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 H₂O and hexane and the resulting H₂O layer was extracted with CHCl₃. The CHCl₃ 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**, C₂₀H₂₂O₅, was established from high resolution (HR)-FAB-MS, ¹³C-NMR, and distortionless enhancement by polarization transfer (DEPT) spectral data. The ¹³C-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 ¹H-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 ¹H-¹H 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''-OCH₃

in the HMBC and a positive NOE effect between H-2'' and C-3''-OCH₃ 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 ¹H-NMR chemical shifts of (*S*)- and (*R*)-MTPA ester derivatives (**1_R**, **1_S**). ¹H-NMR data were assigned based on the ¹H-¹H COSY spectra of **1_R** and **1_S** (Table 1). For **1**, the positive value of Δδ_H(δ_S–δ_R) at H-1 and the negative value of Δδ_H(δ_S–δ_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 C₂₀H₂₄O₄ as determined from HR-FAB-MS and NMR data. The ¹H-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 diphenylether-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 ^1H -NMR Data of Mosher Esters of **1**, **2**, and **3** for Determination of Stereochemistry

Position	1_S δ_S	1_R δ_R	$\Delta\delta$ $\delta_S - \delta_R$	Position	2_S δ_S	2_R δ_R	$\Delta\delta$ $\delta_S - \delta_R$	Position	3_S δ_S	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	<i>R</i>	3	4.56	4.64	<i>R</i>	5	5.51	5.53	<i>S</i>
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 ^1H - ^1H 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"-OCH₃ in the heteronuclear multiple bond connectivity (HMBC) spectrum and a NOE effect between H-2" and C-3"-OCH₃ 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 (2_R , 2_S) of **2** were prepared, and ^1H -NMR data were also assigned based on the ^1H - ^1H COSY spectra (Table 1). For **2**, the negative value of $\Delta\delta_{\text{H}}(\delta_S - \delta_R)$ at H-2 and the positive value of $\Delta\delta_{\text{H}}(\delta_S - \delta_R)$ at H-4 suggested a *R* configuration at C-3 in compound **2**.

^1H - 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 another plant.¹⁴⁾ However, optical rotation value ($[\alpha]_{\text{D}}^{25} -2.52^\circ$) of **3** was different from that of literature ($[\alpha]_{\text{D}}^{25} +1.05^\circ$).¹⁴⁾ To determine the absolute configuration of the hydroxyl group at C-5, Mosher ester derivatives (3_R , 3_S) of **3** were prepared, and ^1H -NMR data of 3_R and 3_S were also assigned based on the ^1H - ^1H COSY spectra (Table 1). For **3**, the positive value of $\Delta\delta_{\text{H}}(\delta_S - \delta_R)$ at H-4 and the negative value of $\Delta\delta_{\text{H}}(\delta_S - \delta_R)$ 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-3-methoxyphenyl)-1(4-hydroxyphenyl)-3-heptanone.

Compound **4** had the molecular formula $\text{C}_{21}\text{H}_{26}\text{O}_5$ as determined from its HR-FAB-MS, ^{13}C -NMR, and DEPT spectral data. The ^1H -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 ^1H - ^1H 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 H-4. 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-OCH₃ and between C-3" and C-3"-OCH₃ and NOE correlations in the NOESY spectrum: that between C-3"-OCH₃ and H-2" and those between C-5-OCH₃ 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** [(*-*)-re-

giolone] were reported from other plants and identified by comparison of physical and spectroscopic data (optical rotation values, ^1H - 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 $\mu\text{g}/\text{ml}$ and >50 $\mu\text{g}/\text{ml}$, 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 F₂₅₄ and Rp-18 F₂₅₄) were purchased from EM Scientific. Spots were detected under UV radiation and by spraying with 10% H₂SO₄, 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 H₂O and hexane. The resulting H₂O layer was extracted with CHCl₃ and the CHCl₃ solution was evaporated to dryness *in vacuo*. The CHCl₃ 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 H₂O (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-H₂O (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-H₂O (gradient from 2:8 to 9:1), affording **1**, **2**, and **4**.

Compound 1: Brown solid (14 mg); $[\alpha]_{\text{D}}^{25} -81.13^\circ$ ($c=0.03$, 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} ; ^1H -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"-OCH₃), 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"-OCH₃), 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); $[\alpha]_D^{25} -39.07^\circ$ ($c=0.21$, 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} ; 1H -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''-OCH₃), 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''-OCH₃), 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); $[\alpha]_D^{25} -2.52^\circ$ ($c=0.09$, MeOH), lit.¹⁴ $[\alpha]_D^{25} +1.05^\circ$ ($c=0.80$, EtOH); 1H - 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); $[\alpha]_D^{21} -7.1^\circ$ ($c=0.42$, 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} ; 1H -NMR (CDCl₃, 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''-OCH₃), 3.67 (1H, m, H-5), 3.28 (3H, s, 5-OCH₃), 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 (CDCl₃, 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-OCH₃), 55.8 (3''-OCH₃), 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); $[\alpha]_D^{25} +6.35^\circ$ ($c=0.35$, MeOH), lit.¹⁵ $[\alpha]_D^{27} +23.5^\circ$ ($c=0.7$, CHCl₃); 1H - 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); $[\alpha]_D^{25} -0.85^\circ$ ($c=0.51$, MeOH), lit.⁵ $[\alpha]_D -3.3^\circ$ ($c=0.077$, EtOH); 1H - 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 CH₂Cl₂ 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–CH₂Cl₂ (1:3). The elute was dried, CH₂Cl₂ (5 ml) was added, and the CH₂Cl₂ was washed using 1%

NaHCO₃ (5 ml×2) and H₂O (5 ml×2). The washed elute was dried *in vacuo* to give the *S*-Mosher esters (**1_S**, **2_S**, **3_S**) of **1**, **2**, and **3**, respectively. Using (*S*)-MTPA chloride afforded the *R*-Mosher esters (**1_R**, **2_R**, **3_R**) of **1**, **2**, and **3**, respectively. Their 1H -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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