quaternary carbon and one carbonyl group, corresponding to the  $^{13}\text{C-NMR}$  and DEPT spectra. Analysis of the  $^1\text{H}$ - and  $^{13}\text{C-NMR}$  data of **2** suggested it was a bisabolane sesquiterpenoid. The spectroscopic data of **2** was very similar to those of bisacurone, which possessed a 2-methyl-2-hepten-4-one structural unit (Ohshiro *et al.*, 1990). The only difference between them was the substituent pattern on C-4. The absence of the signals at  $\delta_{\text{C}}$  70.7 (s) and 23.7 (q), and the presence of the signals at  $\delta_{\text{C}}$  144.8 (s) and 113.6 (t) in the  $^{13}\text{C-NMR}$  spectrum, indicated the existence of an exocyclic double bond on C-4 of **2**. Compound **2** was identified as a new natural bisabolane sesquiterpenoid and named as intermedin B even if it was previously synthesized by Uehara to establish the absolute configuration of bisacurone A (Uehara *et al.*, 1990).

## ACKNOWLEDGEMENTS

We thank Prof. Hao Huang, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, for their generous support on the measurement of the CD spectra.

This project was financially supported by the National Natural Science Foundation of China (No. 20402016), the Foundation of Excellent Ph.D Dissertation of China (200780), the Foundation of Excellent Ph.D Dissertation of Chinese Academy of Sciences (O0602551221), the Program for New Century Excellent Talents on University (NCET-06-0824), and the Young Academic and Technical

Leader Raising Foundation of Yunnan Province (No. 2005py01-32).

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