New Naphthalenyl Glucosides from the Roots of *Juglans mandshurica*

Yoon-Ki Joe and Jong-Keun Son*

College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea

Sung-Hee Park and In-Ja Lee

College of Pharmacy, Hyosung Women's University, Hayang-goon, Kyongbuk 713-702, Korea

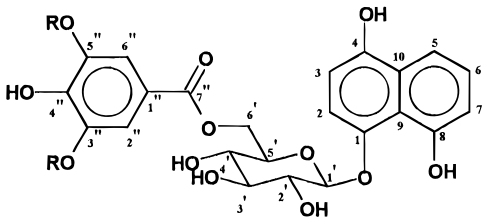
Dong-Chul Moon

College of Pharmacy, Chungbuk University, Chugju 360-763, Korea

Received May 1, 1995[⊗]

Two new gallic acid esters of 1,4,8-trihydroxynaphthalene glucoside were isolated from the roots of *Juglans mandshurica*, and their structures were elucidated on the basis of spectroscopic studies including 2D-NMR.

The roots of *Juglans mandshurica* Maximowicz (Juglandaceae) have been used as a folk medicine for treatment of cancer in Korea. Several naphthoquinones and naphthalenyl glucosides from *Juglans* species have been reported.[1−8] In the course of isolating possible cytotoxic compound(s) from the roots of this plant, we isolated 1,4,8-trihydroxynaphthalenyl 1-O-*β*-D-[6′-*O*-(3″,4″,5″-trihydroxybenzoyl)]glucopyranoside (**1**) and 1,4,8-trihydroxynaphthalenyl 1-*O*-*β*-D-[6′-*O*-(3″,5″-dimethoxy-4″-hydroxybenzoyl)]glucopyranoside (**2**). The structures of these compounds were determined by various NMR experiments including ^{1}H,^{1}H-homonuclear COSY, DEPT, HMQC, and HMBC.



1 R = H
2 R = CH_3

The MeOH extract of the root of *Juglans mandshurica* was partitioned between H_2O and hexane and the H_2O layer extracted with $CHCl_3$ followed by EtOAc. The EtOAc extract was chromatographed twice on Si gel column, which afforded **1** and **2**. In the positive FABMS spectrum of **1**, a M + 1 peak at *m*/*z* 491 (calcd for $C_{23}H_{22}O_{12}$, 490.111) and an intense fragment ion peak due to the galloyl part of **1** at *m*/*z* 153 were recognized. After acid hydrolysis of **1**, gallic acid and D-glucose were identified, separately, on TLC plates with reference samples. Noise-decoupled ^{13}C-NMR and DEPT spectra of **1** showed 21 carbon peaks (Table 1) including 11 methine, 9 quaternary, and 1 methylene carbon peaks. These spectra showed two chemically equivalent peaks (C2″ and C6″ at 110.0 ppm, C3″ and C5″ at 146.1 ppm) and one carbonyl peak (C1″ at 166.5 ppm) due to the galloyl group. Other characteristic peaks, due to the sugar part of **1**, include one anomeric (C1′ at 104.7 ppm), four aliphatic methine (C2′, C3′, C4′, C5′ at 77.9, 74.8, 71.5, 75.6 ppm, respectively), and one aliphatic methylene carbon peak (C6′ at 64.4 ppm). In the ^{1}H,^{1}H-homonuclear COSY spectrum, not only were connectivities among the sugar protons H1′−H2′−H3′−H4′−H5′−H6′ established, but connectivities among the protons on the naphthalene ring, one between H2 and H3 and the other among H5, H6, and H7, were also recognized. In the HMQC spectrum of **1**, all direct ^1J connectivities between carbons and protons were determined. The HMBC spectrum led us to establish the locations to be connected among the naphthalene, sugar, and galloyl moieties of **1** on the basis of the cross peaks; one due to the coupling between H1′ and C1 and the other two due to the couplings between two H6′s and C7″. The position of one hydroxyl group on the naphthalene ring could be deduced from the fact that, on the HMBC spectrum, the hydrogen peak (9.14 ppm) of the hydroxyl group on C-8 showed cross peaks with C-7, C-8, and C-9.

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1** and **2**

	compound			
	1		**2**	
position	δ_C	δ_H (mult. J_{H-H})	δ_C	δ_H (mult. J_{H-H})
1	148.4		148.4	
2	108.3	6.55 (d, 8.4)	107.8	6.55 (d, 8.4)
3	112.6	7.11 (d, 8.4)	112.4	7.24 (d, 8.4)
4	149.8		149.9	
5	113.9	7.54 (dd, 8.4, 1.1)	113.9	7.68 (dd, 8.4, <1.0)
6	126.9	7.15 (dd, 8.4, 8.4)	127.0	7.30 (dd, 8.4, 8.4)
7	111.9	6.67 (dd, 8.4, 1.1)	112.0	6.81 (dd, 8.4, <1.0)
8	154.8		154.8	
9	117.3		117.4	
10	128.3		128.2	
1′	104.7	4.95 (d, 7.5)	104.6	5.12 (d, 7.4)
2′	77.9	3.55 (m)	77.8	3.67 (m)
3′	74.8	3.54 (m)	74.7	3.69 (m)
4′	71.5	3.46 (m)	71.6	3.61 (m)
5′	75.6	3.78 (m)	75.6	3.97 (m)
6′	64.4	4.32 (dd, 12.0, 6.9)	64.7	4.49 (dd, 10.7, 6.4)
		4.85 (dd, 12.0, 2.1)		4.79 (dd, 10.7, 2.6)
7″	166.5		166.4	
1″	121.7		121.1	
2″, 6″	110.0	7.09 (s)	108.0	7.39 (s)
3″, 5″	146.1		148.3	
4″	138.9		141.7	
4-OH		8.61 (s)		8.84 (s)
8-OH		9.14 (s)		9.29 (s)
3″, 5″-OHs		8.16 (s)		
4″-OH				8.12 (s)
3″, 5″-OCH_3s			56.7	3.86 (s)

[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1996.

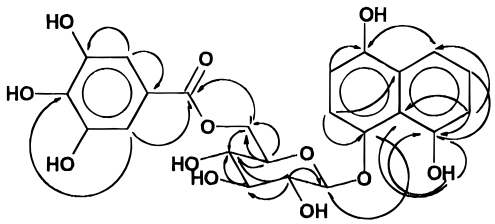


Figure 1. Long-range HMBC correlations of **1**.

The assignment of the ^{1}H- and ^{13}C-NMR chemical shift values (Table 1) on the naphthalene ring of **1** was based on the HMQC and HMBC correlation (Figure 1). The correlations on both the glucose and gallic acid moieties on the HMBC and HMQC spectra of **1** were consistent with the data from ^{1}H,^{1}H-COSY. The configuration of the anomeric proton of the glucose was proposed to be β on the basis of the coupling constant (7.5 Hz) of the proton peak at 4.95 ppm. From the above experimental results, the chemical structure of **1** was proposed.

The 1D ^{1}H-, ^{13}C-, and DEPT-NMR spectra of **2** were similar to those of **1** except that one peak due to two chemically equivalent methoxyl groups was also observed (3.86 ppm in ^{1}H-NMR and 56.7 ppm in ^{13}C-NMR spectra, respectively). Shifts (less than 3 ppm) of the three peaks due to the carbons of the galloyl moiety (δ 108.0 vs. δ 110.0 for C-2″ and C-6″, δ 148.3 vs. δ 146.1 for C-3″ and C-5″ and δ 141.7 vs. δ 138.9 for C-4″) on the ^{13}C-NMR spectrum of **2**, as compared to those of **1**, are ascribable to the substitution of methoxyl groups for the hydroxyl groups of **1**. The chemical shift values and coupling constants of the naphthalenyl-glucosyl part of **2** were essentially identical to those of **1** (Table 1). The positive FABMS showed $M^+ + 1$ peak at *m/z* 519 (calcd for $C_{25}H_{26}O_{12}$, 518.142) and an intense fragment ion peak due to the dimethyl galloyl group at *m/z* 181. After acid hydrolysis of **2**, D-glucose was identified on the TLC plate by comparison with an authentic sample. As for **2**, the configuration of the anomeric proton of the sugar portion was proposed to be β on the basis of the proton coupling constant (7.4 Hz) of the proton peak at 5.12 ppm. Therefore, the structure of **2** was proposed.

Compounds **1** and **2** showed weak cytotoxicities (IC_{50}; 30 μg/mL and 50 μg/mL, respectively) against U937 (histiocytic lymphoma), and no effect (IC_{50}; >100 μg/mL) against Hep3B (hepatocellular carcinoma) by the MTT assay.[9]

Experimental Section

General Experimental Procedures. The NMR spectra were recorded on a 300 MHz (Bruker ARX 300) and a 500 MHz (Bruker AMX 500) spectrometer. Samples were dissolved in acetone-d_6 and the chemical shift values were reported in ppm downfield from TMS. The 2D NMR spectra were recorded with Bruker's standard pulse program using inverse probe. The hetero-long-range-COSY were measured twice with different mixing times (D2), 2.3 ms and 4.572 ms, respectively. The concentrations of **1** and **2** were 0.15 mM and 0.1 mM, respectively. The FABMS were measured by VG TRIO 2A mass spectrometer. Si. gel 60 (70−230 and 270−400 mesh) and TLC plate (Si gel 60 F 254) were purchased from EM Scientific. Gallic acid and sugar standards were purchased from Wako Pure Chemical Industry and Sigma Chemical Company, Ltd., respectively. All other chemicals and solvents were analytical grade and used without further purification.

Plant Material. Roots of *Juglans mandshurica* were collected in September 1993, at a mountainous area of Pyongchang-goon, Gangwon-do, Korea, and dried at room temperature for 2 weeks. A voucher specimen is preserved at the College of Pharmacy, Yeungnam University, Gyongsan, Korea.

Extraction and Isolation. The roots of *Juglans mandshurica* (3 kg) were extracted twice with MeOH by reflux for 12 h. The MeOH solution was evaporated to dryness (300 g) and partitioned between H_2O (3000 mL) and hexane (3000 mL). The resulting H_2O layer was extracted with $CHCl_3$ followed by EtOAc. The EtOAc solution was evaporated to dryness *in vacuo*, and the EtOAc extract (58.9 g) was chromatographed on Si gel (60 g). The column was eluted with $CHCl_3$−MeOH−H_2O (7:2:0.1), and 50-mL fractions were collected. Fractions 11 and 12 were combined, and the solution was taken to dryness *in vacuo* (5.8 g). The residue was chromatographed on a Si gel column and eluted in a stepwise gradient of hexane and EtOAc. Fractions were selected on the basis of TLC and pooled, then worked up to afford **1** (200 mg) and **2** (70 mg) as dark brown powders.

Compound 1: mp 135−145 °C; UV λ max (MeOH) (log ϵ) 224 (5.0), 282 (4.3), 326 (4.1), 340 (4.1); positive FABMS *m/z* 491 ($M^+ + 1$), 315, 297, 175, 153; IR ν max (KBr) cm^{-1} 3407, 1697, 1611, 1448, 1376, 1244, 1069, 758. ^{1}H- and ^{13}C-NMR data are presented in Table 1.

Compound 2: mp 158−162 °C; UV λ max (MeOH) (log ϵ) 240 (4.8), 286 (4.2), 326 (3.9), 342 (3.9); positive FABMS *m/z* 519 ($M^+ + 1$), 343, 181, 176; IR ν max (KBr) cm^{-1} 3396, 1684, 1610, 1231, 1069. ^{1}H- and ^{13}C-NMR data are presented in Table 1.

Hydrolysis of 1 and 2. Each compound (20 mg) was dissolved in 4 N HCl−dioxane (1:1, 10 mL) and the solution was refluxed for 2 h. The resulting mixture was partitioned with ethyl ether (10 mL × 3). The water layer was neutralized with Ag_2CO_3 and filtered, and the filtrate was used for sugar analysis on both cellulose TLC (pyridine−EtOAc−AcOH−H_2O, 36:36:7:21) and silica TLC ($CHCl_3$−MeOH−H_2O, 26:14:5). The sugars on the TLC plate were visualized by the aniline phthalate reagent.[10]

Acknowledgment. This research was supported financially by the Korea Science and Engineering Foundation (941-0700-031-2).

References and Notes

(1) Binder, R. G.; Benson, M. E.; Flash, R. A. *Phytochemistry* **1989**, *28*, 2799.
(2) Gupta, S. R.; Ravindranath, B.; Seshadri, T. R. *Phytochemistry* **1972**, *11*, 2634.
(3) Muller, W.-U.; Leistner, E. *Phytochemistry* **1978**, *17*, 1739.
(4) Matos, M. E. O.; Sousa, M. P.; Machado, M. I. L.; Brazz Filho, R. *Phytochemistry* **1986**, *25*, 1419.
(5) Hirakawa, K.; Ogiue, E.; Motoyoshiya, J.; Yajima, M. *Phytochemistry* **1986**, *25*, 1494.
(6) Talpatra, S. K.; Karmacharta, B.; De, S. C.; Talapatra, B. *Phytochemistry* **1988**, *27*, 3929.
(7) Pardhasaradhi, M.; Hari Babu, M. *Phytochemistry* **1978**, *17*, 2042.
(8) Hedin, P. A.; Collum, D. H.; Langhans, V. E.; Grave, C. H. *J. Agric. Food Chem*. **1980**, *28*, 340.
(9) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res*. **1987**, *47*, 936.
(10) Weissberger, A. *Techniques of Organic Chemistry*: *Thin-Layer Chromatography*; John Wiley and Sons: New York, 1967; Vol. 12, p 152.

NP9600356