Two New Diarylheptanoids from *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,[1-17] and these compounds have been shown cytotoxic activity, topoisomerases I and II inhibitory activity, inhibitory effect on DNA polymerase and on the RNase H activity of HIV-1 reverse transcriptase.[12-17] In the continuation of our studies on this plant,[10-17] we isolated two new diarylheptanoids (**1** and **2**) from the $CHCl_3$ fraction of the MeOH extract. This paper described the structural determination of two new diarylheptanoids (**1** and **2**) on the basis of spectroscopic studies, and the absolute configuration of **1** was elucidated by Mosher's ester method.

Two diarylheptanoids (**1** and **2**) 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** had the molecular formula $C_{20}H_{26}O_5$ as determined from the HREIMS, ^{13}C-NMR and DEPT spectral data. In the aromatic region of the ^{1}H-NMR spectrum, ^2J coupling between H-5" and H-6" and ^3J coupling between H-2" and H-6" indicated an 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

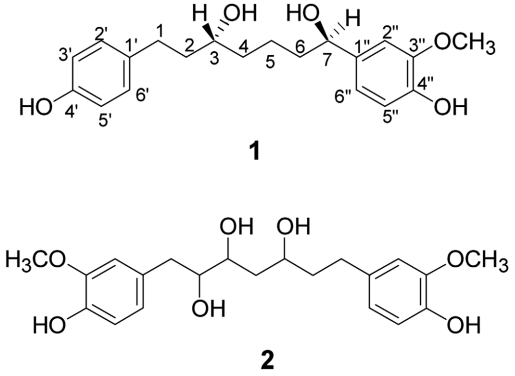

Figure 1. Diarylheptanoids isolated from the roots of *Juglans mandshurica*.

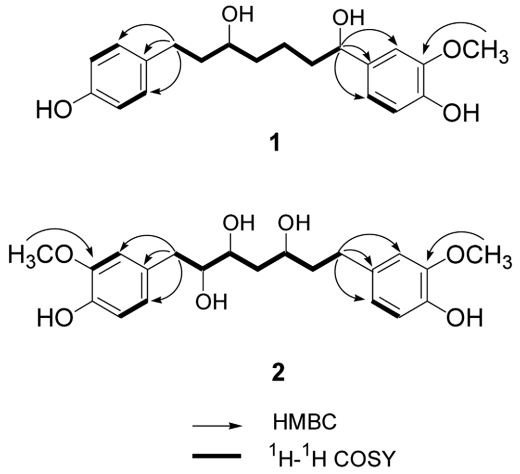

Figure 2. HMBC and ^{1}H-^{1}H COSY correlations of compounds **1** and **2**.

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, two hydroxymethine and five 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, the connectivities of the two aromatic rings with the alkyl chain were indicated by the cross peaks between H-7 and C-

Table 1. Characteristic ^{1}H-NMR data of Mosher esters of **1** for determination of stereochemistry

Position	$\mathbf{2}_S$ δ_S	$\mathbf{2}_R$ δ_R	$\Delta\delta$ δ_S-δ_R
2	1.83	1.92	−0.11
3	5.02	4.96	***R***
4	1.51	1.44	+0.07
5	1.91	1.82	+0.09
	1.74	1.68	+0.06
7	5.78	5.69	***R***
2"	6.98	7.03	−0.05
6"	6.81	6.84	−0.03

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 of **1** was determined by both the HMBC correlation of C-3" with 3"-OCH_3, and the positive NOE effect (6.3%) between H-2" and 3"-OCH_3.[17,18]

The absolute stereochemistry of the chiral center in **1** was determined using the Mosher's ester methodology 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 $\mathbf{1}_R$ derivatives (Table 1). The negative value of $\Delta\delta_H$ (δ_S-δ_R) at H-2 and the positive value of $\Delta\delta_H$ (δ_S-δ_R) at H-4 suggested a *R* configuration at C-3, and the positive value of $\Delta\delta_H$ (δ_S-δ_R) at H-5 and the negative values of $\Delta\delta_H$ (δ_S-δ_R) at H-2" and H-6" suggested a *R* configuration at C-7. It was reported that, in a case of determination of the absolute configuration on alpha carbon from an aromatic group (C-7), chemical shift differences of protons [$\Delta\delta_H$ (δ_S-δ_R)] at beta position from the aromatic group (C-6) could not be used for due to inconsistency of them.[19]

Compound **2** had the molecular formula $C_{21}H_{28}O_7$ determined from the HREIMS, ^{13}C-NMR and DEPT spectral data. The ^{1}H-NMR spectrum of **2** showed signals for two sets of 1,3,4-trisubstituted aromatic rings and two methoxyl groups. The ^{13}C-NMR and DEPT spectra of **2** exhibited a total of 21 carbon signals, including three hydroxymethine groups, four methylene and twelve aromatic carbons. The ^{1}H-^{1}H COSY spectrum showed the connectivities among H-1, H-2, H-3, H-4, H-5, H-6 and H-7, between H-5' and H-6', and between H-5" and H-6". In the HMBC spectrum, 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 two methoxyl groups on the two aromatic rings were determined by the HMBC correlation of C-3" with 3"-OCH_3, and that of C-3' with 3'-OCH_3. In the NOESY spectrum, NOE cross peaks between 3"-OCH_3 and H-2" and between 3'-OCH_3 and H-2' were also observed. However, absolute configuration at chiral center of **2** could not be determined because of the shortage of material.

Compounds **1** and **2** showed neither cytotoxicity against HT-29 and MCF-7 cell lines (IC_{50} of **1** and **2** were more than 100 μM) nor inhibitory activity on both DNA topoisomerases I and II at a concentration of 100 μM.[16]

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, and UV spectra using on a JASCO V-550 spectrophotometer. NMR spectra were recorded on a Bruker 250 MHz (DMX 250) spectrometer using Bruker's standard pulse program. Samples were dissolved in CD_3OD, and chemical shifts were reported in ppm downfield from TMS. The MS spectra were measured by a VG TRIO 2A mass spectrometer. For preparative HPLC, and LC-10AD pump (Shimadzu), SPD-10A detector (Shimadzu) and Shim-Pack Prep-ODS (20 × 250 mm) column were used. Silica gel 60 (70-230 and 270-400 mesh, Merck), Lichroprep RP-18 gel (40-63 μm, Merck), and TLC plate (Silica-gel 60 F_{254} and RP-18 F_{254}) were 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. The 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. The material was confirmed taxonomically by Professor G. H. Bae, at Chungnam National University in Daejeon, Republic of Korea. A voucher specimen has been deposited at the College of Pharmacy, Yeungnam University.

Isolation. The roots of *J. mandshurica* (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 *n*-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 20% to 100% MeOH), which afforded 22 subfractions (F8-1–8-22). Subfraction F8-6 (160 mg) from the column was further purified on a reverse-phase column (60 × 2.0 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 20% to 100% MeOH), affording **1** and **2**. Further purifications of **1** and **2** were carried out using HPLC with MeOH-H_2O gradients.

Compound 1: brown solid (12 mg), $[\alpha]_D^{25}$ –42.19° (*c* = 0.168, MeOH); UV (MeOH) λ_{max} (log ε) 224.8 (4.04), 278.6 (3.50); IR (KBr) ν_{max} 3422, 2931, 1718, 1515, 1457, 1375, 1267, 1075, 1036, 821 cm^{-1}; ^{1}H-NMR (CD_3OD, 250 MHz) δ6.87 (2H, d, *J* = 8.4 Hz, H-2'/H-6'), 6.83 (1H, d, *J* = 1.6 Hz, H-2"), 6.68 (1H, dd, *J* = 8.1, 1.6 Hz, H-6"), 6.62 (1H, d, *J* = 8.1 Hz, H-5"), 6.55 (2H, d, *J* = 8.4 Hz, H-3'/H-5'), 4.15 (1H, m, H-7), 3.73 (3H, s, 3'-OCH_3), 3.31 (1H, m, H-3), 2.51 (2H, m, H-1), 1.75-1.16 (8H, m, H-5a, -2, -6a, -5b, -4a, -6b, -4b); ^{13}C-NMR (CD_3OD, 62.9 MHz) δ156.3 (C-4'), 148.7 (C-3"), 146.8 (C-4"), 136.3 (C-1"), 134.3 (C-1'), 130.3 (C-2'/C-6'), 119.9 (C-6"), 116.0 (C-3'/C-5'), 115.7 (C-5"), 110.0 (C-2"), 81.2 (C-7), 78.3 (C-3), 56.3 (3"-OCH_3), 39.6 (C-2), 34.3 (C-6), 32.4 (C-4), 31.8 (C-1), 25.1 (C-5); HREIMS *m/z* 328.1685 ([M – H_2O]$^+$, calcd. for $C_{20}H_{24}O_4$, 328.1675).

Compound 2: brown solid (6 mg), $[\alpha]_D^{25}$ +15.91° (*c* = 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 280.6 (3.76); IR (KBr) ν_{max} 3385, 2928, 1602, 1509, 1457, 1381, 1273, 1124,

1032, 820 cm^{-1}; ^{1}H-NMR (CD_3OD, 250 MHz) δ6.72 (1H, d, J = 1.7 Hz, H-2'), 6.66 (1H, d, J = 1.7 Hz, H-2"), 6.64 (1H, d, J = 7.8 Hz, H-5'), 6.57 (1H, d, , J = 8.0 Hz, H-5"), 6.56 (1H, dd, , J = 1.7, 7.8 Hz, H-6'), 6.48 (1H, dd, J = 1.7, 8.0 Hz, H-6"), 4.01 (1H, m, H-5), 3.70 (3H, s, 3'-OCH_3), 3.69 (3H, s, 3"-OCH_3), 3.66 (3H, m, H-5, -3, -2), 2.72-2.42 (4H, m, H-1, -7), 1.61 (4H, m, H-4, -6); ^{13}C-NMR (CD_3OD, 62.9 MHz) ä 148.7 (C-3'/C-3"), 145.7 (C-4'), 145.4 (C-4"), 135.2 (C-1'), 132.0 (C-1"), 128.8 (C-6'), 121.7 (C-6"), 116.1 (C-5"), 115.9 (C-5'), 114.1 (C-2'), 113.1 (C-2"), 76.3 (C-2), 72.8 (C-3), 70.6 (C-5), 56.3 (3'-OCH_3/3"-OCH_3), 40.9 (C-4), 40.8 (C-6), 40.0 (C-1), 32.3 (C-7); HREIMS *m/z* 392.1829 ($[M]^+$, calcd. for $C_{21}H_{28}O_7$, 392.1835).

Preparation of Mosher's Esters. To each 1 mg of **1** 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.[20,21] The mixture was left at room temperature overnight and purified over a micro-column (0.6 × 6 cm) of silica gel (230-400 mesh) eluted with 3-4 mL of *n*-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's ester ($\mathbf{1}_S$) of **1**. Using (*S*)-MTPA chloride afforded the *R*-Mosher's ester ($\mathbf{1}_R$) of **1**. Their ^{1}H-NMR chemical shifts are given in Table 1.

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