Cytotoxic Lignans from the Stem Bark of *Magnolia officinalis*

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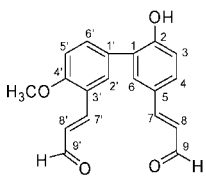
Three new lignans, 4′-methoxymagndialdehyde (**1**), 4′-methoxymagnaldehyde B (**2**), and 4′-methoxymagnaldehyde E (**3**), were isolated from hexane- and EtOAc-soluble fractions of the stem bark of *Magnolia officinalis,* together with eight known compounds (**4**–**11**). The structures of compounds **1**–**3** were determined on the basis of spectroscopic and physicochemical data analysis. Compounds **1**–**11** were tested in vitro for their cytotoxic activity against the K562, HeLa, and A549 cancer cell lines. Among the compounds tested, compound **1** showed the most potent cytotoxic activity against these cancer cell lines, with IC_{50} values of 3.9, 1.5, and 3.7 μg/mL, respectively.

The stem bark of *Magnolia officinalis* Rehd. et Wils. (Magnoliaceae) has been used as a traditional medicine for the treatment of gastrointestinal disorders, anxiety, and allergic diseases, including bronchial asthma, in Korea, mainland China, and Japan.[1] Chemical studies have revealed a variety of neo-lignans and alkaloids as constituents of the plant. These compounds were shown to display muscle relaxation,[2] a central depressant effect,[3] and antigastric ulcer,[4] vasorelaxant,[5] antiallergic,[6] antibacterial,[7,8] and neurotrophic activities.[9] In the course of a phytochemical study on *M. officinalis*, three new lignans (**1**–**3**) along with eight known compounds (**4**–**11**) were isolated. This paper deals with the isolation and structure elucidation of **1**–**3**, as well as the evaluation of **1**–**11** for cytotoxic activity against the HeLa (cervical epitheloid carcinoma), A549 (human nonsmall lung), and K562 (human lymphocytic leukemia) cancer cell lines.



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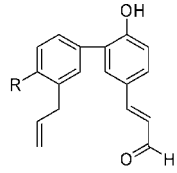

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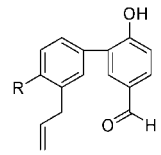
10 R = OH



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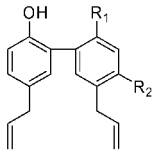
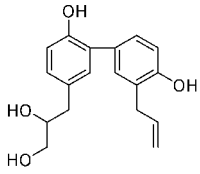
11 R = OH



	R_1	R_2
4	H	OCH_3
5	OH	H
6	H	OH



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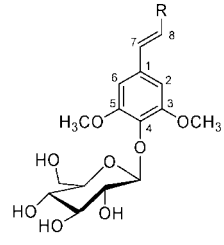

8 R = CH_2OH
9 R = CHO

Repeated chromatography of the hexane- and EtOAc-soluble fractions of the MeOH extract from the stem bark of *M. officinalis* led to the isolation of 11 lignans (**1**–**11**). Among them, eight were identified as the previously known compounds 4-methoxyhonokiol (**4**),[11] magnolol (**5**),[10] honokiol (**6**),[10] magnolignan C (**7**),[10] syringin (**8**),[12] synapic aldehyde 4-*O*-β-D-glucopyranoside (**9**),[13] magnaldehyde B (**10**),[10] and magnaldehyde E (**11**).[10]

Compound **1** was obtained as a yellow powder. Its molecular formula was deduced as sodiated molecular ion $C_{19}H_{16}O_4$, on the basis of the peak at *m/z* 331.0929 $[M + Na]^+$ (calcd for $C_{19}H_{16}O_4Na$, 331.0946) in the HREIMS. The IR spectrum showed the presence of an α,β-unsaturated carbonyl group at 1670 and 1625 cm^{-1}. This observation was further supported by ^1H NMR spectroscopic assignments, such as two sets of α,β-unsaturated aldehyde groups at δ 6.92 (1H, dd, $J = 7.8, 15.6$ Hz), 7.60 (1H, d, $J = 15.6$ Hz), and 9.85 (1H, d, $J = 7.8$ Hz) and at δ 7.13 (1H, dd, $J = 7.8, 16.2$ Hz), 7.97 (1H, d, $J = 16.2$ Hz), and 9.88 (1H, d, $J = 7.8$ Hz). In addition, the ^1H NMR spectrum of **1** showed two sets of ABX-type aromatic signals at δ 7.08 (1H, d, $J = 8.7$ Hz), 7.93 (1H, dd, $J = 2.4, 8.7$ Hz), and 8.19 (1H, d, $J = 2.4$ Hz) and at δ 7.31 (1H, d, $J = 8.4$ Hz), 7.62 (1H, dd, $J = 2.4, 8.4$ Hz), and 7.86 (1H, d, $J = 2.4$ Hz), which were assignable to a neo-lignan moiety, on comparison with data for magnaldehyde B (**10**).[10] Furthermore, the ^{13}C NMR spectrum of **1** exhibited the presence of 19 carbons with two oxygenated aromatic carbons at δ 158.3 and 159.6, four olefinic carbons at δ 126.9, 132.5, 148.6, and 153.0, and two aldehyde carbons at δ 193.9 and 194.7. Long-range correlations between δ_H 7.97 (H-7′)/7.13 (H-8′) with δ_C 123.7 (C-3′) and δ_H 7.60 (H-7)/6.92 (H-8) with δ_C 117.9 (C-5) indicated that two α,β-unsaturated aldehyde groups are located at C-3′ and C-5, respectively, instead of two allyl groups as in honokiol (**6**). Moreover, the ^1H NMR spectrum showed the presence of a methoxy group (δ_H 3.79), which correlated with the aromatic quaternary carbon at δ_C 158.4 (C-4′) in the HMBC spectrum (Figure 1) and indicated the position of this group to be at C-4′. On the basis of the above evidence, **1** was assigned as 2-hydroxy-4′-methoxy-3′,5-dicinnamic aldehyde and may be named 4′-methoxymagndialdehyde.

Compound **2** was obtained as a yellowish oil and established to have a molecular formula of $C_{19}H_{18}O_3$ by HREIMS. The IR absorptions bands at 1675 and 1625 cm^{-1} again suggested the presence of an α,β-unsaturated carbonyl group. The ^1H NMR spectrum of **2** showed two sets of ABX-type aromatic signals at δ 6.99 (1H, d, $J = 8.5$ Hz), 7.22 (1H, d, $J = 2.5$ Hz), and 7.28 (1H, dd, $J = 2.5, 8.5$ Hz) and at δ 7.02 (1H, d, $J = 8.5$ Hz), 7.49 (1H, dd, $J = 2.5, 8.5$ Hz), and 7.44 (1H, d, $J = 2.5$ Hz), signals for an allyl group at δ 3.44 (2H, d, $J = 6.5$ Hz), 5.09 (2H, m), and 6.00 (1H, m), and resonances for an α,β-unsaturated aldehyde group at δ 6.61 (1H, dd, $J = 7.5, 15.5$ Hz), 7.43 (1H, d, $J = 15.5$ Hz), and 9.65 (1H, d, $J = 7.5$ Hz) and for a methoxy group at δ 3.90 (3H, s). The ^{13}C NMR spectrum of **2** exhibited the presence of 19 carbons

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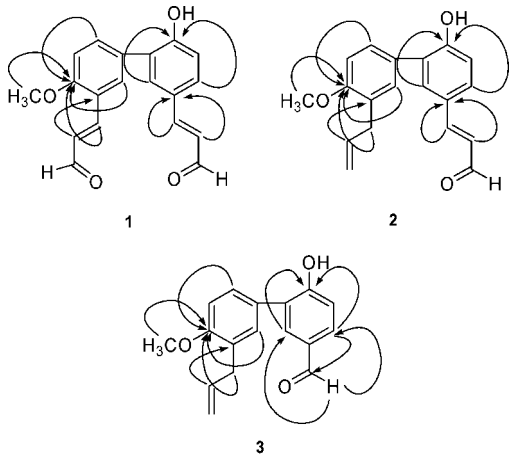

Figure 1. Key HMBC correlations of **1**–**3**.

Table 1. ^{1}H NMR Spectroscopic Data (δ) of Compounds **1**–**3**

proton	**1**[a]	**2**[b]	**3**[c]
3	7.31d (8.4)	7.02 d (8.5)	7.09 d (8.1)
4	7.62 dd (2.4, 8.4)	7.49 dd (2.5, 8.5)	7.77 dd (2.1, 8.1)
6	7.86 d (2.4)	7.44 d (2.5)	7.77 d (2.1)
7	7.60 d (15.6.	7.43 d (16.0)	10.52 s
8	6.92 dd (7.8, 15.6)	6.61 dd (7.5, 15.5)	
9	9.85 d (7.8)	9.65 d (7.5)	
2′	8.19 d (2.4)	7.22 d (2.5)	7.23 d (2.4)
5′	7.08 d (8.7)	6.99 d (8.5)	6.98 d (8.4)
6′	7.93 dd (2.4, 8.7)	7.28 dd (2.5, 8.5)	7.28 dd (2.4, 8.4)
7′	7.97 d (16.2)	3.44 d (6.5)	3.44 d (6.6)
8′	7.13 dd (7.8, 16.2)	6.00–6.02 m	5.94–6.08 m
9′	9.88 d (7.8)	5.09–5.12 m	5.07–5.13 m
OH-2		5.67 s	5.83 s
OCH_3-4′	3.79 s	3.90 s	3.90 s

[a] Spectrum recorded at 600 MHz in pyridine-d_5. [b] Spectrum recorded at 500 MHz in $CDCl_3$. [c] Spectrum recorded at 300 MHz in $CDCl_3$.

Table 2. ^{13}C NMR Spectroscopic Data (δ) of Compounds **1**–**3**

carbon	**1**[a]	**2**[b]	**3**[c]
1	129.5	129.0	128.9
2	130.3	131.1	158.3
3	126.9	127.1	116.4
4	130.2	129.6	131.3
5	117.9	116.6	130.3
6	159.6	155.7	132.5
7	153.0	153.0	191.2
8	132.5	126.8	
9	193.9	193.9	
1′	131.8	127.7	127.3
2′	131.1	130.5	130.5
3′	123.7	130.4	130.5
4′	158.4	157.7	157.8
5′	112.1	111.3	111.4
6′	134.6	128.0	128.0
7′	148.6	34.4	34.4
8′	126.9	136.4	136.4
9′	194.7	116.3	116.3
OCH_3	56.1	55.7	55.8

[a] Spectrum obtained at 150 MHz in pyridine-d_5. [b] Spectrum obtained at 125 MHz in $CDCl_3$. [c] Spectrum obtained at 75 MHz in $CDCl_3$.

with a methoxy group at δ 55.7, two oxygenated aromatic carbons at δ 155.7 and 157.7, a benzylic methylene signal at δ 34.4, two olefinic carbons at δ 116.3 and 136.4, and an aldehyde carbon at δ 193.9 (Tables 1 and 2). These ^{1}H and ^{13}C NMR spectroscopic features were similar to those of a known lignan compound, magnaldehyde B (**10**).[10] In comparison with **10**, compound **2** showed a methoxy proton signal (δ_H 3.90) that was correlated with an aromatic quaternary carbon at δ_C 157.7 (C-4′) in the HMBC experiment. Therefore, compound **2** (4′-methoxymagnaldehyde B) was assigned as 3′-allyl-2-hydroxy-4′-methoxyphenyl-5-cinnamic aldehyde.

Compound **3**, 4′-methoxymagnaldehyde E, was obtained as a yellowish oil, and its molecular formula of $C_{17}H_{16}O_3$ was established in the molecular ion peak at *m/z* 268.1098 $[M]^+$ in the HREIMS. The IR spectrum showed the presence of a hydroxyl group at 3400 cm^{-1}, an α,β-unsaturated carbonyl group at 1665 and 1620 cm^{-1}, and aromatic groups at 1605 cm^{-1}, which matched those of **2**. The ^{1}H and ^{13}C NMR spectra of **3** were quite similar to those of magnaldehyde E (**11**),[10] except for the presence of a methoxy group in **3**. In the HMBC spectrum of **3**, a correlation of the methoxy proton signal (δ_H 3.90) with δ_C 157.8 (C-4′) was observed, suggesting that the methoxy group is located at C-4′. This evidence led to the conclusion that **3** is 3′-allyl-6-hydroxy-4′-methoxybiphenyl-3-carbaldehyde.

Compounds **1**–**11** were tested in vitro for their cytotoxic activity against the HeLa, A549, and K562 tumor cell lines. Of these, compound **1** exhibited cytotoxic activity against these three cell lines, with IC_{50} values of 3.9, 1.5, and 3.7 μg/mL, respectively. All of the remaining compounds were inactive (IC_{50} >5 μg/mL) against all three cell lines. Unlike the structurally related lignans obtained in this investigation, compound **1** has two α,β-unsaturated aldehyde groups present in the molecule, and these appear to play an important role in mediating cytotoxic activity against the cancer cell lines tested.

Experimental Section

General Experimental Procedures. Melting points were measured by using an Electrothermal apparatus. UV spectra were obtained with a Beckman Du-650 UV–vis recording spectrophotometer. IR spectra were recorded on a JASCO Report-100 infrared spectrometer. ^{1}H NMR (300, 500, and 600 MHz) and ^{13}C NMR (75, 125, and 150 MHz) were recorded on Bruker DRX300 and JEOL 400 spectrometers (the chemical shifts were referenced to δ using TMS as an internal standard). Two-dimensional (2D) NMR experiments (HMQC and HMBC) were recorded on a Bruker Avance 500 spectrometer. Mass spectra were obtained with a JEOL JMS-700 Mstation mass 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 (0.25 mm, Merck).

Plant Material. The dried stem bark of *Magnolia officinalis* was purchased from a local market in Daejeon, Korea, in June 2005, and was identified by one of the authors (K.-H.B.). A voucher specimen (CNU 594) was deposited at the herbarium of the College of Pharmacy, Chungnam National University.

Extraction and Isolation. The dried stem bark of *M. officinalis* (5 kg) was extracted with methanol (MeOH) three times under reflux for 4 h. The MeOH solutions were combined, filtered, and concentrated to yield a dried MeOH extract (640 g). The MeOH extract (640 g) was suspended in distilled water and fractionated successively with hexane, EtOAc, and 1-BuOH to give hexane- (180 g), EtOAc- (270 g), and 1-BuOH-soluble fractions (80 g), respectively. 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 H3 was chromatographed on a silica gel column eluting with hexane–EtOAc (50:1 to 20:1) to give compound **4** (8.5 g). Fraction H7 was chromatographed on a silica gel column eluting with hexane–EtOAc (50:1 to 10:1) to obtain crude crystals, which were recrystallized from $CHCl_3$ to give compound **5** (15 g). Fraction H9 was chromatographed on a silica gel column eluting with hexane–EtOAc (50:1 to 10:1) to obtain crude crystals, which were recrystallized from $CHCl_3$ to give compound **6** (10 g). The EtOAc-soluble fraction was chromatographed over a silica gel column eluting with $CHCl_3$–MeOH (100:1 to 2:1) to afford 12 fractions (E1–E12). Fraction E1 was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (50:1 to 5:1) to afford four subfractions (E1.1–E1.4). Subfraction E1.2 was further purified by crystallization from MeOH to give compound **10** (500 mg). Subfraction E1.3 was subjected to HPLC [YMC-pack ODS-A, MeOH–H_2O (40:60)] to yield compounds **1** (5 mg, t_R 90 min), **2** (3 mg, t_R 100 min), and **3** (2 mg, t_R 120 min), respectively. Subfraction E1.4 was recrystallized from $CHCl_3$ to yield

11 (30 mg). Fraction E3 was chromatographed on a silica gel column using $CHCl_3$–MeOH (50:1 to 10:1) to afford three subfractions (E3.1–E3.3). Subfraction E3.2 was subjected to passage over a silica gel column, using hexane–EtOAc (20:1 to 5:1), to give **7** (600 mg). Fraction E5 was chromatographed on a silica gel column using $CHCl_3$–MeOH (50:1 to 10:1) to give **8** (500 mg) and **9** (2.5 mg).

4′-Methoxymagndialdehyde (1): yellow powder (MeOH–$CHCl_3$); mp 168−170 °C; UV (MeOH) λ_{max} (log ε) 294 (3.4), 333 (3.3) nm; IR (KBr) ν_{max} 3500, 1670, 1625, 1605 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HREIMS *m/z* 331.0929 $[M + Na]^+$ (calcd for $C_{19}H_{16}O_4Na$, 331.0946).

4′-Methoxymagnaldehyde B (2): yellowish oil; UV (MeOH) λ_{max} (log ε) 265 (4.3), 300 (4.3) nm; IR (KBr) ν_{max} 3450, 1675, 1625, 1600 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HREIMS *m/z* 294.1257 $[M]^+$ (calcd for $C_{19}H_{18}O_3$, 294.1256).

4′-Methoxymagnaldehyde E (3): yellowish oil; UV (MeOH) λ_{max} (log ε) 271 (4.3) nm; IR (KBr) ν_{max} 3400, 1665, 1620, 1605 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HREIMS *m/z* 268.1098 $[M]^+$ (calcd for $C_{17}H_{16}O_3$, 268.1099).

Cytotoxic Assay. The human tumor cell lines used in this study were purchased from KCLB (Korean Cell Line Bank). Cytotoxicity was determined against the HeLa (cervical epitheloid carcinoma), A549 (human nonsmall lung), and K562 (human lymphocytic leukemia) cancer cell lines using the MTT assay method. The cytotoxicity assays were performed according to a published procedure.[14] Adriamycin was used as a positive control and exihibited IC_{50} values of 1.41 ± 0.1, 0.8 ± 0.1, and 1.2 ± 0.1 μg/mL against the K562, HeLa, and A549 cell lines, respectively.

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