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Two new tetralone glycosides from the green walnut husks of *Juglans mandshurica* Maxim

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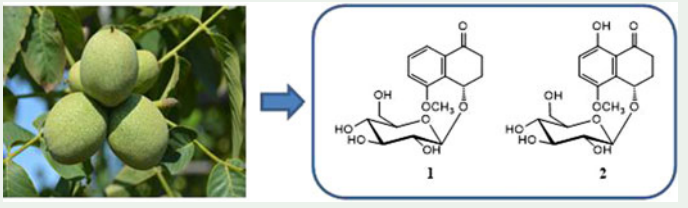
ABSTRACT

Two new tetralone glycosides, 4(*S*)-5-methoxy juglanoside A (**1**), 4(*S*)-5-methoxy juglanoside D (**2**), together with ten known compounds (**3**–**12**) have been isolated from the green walnut husks of *Juglans mandshurica* Maxim. Their structures were elucidated on the basis of their ESI-MS, 1 D and 2 D NMR spectroscopic data. In addition, all compounds were evaluated for their cytotoxic activities against the cancer BGC-823 (human gastric carcinoma), HCT-15 (human colorectal carcinoma) and K562 (human chronic myeloid leukemia) cell lines. The results showed aglycones of naphthoquinones had stronger cytotoxic activities than glycosides of tetralone.



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Juglans mandshurica Maxim; tetralone glycosides; cytotoxicity

1. Introduction

Juglans mandshurica Maxim belongs to the family of Juglandaceae, which is one of the deciduous trees widely distributed in China and other Asia (Wu and Raven 1999). As a kind of traditional Chinese medicine, the green walnut husks of *Juglans mandshurica* Maxim (Chen et al. 2013; Gawlik-Dziki et al. 2014; Nasiry et al. 2017) have dramatic effects on analgesia (Erdemoglu et al. 2003) and antitumor (Xin et al. 2014; Wang et al. 2015; Delaviz et al. 2017). In addition, modern pharmacological studies have demonstrated that this medicinal part has also antibacterial and antioxidant effect (Oliveira et al. 2008; Carvalho et al. 2010; Sun et al. 2017). In previous investigations, a number of naphthoquinones (Chen et al. 2015), triterpenoids (Zhou et al.

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2015) and other compounds (Yao et al. 2014; Zhou et al. 2017) have been isolated from the green walnut husks of *Juglans mandshurica* Maxim. In this study, two new tetralone glycosides together with ten known compounds have been obtained. We mainly reported the structural elucidation of them as well as the cytotoxic activities against three human cancer cell lines.

2. Results and discussion

2.1. Chemistry

Compound **1** was obtained as a red amorphous powder with the molecular formula $C_{17}H_{22}O_8$, as determined by HR-ESI-MS at *m/z* 377.3248 ($[M+Na]^+$, calcd for $C_{17}H_{22}O_8Na$, 377.3283). The ^{1}H-NMR spectrum of **1** indicated a set of proton signals of two methylene protons in the high field at δ_H 2.48 (1 H, ddd, $J = 17.7$, 4.1, 2.2 Hz, H-2a), 3.11-3.20 (1 H, m, H-2b); 2.15 (1 H, tdd, $J = 14.0$, 4.4, 2.6 Hz, H-3a), 2.58 (1 H, ddt, $J = 14.0$, 4.4, 2.6 Hz, H-3b) and a methine proton at δ_H 5.47 (1 H, t, $J = 2.8$ Hz, H-4). DEPT and HSQC experiments showed signals of two methylene carbons at δ_C 30.3 (C-3), 33.8 (C-2), a tertiary carbon with hydroxyl group at δ_C 68.3 (C-4) and a carbonyl carbon at δ_C 200.9 (C-1). All above data further revealed that **1** had an α-tetralone framework, similar to the reference (Liu et al. 2004). Meanwhile, the ^{1}H-NMR spectrum of **1** showed three mutually ortho-coupled aromatic protons at δ_H 7.57 (1 H, dd, $J = 8.0$, 0.9 Hz, H-8), 7.44 (1 H, t, $J = 8.0$ Hz, H-7) and 7.28 (1 H, dd, $J = 8.0$, 0.9 Hz, H-6) in the downfield, which were ascribed to an ABC-type aromatic ring substituted with a methoxy group. The substituent position of the methoxy group was deduced to be C-5 by observation of the HMBC correlations between δ_H 3.91 (3 H, s, H-11) and δ_C 159.0 (C-5) (Figure S6). Moreover, a glucose moiety was showed with the signals of δ_H 4.47 (1 H, d, $J = 7.8$ Hz, H-1′), 3.30 (1 H, m, H-5′), 3.72 (1 H, dd, $J = 4.0$, 12.0 Hz, H-6′a), 3.91 (1 H, m, H-6′b), and δ_C 103.8 (C-1′), 78.1 (C-5′), 62.9 (C-6′). The conjunctive position of sugar to aglycone was located at C-4, as established by HMBC correlations between δ_H 4.47 (1 H, d, $J = 7.8$ Hz, H-1′) and δ_C 68.3. The coupling constants δ_H 4.47 of the anomeric protons suggested that sugar unit was β-configuration. The absolute configuration of the glucose was determined as D-form by acid hydrolysis. Furthermore, the absolute configuration of the chiral center at C-4 of the aglycone was measured by CD (Figure S14) spectrum. It was identified to be *S* configuration where a positive Cotton effect at 240 nm and a negative Cotton effect at 352 nm were observed. These NMR data were almost similar to those of juglanoside A (Liu et al. 2004). Therefore, the structure of **1** was determined to be 4(*S*)-4-hydroxy-5-methoxy-α-tetralone-4-*O*-β-D-glucopyranoside, named 4(*S*)-5-methoxy juglanoside A. The structure was elucidated as shown (Figure 1).

Compound **2** was obtained as a red amorphous powder, which was assigned the molecular formula to be $C_{17}H_{22}O_9$ according to HR-ESI-MS data at *m/z* 393.1162 $[M+Na]^+$ (calcd for $C_{17}H_{22}O_9Na$, 393.1204). The general features of its NMR spectra closely resembled those of **1**, indicating that **2** was a derivative of **1**. There was the minor difference between **1** and **2** was that an additional hydroxy group at C-8 (δ_C 157.5) appeared in **2**. Thus, the ^{1}H-NMR spectrum showed the AB-type aromatic proton signals at δ_H 7.31 (1 H, d, $J = 9.2$ Hz, H-6) and 6.91 (1 H, d, $J = 9.2$ Hz, H-7)

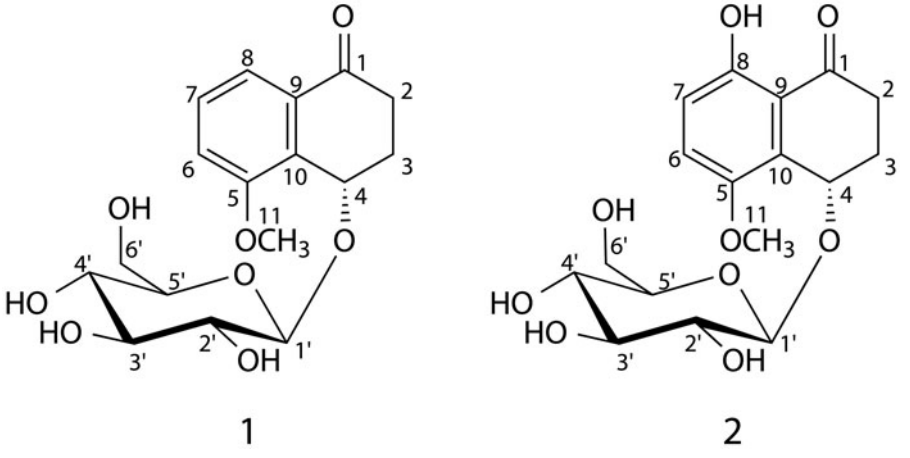

Figure 1. Structures of new compounds **1** and **2**.

in place of ABC-type in the aromatic ring. There were a group of β-D-glucopyranose carbon signals observed at δ_C 103.1 (C-1′), 75.1 (C-2′), 78.1 (C-3′), 71.8 (C-4′), 78.1 (C-5′) and 62.9 (C-6′) in the DEPT and ^{13}C NMR spectrums. The glucopyranosyl unit was also assigned to C-4 by the key HMBC correlation between δ_H 4.42 (1 H, d, $J = 7.8$ Hz, H-1′) and δ_C 67.8 (C-4) (Figure S12). What's more, the absolute configuration of the chiral center at C-4 of the aglycone was identified to be *S* configuration from the CD (Figure S14) spectrum with a positive Cotton effect at 249 nm and a negative Cotton effect at 383 nm. In addition to C-5 as methoxy, the spectroscopic data of **2** were nearly similar to those of juglanoside D (Liu et al. 2004). Thus, the structure of **2** was elucidated to be 4(*S*)-4,8-dihydroxy-5-methoxy-α-tetralone-4-*O*-β-D-glucopyranoside, and named as 4(*S*)-5-methoxy juglanoside D. The structure of **2** was shown in Figure 1.

By comparing the spectral data in the literature, the remaining ten known compounds were respectively identified as 4(*S*)-4-hydroxy-α-tetralone-4-*O*-β-D-glucopyranoside (**3**) (Zhou et al. 2015), 4(*S*)-4,5-dihydroxy-α-tetralone-4-*O*-β-D-glucopyranoside (**4**) (Zhou et al. 2015), 1,4,8-trihydroxy-3-naphthalenecarboxylic acid-1-*O*-β-D-glucopyranoside ethyl ester (**5**) (Zhou et al. 2015), 4(*S*)-4,5,8-trihydroxy-α-tetralone-5-*O*-β-D-[6′-O-(3",4",5"-trihydroxybenzoyl)] glucopyranoside (**6**) (Zhou et al. 2015), juglone (**7**) (Luo et al. 2012), 3-ethoxy juglone (**8**) (Zhou et al. 2015), 2-hydroxy-1,4-naphthoquinone (**9**) (Dong et al. 2011), 2,5-dihydroxy-1,4-naphthoquinone (**10**) (Dong et al. 2011), 5,8-dihydroxy-1,4-naphthoquinone (**11**) (Zhou et al. 2015) and 3,5-dihydroxy-1,4-naphthoquinone (**12**) (Zhou et al. 2015).

2.2. Anti-tumour activity in vitro

All compounds (**1**–**12**) were separated then tested for cytotoxicity against BGC-823 cells, HCT-15 cells and K562 cells by methyl thiazolyl tetrazolium (MTT) assay in *vitro*. BGC-823 cells were shown obvious inhibition by compounds **7**, **8**, **10**–**12**. Compounds (**7**–**9**) were potently against HCT-15 cells. While K562 cells were inhibited by compounds **7** and **10**. However, other compounds including two new naphthoquinones were inactive. All results were shown in Table S3.

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained with a JASCO P2000 digital polarimeter (Jasco, Tokyo, Japan). High-resolution electrospray ionization (HR-ESI) mass spectra were obtained on a micromass LCT spectrometer (Waters, Milford, USA). Circular dichroism spectra were taken on an Applied Photophysics Spectropolarimeter (Agilent, USA). The NMR spectra were recorded on Bruker DPX 400 spectrometer (Bruker, Rheinstetten, Germany) operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C, using TMS as an internal standard. GC was performed on Agilent 7890 A Gas Chromatograph System (Agilent Technologies, Santa Clara, CA, USA). Thin-layer chromatography (TLC) was carried out on precoated silica gel GF_{254} (Qingdao Haiyang Chemical Co. Ltd., Qingdao, China). Column chromatography (CC) was performed using 200–300 mesh Si gel (HaiYang Co. Ltd, Qingdao, China). Octadecylsilanized (ODS) silica gel (YMC Ltd., Kyoto, Japan) and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden) were used for open column chromatography. HPLC chromatograms were obtained with an Agilent Technologies 1260 infinity HPLC system (Agilent Technologies, Germany) and semi-preparative HPLC (Waters, 515–2414, Milford, USA) was performed using a Hypersil-ODS II column (300 × 20 mm i.d., 10 μm, Ylite, Dalian, China).

3.2. Plant material

The green walnut husks of *Juglans Mandshurica* Maxim were collected from Changbai Mountain (Jilin, China), which were identified by Prof. B.Y. Yang. The specimen (No.20170902) has been deposited in College of Pharmacy, Heilongjiang University of Chinese Medicine.

3.3. Extraction and isolation

The air-dry green walnut husks of *Juglans mandshurica* Maxim (5.0 kg) were powdered and extracted with 95% EtOH (40 L × 7d × 3) at room temperature. After vacuum concentration, we got 95% EtOH extract (330.6 g). Then, the residue was scattered in water and extracted successively by CH_2Cl_2, EtOAc and *n*-BuOH to get these three parts. The CH_2Cl_2 part (52.2 g) was subjected to silica gel column chromatography (CC) with a stepwise gradient of PE-EtOAc (40:1-1:1, *v/v*) to get ten fractions (Fr.C1-C10). Compound **7** (61.2 mg) was obtained from the fraction C1 (3.5 g) by recrystallization from dichloromethane. From the fraction C4 (5.5 g), compounds **8** (10.8 mg) and **9** (9.6 mg) were isolated by using CC with PE-EtOAc (40:1-1:1, *v/v*). Fraction C5 (69.0 mg) was subjected to CC with PE-EtOAc (20:1-3:1, *v/v*) to afford compounds **10** (8.4 mg), **11** (5.0 mg) and **12** (7.2 mg). The *n*-BuOH part (65.1 g) was subjected to CC using CH_2Cl_2-MeOH (15:1-0:1, *v/v*) to purified eight fractions (Fr.B1-B8). Fraction B1 (13.5 g) was performed repeatedly over the CC with CH_2Cl_2-MeOH (10:1-0:1, *v/v*) and the Sephadex LH-20 chromatography eluting with MeOH-H_2O (40:60, *v/v*) to get compounds **3** (4.3 mg), **1** (6.2 mg) and **4** (7.1 mg). Fraction B2 (3.7 g) was subjected to CC with CH_2Cl_2-MeOH (8:1-0:1, *v/v*) repeatedly to give compound **2** (4.8 mg). Fraction B5 (6.4 g)

was purified by the CC with CH_2Cl_2-MeOH (6:1-0:1, *v/v*) to get six fractions (Fr.B5a-Fr.B5f), and then semi-preparative HPLC chromatography (MeOH-H_2O 70:30, *v/v*, 3.0 mL/min) to give **6** (4.2 mg, $t_R = 26.0$ min) and **5** (3.8 mg, $t_R = 32.0$ min) from fraction B5b (312.2 mg).

3.3.1. 4(S)-5-methoxy juglanoside A (1)

Red powder; $[\alpha]_D^{25}$-56 (c 0.60, MeOH); CD (c 1.31×10^{-5}, MeOH) $\triangle\varepsilon$ (nm): +7.81 (240), −2.48 (352); ^{1}H and ^{13}C-NMR spectral data, see Table S1; HR-ESI-MS *m/z* 377.3248 $[M + Na]^+$ (calcd for $C_{17}H_{22}O_8Na$, 377.3283).

3.3.2. 4(S)-5-methoxy juglanoside D (2)

Red powder; $[\alpha]_D^{25}$-25 (c 0.80, MeOH); CD (c 1.08×10^{-5}, MeOH) $\triangle\varepsilon$ (nm): +8.38 (249), −2.10(383); ^{1}H and ^{13}C-NMR spectral data, see Table S2; HR-ESI-MS *m/z* 393.1162 $[M + Na]^+$ (calcd for $C_{17}H_{22}O_9Na$, 393.1204).

3.4. Acid hydrolysis of 1 and 2

Compounds **1** and **2** (each of 1.5 mg) were treated with 1.0 mol/L HCl (5 mL) and refluxed for 6 h at 90 °C. After cooling to room temperature, these two mixed solutions were extracted with CH_2Cl_2:H_2O (each 1:1, *v/v*, 1 mL) thrice. Then, each mixture was added $NaHCO_3$ to neutralize and concentrated to dryness. The monosaccharide of compounds **1** and **2** was examined by co-TLC [CH_2Cl_2-MeOH-HOAc-H_2O (25:10:2:2)]. After sprayed with 10% H_2SO_4 followed by heating, the sugars in compounds **1** and **2** were D-glucose by the same retention factor value (R_f) with authentic sample (R_f value of D-glucose was 0.24). At the same time, the residue was dissolved in anhydrous pyridine and then added 1.5 mg hydroxylamine hydrochloride The solution was stirred at 90 °C in water bath for 30 min. After cooling to room temperature, the mixture was added acetic anhydride (150 μL) and kept for another 30 min at 90 °C in water bath with stirring. After centrifuged off and filtered through 0.45 μm millipore filtration, the supernatant was ready subjected to GC analysis (Jia et al. 2017, Yang et al. 2018) under the following conditions: column temperature, 260 °C; injection temperature, 250 °C; flow rate, 1.0 mL/min; carrier gas, N_2. Comparing the retentions times (R_t) of standard D-glucose (R_t at 20.4 min), the absolute configuration of sugar of compounds **1** and **2** was determined as D-glucose.

3.5. Antitumor activity in vitro

Three human cancer cell lines BGC-823, HCT-15 and K562 (Institute of Biochemistry and Cell Biology, Shanghai, China) maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 5% fetal bovine serum (Gibco, New York, USA) and 1% antibiotic mixture comprising penicillin-streptomycin (Sigma, Missouri, USA) were grown in 96-well microtiter plates with a density of 4×10^4 cells/mL in an atmosphere containing at 37 °C with 5% CO_2. After 24 h, different concentrations of test samples (10 μL) and cisplatin which was as a positive control were added and cultivated in the above

environment for 48 h. Before the end of cultivation, MTT (10 μL, 5 mg/mL) was added into each well and continuously incubated for 4 h. Then, the DMSO (100 μL) was added to each well to suspended. And the absorbance at 490 nm of each well was detected and inhibition rate was counted on a multiscan microplate reader (Thermo Labsystems, Helsinki, Finland). The results were expressed as the 50% inhibitory concentration (IC_{50} value). Data was represented as mean ± SD of three independent experiments.

4. Conclusions

In summary, this work separated seven naphthoquinones and five tetralone from the green walnut husks of *Juglans mandshurica* Maxim including two new tetralone glycosides (compounds **1** and **2**) together with ten known compounds. All compounds were tested against BGC-823 cells, HCT-15 cells and K562 cells to survey their cytotoxic activities. Compounds **7**, **8**, **10**–**12** exhibited obvious anticancer activities against BGC-823 cells with the IC_{50} value ranges of 9.6 ± 2.35 to 33.8 ± 2.06 μM. Compounds **7**–**9** showed IC_{50} value of 27.8 ± 2.66 to 37.4 ± 5.10 μM on HCT-15 cells. In addition, compounds **7** and **10** inhibited significant toxicities with the IC_{50} value ranges of 35.5 ± 5.11 and 39.7 ± 1.21 μM on K562 cells. Obviously, aglycones of naphthoquinones had stronger cytotoxic activities than tetralone including aglycones and glycosides.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Carvalho M, Ferreira PJ, Mendes VS, Silva R, Pereira JA, Jerónimo C, Silva BM. 2010. Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. Food Chem Toxicol. 48(1):441–447. doi:10.1016/j.fct.2009.10.043

Chen CM, Han SJ, Yuan SS, Wang CJ, Yu JH. 2013. Isolation and characterization of 20 polymorphic microsatellite markers for *Juglans mandshurica* (Juglandaceae). Appl Plant Sci. 1(7):1200009.

Chen G, Pi XM, Yu CY. 2015. A new naphthalenone isolated from the green walnut husks of *Juglans mandshurica* Maxim. Nat Prod Res. 29(2):174–179.

Delaviz H, Mohammadi J, Ghalamfarsa G, Mohammadi B, Farhadi N. 2017. A review study on phytochemistry and pharmacology applications of *Juglans regia* Plant. Pharmacogn Rev. 11(22):145–152.

Dong M, Yuan R, Qi FQ, Li TL. 2011. Chemical constituents of pericarpium *Juglantis*. Nat Prod Res Dev. 23:805–808.

Erdemoglu N, Küpeli E, Yeşilada E. 2003. Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine. J Ethnopharmacol. 89(1): 123–129.

Gawlik-Dziki U, Durak A, Pecio L, Kowalska I. 2014. Nutraceutical potential of tinctures from fruits, green husks, and leaves of *Juglans regia* L. Sci World J. 2014:501392.

Jia W, Mao SM, Jin J, Yan GL, Li J, Liu YH. 2017. Study on monosaccharide components of polysaccharide from *Lonicera japonica* thumb by thin layer chromatography combined with gas chromatography. Shandong J Trad Chin Med. 36:326–328.

Liu LJ, Li W, Koike K, Zhang SJ, Nikaido T. 2004. New α-tetralonyl glucosides from the fruit of *Juglans mandshurica*. Chem Pharm Bull. 52(5):566–569.

Luo JJ, Yang B, Zeng Y, Li C. 2012. Chemical constituents from the flower of *Juglans regia*. J Chin Med Mater. 35:1614–1616.

Nasiry D, Khalatbary AR, Ahmadvand H, Talebpour Amiri F, Akbari E. 2017. Protective effects of methanolic extract of *Juglans regia* L. leaf on streptozotocin-induced diabetic peripheral neuropathy in rats. BMC Complement Altern Med. 17(1):476.

Oliveira I, Sousa A, Ferreira IC, Bento A, Estevinho L, Pereira JA. 2008. Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. Food Chem Toxicol. 46(7):2326–2331.

Sun ZL, Dong JL, Wu J. 2017. Juglanin induces apoptosis and autophagy in human breast cancer progression via ROS/JNK promotion. Biomed Pharmacother. 85:303–312.

Wang RJ, Wang S, Xia YJ, Tu MWLJ, Zhang LJ, Wang YM. 2015. Antitumor effects and immune regulation activities of a purified polysaccharide extracted from *Juglan regia*. Int J Biol Macromol. 72:771–775.

Wu ZY, Raven PH. 1999. Flora of China. Vol. 4. Peking: Science Press. 282.

Xin N, Hasan M, Li W, Li Y. 2014. *Juglans mandshurica* Maxim extracts exhibit antitumor activity on HeLa cells in vitro. Mol Med Rep. 9(4):1313–1318.

Yang BY, Chen ZL, Liu Y, Guo JT, Kuang HX. 2018. New lignan from the rattan stems of *Schisandra chinensis*. Nat Prod Res. 2018:1–7.

Yao D, Jin M, Zhang C, Luo J, Li R, Zheng M, Cui J, Li G. 2014. A new phenolic glycoside from *Juglans mandshurica*. Nat Prod Res. 28(13):998–1002.

Zhou YY, Liu YX, Jiang YA, Liu ZX, Niu F, Yang BY, Xiao HB, Zhao H. 2015. Chemical constituents from pericarp of *Juglans mandshurica* Maxim. Chin Trad Patent Med. 37:2669–2673.

Zhou YY, Liu QY, Yang BY, Jiang YQ, Liu YX, Wang Y, Guo S, Kuang HX. 2017. Two new cytotoxic glycosides isolated from the green walnut husks of *Juglans mandshurica* Maxim. Nat Prod Res. 31(11):1237–1244.

Zhou YY, Yang BY, Jiang YQ, Liu ZX, Liu YX, Wang XL, Kuang HX. 2015. Studies on cytotoxic activity against HepG-2 Cells of naphthoquinones from green walnut husks of *Juglans mandshurica* Maxim. Molecules. 20(9):15572–15588.

Zhou YY, Yang BY, Liu ZX, Jiang YQ, Liu YX, Fu L, Wang XL, Kuang HX. 2015. Cytotoxicity of triterpenes from green walnut husks of *Juglans mandshurica* Maxim in HepG-2 cancer cells. Molecules. 20(10):19252–19262.