Cytotoxic Compounds from the Roots of *Juglans mandshurica*

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Received September 5, 1997

Three new compounds, a diarylheptanone glucoside (**1**), 4,5,8-trihydroxy-α-tetralone 5-*O*-*β*-D-[6′-*O*−(3″,5″-dimethoxy-4″-hydroxybenzoyl)]glucopyranoside (**2**), and 1,4,8-trihydroxy-3-naphthalenecarboxylic acid 1-*O*-*β*-D-glucopyranoside methyl ester (**3**), 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.[1−7] Previously, we isolated 1,4,8-trihydroxynaphthalene 1-*O*-*β*-D-[6′-*O*−(4″,5″,6″-trihydroxybenzoyl)]glucopyranoside, 1,4,8-trihydroxynaphthalene 1-*O*-*β*-D-[6′-*O*−(4″,6″-dimethoxy-5″-hydroxybenzoyl)]glucopyranoside, 1,5,8-trihydroxy-α-tetralone 5-*O*-*β*-D-glucopyranoside, and 1,4,8-trihydroxynaphthalene-1-*O*-*β*-D-glucopyranoside from the roots of this plant.[8,9] As continuation of this work, we now report the isolation of three additional new compounds from this plant. The structures of these compounds were determined by various NMR experiments including ^{1}H,^{1}H-homonuclear COSY, DEPT, HMQC, and HMBC.

The MeOH extract of roots of *Juglans mandshurica* was partitioned between H_2O and hexane, and the resulting H_2O layer was extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on a Si gel column, and fraction 9 was eluted through a reversed-phase column. The three major fractions from the reversed-phase column were separated further by Sephadex-LH-20 CC, which afforded compounds **1**−**3**. Fraction 4 from the above Si gel column chromatography was further fractionated on a Si gel column to give compound **4**.

Compound **1** showed the $M^+ - 1$ peak at *m*/*z* 503.1 (calculated for $C_{26}H_{32}O_{10}$, 504.1). Hydrolysis of **1** yielded glucose, which was identified on a TLC plate by comparison with a reference sample. Noise-decoupled ^{13}C NMR and DEPT spectra of **1** showed 26 carbon signals (Table 1), including one methyl, six methylene, 12 methine, and seven quaternary. These spectra showed peaks characteristic of a carbonyl group at *δ* 212.3, 12 signals due to two benzene rings, and six peaks due to the sugar part of **1**. In the aromatic region of the ^{1}H spectrum, a peak due to H-6 (*δ* 5.45) appeared abnormally upfield from peaks due to other protons on the two benzene rings. A Dreiding model of **1** showed that the two benzene rings are perpendicular to each other, and H-6 is located above the plane of the other benzene ring. Thus, H-6 is shielded more than the other protons by the anisotropic effect of the ring current.[10] Such shielding effects have been reported in diarylheptanoids, which have an ether linkage between C-1 and C-17, and

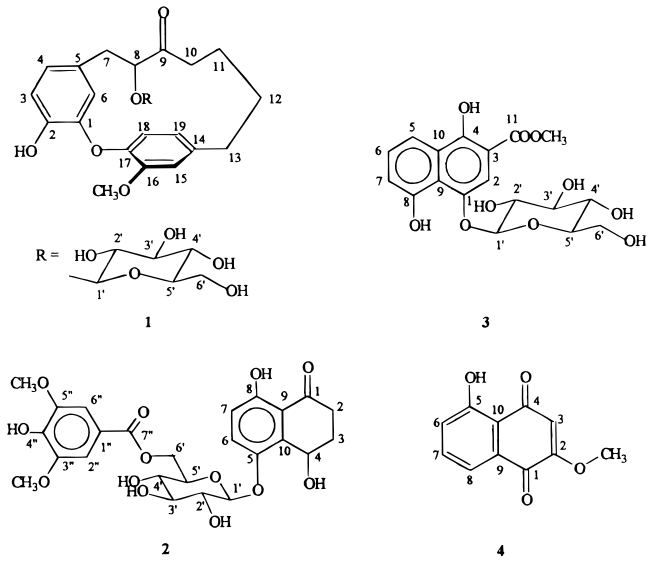

Table 1. ^{1}H and ^{13}C NMR Data and HMBC Correlation of 1 in CD_3OD

position	*δ* ^{13}C	*δ* ^{1}H (m, *J* in Hz)	HMBC
1	149.4		H3, H6
2	145.6		H3, H4, H6
3	117.1	6.63 (d, 7.8)	H4
4	124.2	6.49 (d, 7.8)	H3
5	128.9		H3, H7, H8
6	115.6	5.45 (s)	H4, H7
7	36.7	2.87 (br.d, 14.5)	H4, H6, H8
		2.93 (br.d, 8.4)	
8	82.6	4.01 (d, 4.5)	H7, H1
9	212.3		H7, H8
10	44.0	1.88 (m)	
11	20.3	1.43 (br.s)	H13
12	28.2	1.61 (br.s)	H13
13	34.9	2.63 (m)	H15, H19
14	141.2		H18
15	116.3	6.93 (s)	H13, H19
16	153.8		H15, H18, 16-OCH_3
17	145.0		H15, H18, H19
18	149.4	6.83 (d, 7.8)	H15, H18
19	123.9	6.76 (d, 7.8)	H15
16-OCH_3	56.7	3.65(s)	
1′	103.0	4.14 (d, 7.7)	
2′	74.9	2.97 (t, 8.0)	H3′
3′	77.6	3.17 (m)	H2′, H5′
4′	71.3	3.15 (m)	
5′	77.9	3.20 (m)	
61′	62.5	3.51 (dd, 4.4, 15.9)	
		3.70 (br.d, 11.4)	

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S0163-3864(97)00413-8 CCC: $15.00
Published on Web 05/01/1998

Table 2. ^{1}H and ^{13}C NMR Data and HMBC Correlation of **2** in CD_3OD

position	δ ^{13}C	δ ^{1}H (m, *J* in Hz)	HMBC
1	209.1		H2, H3
2	36.3	2.35 (br.d, 17.5)	H4
		2.88 (m)	
3	33.0	2.05 (m)	H2
4	64.0	5.20 (s)	H2
5	151.1		H6, H7, H1′
6	131.1	7.27 (d, 9.1)	
7	121.6	6.51 (d, 9.1)	
8	162.6		H6, H7
9	118.9		H4, H7
10	138.2		H6
1′	107.3	4.72 (d, 6.9)	H5′
2′	78.5	3.45	H4′, H6′
3′	80.6	3.43	H2′, H4′
4′	74.7	3.34 (t, 8.2)	H3′, H5′
5′	78.0	3.61 (br.t, 7.5)	H3′, H6′
6′	67.8	4.37 (dd, 7.3, 11.5)	H3′, H5′
		4.54 (br.d, 11.1)	
1″	123.9		H2″, H6″
2″	111.3	7.16 (s)	H6″
3″	151.7		H2″, H6″, H3″-OCH_3
4″	145.0		H2″, H6″
5″	151.7		H2″, H6″, H5″-OCH_3
6″	111.3	7.16 (s)	H2″
7″	170.5		H6′, H2″, H5″-OCH_3
3″-OCH_3	59.7	3.74 (s)	
5″-OCH_3	59.7	3.74 (s)	

therefore, an ether linkage between C-1 and C-17 of **1** was indicated.[11] In the ^{1}H,^{1}H-homonuclear COSY spectrum, not only were connectivities among the sugar protons established, but connectivities among the protons on the aliphatic chain were recognized and assigned. Two separate coupling systems on the aromatic rings, one between H-3 and H-4 and the other between H-18 and H-19, were also identified. In the HMQC spectrum of **1**, all direct ^1J connectivities between carbons and protons were determined. In the HMBC spectrum, connectivities among C-8, C-9, and C-10 on the aliphatic chain of **I** were recognized by the cross-peaks: one between H-10 and C-9 and another between H-8 and C-9. Other connectivities were established on the basis of cross-peaks due to ^2J and ^3J couplings (Table 1). Location of the link between the aliphatic chain and the sugar moiety of **1** was established on the basis of a cross-peak between H-1′ and C-8. The position of methoxyl group was determined by the cross-peak between C-16 and C-16-OMe protons. ^{1}H and ^{13}C NMR chemical shift assignments (Table 1) were based on HMQC and HMBC correlations. The configuration of the glucose anomeric proton was deduced to be β from the coupling constant (7.7 Hz) of the proton peak at δ 4.14. Thus, the structure of **1** was proposed as indicated. The configuration at C-8 remains unknown.

Compound **2** showed an $M^+ - 1$ peak at *m*/*z* 535.1 (calcd for $C_{25}H_{28}O_{13}$, 536.1) and an intense fragment ion peak due to the tetralone moiety at *m*/*z* 193 in negative FABMS. After hydrolysis of **2**, glucose was identified on a TLC plate by comparison with a reference sample. Noise-decoupled ^{13}C NMR and DEPT spectra of **2** showed 25 peaks (Table 2): two methyl, three methylene, 10 methine, and 10 quaternary carbons. These spectra showed three sets of chemically equivalent peaks and one carbonyl peak due to the galloyl group. Other characteristic peaks due to the sugar part of **2**, one anomeric, four methine, and one methylene carbons,

Table 3. ^{1}H and ^{13}C NMR Data and HMBC Correlation of 3 in CD_3OD

position	δ ^{13}C	δ ^{1}H (m, *J* in Hz)	HMBC
1	147.9		H2, H1′
2	109.0	7.58 (s)	
3	105.6		H2
4	155.0		H2, H5
5	116.0	7.74 (dd, 0.8, 7.8)	H7
6	128.4	7.29 (t, 8.7)	
7	116.1	6.89 (dd, 0.6, 7.8)	H5
8	157.4		H6, H7
9	120.1		H2, H5, H7
10	128.5		H6
11	172.1		H2, 11-$COCH_3$
1′	105.3	4.89 (d, 7.8)	
2′	78.8	3.38 (m)	H3′
3′	75.0	3.52 (m)	H4′
4′	71.4	3.34 (dd, 8.7, 17.5)	
5′	78.1	3.43 (m)	H6′
6′	62.5	3.66 (dd, 5.6, 11.9)	
11-$COCH_3$	52.9	3.86 (s)	

were also recognized. The peak due to C-1, the ketone conjugated with the adjacent aromatic ring, appeared unusually downfield at δ 206.3 because this ketone has a strong intramolecular hydrogen bond with the OH group on C-8.[12] The ^{1}H,^{1}H-homonuclear COSY spectrum established connectivities among the sugar, and the dihydronaphthoquinone protons. The HMBC spectrum established linkages between the dihydronaphthoquinone, sugar, and galloyl moieties of **2** on the basis of the cross-peaks; one due to the coupling between H-1′ and C-5 and another two due to the couplings between two H-6's and C-7″. Assignment of the ^{1}H and ^{13}C NMR chemical shift values for **2** was based on HMQC and HMBC correlations (Table 2). The configuration of the anomeric proton of glucose was proposed to be β from the coupling constant (6.9 Hz) of the proton peak at δ 4.72. The configuration at C-4 remains unknown.

Compound **3** showed an M − 1 peak at *m*/*z* 395.1 (calcd for $C_{18}H_{20}O_{10}$, 396.1) and an intense fragment ion peak due to the naphthalene moiety ($M^+ - C_6H_{11}O_5$) at *m*/*z* 232.9. After hydrolysis of **3**, glucose was identified by TLC. Noise-decoupled ^{13}C NMR and DEPT spectra of **3** showed 18 carbon peaks (Table 3), including one methyl, one methylene, nine methine, and seven quaternary. Ten peaks due to the naphthalene ring and six peaks due to the glucose are evident along with characteristic peaks due to the carbonyl ketone (δ 172.1), an anomeric carbon (δ 105.3), and a methoxyl group (δ 52.9). In the ^{1}H spectrum, peaks due to the H-5, H-6, and H-7, which couple among themselves, one isolated peak due to H-2 (δ 7.58), and a peak at δ 4.89 due to the anomeric proton were evident. In the HMBC spectrum of **3** (Table 3) a cross-peak between H-1′ and C-1 and a cross-peak between H-2 and C-11 allowed determination of the locations of both the glucose moiety and the methoxy carbonyl group on the naphthalene ring. The HMQC and HMBC correlations were consistent with structure **3** as indicated. The configuration of the glucose anomeric proton was proposed to be β on the basis of the coupling constant (7.8 Hz) of the proton peak at δ 4.89.

The structure of **4** was determined by comparison of the MS and ^{1}H and ^{13}C NMR data of 5-hydroxy-2-methoxy-1,4-naphthoquinone, which was synthesized by Barre et al.[13,14] and recently reported from *Platycarya*

Table 4. IC_{50} Values of Compounds against HT-29 and A549 Cell Lines

	IC_{50} (μg/mL)	
	HT-29	A549[b]
1	>50	>50
2	>50	>50
3	8.06	7.79
4	2.63	0.08
altromycin B	3.98	4.17

[a] HT-29: human colon carcinoma. [b] A549: human lung carcinoma.

strobilacea (Juglandaceae).[15] Compounds **3** and **4** showed strong cytotoxicities against human colon carcinoma and human lung carcinoma cell lines (Table 4).

Experimental Section

General Experimental Procedures. The NMR spectra were recorded on Bruker 300 MHz (ARX 300) and Bruker 600 MHz (DMX 600) spectrometers. Samples dissolved in CD_3OD were reported in δ (ppm) downfield from TMS. The 2D NMR spectra were recorded by using Bruker's standard pulse program. The FABMS were measured by a VG TRIO 2A mass spectrometer. Silica gel 60 (70−230 and 270−400 mesh) and TLC plates (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 Co., Ltd., respectively. All other chemicals and solvents were analytical grade and used without further purification.

Plant Materials. Roots of *J. mandshurica* were collected during September 1993 at a mountain 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.

Cytotoxicity Bioassays. The tetrazolium-based colorimetric assay (MTT assay) was used for the in vitro assay of cytotoxicity to human lung carcinoma (A549) and human colon carcinoma (HT-29) cells.[16] Altromycin B was used as a control experiment.

Isolation. The roots of *J. mandshurica* (3 kg) were extracted twice with MeOH by reflux for 12 h. The MeOH solution was evaporated to dryness (300 g). The MeOH extract of the roots of *J. mandshurica* was partitioned between H_2O (3000 mL) and hexane (3000 mL). The resulting H_2O layer was extracted with $CHCl_3$, EtOAc, and *n*-BuOH, succesively, and the $CHCl_3$ solution was evaporated to dryness in vacuo. The $CHCl_3$ extract (50 g) was loaded on a silica gel column (60 × 9 cm, Si gel 70−230 mesh, Merck). The column was eluted using a stepwise gradient of $CHCl_3$, MeOH, and H_2O, and the eluent was combined, on the basis of TLC, to gave 17 fractions. Fraction 9 (2.3 g) was chromatographed on a reversed-phase column (75 × 2.6 cm, Lichroprep Rp-18, Merck) with a stepwise gradient of MeOH and H_2O. Fractions 7, 9, and 13 from the reversed-phase column were further purified on a Sephadex LH-20 column (100 × 1.9 cm, Pharmacia) eluted with MeOH, which afforded **1** (40 mg), **2** (25 mg), and **3** (30 mg). Fraction 4, the other major fraction obtained from the first chromatography of the $CHCl_3$ extract, was rechromatographed on a Si gel column with stepwise gradient of hexane and ethyl acetate, which gave **4** (35 mg).

Compound 1: colorless powder; mp 65−70 °C; $[\alpha]_D$ +6.5 (*c* 0.59, MeOH); UV (MeOH) λ_{max} (log ϵ) 204.8 (4.49), 280.6 (3.53); IR (KBr) ν_{max} 3422, 2925, 1654, 1458; ^1H and ^{13}C NMR see Table 1; negative FABMS *m/z* $[M - 1]^+$ 503.1 (31), 341 (8), 297 (40); positive HRFABMS *m/z* 504.1993 (calcd for $C_{26}H_{32}O_{10}$ 504.1995).

Compound 2: colorless powder; mp 127−133 °C; $[\alpha]_D$ −29.2 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ϵ) 215.8 (4.47), 262.2 (4.01), 345.4 (3.49); IR (KBr) ν_{max} 3396, 2926, 1718, 1508 cm^{-1}; ^1H and ^{13}C NMR see Table 2; negative FABMS *m/z* $[M - 1]^+$ 535.1 (35), 518.1 (8), 391.3 (5), 297.2 (26), 295.2 (12); positive HRFABMS *m/z* 536.1520 (calcd for $C_{25}H_{28}O_{13}$, 536.1530).

Compound 3: colorless powder; mp 133−137 °C; $[\alpha]_D$ −10.5 (*c* 0.47, MeOH); UV (MeOH) λ_{max} (log ϵ) 213.6 (4.33), 246.6 (3.39); IR (KBr) ν_{max} 3421, 2961, 1654, 1458 cm^{-1}; ^1H and ^{13}C NMR see Table 3; negative FABMS *m/z* $[M - 1]^+$ 395.1 (100), 297.1 (38), 232.9 (75), 222.9 (60), 148.0 (100); positive HRFABMS *m/z* 396.1082 (calcd for $C_{18}H_{20}O_{10}$ 396.1056).

Compound 4: colorless powder; ^1H and ^{13}C NMR data in good agreement with published data;[13−15] negative FABMS *m/z* 205.0 $[M - 1]^+$ (100), 154.0 (55), 136.0 (43).

Hydrolysis of 1−3. Each compound (2 mg) was dissolved in 4 NHCl−dioxane (1:1, 2 mL), and the solution was refluxed for 2 h. The H_2O layer was neutralized with Ag_2CO_3 and filtered, and the filtrate was used to compare it with standard sugars on both cellulose TLC (pyridine−EtOAc−HOAc−H_2O = 36:36:7:21) and silica TLC ($CHCl_3$−MeOH−H_2O = 26:14:5).

Acknowledgment. The authors are grateful to Dr. Jong-Shin Yoo of Korea Basic Science Institute, Taejeon, for measuring FABMS spectra of these compounds. This research was financially supported 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*, 2799−2801.
(2) Gupta, S. R.; Ravindranath, B.; Seshadri, T. R. *Phytochemistry* **1972**, *11*, 2634−2636.
(3) Muller, W.-U.; Leistner, E. *Phytochemistry* **1987**, *17*, 1739−1741.
(4) Hirakawa, K.; Ogiue, E.; Motoyoshiya, J.; Yajima, M. *Phytochemistry* **1986**, *25*, 1494−1495.
(5) Talapatra, S. K.; Karmacharta, B.; De, S. C.; Talapatra, B. *Phytochemistry* **1988**, *27*, 3929−3932.
(6) Pardhasaradhi, M.; Hari Babu, M. *Phytochemistry* **1978**, *17*, 2042−2043.
(7) Hedin, P. A.; Collum, D. H.; Langhans, V. E.; Grave, C. H. *J. Agric. Food Chem*. **1980**, *28*, 340−342.
(8) Joe, Y. K.; Son, J. K.; Park, S. H.; Lee, I. J.; Moon, D. C. *J. Nat. Prod*. **1996**, *59*, 159−160.
(9) Son, J. K. *Arch. Pharm. Res*. **1995**, *18*, 203−205.
(10) Silverstein, R. M.; Bassler, G. C.; Morrill T. C. *Spectrometric Identification of Organic Compounds*, 5th ed.; John Wiley & Sons: New York, 1991; Chapter 4, pp 174−175.
(11) Nagai, M.; Kubo, M.; Fugita, M.; Inoue, T.; Matsuo M. *Chem. Pharm. Bull*. **1978**, *26*, 2806−2810.
(12) Atta-ur-Rahman. *Nuclear Magnetic Resonance; Basic Principles*, Springer-Verlag: New York, 1986; pp 148.
(13) Barre, G.; Hocquaux, M.; Jacquet, B.; Min, N.; Maurette, M. T.; Oliveros, E.; *Tetrahedron Lett*. **1986**, *51*, 6197−6200.
(14) Khanna, R. N.; Singh K. P.; Yadav, S. K.; Srinivas, S. *Synth. Commun*. **1989**, *19*, 3151−3157.
(15) Kim, Y. I.; Lee, S. H.; Cho, T. S. *Kor. J. Pharmacogn*. **1996**, *27*, 238−245.
(16) Rubinstein, L. V.; Shoemaker, R. H.; Paul, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero D. A.; Monks, A.; Boyd, M. R. *J. Nat. Cancer Inst*. **1990**, *82*, 1113−1118.

NP970413M