Juglanones A and B: Two Novel Tetralone Dimers from Walnut Pericarp (*Juglans regia*)

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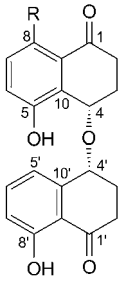
Two novel tetralone dimers, with an O-bridge, named juglanones A and B (**1** and **2**, resp.), were isolated from the AcOEt extract of walnut pericarps. These compounds are the first examples of O-bridged dimeric tetralones. Their structures were determined by spectroscopic methods, including HR-TOF-MS, and 1D- and 2D-NMR. Biological evaluation of these two isolates against a number of human cancer cell lines is also described.

Introduction. – The genus *Juglans* (Juglandaceae), which comprises *ca.* 20 species, is widely distributed in temperate and subtropical areas [1]. The fresh pericarp of some species, *e.g.*, *J. mandshurica* and *J. regia*, known locally as '*Qing Long Yi*', have been used in China, Japan, and Korea due to their purported anticancer and antioxidant properties, and for treating pain and inflammation [2]. The roots and leaves of *Juglans* plants are also used as folk medicine for the treatment of cancer, rheumatic pains, and eczema [3][4]. Diverse classes of secondary metabolites, including naphthoquinones, flavonoids, diarylheptanoids, phenolic acids, tetralones, and terpenoids, are reportedly responsible for the biological activities associated with this genus [5–8]. A series of tetralones and derivatives have been isolated from members of the *Juglans* genus, and several are considered important chemotaxonomic indicators. Members of this group of natural products are of particular interest because of their cytotoxic and antitumor activities. In an ongoing project to search for new antitumor agents from natural products, two new tetralone dimers were isolated from '*Qing Long Yi*'. The characterization of the new compounds and their cytotoxic effects against seven human cancer cell lines (A549, MCF-7, BEL-7402, HeLa, COLO205, BGC-823, and SK-OV-3) are reported in this study.

Results and Discussion. – Juglanone A (**1**) was obtained as a brown amorphous powder. Its HR-TOF mass spectrum exhibited a quasimolecular-ion peak at *m*/*z* 339.1243 ($[M+H]^+$; calc. 339.1232), indicating the molecular formula $C_{20}H_{18}O_5$ that corresponds to twelve C=C-bond equivalents. The UV spectrum of **1** (λ_{max} (log ε): 220 (2.43), 259 (2.74), 325 (3.19)) indicated an acetophenone type chromophore [9]. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed a set of signals due to one chelated phenolic H-atom (δ(H) 12.36 (*s*, 1 H)), two CH–O (δ(H) 5.20 (br. *s*, 1 H) and 4.84 (*dd*, $J = 7.8$,

3.6, 1 H)) and two CH_2 groups (δ(H) 2.45–2.47 (*m*, 2 H) and 2.12–2.22 (*m*, 2 H)), and four non-equivalent CH_2 H-atom *multiplet*s (δ(H) 2.81–2.97 (*m*, 1 H), 2.43–2.45 (*m*, 1 H), 2.69 (*ddd*, $J = 17.4, 8.4, 4.2$, 1 H), and 2.83 (*ddd*, $J = 17.4, 7.8, 4.2$, 1 H)). Moreover, the ^{13}C-NMR spectrum, in the HSQC experiments, showed signals for two C=O C-atoms (δ(C) 205.5 and 198.1), four CH_2 C-atoms (δ(C) 28.1, 28.9, 32.9, and 35.2), and two CH–O C-atoms (δ(C) 72.8 and 66.1). These data indicated that **1** is an α-tetralone dimer [10]. Subsequently, the ^{1}H-NMR spectrum showed two sets of *ABC*-type aromatic H-atom signals (δ(H) 6.83 (*dd*, $J = 8.4$, < 1.0, 1 H), 6.87 (*dd*, $J = 7.8$, < 1.0, 1 H), 7.46 (*dd*, $J = 8.4, 7.8$, 1 H); 7.13 (*dd*, $J = 7.8$, < 1.0, 1 H), 7.30 (*dd*, $J = 7.8, 7.8$, 1 H), 7.36 (*dd*, $J = 7.2$, < 1.0, 1 H)), which indicated the presence of a OH group on the aromatic ring. The position of one OH group on the aromatic ring was assigned as C(5) based on the HMBC between δ(H) 5.20 (H–C(4)) and δ(C) 155.28 (C(5)). The position of the other OH group was deduced as C(8′) based on the downfield-shifted H-atom signal at δ(H) 12.36. The connection of the two substructures from C(4) to C(4′) *via* an O-bridge was evidenced by the correlation between δ(H) 5.20 (H–C(4)) and δ(C) 72.8 (C(4′)), and further confirmed by the cross-peaks between δ(H) 4.84 (H–C(4′)) and δ(C) 66.1 (C(4′)) in the HMBC experiments.

The absolute configuration of **1** was deduced from CD spectrum. The CD *Cotton* effect of 4-hydroxy-α-tetralones is strongly dominated by the conformation of the cyclohexanone ring, and the sign of the *Cotton* effect in the $\pi \rightarrow \pi^*$ region may be attributed to the axial chirality effect of axial H (and/or quasi-axial) or a substituent in the α-position, adjacent to the C=O group [11][12]. The CD spectrum of **1** showed a strong positive *Cotton* effect at 222 nm, a medium negative one at 262 nm, and another broad weak negative band at 326 nm, which was confirmed by comparison of the CD spectral data and optical rotation with those of (4*S*)- and (4*R*)-configured isosclerone, sclerone, and analogous compounds [13][14]. Consequently, the absolute configurations at C(4) and C(4′) in **1** were assigned as (*S*). Therefore, the structure of **1**, based on the aforementioned evidence, was elucidated as shown in the *Figure*.



1 R = H
2 R = OH

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

Juglanone B (**2**) was obtained as a brown amorphous powder. Its molecular formula was established as $C_{20}H_{18}O_6$ from its quasimolecular-ion peak in its HR-TOF mass spectrum (*m*/*z* 353.1027 ($[M - H]^-$; calc. 353.1025)). The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table 1*) indicated that it was also an α-tetralone dimer. In the ^{1}H-NMR spectrum of **2** compared with that of **1**, the H-atom signals due to an aromatic ring was changed from an *ABX*-type to an *AB*-type at δ(H) 7.15 (*d*, $J = 9.2$, 1 H) and 6.81 (*d*, $J = 8.8$,

Table 1. *^{1}H- and ^{13}C-NMR* ((D$_6$)DMSO) *Data of* **1** and **2**. Atom numbering as indicated in the *Figure*; δ in ppm, J in Hz.

Position	**1**			**2**		
	δ(H)[a])	δ(C)	HMBC	δ(H)[b])	δ(C)	HMBC
1		198.1			205.9	
2	2.81–2.97 (*m*), 2.43–2.45 (*m*)	32.9	3, 10	2.89–2.95 (*m*), 2.48–2.50 (*m*)	33.0	4, 2′
3	2.45–2.47 (*m*)	28.1	1, 4	2.24–2.26 (*m*)	27.2	3, 4
4	5.20 (br. *s*)	66.1	2, 4′, 5, 9, 10	5.13 (br. *s*)	65.3	4′
5		155.8			147.1	
6	7.13 (*dd*, J = 7.8, < 1.0)	120.8	4, 5, 8, 10	7.15 (*d*, J = 9.2)	126.0	5, 8, 10
7	7.30 (*dd*, J = 7.8, 7.8)	129.7	1, 5, 6, 9	6.81 (*d*, J = 8.8)	118.2	5, 8, 9
8	7.36 (*dd*, J = 7.2, < 1.0)	117.3	1, 4, 5, 6, 10		155.0	
9		133.4			115.9	
10		129.2			127.1	
1′		205.5			205.8	
2′	2.69 (*ddd*, J = 17.4, 8.4, 4.2), 2.83 (*ddd*, J = 17.4, 7.8, 4.2)	35.2	1′, 3′, 4′, 9′	2.96–2.98 (*m*), 2.58–2.60 (*m*)	34.1	3′
3′	2.12–2.22 (*m*)	28.9	1′, 2′, 4′, 10′	2.32–2.40 (*m*), 2.02–2.04 (*m*)	26.0	
4′	4.84 (*dd*, J = 7.8, 3.6)	72.8	2′, 3′, 4, 6′, 7′	4.89 (*t*, J = 4.0)	71.7	4, 2′, 5′, 9′, 10′
5′	6.83 (*dd*, J = 8.4, < 1.0)	116.9	1′, 7′, 8′, 9′, 10′	7.09 (*dd*, J = 7.2, < 1.0)	119.2	7′, 9′
6′	7.46 (*dd*, J = 8.4, 7.8)	137.0	7′, 8′, 10′	7.57 (*dd*, J = 8.0, 8.0)	137.5	8′, 9′, 10′
7′	6.87 (*dd*, J = 7.8, < 1.0)	118.4	1′, 5′, 6′, 8′, 9′	6.90 (*dd*, J = 8.4, < 1.0)	117.4	5′, 8′, 9′
8′		162.0			162.0	
9′		115.8			115.8	
10′		145.9			145.0	
HO–C(8′)	12.36 (*s*)		6′, 8′, 9′	12.36 (*s*)		7′, 8′, 9′
HO–C(8)				11.85 (*s*)		7, 8, 9

[a]) Recorded at 600 MHz. [b]) Recorded at 400 MHz.

1 H). Further analysis of its HMBC suggested that one subunit of **2** is 5,8-dihydroxy-α-tetralone, as shown in the *Figure*. The two constructed tetralone units in **1** are connected at C(4) and C(4′) through an O-bridge due to the HMBC of H–C(4) to C(4′) and of H–C(4′) to C(4). The CD behavior and optical rotation of **2** were similar to those of **1**, which permitted us to assign the absolute configuration at C(4) and C(4′). Thus, the structure of **2** was determined as shown in the *Figure*.

Isolates **1** and **2** were evaluated for their *in vitro* cytotoxicities against seven human cancer cell lines (A549, MCF-7, BEL-7402, HeLa, COLO205, BGC-823, and SK-OV-3). Juglanone B (**2**) exhibited weak cytotoxicity against the human breast cancer cell line MCF-7 (inhibition percentage, 66.1 ± 0.05%) and the human gastric cancerous cell line BGC-823 (55.87 ± 6.28%). Both juglanones A and B (**1** and **2**, resp.) weakly inhibited the other cell lines (0.22 to 47.4%) at concentrations as high as 100 μM (*cf. Table 2*).

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Table 2. *Inhibition Rate of Compounds* **1** *and* **2** *against Cell Lines Listed.* Final concentration [M], inhibition rate [%].

	1[a])		**2**[a])		Adriamycin[a])[b])
	Final concentration	Inhibition rate	Final concentration	Inhibition rate	IC_{50} [μM]
A549	1.00×10^{-3}	86.40 ± 0.57	1.00×10^{-3}	84.74 ± 0.01	0.66
	1.00×10^{-4}	1.57 ± 3.05	1.00×10^{-4}	24.52 ± 0.59	
	1.00×10^{-5}	0	1.00×10^{-5}	0	
MCF-7	1.00×10^{-3}	94.16 ± 0.37	1.00×10^{-3}	81.16 ± 0.40	0.45
	1.00×10^{-4}	2.35 ± 0.68	1.00×10^{-4}	66.10 ± 0.05	
	1.00×10^{-5}	0	1.00×10^{-5}	0	
BEL-7402	1.00×10^{-3}	80.82 ± 2.08	1.00×10^{-3}	64.84 ± 4.50	0.26
	1.00×10^{-4}	8.87 ± 3.30	1.00×10^{-4}	35.78 ± 2.93	
	1.00×10^{-5}	4.44 ± 3.40	1.00×10^{-5}	11.53 ± 3.87	
HeLa	1.00×10^{-3}	77.46 ± 14.96	1.00×10^{-3}	92.62 ± 2.00	0.66
	1.00×10^{-4}	0	1.00×10^{-4}	47.40 ± 13.57	
	1.00×10^{-5}	0	1.00×10^{-5}	0	
COLO205	1.00×10^{-3}	74.76 ± 14.39	1.00×10^{-3}	86.29 ± 2.29	1.67
	1.00×10^{-4}	14.98 ± 10.21	1.00×10^{-4}	0	
	1.00×10^{-5}	0	1.00×10^{-5}	0	
BGC-823	1.00×10^{-3}	83.32 ± 14.66	1.00×10^{-3}	88.96 ± 7.57	0.58
	1.00×10^{-4}	27.78 ± 5.37	1.00×10^{-4}	55.87 ± 6.28	
	1.00×10^{-5}	5.31 ± 4.45	1.00×10^{-5}	15.90 ± 2.49	
SK-OV-3	1.00×10^{-3}	62.38 ± 25.62	1.00×10^{-3}	75.64 ± 20.18	0.62
	1.00×10^{-4}	0.77 ± 0.25	1.00×10^{-4}	22.10 ± 18.71	
	1.00×10^{-5}	0	1.00×10^{-5}	0	

[a]) Samples dissolved in DMSO. [b]) Adriamycin used as positive control.

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; 100–200 and 200–300 mesh; *Qingdao Marine Chemical Ltd.*); *Sephadex LH-20*, *Toyopearl HW-40F* gel (*TOSOH Co., Ltd.*); and *MCI* gel *CHP-20P* (*Mitisubishi Kasei Industry Co., Ltd.*). TLC: Precoated plates of SiO_2 GF_{254}; detection under UV light and spraying with anylsaldehyde/H_2SO_4 in EtOH or H_2O/H_2SO_4. Optical rotation: *WZZ-3* polarimeter (*Shanghai Shenguang Co., Ltd.*) at 25°. UV Spectra: *UV-2100 UV* spectrophotometer (*RAYLEIGH Co., Ltd.*) in MeOH; λ_{max} (log ε) in nm. CD Spectrum: *Chirascan*™ CD spectroscopy (*Applied Photophysics Ltd.*) in MeOH at 25°; λ_{max} ($\Delta\varepsilon$) in nm. ^{1}H-, ^{13}C-, and 2D-NMR: *Bruker AV II-400 and AV II-600* spectrometers; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-TOF-MS: quadrupole time of flight (Q-TOF) premier spectrometer coupled with an ESI source (*Micromass Co., Ltd.*); in m/z.

Plant Material. Walnut pericarps (*Juglans regia*) were purchased in November 2009 from a local herbal-medicine store (Chengdu, Sichuan Province, China) and identified by *Y.-F. L.* (Sichuan

University). A voucher specimen (D201002) was deposited with the Department of Pharmaceutics and Bioengineering, Sichuan University, Chengdu, China.

Extraction and Isolation. Dried and powdered walnut pericarps (5.4 kg) were extracted three times with 95% EtOH (15 l) at r.t. for 3 d. The solvents were pooled and evaporated under reduced pressure to give the crude EtOH extract (126.5 g), which was redissolved in H_2O and partitioned with petroleum ether (1 l), AcOEt (1 l), and BuOH (1 l) to obtain 36, 37, and 30 g of extract, resp. The AcOEt-soluble extract (37 g) was subjected to CC (SiO_2; (petroleum ether/AcOEt 5 : 1 → 0 : 1): *Frs. A – L*. *Fr. C* (4.32 g) was purified by CC (*MCI* column, acetone/H_2O 3 : 7 → 9 : 1): *Frs. C1 – C6*. *Fr. C6* (256 mg) was separated by CC (*Sephadex LH-20* CC; MeOH; and *Toyopearl HW-40*; MeOH) to afford **2** (4.9 mg) and **1** (4.8 mg).

Juglanone A (=*(4*S*)-3,4-Dihydro-5-hydroxy-4-{[(1*R*)-1,2,3,4-tetrahydro-5-hydroxy-4-oxonaphthalen-1-yl]oxy}naphthalen-1(2*H*)-one*; **1**). Brown amorphous powder. $[\alpha]_D^{20} = +74.2$ ($c = 0.6$, MeOH). UV: 220 (2.43), 259 (2.74), 325 (3.19). CD ($c = 0.004$, MeOH): 262 (−1.56), 240 (+0.57), 222 (+2.32). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-TOF-MS: 339.1243 ($[M+H]^+$, $C_{20}H_{19}O_5^+$; calc. 339.1232).

Juglanone B (=*(4*S*)-3,4-Dihydro-5,8-dihydroxy-4-{[(1*R*)-1,2,3,4-tetrahydro-5-hydroxy-4-oxonaphthalen-1-yl]oxy}naphthalen-1(2*H*)-one*; **2**). Brown amorphous powder. $[\alpha]_D^{20} = +35.6$ ($c = 0.61$, MeOH). UV: 218 (3.08), 259 (2.35), 325 (1.96). CD ($c = 0.003$, MeOH): 269 (−8.16), 215 (+19.58). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-TOF-MS: 353.1027 ($[M-H]^-$, $C_{20}H_{17}O_6^-$; calc. 353.1025).

Cytotoxicity Assay. The cytotoxic activities of **1** and **2** against the human cancer cell lines A-549 (lung adenocarcinoma cancer), MCF-7 (breast cancer), BEL-7402 (liver cancer), HeLa (cervical cancer), COLO205 (colon adenocarcinoma), BGC-823 (gastric cancer), and SK-OV-3 (ovarian carcinomas) were determined. The cytotoxicity assay was performed according to the sulforhodamine B (SRB) assay in 96-well microplates as described in [15], and expressed as IC_{50} values (50% inhibition of cell proliferation, mg/ml), which was calculated by GraphPad Prism. The OD value was read on a *SYNERY™ 4* multi-mode microplate reader (*BioTek Instrument Inc.*, USA) at 570 nm.

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