Four New Diarylheptanoids from the Roots of *Juglans mandshurica*

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Four new diarylheptanoids (1—4), along with two known tetralones (5, 6), were isolated from the roots of *Juglans mandshurica* and their structures were elucidated on the basis of spectroscopic studies.

Key words *Juglans mandshurica*; Juglandaceae; diarylheptanoid; tetralone

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] In the course of isolating cytotoxic compounds from the roots of this plant, we have isolated six naphthalene glycosides, two tetralone glucosides, one naphthalene carboxylic acid glucoside, and five diarylheptanoids.[8—12] In this paper, we report four new diarylheptanoids, along with two known tetralones, from the roots of *J. mandshurica.*

Results and Discussion

The MeOH extract of the 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 silica gel column. The two major fractions were chromatographed on a reverse-phase column, which afforded compounds **1**—**6**.

The molecular formula of **1**, $C_{20}H_{22}O_5$ was established from high resolution (HR)-FAB-MS, ^{13}C-NMR, and distortionless enhancement by polarization transfer (DEPT) spectral data. The ^{13}C-NMR and DEPT spectra showed one methyl, five methylene, seven methine, and seven quaternary carbon signals including characteristic peaks of a carbonyl group and 12 aromatic carbons. In the ^{1}H-NMR spectrum, signals on the aromatic region showed coupling patterns due to two sets of 1,3,4-trisubstituted benzene rings: $^2J_{H5'H6'}$ (8.1 Hz) and $^3J_{H2'H6'}$ (2.1 Hz), and $^2J_{H5''H6''}$ (8.0 Hz) and $^3J_{H2''H6''}$ (1.9 Hz), respectively. The H-2′ signal (δ 5.67) appeared abnormally upfield from other proton signals of the two benzene rings, and this shielding effect is characteristic of diphenylether-type diarylheptanoids that have an ether linkage between C-3′ and C-4″.[13] The correlations in the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum displayed connectivities between H-1 and H-2, between H-4–H-5–H-6–H-7, between H-5′ and H-6′, and between H-5″ and H-6″. In addition, a weak cross-peak due to the long-range coupling between H-2′ and H-6′ was recognized. In the HMBC spectrum of **1**, the linkages of two benzene rings on the alkyl chain were established by cross-peaks between H-1 and C-1′, C-2′, and C-6′, and those between H-7 and C-1″, C-2″, and C-6″, respectively. The position of the carbonyl group on the aliphatic chain was established by the correlation of C-3 with H-1 and H-4. The location of the methoxyl group was identified by both a correlation between the C-3″ and C-3″-OCH_3 in the HMBC and a positive NOE effect between H-2″ and C-3″-OCH_3 in the one dimensional nuclear Overhauser effect (1D-NOE) difference spectrum of **1**.

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

Compound **2** had the molecular formula $C_{20}H_{24}O_4$ as determined from HR-FAB-MS and NMR data. The ^{1}H-NMR spectrum of **2** was very similar to that of **1**, exhibiting a H-2′ signal upfield (δ 5.71) from the other aromatic ones, a characteristic of diphenyether-type diarylheptanoids, and coupling patterns due to two sets of 1,3,4-trisubstituted aromatic groups. However, no carbonyl signal was observed in the

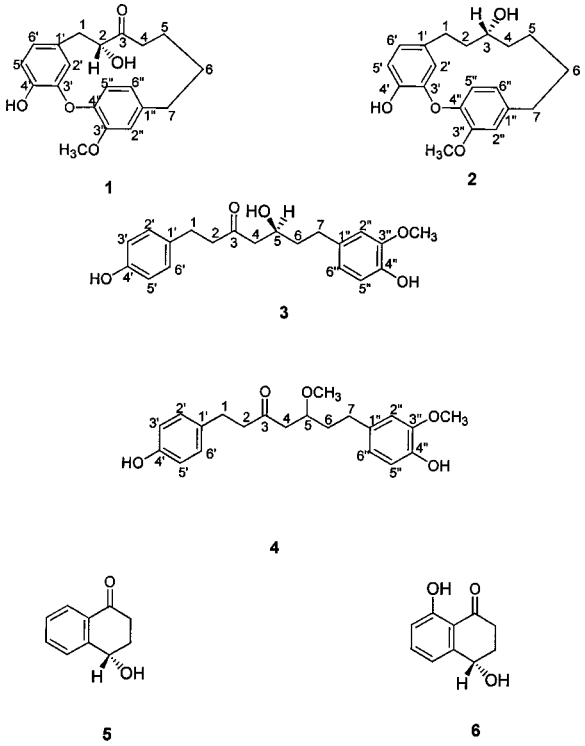

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Table 1. Characteristic ^{1}H-NMR Data of Mosher Esters of **1**, **2**, and **3** for Determination of Stereochemistry

Position	$\mathbf{1}_S$ δ_S	$\mathbf{1}_R$ δ_R	$\Delta\delta$ $\delta_S-\delta_R$	Position	$\mathbf{2}_S$ δ_S	$\mathbf{2}_R$ δ_R	$\Delta\delta$ $\delta_S-\delta_R$	Position	$\mathbf{3}_S$ δ_S	$\mathbf{3}_R$ δ_R	$\Delta\delta$ $\delta_S-\delta_R$
1	3.60	3.39	+0.21	2	1.68	1.78	−0.10	4	2.80	2.77	+0.03
	3.24	3.10	+0.14						2.59	2.49	+0.10
2	5.36	5.41	*R*	3	4.56	4.64	*R*	5	5.51	5.53	*S*
4	1.71	1.89	−0.18	4	1.51	1.38	+0.13	6	1.90	1.99	−0.09
	1.37	1.74	−0.37		1.15	0.93	+0.22				

^{13}C-NMR spectrum of **2** as seen in that of **1**. Cross-peaks in the ^{1}H–^{1}H COSY spectrum indicated the connectivities from H-1 to H-7 on the aliphatic chain. The location of the methoxyl group was identified by both a cross-peak between the C-3″ and C-3″-OCH_3 in the heteronuclear multiple bond connectivity (HMBC) spectrum and a NOE effect between H-2″ and C-3″-OCH_3 in the nuclear Overhauser effect spectroscopy (NOESY) spectrum of **2**. To determine the absolute configuration of the hydroxyl group at C-3, Mosher ester derivatives ($\mathbf{2}_R$, $\mathbf{2}_S$) of **2** were prepared, and ^{1}H-NMR data were also assigned based on the ^{1}H–^{1}H COSY spectra (Table 1). For **2**, 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 in compound **2**.

^{1}H- and ^{13}C-NMR data of **3** were identical with those of the reported compound (5*R*)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1(4-hydroxyphenyl)-3-heptanone from an other plant.[14] However, optical rotation value ($[\alpha]_D^{25}$ −2.52°) of **3** was different from that of literature ($[\alpha]_D^{25}$ +1.05°).[14] To determine the absolute configuration of the hydroxyl group at C-5, Mosher ester derivatives ($\mathbf{3}_R$, $\mathbf{3}_S$) of **3** were prepared, and ^{1}H-NMR data of $\mathbf{3}_R$ and $\mathbf{3}_S$ were also assigned based on the ^{1}H–^{1}H COSY spectra (Table 1). For **3**, the positive value of $\Delta\delta_H(\delta_S-\delta_R)$ at H-4 and the negative value of $\Delta\delta_H(\delta_S-\delta_R)$ at H-5 suggested a *S* configuration at C-5 in compound **3** and the structure of **3** was determined to be (5*S*)-5-hydroxy-7-(4-hydroxy-3methoxyphenyl)-1(4-hydroxyphenyl)-3-heptanone.

Compound **4** had the molecular formula $C_{21}H_{26}O_5$ as determined from its HR-FAB-MS, ^{13}C-NMR, and DEPT spectral data. The ^{1}H-NMR spectrum of **4** showed signals for a 1,3,4-trisubstituted and a 1,4-disubstituted aromatic group. The ^{13}C-NMR spectrum of **4** exhibited a total of 21 carbon signals, including characteristic signals due to a carbonyl group (C-3) and two sets of chemically equivalent aromatic carbons (C-2′, C-6′ and C-3′, C-5′). The ^{1}H–^{1}H COSY spectrum of **4** showed 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 **4**, the connectivities among two aromatic rings and 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′ and the location of the carbonyl group in the chain was established by the correlation of C-3 with H-1, H-2, and H4. The positions of two methoxyl groups on C-5 and C-3″ of **4** were determined based on both HMBC correlations between C-5 and C-5-OCH_3 and between C-3″ and C-3″-OCH_3 and NOE correlations in the NOESY spectrum: that between C-3″-OCH_3 and H-2″ and those between C-5-OCH_3 and H-4, H-6, and H-7. Absolute configuration on the chiral center of **4** was not determined.

Compounds **5** [(*S*)-(+)-4-hydroxytetralone] and **6** [(−)-regiolone] were reported from other plants and identified by comparison of physical and spectroscopic data (optical rotation values, ^{1}H- and ^{13}C-NMR) with those in the literature.[5,15,16]

Only **4** among these compounds showed weak cytotoxicities against the HT-29 and MCF-7 cell lines (IC_{50}: 41.3 μg/ml and >50 μg/ml, respectively).

Experimental

General Experimental Procedures Optical rotations were measured using a JASCO DIP-1000 (Tokyo, Japan) automatic digital polarimeter. FT-IR spectra were recorded on a JASCO FT-IR 300E spectrophotometer, and UV spectra on a JASCO V-550 spectrophotometer. The NMR spectra were recorded on Bruker 250 MHz (DMX 250) spectrometer using Bruker's standard pulse program. Samples were dissolved in either chloroform-d_1 or acetone-d_6, and chemical shifts were reported in ppm downfield from TMS. The FAB-MS spectra were measured with a VG TRIO 2A mass spectrometer. Stationary phases for column chromatography (Silica gel 60, 70—230 and 270—400 mesh and Lichroprep Rp-18 gel, 40—63 μm, Merck) and TLC plates (Si-gel 60 F_{254} and Rp-18 F_{254}) were purchased from EM Scientific. Spots were detected under UV radiation and by spraying with 10% H_2SO_4, followed by heating. For preparative HPLC, a LC-10AD pump (Shimadzu), SPD-10A detector (Shimadzu), and Shim-Pack Preparative ODS (20×250 mm) column were used. All other chemicals and solvents were of analytical grade and used without further purification.

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

Extraction and 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) and partitioned between H_2O and 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, Si-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. Fraction 6 (5.7 g) was chromatographed on a silica gel column (60×4 cm, Si-gel 70—230 mesh) with *n*-hexane–EtOAc (gradient from 85 : 15 to 10 : 90). The major fraction 7 from the column was further purified on a reverse-phase column (75×2.6 cm, LiChroprep Rp-18) with MeOH–H_2O (gradient from 1 : 9 to 9 : 1), affording **3**, **5**, and **6**. Fraction 7 (4.3 g) was chromatographed on a silica gel column (60×4 cm, Si-gel 70—230 mesh) with *n*-hexane–EtOAc (gradient from 40 : 60 to 10 : 90) and the subfraction 3 from the column was further purified on a reverse-phase column (60×2 cm, LiChroprep Rp-18) with MeOH–H_2O (gradient from 2 : 8 to 9 : 1), affording **1**, **2**, and **4**.

Compound **1**: Brown solid (14 mg); $[\alpha]_D^{25}$ −81.13° (*c*=0.03, MeOH); UV (MeOH) λ_{max} (log ε) 282.2 (3.53); IR (KBr) ν_{max} 3447, 2925, 1637, 1595, 1508, 1383, 1270, 1030 cm^{-1}; ^{1}H-NMR (acetone-d_6, 250 MHz) δ 7.05 (1H, d, *J*=1.9 Hz, H-2″), 7.00 (1H, d, *J*=8.0 Hz, H-5″), 6.87 (1H, dd, *J*=8.0, 1.9 Hz, H-6″), 6.71 (1H, d, *J*=8.1 Hz, H-5′), 6.55 (1H, dd, *J*=8.1, 2.1 Hz, H-6′), 5.66 (1H, d, *J*=2.1 Hz, H-2′), 4.01 (1H, dd, *J*=6.9, 2.4 Hz, H-2), 3.69 (3H, s, 3″-OCH_3), 2.94 (1H, dd, *J*=14.8, 2.4 Hz, H-1a), 2.83 (1H, dd, *J*=14.8, 6.9 Hz, H-1b), 2.72 (2H, t, *J*=6.1 Hz, H-7), 1.93 (1H, m, H-4a), 1.75—1.40 (5H, m, H-6a, -4b, -6b, -5a, -5b); ^{13}C-NMR (acetone-d_6, 62.9 MHz) δ 211.8 (C-3), 152.6 (C-3″), 148.4 (C-3′), 145.2 (C-4′), 144.7 (C-4″), 140.3 (C-1″), 128.1 (C-1′), 124.6 (C-5″), 124.0 (C-6′), 122.1 (C-6″), 115.8 (C-2″), 115.8 (C-5′), 115.7 (C-2′), 76.4 (C-2), 55.7 (3″-OCH_3), 41.4 (C-4), 37.5 (C-1), 36.1 (C-7), 27.9 (C-6), 20.0 (C-5); HR-FAB-MS *m/z*

343.1542 (Calcd for $C_{20}H_{23}O_5$ $[M+H]^+$, 343.1545).

Compound **2**: Brown oil (27 mg); $[\alpha]_D^{25}$ −39.07° (c=0.21, MeOH); UV (MeOH) λ_{max} (log ε) 278.6 (3.60); IR (KBr) ν_{max} 3420, 2926, 1594, 1515, 1455, 1265, 1151, 1119, 1031, 835 cm^{-1}; ^{1}H-NMR (acetone-d_6, 250 MHz) δ 7.05 (1H, d, J=8.0 Hz, H-5″), 6.99 (1H, d, J=1.8 Hz, H-2″), 6.91 (1H, dd, J=8.0, 1.8 Hz, H-6″), 6.72 (1H, d, J=8.0 Hz, H-5′), 6.53 (1H, dd, J=8.0, 1.8 Hz, H-6′), 5.71 (1H, d, J=1.8 Hz, H-2′), 3.65 (3H, s, 3″-OCH_3), 3.00 (1H, m, H-3), 2.76—2.40 (4H, m, H-7a, -1a, -1b, -7b), 1.74 (1H, m, H-6a), 1.52 (1H, m, H-6b), 1.40 (2H, m, H-2), 1.27—1.00 (3H, m, H-4a, -5a, -5b), 0.82 (1H, m, H-4b); ^{13}C-NMR (acetone-d_6, 62.9 MHz) δ 153.1 (C-3″), 149.3 (C-3′), 144.5 (C-4″), 144.4 (C-4′), 141.8 (C-1″), 134.0 (C-1′), 124.8 (C-5″), 122.8 (C-6′), 122.7 (C-6″), 116.7 (C-2″), 116.4 (C-5′), 114.0 (C-2′), 71.5 (C-3), 56.4 (3″-OCH_3), 39.7 (C-4), 37.5 (C-2), 36.1 (C-7), 31.1 (C-6), 29.1 (C-1), 23.4 (C-5); HR-FAB-MS m/z 329.1756 (Calcd for $C_{20}H_{25}O_4$ $[M+H]^+$ 329.1753).

Compound **3**: Yellow oil (10 mg); $[\alpha]_D^{25}$ −2.52° (c=0.09, MeOH), lit.[14] $[\alpha]_D^{25}$ +1.05° (c=0.80, EtOH); ^{1}H- and ^{13}C-NMR data are consistent with literature values;[14] HR-FAB-MS m/z 345.1699 (Calcd for $C_{20}H_{25}O_5$ $[M+H]^+$ 345.1702).

Compound **4**: Brown oil (15 mg); $[\alpha]_D^{21}$ −7.1° (c=0.42, MeOH); UV (MeOH) λ_{max} (log ε) 224.2 (3.99), 280.6 (3.48); IR (KBr) ν_{max} 3426, 2931, 1700, 1654, 1617, 1509, 1457, 1364, 1270, 1032, 825 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 6.99 (2H, d, J=8.4 Hz, H-2′/H-6′), 6.80 (1H, d, J=8.0 Hz, H-5″), 6.70 (2H, d, J=8.4 Hz, H-3′/H-5′), 6.64 (1H, s, H-2″), 6.63 (1H, d, J=8.0 Hz, H-6″), 3.84 (3H, s, 3″-OCH_3), 3.67 (1H, m, H-5), 3.28 (3H, s, 5-OCH_3), 2.79 (2H, t, J=6.3 Hz, H-1), 2.69 (2H, t, J=6.1 Hz, H-2), 2.66 (1H, d, J=15.8 Hz, H-4a), 2.59 (2H, m, H-7), 2.41 (1H, dd, J=15.8, 5.3 Hz, H-4b), 1.75 (2H, m, H-6); ^{13}C-NMR ($CDCl_3$, 62.9 MHz) δ 209.0 (C-3), 154.0 (C-4′), 146.3 (C-3″), 143.6 (C-4″), 133.7 (C-1″), 132.8 (C-1′), 129.3 (C-2′/C-6′), 120.8 (C-6″), 115.2 (C-3′/C-5′), 114.2 (C-5″), 110.9 (C-2″), 76.6 (C-5), 57.0 (5-OCH_3), 55.8 (3″-OCH_3), 47.3 (C-4), 45.6 (C-2), 36.0 (C-6), 31.0 (C-7), 28.6 (C-1); HR-FAB-MS m/z 359.1860 (Calcd for $C_{21}H_{27}O_5$ $[M+H]^+$ 359.1858).

Compound **5**: Brown oil (16 mg); $[\alpha]_D^{25}$ +6.35° (c=0.35, MeOH), lit.[15] $[\alpha]_D^{27}$ +23.5° (c=0.7, $CHCl_3$); ^{1}H- and ^{13}C-NMR data are consistent with literature values;[16] positive FAB-MS m/z 163.0 ($C_{10}H_{10}O_2$ $[M+H]^+$).

Compound **6**: Brown amorphous powder (40 mg); $[\alpha]_D^{25}$ −0.85° (c=0.51, MeOH), lit.[5] $[\alpha]_D$ −3.3° (c=0.077, EtOH); ^{1}H- and ^{13}C-NMR data are consistent with literature values;[5] positive FAB-MS m/z 179.0 ($C_{10}H_{10}O_3$ $[M+H]^+$).

Preparation of Mosher Esters A previously described method was used.[17,18] To each 1 mg of **1**, **2**, 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. 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 ($\mathbf{1}_S$, $\mathbf{2}_S$, $\mathbf{3}_S$) of **1**, **2**, and **3**, respectively. Using (*S*)-MTPA chloride afforded the *R*-Mosher esters ($\mathbf{1}_R$, $\mathbf{2}_R$, $\mathbf{3}_R$) of **1**, **2**, and **3**, respectively. Their ^{1}H-NMR chemical shifts are given in Table 1.

Cytotoxicity Bioassays 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.[19]

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