Cytotoxic Diarylheptanoids from the Roots of *Juglans mandshurica*

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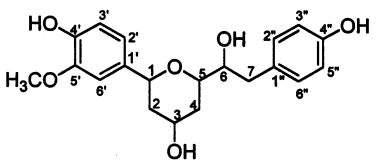
A new (**2**) and three known diarylheptanoids (**1**, **3**, and **4**), along with one known sesquiterpenoid (**5**), were isolated from the roots of *Juglans mandshurica*, and their structures were elucidated on the basis of spectroscopic studies. Four of these compounds (**2**−**5**) exhibited moderate cytotoxicities against human colon carcinoma and human lung carcinoma cell lines with IC_{50}'s ranging from 2 to 25 μg/mL.

The roots of *Juglans mandshurica* Maximowicz (Juglandaceae) have been used as a folk medicine for treatment of cancer in Korea.[1] Several naphthoquinones and naphthalenyl glucosides from *Juglans* species have been reported.[2−8] 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 one diarylheptanoid glucoside.[9−12] In this paper, we report a new diarylheptanoid along with three known diarylheptanoids and one known sesquiterpene from the roots of *J. mandshurica*.

The MeOH extract of the roots of *J. 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. The three major fractions were chromatographed on a reversed-phase column, which afforded compounds **1**−**5**. Compound **2** had the molecular formula $C_{20}H_{22}O_4$, determined from its HR-FABMS. The ^{13}C NMR spectrum exhibited a total of 20 carbon signals including characteristic signals due to a carbonyl group (C3) and two pairs of chemically equivalent aromatic carbons (C2′, C6′ and C3′, C5′). The $^1H-^1H$ COSY spectrum showed the connectivities among H4, H5, H6, and H7, between H1 and H2, and between H2′ (H6′) and H3′ (H5′). In the HMBC spectrum of **2**, the correlation of C3 with H1, H2, H4, and H5 established the location of the carbonyl group in the chain, and the correlation of C3″ with C3″-OCH_3 determined the position of a methoxyl group on the aromatic ring. The 1D NOE difference experiment of **2**, irradiating the methoxyl group, showed a positive NOE enhancement of the H2″ signal but no enhancement of other proton signals. The connectivity among two aromatic rings and the alkyl chain were indicated by the cross-peaks between H7 and C1″, C2″, and C6″ and those between H1 and C1′, C2′, and C6′.

Compounds **1**, **3**, **4** (galleon), and **5** (eudesmane-4α,11-diol) were reported from other plants and identified by comparison of physical and spectroscopic data (optical rotation values, UV, IR, ^1H and ^{13}C NMR) with literature values.[13−17] Compounds **2**, **3**, **4**, and **5** showed moderate cytotoxicities against human colon carcinoma and human lung carcinoma cell lines (Table 1).



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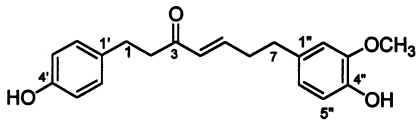

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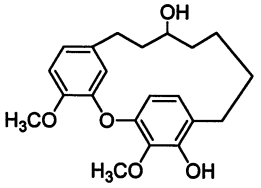

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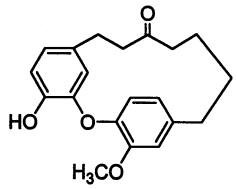

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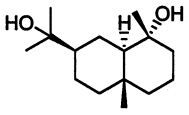

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Table 1. IC_{50} Values of Compounds against HT-29 and A549 Cell Lines

	IC_{50} (μg/mL)	
	HT-29[a]	A549[b]
1	>50	>50
2	8.2 ± 0.62	5.4 ± 0.01
3	23.9 ± 2.09	11.5 ± 0.39
4	5.3 ± 1.14	2.2 ± 0.12
5	12.9 ± 1.08	9.9 ± 0.44
altromycin B	3.9 ± 0.98	4.2 ± 0.54

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

Experimental Section

General Experimental Procedures. The NMR spectra were recorded on Bruker 250 MHz (DMX 250), Bruker 300 MHz (ARX 300), and Bruker 600 MHz (DMX 600) spectrometers. Samples were dissolved in either chloroform-d_1 or CD_3-OD, and chemical shifts were reported in ppm downfield from

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TMS. The 2D NMR spectra were recorded by using Bruker's standard pulse program. The FABMS spectra were measured by VG TRIO 2A mass spectrometer. Si gel 60 (70−230 and 270−400 mesh), reversed-phase support (LiChroprep RP-18), and TLC plates (Si gel 60 F_{254}) were purchased from EM Scientific. All other chemicals and solvents were analytical grade and used without further purification.

Plant Materials. Roots of *Juglans mandshurica* were collected in September 1993 in 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.

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 Si 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%). Eluent was combined on the basis of TLC, giving 17 fractions. Fraction 6 (5.7 g) was chromatographed on a Si gel column (60 × 4 cm, Si gel 70−230 mesh) with *n*-hexane−EtOAc (gradient from 85:15 to 10:90). Fractions 3, 8, and 10 were each further purified on reversed-phase columns (75 × 2.6 cm, LiChroprep RP-18) with MeOH−H_2O (gradient from 2:8 to 8:2), affording **3** from fraction 3, **1**, **2**, and **4** from fraction 8, and **5** from fraction 10, respectively.

Compound 1: brown powder (14 mg); mp 104−107 °C; $[\alpha]^{25}_D$ 69.5° (*c* 0.18, MeOH) {lit.[13] $[\alpha]^{12}_D$ 97° (*c* 0.3, MeOH)}; UV, IR (KBr), ^{1}H and ^{13}C NMR data are consistent with literature values;[13] negative FABMS *m/z* 359, 297, 148, 146; HRFABMS *m/z* 360.1570 ($[M]^+$, calcd for $C_{20}H_{24}O_6$, 360.1573).

Compound 2: colorless oil (12 mg); UV ($CHCl_3$) λ_{max} (log ϵ) nm 240 (3.99), 281 (3.57); IR ν_{max} (KBr) cm^{-1} 3382, 2933, 1652, 1515; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.00 (2H, d, J = 8.2 Hz, H-2′/ H-6′), 6.82 (1H, s, H-5″), 6.80 (1H, d, J = 13.2 Hz, H-5), 6.72 (2H, d, J = 8.2 Hz, H-3′/ H-5′), 6.63 (1H, s, H-2″), 6.62 (1H, s, H-6″), 6.08 (1H, d, J = 13.2 Hz, H-4), 5.76 (1H, brs, OH), 5.59 (1H, brs, OH), 3.83 (3H, s, 3″-OCH_3), 2.84 (2H, t, J = 6.7 Hz, H-1), 2.80 (2H, t, J = 6.7 Hz, H-2), 2.67 (2H, t, J = 7.3 Hz, H-7), 2.47 (2H, ddd, J = 7.2, 7.2, 7.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 200.3 (C-3), 154.1 (C-4′), 146.8 (C-5), 146.4 (C-3″), 143.9 (C-4″), 132.9 (C-1′), 132.6 (C-1″), 130.6 (C-4), 129.4 (C-2′/C-6′), 120.9 (C-6″), 115.3 (C-3′/ C-5′), 114.3 (C-5″), 110.9 (C-2″), 55.9 (3″-OCH_3), 41.9 (C-2), 34.4 (C-6), 34.1 (C-7), 29.3 (C-1); positive FABMS (%) *m/z* $[M + 1]^+$ 327 (20), $[M]^+$ 326 (15), 185 (12), 137 (100) 107 (40); HRFABMS *m/z* 326.1522 ($[M]^+$, calcd for $C_{20}H_{22}O_4$, 326.1518).

Compound 3: white powder (16 mg); $[\alpha]^{25}_D$ −74.23° (*c* 0.2, $CHCl_3$) {lit.[14] $[\alpha]^{15}_D$ −58.5° (*c* 0.3, $CHCl_3$)}; UV, IR (KBr), ^{1}H and ^{13}C NMR data are consistent with literature values;[14] HRFABMS *m/z* 358.1770 ($[M]^+$, calcd for $C_{21}H_{26}O_5$, 358.1780).

Compound 4: white powder (10 mg); UV, IR (KBr), ^{1}H and ^{13}C NMR data are consistent with literature values;[15,16] HRFABMS *m/z* 326.1522 ($[M]^+$, calcd for $C_{20}H_{22}O_4$, 326.1518).

Compound 5: white powder (20 mg); $[\alpha]^{25}_D$ −17.06° (*c* 0.2, $CHCl_3$) {lit.[17] $[\alpha]^{25}_D$ −25.3° (*c* 1.1)}; IR (KBr), ^{1}H and ^{13}C NMR data are consistent with literature values;[17] HRFABMS *m/z* $[M]^+$ 240.1933 (calcd for $C_{15}H_{28}O_2$, 240.1959).

Cytotoxicity Bioassays. The tetrazolum-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.[18]

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Supporting Information Available: Spectra of ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and ROSEY spectra of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Kim, Y. I.; Lee, S. H.; Cho, T. S. *Kor. J. Pharmacogn*. **1996**, *27*, 238−245.
(2) Binder, R. G.; Benson, M. E.; Flash, R. A. *Phytochemistry* **1989**, *28*, 2799−2801.
(3) Gupta, S. R.; Ravindranath, B.; Seshadri, T. R. *Phytochemistry* **1972**, *11*, 2634−2636.
(4) Muller, W.-U.; Leistner, E. *Phytochemistry* **1978**, *17*, 1739−1742.
(5) Hirakawa, K.; Ogiue, E.; Motoyoshiya, J.; Yajima, M. *Phytochemistry* **1986**, *25*, 1494−1495.
(6) Talpatra, S. K.; Karmacharta, B.; De, S. C.; Talapatra, B. *Phytochemistry* **1988**, *27*, 3929−3932.
(7) Pardhasaradhi, M.; Hari Babu, M. *Phytochemistry* **1978**, *17*, 2042−2043.
(8) Hedin, P. A.; Collum, D. H.; Langhans, V. E.; Grave, C. H. *J. Agric. Food Chem*. **1980**, *28*, 340−342.
(9) Joe, Y. K.; Son, J. K.; Park, S. H.; Lee, I. J.; Moon, D. C. *J. Nat. Prod*. **1996**, *59*, 159−160.
(10) Son, J. K. *Arch. Pharm. Res*. **1995**, *18*, 203−205.
(11) Kim, S. H.; Lee, K. S.; Son, J. K.; Je, G. H.; Lee J. S.; Lee, C. H.; Cheong, C. J. *J. Nat. Prod*. **1998**, *61*, 643−645.
(12) Lee, S. W.; Lee, K. S.; Son, J. K. *Planta Med*. **2000**, *66*, 184−186.
(13) Jiang, Z. H.; Tanaka, T.; Hirata, H.; Fukuoka, R.; Kouno, I. *Phytochemistry* **1966**, *43*, 1049−1054.
(14) Jiang, Z.; Tanaka, T.; Inutsuka, C.; Kouno, I. *Chem. Pharm. Bull*. **2001**, *49*, 737−740.
(15) Malterud, K. E.; Anthosen, T.; Hjortas, J. *Tetrahedron Lett*. **1976**, *35*, 3069−3072.
(16) Morihara, M.; Sakurai, N.; Inoue, T.; Kawai, K.; Nagai, M. *Chem. Pharm. Bull*. **1997**, *45*, 820−823.
(17) Ando, M.; Arai, K.; Kikuchi, K.; Isogai, K. *J. Nat. Prod*. **1994**, *57*, 1189−1199.
(18) 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.

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