A New Triterpene and Dibenzocyclooctadiene Lignans from *Schisandra propinqua* (WALL.) BAILL

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A new triterpene and two new natural dibenzocyclooctadiene lignans were isolated from the stems of *Schisandra propinqua*. In addition, three known lignans, octadecanoic acid, 2,3-dihydroxypropyl ester and β-sitosterol were isolated. The structures of the new triterpene and new natural products were elucidated base on spectral analysis, including 1D and 2D NMR experiments. The isolates were tested for their cytotoxic effects against several tumor cell lines by MTT assay.

Key words *Schisandra propinqua*; Schisandraceae; triperpene; dibenzocyclooctadiene lignan; cytotoxicity

Schisandra propinqua (WALL.) BAILL is widely distributed in southwest of China. Its stems were used in folk medicine for the treatment of rheumatism, traumatic injury, ulcer with pyogenic infections, stomachache and neurasthenia. Previous phytochemical investigation revealed that this plant mainly contained lignans[1—5] and triterpenoids.[6—8] As a part of our search for bioactive materials, the petroleum ether extract of the stems of *S. propinqua* showed cytotoxic activity against tumor cell lines during preliminary screening (HepG2, IC_{50}=206 $\mu g \cdot ml^{-1}$; Bel-7402, IC_{50}=191 $\mu g \cdot ml^{-1}$). Subsequent chromatographic separation and purification led to the isolation of one new triterpene, schisanterpene B (**1**) as well as 5 dibenzocyclooctadiene lignans, propinquanin E (**2**), schisantherin G[9] (**3**), propinquanin F (**4**), schisantherin F[10] (**5**) and Kadsurarin II[11] (**6**). Compounds **2** and **4** are first reported occurrence from a natural source. In addition, octadecanoic acid, 2,3-dihydroxypropyl ester[12] (**7**) and β-sitosterol (**8**) were isolated. This paper deals with the isolation and the characterization of the new triterpenoid and the two new natural products. The evaluation of the cytotoxic activity of the isolates by MTT assay was also described herein.

Results and Discussion

Compound **1** was afforded as colorless needles, mp: 193—194 °C, $[\alpha]_D^{20}$: +20° (c=0.1, $CHCl_3$). The molecular formula was established as $C_{27}H_{40}O_4$ by HR-EI-MS at m/z 428.2927 (Calcd for 428.2938). The IR spectrum showed strong absorption at 1760 (γ-lactone ring), 1708 (ketone) and 3600 cm^{-1} (hydroxyl group). The ^{1}H-NMR spectrum of **1** (Table 1) revealed a pair of doublets at δ 0.61 and 0.95 (1H each, d, J=4.4 Hz), which correlated with the methylene carbon at δ 28.34 in the HMBC, indicating the presence of a cyclopropane methylene group in the molecule. The ^{1}H-NMR spectrum also exhibited signals of five tertiary methyl groups at δ 1.00, 1.06, 1.11, 1.19, 1.46 (3H each, s). In addition, the ^{13}C-NMR and DEPT spectra confirmed the presence of five methyl, ten methylene, four methine and eight quaternary carbons (including a ketone at δ 216.17). The aforementioned NMR data together with a mass spectral peak at m/z 428 suggested a cycloartanone-type triterpene skeleton with one hydroxyl group. The mass spectrum of **1** showed an important base peak at m/z 99, indicating the side chain of **1** was a γ-lactone ring.[13] This was confirmed by the correlations of 1.46/34.06 (H-21/C-22), 1.46/54.75 (H-21/C-17), 2.48/88.54 (H-23/C-20) and 2.57/ 177.57 (H-23/ C-24) in the HMBC spectrum. The β configuration was confirmed by the obvious cross peak at δ 2.10/1.00 (H-17α/Me-28α) in the NOESY spectrum. In the ^{1}H–^{1}H COSY spectrum, the cross peaks at δ 3.63/1.68 (H-7/H-8), δ 1.90/1.17 (H-5/H-6) and δ 1.17/3.63 (H-6/H-7) indicated that there was a hydroxyl group at C-7 ($\delta_{C\text{-}7}$ 70.68, $\delta_{H\text{-}7}$ 3.63 in $CDCl_3$). And this substitution was confirmed by the correlations of 1.17/49.70 (H-6/C-4) in the HMBC spectrum (Fig. 2). The β configuration was confirmed by cross peaks at δ 3.63/1.00 (H-7α/Me-28α), δ 3.63/1.90 (H-7α/H-5), δ 3.63/1.59 (H-7α/H-15α)

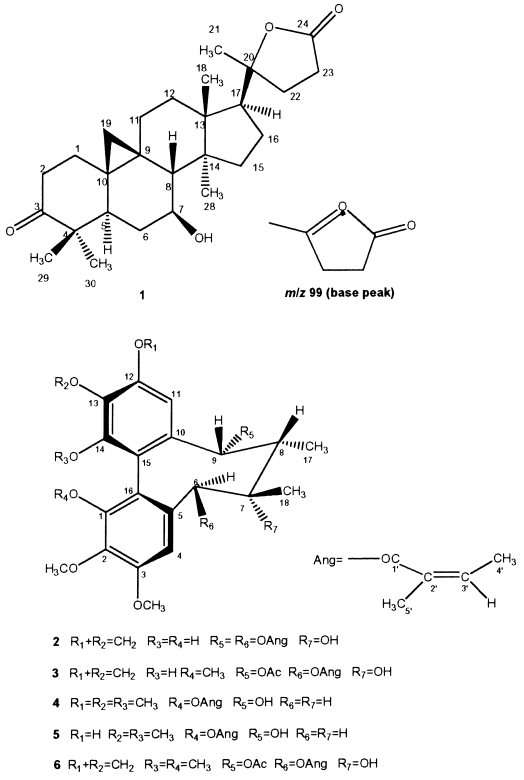

Fig. 1. Chemical Structures of Schisanterpene B and Dibenzocyclooctadiene Lignans from *S. propinqua*

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Table 1. ^{1}H- (400 MHz), ^{13}C-NMR (100 MHz) of Compound **1** in $CDCl_3$

C	δ_H	δ_C
1	1.54, 1.78, 1H each, m	32.92
2	2.32, 2.70, 1H each, m	37.23
3		216.17
4		49.70
5	1.90, 1H, dd, J=3.2/12.8 Hz	46.79
6	1.17, 2H, overlapped	31.38
7	3.63, 1H, m	70.68
8	1.68, 1H, d, J=7.2 Hz	54.48
9		20.45
10		26.61
11	1.32, 1.97, 1H each, m	26.77
12	1.71, 1.76, 1H each, overlapped	32.57
13		46.53
14		48.27
15	1.59, 1.64, 1H each, overlapped	36.47
16	1.69, 1.81, 1H each, overlapped	22.87
17	2.10, t, 1H, J=9.2 Hz	54.75
18	1.19, 3H, s	18.97
19	0.61, 0.95, 1H each, d, J=4.4 Hz	28.34
20		88.54
21	1.46, 3H, s	26.52
22	2.22, 1.87, 1H each, m	34.06
23	2.48, 2.57, 1H each, dq	27.98
24		177.57
28	1.00, 3H, s	18.97
29	1.11, 3H, s	20.67
30	1.06, 3H, s	22.18

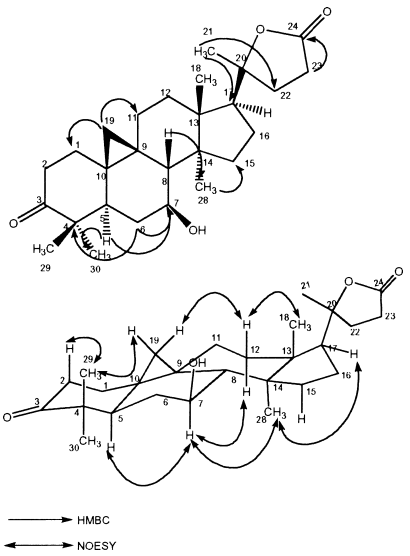

Fig. 2. Key HMBC and NOESY Correlations of Schisanterpene B

and δ 3.63/1.71 (H-7α/H-12α) in the NOESY spectrum. Therefore, the structure of **1** was established as depicted in Fig. 1.

Propinquanin E (**2**) gave a HR-EI-MS molecular ion at *m*/*z* 584.2249 (Calcd 584.2258), corresponding to a molecular formula of $C_{31}H_{36}O_{11}$. The UV, IR and NMR spectra revealed that **2** possessed a C18 lignan skeleton. The ^{1}H-NMR spectrum of **2** displayed signals of two methyl groups, two angelic esters at C-7, 8, 6 and 9, respectively. The protons signals at δ 5.97, 5.94 (1H each, br s-like) and carbon signal at δ_C 101.72 were attributable to the methylenedioxy moiety on

Table 2. ^{1}H-NMR Data for Compounds **2** and **4** (Measured in $CDCl_3$ at 400 MHz)

	2	**4**
4	6.71 (1H, s)	6.73 (1H, s)
11	6.45 (1H, s)	6.39 (1H, s)
6	5.55 (1H, s)	2.65 (1H, m)
9	5.63 (1H, s)	4.79 (1H, s)
7		2.05 (1H, m)
8	2.25 (1H, m)	1.86 (1H, m)
7-Me	1.41 (3H, s)	1.03 (3H, d, J=7.2)
8-Me	1.31 (3H, d, J=6.8)	1.22 (3H, d, J=7.2)
2-OMe	3.90 (3H, s)	3.80 (3H, s)
3-OMe	3.93 (3H, s)	3.90 (3H, s)
12-OMe		3.86 (3H, s)
13-OMe		3.83 (3H, s)
14-OMe		3.59 (3H, s)
OCH_2O	5.96, 5.94, 1H each, br s-like	
Ang 3′	5.96 (1H, dd, J=7.2)	5.96 (1H, dd, J=7.2)
4′	1.85 (3H, d, J=7.2)	1.77 (3H, d, J=7.2)
5′	1.45 (3H, s)	1.82 (3H, s)
Ang 3″	5.96 (1H, dd, J=7.2)	
4″	1.88 (3H, d, J=7.2)	
5″	1.33 (3H, s)	

Table 3. ^{13}C-NMR Data for Compounds **2** and **4** (Measured in $CDCl_3$ at 100 MHz)

	2	**4**
1	146.14	141.61
2	134.89	138.98
3	151.11	151.49
4	108.55	112.96
5	132.18	134.62
6	85.41	38.93
7	73.95	35.32
8	43.23	43.07
9	83.80	83.11
10	133.79	140.93
11	101.28	106.74
12	148.79	152.82
13	134.30	140.36
14	136.91	151.31
15	115.51	119.46
16	112.35	123.41
17	28.99	20.30
18	17.28	15.21
2-OMe	60.54	61.18
3-OMe	56.04	60.73
12-OMe		56.01
13-OMe		55.95
14-OMe		60.73
Ang 1′	166.14	167.24
2′	126.70	127.26
3′	141.41	138.80
4′	19.91	20.30
5′	15.77	15.41
Ang 1″	165.15	
2″	125.62	
3″	140.70	
4″	19.86	
5″	15.75	

aromatic ring. Two methoxyl signals at δ 3.90, 3.93 were assigned for 2-OCH_3, 3-OCH_3, on the basis of the HMBC spectrum. The two angeloyloxy groups were identified by the EI-MS signals at *m*/*z* 484 [$M^+ - C_4H_7COOH$] and *m*/*z* 384 [$M^+ - C_4H_7COOH - C_4H_7COOH$], respectively. Based on the

Table 4. Cytotoxicities of Isolates from the *Schisandra propinqua*[a)]

Compounds	Cell lines[b)]			
	HepG2	KB	HL-60	Bel-7402
1	61.32	—	58.07	72.93
2	35.95	46.23	32.53	39.38
3	45.95	49.35	38.60	48.01
4	59.91	42.98	60.02	—
5	—	—	—	—
6	50.69	—	48.95	—
7	50.44	—	37.20	39.47
Camptothecin[c)]	1.23	1.78	1.35	1.02

a) Results are expressed as IC_{50} values in μM. For effective of a compound, an IC_{50} value $\leq 80\,\mu$M is required. *b*) Cell lines: Hep-G2=human hepatocellular carcinoma; KB=human oropharyngeal epidermoid carcinoma; HL-60=human acute promyelocytic leukemia; Bel-7402=hepatocellular carcinoma. *c*) Camprotohecin was used as a positive control.

NOESY spectrum, the configuration of **2** was determined to be the same as that of **3**. Thus the structure of **2** was similar to schisantherin H,[9)] except for a hydroxyl at C-14 instead of an methoxyl in schisantherin H.

Propinquanin F (**4**) had a molecular formula of $C_{28}H_{36}O_8$, as established from the molecular ion peak at *m*/*z* 500.2394 (Calcd 500.2410) in the HR-EI-MS. Its IR, UV, CD and NMR spectra were very closely to those of **5**, which indicated **4** was an analogue of **5**. The ^{1}H- and ^{13}C-NMR spectra of **4** showed signals for five methoxyl groups, while the molecular ion at *m*/*z* 500 was 14 units greater than that of **5** (*m*/*z* 486), indicating that one hydroxyl in **5** was replaced by an methoxyl in **4**. Based on the HMBC correlations, the structure of **4** was established to the known schisantherin F (**5**) except for a methoxyl at C-12 instead of a hydroxyl in **5**.

The configuration of the biphenyl groups in all isolated dibenzocyclooctadiene lignans were determined based on their characteristic circular dichroism (CD) spectra. The CD spectra of **2** and **4** showed a positive cotton effect at 217 nm and 218 nm, respectively, and a negative cotton effect at 255 nm and 253 nm, respectively, which suggested that they all possessed an *S*-biphenyl configuration as gomisin B.[14,15)]

The *in vitro* cytotoxicty of isolated compounds against Hep-G2, KB, HL-60 and Bel-7402 cell lines were determined using the MTT-assay with camptothecin as the positive control (Table 4). The isolates **1**—**7** showed moderate cytotoxic activity on the tested cell lines, except for **5**. As far as we know, this is the first time to report the cytotoxic effects of **1**—**7**.

Experimental

General Procedure The CD spectra were recorded on a J-715 (JASCO) spectropolarimeter. Optical rotations were performed on a Perkin-Elmer digital polarimeter. Melting points were measured on a Fisher-Johns apparatus and were uncorrected. IR spectra were run on a Shimadzu FTIR 8400 infrared spectrometer recorded as KBr patches. UV spectra were measured on Perkin-Elmer Lambda 35 UV/VIS spectrometer. NMR spectra were measured on a Bruker AV 400 spectrometer with TMS as an internal standard. EI-MS spectra were obtained using a Micromass ZabSpec high-resolution mass spectrometer. Silica gel 60H (400—500 mesh) and silica gel GF_{254} sheets (0.20—0.25 mm) (both from Qingdao Haiyang Chemical Group Co., Shandong Province, People's Republic of China) were used for column chromatography and TLC, respectively.

Plant Material *Schisandra propinqua* (Wall.) Baill (Schisandraceae) was collected at Shennongjia, Hubei Province, People's Republic of China, in July 2003, and identified by Prof. Si-bao Chen, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (W-06) has been deposited in the Herbarium of this institute.

Extraction and Isolation The dried stems of *Schisandra propinqua* (Wall.) Baill (10 kg) were pulverized and extracted with 95% EtOH under reflux (2 h×3). The ethanol extract was concentrated *in vacuo* to yield a residue (870 g), 800 g of which was suspended in water and successively partitioned with petroleum ether (4×2 l), $CHCl_3$ (5×2 l), EtOAc (4×2 l) and *n*-BuOH (3×2 l). The solvents were evaporated under vacuum to afford a petroleum ether fraction (121 g), a $CHCl_3$ fraction (63 g), an EtOAc fraction (89 g) and an *n*-BuOH fraction (352 g). The petroleum ether part (121 g) was chromatographed on silica gel using a gradient system of petroleum ether–acetone (20 : 1, 10 : 1, 4 : 1, 1 : 1, acetone) yielding 10 pooled fractions: P1—10. P2 was purified by Sephadex LH-20 column to give **7** (872 mg). P3 (2.4 g) was purified by Sephadex LH-20 column, eluted with $CHCl_3$–MeOH to give **1** (9 mg) and **8** (932 mg). P6 (3.2 g) was subjected to silica gel column chromatography, eluted with cyclohexane–acetone (97 : 3) to obtain 38 fractions: P6-1—38. **2** (79 mg) was obtained from P6-8—12 and **3** (55 mg) was obtained from P6-32—36. P8 (6.2 g) was chromatographed on silica gel-H, eluted with petroleum ether–EtOAc (92 : 8) to afford 20 fractions: P8-1—20. Fraction P8-5—7 (1.1 g) was then purified by sephadex LH-20 column to give **5** (682 mg). Fraction P8-16—18 (2.2 g) was subjected to silica gel-H, eluted with *n*-hexane–aceton (95 : 5) to afford 35 fractions P8-16-18-1—35, **4** (341 mg) was obtained from P8-16-18-12—17 and **6** (22 mg) was obtained from P8-16-18-29—33.

Schisanterpene B (**1**): Colorless needles, mp: 193—194 °C; $[\alpha]_D^{20}$: +20 °C (c=0.1, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3600, 1708, 1760. HR-EI-MS *m*/*z*: 428.2927 (Calcd 428.2938 for $C_{27}H_{40}O_4$). EI-MS: 428 [M^+], 410, 395, 324, 99. ^{1}H- and ^{13}C-NMR: see Table 1.

Propinquanin E (**2**): Colorless needles, mp: 110—112 °C; $[\alpha]_D^{20}$: +34°C (c=0.22, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3487, 2939, 1708, 1589, 1458; UV (EtOH): λ_{max} (log ε)=219 (4.05), 255 (sh 2.87), 283 (sh 3.02); CD (MeOH) $\Delta\varepsilon$ nm: +12.28 (217), −4.91 (255); HR-EI-MS *m*/*z*: 584.2249 (Calcd 584.2258 for $C_{31}H_{36}O_{11}$). EI-MS: 584 [M^+], 522, 484, 384, 341, 301, 105, 83. ^{1}H- and ^{13}C-NMR: see Tables 2 and 3.

Propinquanin F (**4**): Colorless needles, mp: 109 °C; $[\alpha]_D^{20}$: +8.5° (c=1.48, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3386, 3136, 2947, 1701, 1593, 1504; UV (EtOH): λ_{max} (log ε)=214 (3.97), 257 (sh 3.01), 282 (sh 3.11); CD (MeOH) $\Delta\varepsilon$ nm: +8.23 (218), −3.01 (253); HR-EI-MS *m*/*z*: 500.2394 (Calcd 500.2410 for $C_{28}H_{36}O_8$). EI-MS: 500 [M^+], 482, 400, 369, 83. ^{1}H- and ^{13}C-NMR: see Tables 2 and 3.

Cytotoxicity Assay The cytotoxicity test of the compounds was performed according to a slight modification of the procedure reported previously.[16)] All the cells were seeded at a density of 2×10^3 cells/0.1 ml/well onto 96-well tissue culture plates (Falcon). All compounds (**1**—**7**) dissolved in dimethyl sulfoxide (DMSO) were dispensed to the cultures at designed concentrations. DMSO was added to each culture as the solvent control. The final concentration of DMSO did not exceed 0.2% in any experiment. After 48 h of treatment, the cells were reacted with MTT (Sigma Cat. M2128). The reaction product, formazan, was extracted with DMSO, and the absorbance was read at 540 nm. The IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared to untreated cells in the MTT assay. Data represent the mean values and standard deviations of triplicate assays in at least one experiment.

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