Two New Lignans from the Stem Bark of *Magnolia obovata* and Their Cytotoxic Activity

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Two new lignans, 4-methoxymagnaldehyde B (1) and coumanolignan (2), were isolated from the stem bark of *Magnolia obovata*, together with 11 known compounds (3—13). The structures of compounds 1 and 2 were determined to be 5′-allyl-2′-hydroxyphenyl-4-methoxy-3-cinnamic aldehyde (1) and 6-allyl-8-(5′-allyl-2′-hydroxyphenyl)coumarin (2) on the basis of spectroscopic and physicochemical analyses including 2D NMR and high-resolution EI-MS. Compounds 1—8, 11, 12, and 13 were tested *in vitro* for their cytotoxic activities against the HeLa, A549, and HCT116 cancer cell lines. Among the compounds tested, compound 1 showed the strongest cytotoxic activity against the HCT116 cancer cell line, with an IC_{50} value of 1.3 μg/ml.

Key words *Magnolia obovata*; Magnoliaceae; 4-methoxymagnaldehyde B; coumanolignan; cytotoxic activity

The stem bark of *Magnolia obovata* THUNB. (Magnoliaceae) has been used as traditional medicine for the treatment of gastrointestinal disorders, anxiety, and allergic diseases including bronchial asthma in Korea, Japan, and China.[1)] Previous chemical studies have revealed a variety of neolignans, sesquiterpenes, sesquiterpene-neolignans, phenylpropanoids, and alkaloids. These compounds were reported to possess muscle relaxation,[2)] central depressant,[3)] antigastric ulcer,[4)] vasorelaxant,[5)] antiallergic,[6)] antibacterial,[7,8)] and neurotrophic activities.[9)] As a part of our continuing study on cytotoxic compounds from natural sources, two new lignans along with 11 known compounds were isolated from *M. obovata*. This paper deals with the isolation and structure elucidation of these compounds, as well as their cytotoxic activity against the HeLa (cervical epitheloid carcinoma), A549 (human nonsmall lung), and HCT116 (human colorectal carcinoma) cancer cell lines.

Repeated chromatography of the hexane- and EtOAc-soluble fractions of MeOH extracts from the stem bark of *M. obovata* on silica gel and YMC-pack RP-C_{18} columns led to the isolation of 13 compounds (**1**—**13**). Among them, 11 known compounds were identified, as follows: fargesone C (**3**),[10)] 4-methoxyhonokiol (**4**),[11)] magnolol (**5**),[12)] honokiol (**6**),[12)] obovatol (**7**),[13)] syringin (**8**),[14)] β-sitosterol (**9**),[15)] daucosterol (**10**),[15)] magnaldehyde B (**11**),[12)] magnolignan C (**12**),[12)] and magnaldehyde E (**13**).[12)]

Compound **1**, 4-methoxymagnaldehyde B, was obtained as a yellowish oil and its molecular formula of $C_{19}H_{18}O_3$ was established by the molecular ion peak at *m*/*z* 294.1257 $[M]^+$ in the HR-EI-MS. The IR absorption band at 1675 and 1625 cm^{-1} suggested the presence of an α,β-unsaturated carbonyl group. The ^{1}H- and ^{13}C-NMR spectra of **1** were quite similar to those of 4-methoxyhonokiol (**4**), except for the presence of an α,β-unsaturated aldehyde group in **1**. Long-range correlations between δ_H 7.92 (H-7) and δ_C 159.0 (C-4), and δ_H 6.84 (H-8) and δ_C 123.6 (C-3) indicated that an α,β-unsaturated aldehyde group was located at C-3 instead of an allyl group in 4-methoxyhonokiol (**4**) (Fig. 2). On the basis of the above evidence, compound **1** was established as 5′-allyl-2′-hydroxyphenyl-4-methoxy-3-cinnamic aldehyde.

Compound **2** was obtained as an amorphous powder. The molecular formula of **2** was deduced to be $C_{21}H_{18}O_3$, on the basis of the peak at *m*/*z* 318.1258 $[M]^+$ (Calcd for $C_{21}H_{18}O_3$, 318.1256) in the HR-EI-MS. The ^{1}H-NMR spectrum of **2** showed signals for two olefenic protons at δ 6.42 (d, J=9.4 Hz) and 7.71 (d, J=9.4 Hz), and two aromatic protons at δ 7.32 (d, J=2.4 Hz) and 7.38 (d, J=2.4 Hz), and indicated the presence of 6,8-disubstituted coumarin, compared with that of synthesized 6,8-dimethylcoumarin.[16)] In addition, it showed signals for allyl protons at δ 3.48 (d, J=6.9 Hz), 5.12 (m), and 5.98 (m) in the ^{1}H-NMR spectrum. Long-range correlations between δ_H 3.48 (H-9) and δ_C 127.4 (C-5)/134.7 (C-7), and δ_H 5.98 (H-10) and δ_C 136.7 (C-6) in the HMBC spectrum, indicated that the allyl group was attached at C-6 of the coumarin moiety. Furthermore, the ^{1}H-NMR spectrum exhibited signals for an allyl proton at δ 3.38 (d, J=6.9 Hz), 5.12 (m) and 5.98 (m), and a set of ABX type aromatic protons at δ 6.89 (1H, d, J=8.1 Hz), 7.05 (1H, d, J=2.1 Hz) and 7.10 (1H, dd, J=2.1, 8.1 Hz), which were assignable to a *para*-allylphenyl group, compared with that of magnolol (**5**). This result was further confirmed by the ^{13}C-NMR spectral assignments (a methylene carbon at δ 39.5, two olefinic carbons at δ 115.9 and 136.5, and six aromatic carbons at δ 116.8, 123.0, 130.2, 131.3, 132.6 and 151.6) coupled to DEPT and 2D NMR. The connectivity of the two partial structures determined on the basis of the HMBC correlations between δ_H 7.38 (H-7) and δ_C 123.0 (C-1′), and δ_H 7.05 (H-6′) and δ_C 126.8 (C-8), confirmed that the allylphenol was linked to the coumarin moiety by C-8 (Fig. 2). Therefore, the structure of **2** was elucidated as 6-allyl-8-(5′-allyl-2′-hydroxyphenyl)coumarin, and was named coumanolignan.

Compounds **1**—**8**, **11**, **12**, and **13** were tested *in vitro* for their cytotoxic activities against the HeLa, A549, and HCT116 cancer cell lines (Table 2). Compounds **4**—**7** and **11** showed moderate cytotoxicity against the HeLa, A549, and HCT116 cancer cell lines. Compounds **2**, **3**, **8**, **12**, and **13** showed no cytotoxic activities against the 3 cell lines. Among the compounds tested, compound **1** showed the strongest cytotoxic activity against the HCT116 cancer cell line, with an IC_{50} value of 1.3 μg/ml.

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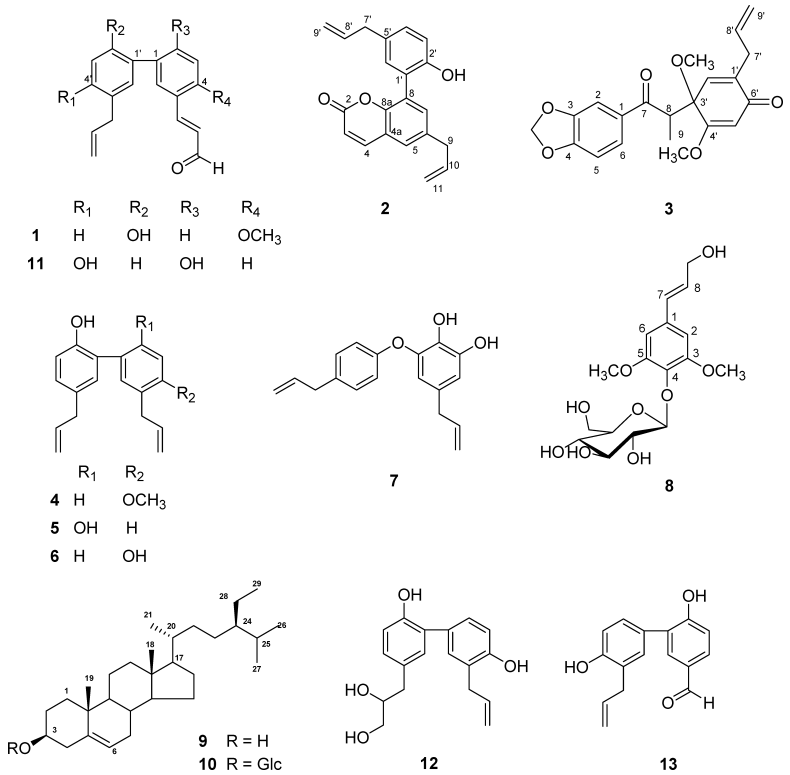

Fig. 1. Chemical Structures of Compounds **1**—**13** from Stem Bark of *M. obovata*

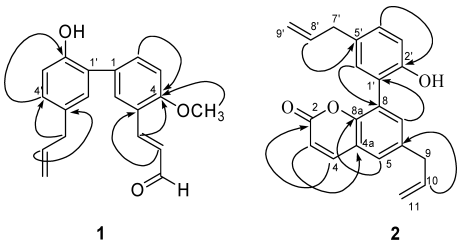

Fig. 2. Key HMBC Correlations of **1** and **2**

Table 2. Cytotoxicity of Compounds against Cultured HeLa, A549, and HCT116 Cancer Cell Lines

Compounds	IC_{50} (μg/ml)[a]		
	HeLa	A549	HCT116
1	8.2±0.7	7.3±1.1	1.3±0.3
2	>30[b]	>30	>30
3	>30	>30	>30
4	12.4±1.0	14.1±0.9	14.4±0.6
5	8.6±1.4	7.7±1.2	12.2±1.5
6	11.1±1.2	11.2±0.7	11.4±0.7
7	15.8±2.2	8.1±1.0	16.4±1.7
8	>30	>30	>30
11	9.1±1.4	19.2±0.8	14.5±2.0
12	>30	>30	>30
Adriamycin[c]	0.8±0.1	1.2±0.1	0.7±0.1

a) IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number and the results are means±standard deviation of three independent replicates. *b*) The IC_{50} greater than 30 μg/ml was considered to be no cytotoxicity. *c*) Positive control substance.

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data (δ) of Compounds **1** and **2** (in $CDCl_3$)

Carbon	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		133.2		
2	7.81 d (2.1)	131.4		160.8
3		123.6	6.42 d (9.4)	116.9
4		159.0	7.71 d (9.4)	143.9
4a				119.4
5	7.01 d (8.4)	112.4	7.32 d (2.4)	127.4
6	7.63 dd (2.1, 8.4)	135.2		136.7
7	7.92 d (16.2)	151.2	7.38 d (2.4)	134.7
8	6.89 dd (7.8, 16.2)	130.1		126.8
8a				150.1
9	9.62 d (7.8)	197.0	3.48 d (6.9)	39.5
10			5.98 m	137.8
11			5.12 m	117.1
1′		128.5		123.0
2′		153.8		151.6
3′	6.80 d (8.1)	117.1	6.89 d (8.1)	116.8
4′	6.96 dd (2.1, 8.1)	129.7	7.10 dd (2.1, 8.1)	130.2
5′		132.8		132.6
6′	7.06 d (2.1)	131.5	7.05 d (2.1)	131.3
7′	3.45 d (6.3)	40.5	3.38 d (6.9)	39.5
8′	5.97 m	139.6	5.98 m	136.5
9′	5.04 m	115.6	5.12 m	115.9
4-OCH_3	3.96 s	56.4		

Experimental

Melting points were measured by using an Electrothermal apparatus. Optical rotation was determined on a JASCO DIP-100 KUY polarimeter. UV spectra were obtained with a Beckman Du-650 UV/VIS recording spectrophotometer. IR spectra were recorded on a Jasco Report-100 infrared spectrometer. Mass were carried out with a JEOL JMS-700 Mstation mass spectrometer. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) were recorded on Bruker DRX300 and JEOL 400 spectrometers. Two-dimensional (2D) NMR experiments (HMBC) were recorded on a Bruker Avance 500 spectrometer. For column chromatography, silica gel (Kieselgel 60, 70—230 mesh and 230—400 mesh, Merck) was used. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F254 plates (0.25 mm, Merck).

Plant Material The dried stem bark of *Magnolia obovata* was purchased from Uchida Co., Ltd., Tokyo, Japan on March 2005. The plant was identified by one of the authors, K. Bae. The voucher specimen (CNU-594) was deposited at the herbarium of the College of Pharmacy, Chungnam National University, Daejeon, Korea.

Extraction and Isolation The dried stem bark of *Magnolia obovata* (20 kg) was extracted with methanol (MeOH) three times under reflux for 4 h. The MeOH solutions were combined, filtrated, and concentrated to yield a MeOH extract (4000 g). The MeOH extract (4000 g) was suspended in distilled water and fractionated with hexane, EtOAc and BuOH to give hexane (1000 g), EtOAc (1200 g) and BuOH-soluble fractions (800 g), successively. The hexane-soluble fraction was chromatographed over a silica gel column eluting with hexane–EtOAc (100 : 0 to 50 : 50) to afford nine fractions (H1—H9). Fraction H2 was chromatographed on a silica gel column eluting with hexane–EtOAc (100 : 1 to 50 : 1) to give **9** (700 mg). Fraction H3 was chromatographed on a silica gel column eluting with hexane–EtOAc (100 : 1 to 20 : 1) to give compound **4** (8.5 g). Fraction H4 was chromatographed on a silica gel column eluting with hexane–EtOAc (100 : 1 to 20 : 1) to give compound **7** (5 g). Fraction H9 was subjected to a silica gel column eluting with hexane–EtOAc (50 : 1 to 10 : 1) to give three subfractions (H9.1—H9.3). Subfraction H9.2 was subjected to a silica gel column eluting with hexane–EtOAc (50 : 1 to 10 : 1) to give **5** (80 g) and **6** (50 g). The EtOAc-soluble fraction was chromatographed over a silica gel column eluting with $CHCl_3$–MeOH (100 : 1 to 2 : 1) to afford nine fractions (E1—E9). Subfraction E4 was chromatographed over a silica gel column eluting with $CHCl_3$–MeOH (20 : 1 to 10 : 1) to give **10** (200 mg). Subfraction E6 was chromatographed over a silica gel column eluting with $CHCl_3$–MeOH (20 : 1 to 10 : 1) to give three subfractions (E6.1—E6.3). Subfraction E6.1 was subjected to HPLC [YMC-pack ODS-A, MeOH–H_2O (40 : 60)] to yield **2** (4 mg, t_R 60 min), **3** (2 mg, t_R 80 min), and **1** (8 mg, t_R 100 min), respectively. Subfraction E6.3 was subjected to a silica gel column using $CHCl_3$–MeOH (20 : 1 to 5 : 1) to give **11** (300 mg), **12** (200 mg), and **13** (30 mg). Fraction E8 was subjected to a silica gel column using $CHCl_3$–MeOH (20 : 1 to 5 : 1) to give four subfractions (E8.1—E8.4). Subfraction H8.2 was chromatographed on a silica gel column eluting with hexane–EtOAc (50 : 1 to 10 : 1) to obtain crude powder, which was purified with crystallization in $CHCl_3$ to give compound **8** (500 mg).

4-Methoxymagnaldehyde B (**1**): Yellowish oil. UV λ_{max} nm (log ε): 265 (4.3), 300 (4.3). IR ν_{max} cm^{-1}: 3450, 1675, 1625, 1600. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz): see Table 1. HR-EI-MS *m*/*z*: 294.1257 $[M]^+$ (Calcd for $C_{19}H_{18}O_3$: 294.1256).

Coumanolignan (**2**): Amorphous powder. mp 156—158 °C. UV λ_{max} nm (log ε): 240 (4.5), 290 (3.9). IR ν_{max} cm^{-1}: 3430, 1705, 1620. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz): see Table 1. HR-EI-MS *m*/*z*: 318.1258 $[M]^+$ (Calcd for $C_{21}H_{18}O_3$: 318.1256).

Cytotoxicity Assay The cancer cell lines (HeLa, A549, and HCT116) were maintained in RPMI 1640 that included L-glutamine (JBI) with 10% FBS (JBI) and 2% penicillin–streptomycin (GIBCO). Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxicity was measured using a modified MTT assay. Viable cells were seeded in the growth medium (180 μl) into 96-well microtiter plates (1×10^4 cells per well) and incubated at 37 °C in a 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to final sample concentrations ranging from 5.0 to 100 μM by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to $<0.1\%$. After standing for 2 h, 20 μl of the test sample was added to each well. The same volume of DMSO was added to the control wells. Forty-eight hours after the test sample was added, MTT 20 μl was also added to each well (final concentration, 5 μg/ml). Two hours later, the plate was centrifuged for 5 min at 1500 rpm, the medium was removed, and the resulting formazan crystals were dissolved with DMSO 150 μl. The optical density (O.D.) was measured at 570 nm using a Titertek microplate reader (Multiskan MCC/340, Flow). The IC_{50} value was defined as the concentration of sample which reduced absorbance by 50% relative to the vehicle-treated control.

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