Diarylheptanoids from the Roots of *Juglans mandshurica*

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The roots of *Juglans mandshurica* Maximowicz (Juglandaceae) have been used as a folk medicine for the treatment of cancer in Korea. Several naphthoquinones, naphthalenyl glucosides, tetralones, flavonoids, diarylheptanoid, and galloyl glycosides have been isolated from *Juglans* species.[1-16] These compounds have shown cytotoxic activity, topoisomerases I and II inhibitory activity, and inhibitory effect on both DNA polymerase and RNase H activity of HIV-1 reverse transcriptase.[12-16] In the continuation of our studies on this plant, we isolated three new diarylheptanoids (**1-3**) from the $CHCl_3$ fraction of the MeOH extract. This paper describes the structural determination of three new diarylheptanoids, and the absolute configurations of **1** and **3** were elucidated by Mosher's esters.

Three diarylheptanoids (**1-3**) were isolated from a $CHCl_3$ fraction of the roots of *J. mandshurica* by repetitive column chromatography and preparative HPLC using a RP-18 column.

Compound **1** has the molecular formula $C_{20}H_{26}O_4$ as determined by the HREABMS, ^{13}C-NMR, and DEPT spectral data. In the aromatic region of the ^{1}H-NMR spectra of **1**, ^2J coupling between H-5" and H-6", and ^3J coupling between H-2" and H-6" indicated a 1,3,4-trisubstituted benzene ring, and ^2J coupling between two sets of chemically equivalent protons (H-2'/H-6' and H-3'/H-5') suggested an 1,4-disubstituted aromatic ring. The ^{13}C NMR spectrum of **1** exhibited a total of 20 carbon signals, including characteristic signals due to a methoxyl group (3"-OCH_3) and two chemically equivalent aromatic carbons (C-2'/C-6' and C-3'/C-5'). In the aliphatic region of DEPT spectra, one hydroxymethine and six methylene signals were exhibited. The ^{1}H-^{1}H COSY spectrum showed connectivities among H-1, H-2, H-3, H-4, H-5, H-6, and H-7, between H-2' (H-6') and H-3' (H-5'), and between H-5" and H-6". In the HMBC spectrum of **1** (Figure 2), the connectivities of the two aromatic rings with the alkyl chain were indicated by the cross peaks between H-7 and C-1", C-2" and C-6", and those between H-1 and C-1', C-2', and C-6'. The position of the methoxyl group was determined by both the HMBC correlation of C-3" with 3"-OCH_3 and the positive NOE effect (8.4%) between H-2" and 3"-OCH_3.[17,18]

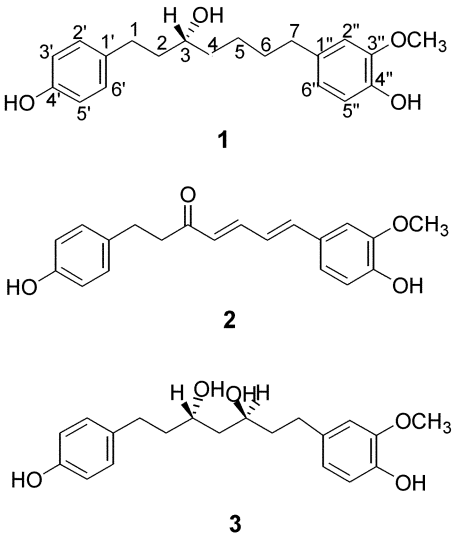

Figure 1. Diarylheptanoids isolated from the roots of *Juglans mandshurica*.

The absolute stereochemistry of the chiral center in **1** was determined using the Mosher's ester based on the differences between the ^{1}H-NMR chemical shifts of (*S*)- and (*R*)-MTPA ester derivatives. ^{1}H-NMR data were assigned based on the ^{1}H-^{1}H COSY spectra of $\mathbf{1}_S$ and $\mathbf{1}_R$ (Table 1). For **1**, the negative value of $\Delta\delta_H(\delta_S-\delta_R)$ at H-2 and the positive value of $\Delta\delta_H(\delta_S-\delta_R)$ at H-4 suggested a *R* configuration at C-3.

Compound **2** has the molecular formula $C_{20}H_{20}O_4$ as determined by the HREABMS, ^{13}C-NMR, and DEPT spectral data. The ^{1}H-NMR spectrum of **2** showed signals for a 1,3,4-trisubstituted and a 1,4-disubstituted aromatic group same as compound **1**. The ^{1}H-^{1}H COSY spectrum of **2** showed the connectivities among H-4, H-5, H-6, and H-7, between H-1 and H-2, between H-2' (H-6') and H-3' (H-5'), and between H-5"and H-6". In the HMBC spectrum of **2** (Figure 2), the location of a carbonyl group in the chain was established by the correlations from C-3 to H-2, H-4, and H5, and the connectivities of the two aromatic rings with the alkyl chain were indicated by the cross peaks from H-7 to C-2" and C-6" and from H-1 to C-2' and C-6'. The position of the methoxyl group on the aromatic ring was also determined by both the

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Table 1. Characteristic ^{1}H-NMR data of MTPA esters of **1** and **3**

Position	$\mathbf{1}_S$ δ_S	$\mathbf{1}_R$ δ_R	$\Delta\delta$ δ_S-δ_R	Position	$\mathbf{3}_S$ δ_S	$\mathbf{3}_R$ δ_R	$\Delta\delta$ δ_S-δ_R
1	2.51	2.72	-0.09	1	2.57	2.62	-0.05
2	1.91	2.01	-0.10	2	2.00	2.07	-0.07
3	5.13	5.17	***R***	3	5.46	5.32	***S***
4	1.79	1.71	+0.08	4	2.18	2.06	+0.12
5	1.45	1.32	+0.13	5	5.46	5.32	***S***
6	1.68	1.59	+0.09	6	1.97	2.00	-0.03
7	2.65	2.55	+0.10	7	2.57	2.62	-0.05

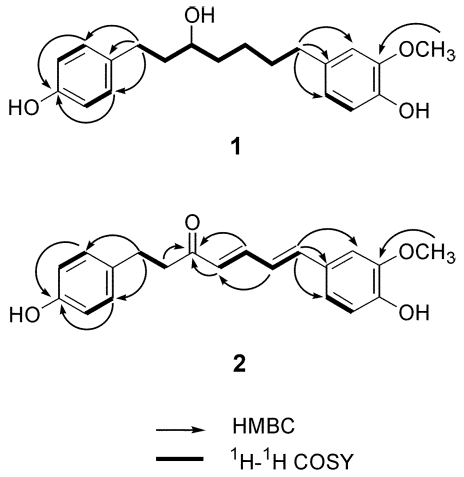

Figure 2. HMBC and ^{1}H-^{1}H COSY correlations of compounds **1** and **2**.

HMBC correlation between C-3" with C-3"-OCH_3, and the positive NOE effect (6.8%) between H-2" and C-3"-OCH_3 in the 1D-NOE difference spectrum of **2**.

^{1}H and ^{13}C NMR data of **3** was identical with those of reported compound which is an enantiomer of **3**.[19] To determine the absolute configuration of the hydroxyl groups at C-3 and C-5, MTPA esters ($\mathbf{3}_R$ and $\mathbf{3}_S$) of **3** were prepared, and ^{1}H-NMR data was also assigned based on the ^{1}H,^{1}H-COSY spectra (Table 1). For **3**, the negative value of $\Delta\delta_H$ (δ_S-δ_R) at H-1 and H-2, and the negative value of $\Delta\delta_H$ (δ_S-δ_R) at H-6 and H-7 suggested both *S* configurations at C-3 and C-5.

Among these compounds, only **1** showed weak cytotoxicities against the HT-29 and MCF-7 cell lines (Table 2, IC_{50}: >50 μg/mL and 47.7 μg/mL, respectively).

Table 2. IC_{50} values of the compounds against HT-29 and MCF-7 cell lines

	IC_{50} (μg/mL)	
	HT-29[a]	MCF-7[b]
1	>50	47.7
2	>50	>50
3	>50	>50
CPT[c]	0.035	3.5

[a]HT-29: Human colon carcinoma. [b]MCF-7: Human breast carcinoma. [c]camptothesin: positive control.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-1000 (Tokyo, Japan) automatic digital polarimeter, and FT-IR spectra were recorded on a JASCO FT-IR 300E spectrophotometer. UV spectra were recorded on a JASCO V-550 spectrophotometer. For preparative HPLC, LC-10AD pump (Shimadzu), SPD-10A detector (Shimadzu), and Shim-Pack Prep-ODS (20 × 250 mm) column were used. NMR spectra were recorded on a Bruker 250 MHz (DMX 250) spectrometer using Bruker's standard pulse program. Samples were dissolved in either acetone-d_6 or CD_3OD, and chemical shifts were reported in ppm downfield from TMS. The MS spectra were measured by a VG TRIO 2A mass spectrometer. Silica gel 60 (70-230 and 270-400 mesh, Merck) and Lichroprep RP-18 gel (40-63 μm, Merck) were used for column chromatography. TLC plate (Silica-gel 60 F_{254} and RP-18 F_{254}) was purchased from EM Scientific. (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl [(*R*)-MTPA] chloride and (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl [(*S*)-MTPA] chloride were purchased from Sigma Chemicals Co. Ltd. (St. Louis, MO, USA). All other chemicals and solvents were analytical grade, and used without further purification.

Plant Material. *J. mandshurica* roots were collected in September 1993 in a mountainous area of Pyongchang-goon, Gangwon-do, Korea, and dried at room temperature for 2 weeks. The material was confirmed taxonomically by Professor Gi-Hwan Bae, at Chungnam National University in Taejeon, Republic of Korea. A voucher specimen has been deposited at the College of Pharmacy, Yeungnam University.

Isolation. *J. mandshurica* roots (3 kg) were extracted with MeOH two times under reflux for 12 h yielding 300 g of a dark solid extract, 280 g of which was then suspended in H_2O, and extracted with hexane. The resulting H_2O layer was extracted with $CHCl_3$, 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, Silica gel 70-230 mesh), and the column was eluted with MeOH-EtOAc saturated with H_2O (gradient from EtOAc 100% to MeOH 100%). The eluent was combined on the basis of TLC, giving 17 fractions (F1-17). Fraction F8 (1.5 g) was chromatographed on a reverse phase column (60 × 3.0 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 2 : 8 to 100% MeOH), which afforded 22 subfractions (F8-1~8-22). Subfraction F8-3 (250 mg) from the column was further purified on a reversed-phase column (75 × 2.0 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 10% to 90% MeOH), affording **1**. Subfraction F8-6 (160 mg) from the column was further purified on a reversed-phase column (60 × 2.0 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 20% to 100% MeOH), affording compounds **2** and **3**. Further purifications of **1-3** were carried out using HPLC with MeOH-H_2O gradients.

Compound 1: yellow solid (15 mg), $[\alpha]_D^{25}$ -12.3° (*c* = 0.312, MeOH); UV (MeOH) λ_{max} (log ε) 223.2 (4.13), 280.4 (3.64), 347.0 (2.60); IR (KBr) ν_{max} 3391, 2933, 1613, 1514,

1455, 1363, 1233, 1151, 1123, 1033, 825 cm^{-1}; ^{1}H-NMR (acetone-d_6, 250 MHz) δ 7.01 (2H, d, J = 8.4 Hz, H-2'/H-6'), 6.77 (1H, d, J = 1.8 Hz, H-2"), 6.72 (2H, d, J = 8.4 Hz, H-3'/H-5'), 6.71 (1H, d, J = 8.0 Hz, H-5"), 6.61 (1H, dd, J = 8.0, 1.8 Hz, H-6"), 3.79 (3H, s, 3"-OCH_3), 3.31 (1H, br s, H-3), 2.61 (2H, m, H-1), 2.50 (2H, t, J = 7.5 Hz H-7), 1.68-1.43 (8H, m, H-2, 6, 4, 5); ^{13}C-NMR (acetone-d_6, 62.9 MHz) δ 156.2 (C-4'), 148.1 (C-3"), 145.4 (C-4"), 134.9 (C-1'), 134.3 (C-1"), 130.1 (C-2'/C-6'), 121.5 (C-6"), 115.9 (C-3'/C-5'), 115.6 (C-5"), 112.8 (C-2"), 70.9 (C-3), 56.2 (3"-OCH_3), 40.8 (C-2), 38.3 (C-4), 36.2 (C-7), 32.8 (C-6), 31.9 (C-1), 26.2 (C-5); HRFABMS m/z 331.1911, (calcd. for $C_{20}H_{27}O_4$ $[M + H]^+$, 331.1909).

Compound 2: yellow solid (14 mg); UV (MeOH) λ_{max} (log ε) 272.4 (4.01), 361.2 (4.21); IR (KBr) ν_{max} 3419, 2927, 1654, 1610, 1591, 1514, 1455, 1384, 1280, 1171, 1123, 1031, 827 cm^{-1}; ^{1}H-NMR (acetone-d_6, 250 MHz) δ 7.42 (1H, dd, J = 15.4, 9.4 Hz, H-5), 7.21 (1H, d, J = 1.8 Hz, H-2"), 7.05 (2H, d, J = 8.4 Hz, H-2'/H-6'), 7.01 (1H, dd, J = 8.4, 1.8 Hz, H-6"), 6.98 (1H, dd, J = 15.4, 9.4 Hz, H-6), 6.95 (1H, d, J = 15.4 Hz, H-7), 6.81 (1H, d, J = 8.4 Hz, H-5"), 6.72 (2H, d, J = 8.4 Hz, H-3'/H-5'), 6.23 (1H, d, J = 15.4 Hz, H-4), 3.87 (3H, s, 3"-OCH_3), 2.84 (2H, t, J = 5.3 Hz, H-2), 2.81 (2H, t, J = 5.3 Hz, H-1); ^{13}C-NMR (acetone-d_6, 62.9 MHz) δ 199.3 (C-3), 156.4 (C-4'), 149.0 (C-3"), 148.7 (C-4"), 143.8 (C-5), 142.3 (C-7), 133.0 (C-1"), 130.0 (C-2'/C-6'), 129.3 (C-1'), 129.2 (C-4), 125.2 (C-6), 122.6 (C-6"), 116.0 (C-5"), 115.9 (C-3'/C-5'), 110.5 (C-2"), 56.2 (3"-OCH_3), 42.8 (C-2), 30.1 (C-1); HRFABMS m/z 325.1438 (calcd. for $C_{20}H_{21}O_4$ $[M + H]^+$, 325.1440).

Compound 3: colorless amorphous solid (10 mg); $[\alpha]_D^{25}$ +4.3° (c = 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 223.0 (3.93), 280.0 (3.48); IR (KBr) ν_{max} 3340, 2934, 1613, 1517, 1454, 1363, 1233, 1157, 1032, 824 cm^{-1}; ^{1}H-NMR (CD_3OD, 250 MHz) δ 6.97 (2H, d, J = 8.4 Hz, H-2'/H-6'), 6.74 (1H, d, J = 1.6 Hz, H-2"), 6.66 (1H, d, J = 8.0 Hz, H-5"), 6.65 (2H, d, J = 8.4 Hz, H-3'/H-5'), 6.59 (1H, dd, J = 8.0, 1.6 Hz, H-6"), 3.79 (3H, s, 3"-OCH_3), 3.79 (2H, m, H-3, 5), 2.70-2.46 (4H, m, H-1, 7), 1.70-1.60 (4H, m, H-2, 6), 1.51 (2H, t, J = 6.1 Hz, H-4); ^{13}C-NMR (CD_3OD, 62.9 MHz) δ 156.3 (C-4'), 148.8 (C-3"), 145.7 (C-4"), 135.2 (C-1"), 134.4 (C-1'), 130.3 (C-2'/C-6'), 121.8 (C-6"), 116.1 (C-3'/C-5'), 116.0 (C-5"), 113.2 (C-2"), 68.6 (C-3/C-5), 56.3 (3"-OCH_3), 45.6 (C-4), 41.4 (C-6), 41.3 (C-2), 32.6 (C-1), 32.1 (C-7); HRFABMS m/z 347.1814 (calcd. for $C_{20}H_{21}O_4$ $[M + H]^+$, 347.1858).

Preparation of Mosher's Esters. To each 1 mg of **1** and **3** in 0.5 mL of CH_2Cl_2 were added sequentially 0.2 mL of pyridine, 0.5 mg of 4-(dimethylamino)pyridine, and 12.5 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl [(*R*)-MTPA] chloride, separately.[20-22] The mixture was left at room temperature overnight and purified over a microcolumn (0.6 × 6 cm) of silica gel (230–400 mesh) eluted with 3–4 mL of hexane-CH_2Cl_2 (1 : 3). The elute was dried, CH_2Cl_2 (5 mL) was added, and the CH_2Cl_2 was washed using 1% $NaHCO_3$ (5 mL × 2) and H_2O (5 mL × 2). The washed elute was dried *in vacuo* to give the *S*-Mosher esters ($\mathbf{1}_S$ and $\mathbf{3}_S$) of **1** and **3**, respectively. The *R*-Mosher esters ($\mathbf{1}_R$ and $\mathbf{3}_R$) of **1** and **3** were prepared from (*S*)-MTPA chloride, respectively.

Cytotoxicity Bioassay. The tetrazolum-based colorimetric assay (MTT assay) was used for the *in vitro* assay of cytotoxicity against human colon carcinoma (HT-29) and human breast carcinoma (MCF-7) cells.[23]

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