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Anti-complement Activity of Phenolic Compounds from the Stem Bark of *Magnolia obovata*

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Abstract – Five neolignans (**1** - **4**, **8**), two sesquiterpene-lignans (**5** - **6**), and two phenylpropanoids (**7**, **9**) were isolated from the stem bark of *Magnolia obovata* Thunberg (Magnoliaceae) by repeated column chromatography. The structures of isolated compounds were identified as 4-methoxyhonokiol (**1**), obovatol (**2**), magnolol (**3**), honokiol (**4**), eudeshonokiol B (**5**), eudesobovatol B (**6**), coumaric acid (**7**), magnaldehyde B (**8**), and *p*-coumaric acid (**9**) on the basis of spectroscopic analysis including 2D-NMR and MS data. Compounds **1** - **9** were evaluated for their anti-complement activities against the classical pathway of the complement system. Of them, compound **8** showed significant anti-complement activity on the classical pathway with IC_{50} value of 102.7 μM, whereas compounds **1** - **7** and **9** were inactive. This result indicated that an aldehyde group in the neolignan is important for the anti-complement activity against the classical pathway.
Keywords – *Magnolia obovata*, Magnoliaceae, neolignan, anti-complement activity

Introduction

The complement system has an essential role in innate immune defenses against infectious agents and the inflammatory process. Activation of the complement system provides efficient means to protect the host from the actions of invading antigens (Kirschfink, 1997). This complement system is activated by one of three different pathways including classical pathway, alternative pathway, and lectin pathway. Of these, the classical pathway is referred to antibody-dependent due to its activation by the binding of the first component C1 to antibodies bound to invasive agents (Ember and Hugli, 1997). However, if the complement system is activated in excess, it may cause a variety of inflammatory injuries such as allergies, osteoarthritis, atopic dermatitis, atherosclerosis, and rheumatoid arthritis (Kirschfink, 1997). Therefore, it is proposed that the modulation of complement activity could be useful in the therapeutic treatment of inflammatory diseases.

In our study for the anti-complement activities from natural sources, a methanol extract of the stem bark of *Magnolia obovata* Thunberg (Magnoliaceae) was examined. This species is an endemic to Northeast Asia and is important medicinal plant that has attracted considerable attention on account of their biological and chemical diversity. The stem bark of this plant has been used as traditional medicine for the treatment of gastrointestinal disorders, anxiety, and allergic diseases including bronchial asthma, in Korea, China, and Japan (Fujita *et al*., 1972). Previous chemical studies have revealed a variety of neolignans, sesquiterpenes, sesquiterpene-neolignans, phenylpropanoids, and alkaloids (Shoji *et al*., 1991). These compounds were shown to display muscle relaxation (Watanabe *et al*., 1975), central depressant effect (Watanabe *et al*., 1983), anti-gastriculcer (Watanabe *et al*., 1986), vasorelaxant (Yamahara *et al*., 1990), antiallergic (Hamasaki *et al*., 1999), antibacterial (Namba *et al*., 1982; Bae *et al*., 1998), and neurotrophic activities (Fukuyama *et al*., 1992). It has also been reported to exhibit a variety of bioactivities of major compounds, magnolol and honokiol, such as anti-inflammation (Wang *et al*., 1995), antimicrobial (Chang *et al*., 1998), and antitumor (Ikeda *et al*., 2003; Youn *et al*., 2007; Youn *et al*., 2008a; Youn *et al*., 2008b). This paper deals the isolation and structure elucidation of neolignans, sesquiterpene-lignans and phenylpropanoids from *M. obovata*, and anti-complement activity using the classical pathway of the complement system.

Experimental

General experimental procedures – Melting points were measured by using an Electrothermal apparatus and

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are not corrected. 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 and 400 MHz) and ^{13}C-NMR (75 and 100 MHz) were recorded on Bruker DRX300 and JEOL 400 spectrometers. Two-dimensional (2D) NMR spectra (^{1}H-^{1}H COSY, HMQC, and 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 F_{254} (0.25 mm, Merck) and RP-18 F_{254} S (0.25 mm, Merck), and spot were detected under an UV light and by spraying with 10% H_2SO_4.

Plant material – The dried stem bark of *M. obovata* was purchased from Uchida Co., Ltd., Tokyo, Japan on March 2005. The crude drug was identified by K. Bae, Chungnam National University, Daejeon, Republic of Korea. 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 *M. ovobata* (20 kg) was extracted with MeOH three times under reflux for 4 h. The MeOH solutions were combined, filtered, and concentrated to yield a dry MeOH extract (4 kg). The MeOH extract (4 kg) 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 on a silica gel column eluting with hexaneEtOAc (100 : 0 to 50 : 50) to yield nine fractions (H1 - H9). Fraction H3 was chromatographed on a silica gel column eluting with hexaneEtOAc (100 : 1 to 20 : 1) to give compound **1** (8.5 g). Fraction H4 was chromatographed on a silica gel column eluting with hexaneEtOAc (100 : 1 to 20 : 1) to afford compound **2** (5.0 g). Fraction H9 was chromatographed on a silica gel column eluting with hexaneEtOAc (50 : 1 to 10 : 1) to give three subfractions (H9.1 - H9.3). Subfraction H9.1 was chromatographed on a silica gel column eluting with hexaneEtOAc (50 : 1 to 10 : 1) to give compound **5** (100 mg) and **6** (50 mg). Subfraction H9.2 was chromatographed on a silica gel column eluting with hexaneEtOAc (50 : 1 to 10 : 1) to give compound **3** (80.0 g) and **4** (50.0 g). The EtOAc-soluble fraction was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (100 : 0 to 2 : 1) to afford twenty-five fractions (E1 - E25). Fraction E8 was subjected to a silica gel column eluting with $CHCl_3$-MeOH (20 : 1 to 10 : 1) to yield compound **7** (30 mg). Fraction E17 was subjected to a silica gel column eluting with $CHCl_3$-MeOH (20 : 1 to 10 : 1) to afford five subfracitons (E17.1 - E17.5). Subfraction E17.5 was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (20 : 1 to 5 : 1) to afford **8** (300 mg). Fraction E24 was subjected to a silica gel column eluting with hexaneacetone (50 : 1 to 10 : 1) to give six subfracitons (E24.1 - E24.6). Compound **9** (500 mg) was obtained by column chromatography eluting with hexane-EtOAc (50 : 1 to 5 : 1) and solvent recrystralization with $CHCl_3$ from subfraction E24.4.

4-Methoxyhonokiol (1) – colorless oil; UV (MeOH) λ_{max} nm: 225, 290; IR ν_{max} cm^{-1} (KBr): 3350, 1645, 1610; EIMS *m/z*: 280 $[M]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.08 (1H, d, J = 2.1 Hz, H-2), 6.91 (1H, d, J = 8.0 Hz, H-5), 7.06 (1H, dd, J = 8.0, 2.1 Hz, H-6), 3.35 (1H, d, J = 6.0, H-7), 6.01 (1H, m, H-8), 5.09 (1H, m, H-9), 6.96 (1H, d, J = 8.2, H-3'), 7.30 (1H, dd, J = 8.2, 2.5 Hz, H-4'), 7.26 (1H, d, J = 2.5 Hz, H-6'), 3.45 (1H, d, J = 6.0, H-7'), 6.01 (1H, m, H-8'), 5.15 (1H, m, H-9'), 3.90 (3H, s, OCH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 129.3 (C-1), 128.1 (C-2), 128.9 (C-3), 157.2 (C-4), 116.0 (C-5), 130.7 (C-6), 34.8 (C-7), 136.7 (C-8), 115.4 (C-9), 128.0 (C-1'), 151.0 (C-2'), 111.1 (C-3'), 129.9 (C-4'), 132.3 (C-5'), 130.4 (C-6'), 39.6 (C-7'), 138.0 (C-8'), 115.7 (C-9'), 55.7 (OCH_3).

Obovatol (2) – colorless oil; UV (MeOH) λ_{max} (log ε) nm: 272 (3.3); IR ν_{max} cm^{-1} (KBr): 3600, 1645, 1610, 1500; EIMS *m/z*: 282 $[M]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.57 (1H, d, J = 1.8 Hz, H-4), 6.29 (1H, d, J = 1.8 Hz, H-6), 3.19 (1H, d, J = 6.6, H-7), 5.92 (1H, m, H-8), 5.05 (1H, m, H-9), 7.14 (1H, d, J = 8.4, H-2'), 6.78 (1H, d, J = 8.4 Hz, H-3'), 6.78 (1H, d, J = 8.4 Hz, H-5'), 7.14 (1H, d, J = 8.4, H-6'), 3.36 (1H, m, H-7'), 5.92 (1H, m, H-8'), 5.04 (1H, m, H-9'); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 144.7 (C-1), 132.8 (C-2), 143.6 (C-3), 111.1 (C-4), 132.5 (C-5), 111.5 (C-6), 39.3 (C-7), 137.2 (C-8), 115.7 (C-9), 154.9 (C-1'), 117.8 (C-2'), 129.8 (C-3'), 133.3 (C-4'), 129.8 (C-5'), 117.8 (C-6'), 39.6 (C-7'), 137.4 (C-8'), 115.8 (C-9').

Magnolol (3) – brownish colorless plate; mp: 99 - 101 °C; UV (MeOH) λ_{max} nm; 208, 290; IR ν_{max} cm^{-1} (KBr): 3600, 1640, 1605; EIMS *m/z*: 266 $[M]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.94 (1H, d, J = 8.0 Hz, H-3), 7.15 (1H, dd, J = 8.0, 2.1 Hz, H-4), 7.10 (1H, d, J = 2.1 Hz, H-6), 3.37 (1H, d, J = 6.0, H-7), 5.94 (1H, m, H-8), 5.01 (1H, m, H-9), 6.94 (1H, d, J = 8.0, H-3'), 7.15 (1H, dd, J = 8.0, 2.1 Hz, H-4'), 7.10 (1H, d, J = 2.1 Hz, H-6'), 3.37 (1H, d, J = 6.0 Hz, H-7'), 5.94 (1H, m, H-8'), 5.01 (1H, m, H-9');

^{13}C-NMR (75 MHz, $CDCl_3$): δ 124.5 (C-1), 151.0 (C-2), 116.8 (C-3), 129.8 (C-4), 133.3 (C-5), 131.4 (C-6), 39.5 (C-7), 137.2 (C-8), 115.2 (C-9), 124.5 (C-1'), 151.0 (C-2'), 116.8 (C-3'), 129.8 (C-4'), 133.3 (C-5'), 131.4 (C-6'), 39.5 (C-7'), 137.2 (C-8'), 115.2 (C-9').

Honokiol (4) – white needle; mp: 87 - 88 °C; UV (MeOH) λ_{max} nm; 208, 253, 290; IR ν_{max} cm^{-1} (KBr): 3580, 1640, 1605; EIMS *m/z*: 266 [M]$^+$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.00 (1H, d, J = 2.1 Hz, H-3), 6.74 (1H, d, J = 8.0 Hz, H-5), 6.97 (1H, dd, J = 8.0, 2.1 Hz, H-6), 3.27 (1H, d, J = 6.6, H-7), 5.94 (1H, m, H-8), 5.10 (1H, m, H-9), 6.91 (1H, d, J = 8.2, H-3'), 7.15 (1H, dd, J = 8.2, 2.2 Hz, H-4'), 7.17 (1H, d, J = 2.2 Hz, H-6'), 3.36 (1H, d, J = 6.6 Hz, H-7'), 5.94 (1H, m, H-8'), 5.20 (1H, m, H-9'); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 126.5 (C-1), 130.3 (C-2), 128.8 (C-3), 153.9 (C-4), 116.5 (C-5), 128.6 (C-6), 35.0 (C-7), 136.1 (C-8), 115.7 (C-9), 127.7 (C-1'), 150.7 (C-2'), 116.8 (C-3'), 129.6 (C-4'), 132.4 (C-5'), 131.1 (C-6'), 39.4 (C-7'), 137.8 (C-8'), 115.8 (C-9').

Eudeshonokiol B (5) – Colorless oil; $[\alpha]_D^{25}$ –70.5° (*c* 1.05, MeOH); UV: λ_{max} 208, 256; EIMS *m/z*: 488 [M]$^+$; IR ν_{max} cm^{-1} (KBr): 3500, 1640, 1600, 1450; ^{1}H-NMR (300 MHz, $CDCl_3$): 2.17 (1H, d, J = 10.8 Hz, H-6α), 1.63 (1H, d, J = 10.8 Hz, H-6β), 0.84 (3H, s, H-11), 1.02 (3H, s, H-12), 1.32 (3H, s, H-13), 1.24 (3H, s, H-14), 7.16 (1H, d, J = 2.1 Hz, H-2'), 7.09 (1H, d, J = 6.9 Hz, H-3'), 7.14 (1H, dd, J = 6.9, 2.1 Hz, H-6'), 3.48 (2H, d, J = 6.0 Hz, H-7'), 5.92 (2H, m, H-8',8''), 5.02 (4H, m, H-9',9''), 6.99 (1H, d, J = 9.0 Hz, H-3''), 6.94 (1H, dd, J = 9.0, 2.4 Hz, H-4''), 7.11 (1H, d, J = 2.4 Hz, H-6''), 3.38 (2H, d, J = 6.0 Hz, H-7''); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 40.5 (C-1), 20.0 (C-2), 37.7 (C-3), 88.3 (C-4), 51.1 (C-5), 22.1 (C-6), 50.0 (C-7), 22.7 (C-8), 44.9 (C-9), 35.1 (C-10), 19.2 (C-11), 21.0 (C-12), 73.0 (C-13), 31.0 (C-14), 27.2 (C-15), 132.5 (C-1'), 132.7 (C-2'), 128.5 (C-3'), 152.1 (C-4'), 115.5 (C-5'), 129.4 (C-6'), 39.5 (C-7'), 138.3 (C-8'), 116.1 (C-9'), 136.6 (C-1''), 148.9 (C-2''), 124.7 (C-3''), 128.8 (C-4''), 134.9 (C-5''), 131.3 (C-6''), 39.7 (C-7''), (C-8''), 118.5 (C-9'').

Eudesobovatol B (6) – Colorless oil; $[\alpha]^{25}$ –20.5° (*c* 1.15, MeOH); UV: λ_{max} 205, 275; IR ν_{max} cm^{-1} (KBr): 3500, 1600; EIMS *m/z*: 504 [M]$^+$; IR (KBr) 3550, 1640, 1610 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.01 (1H, d, J = 12.0 Hz, H-6α), 1.38 (1H, d, J = 12.0 Hz, H-6β), 0.85 (3H, s, H-11), 1.32 (3H, s, H-12), 1.00 (3H, s, H-14), 1.02 (3H, s, H-15), 6.27 (1H, d, J = 2.0 Hz, H-3'), 6.57 (1H, d, J = 2.0 Hz, H-5'), 3.31 (2H, d, J = 6.8 Hz, H-7'), 5.82 (2H, m, H-8',8''), 4.98 (4H, m, H-9',9''), 6.79 (2H, d, J = 8.4 Hz, H-2'', 6''), 7.06 (2H, d, J = 8.4 Hz, H-3'', 5''), 3.19 (2H, d, J = 6.8 Hz, H-7''); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 40.7 (C-1), 20.4 (C-2), 38.6 (C-3), 87.7 (C-4), 53.1 (C-5),

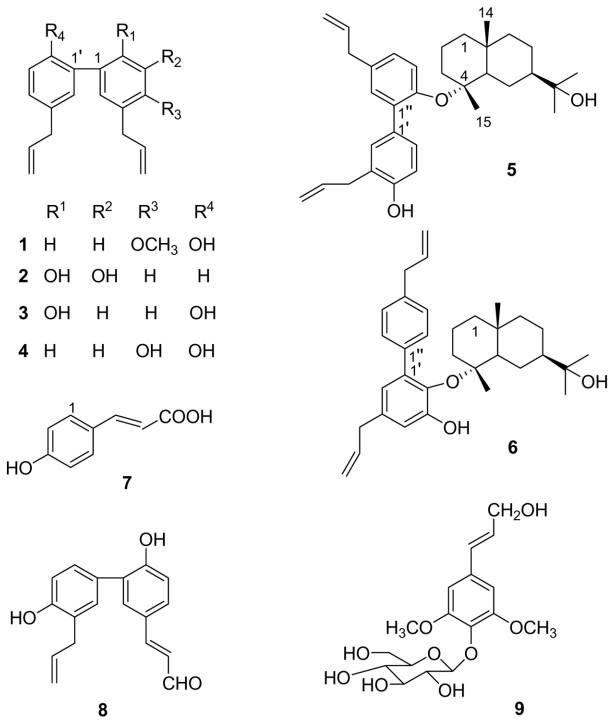

Fig. 1. Structures of compounds **1** - **9**.

21.9 (C-6), 49.7 (C-7), 22.6 (C-8), 45.1 (C-9), 35.2 (C-10), 19.3 (C-11) , 21.2 (C-12), 72.9 (C-13), 27.3 (C-14), 26.6 (C-15), 132.6 (C-1'), 152.4 (C-2'), 111.2 (C-3'), 136.8 (C-4'), 112.6 (C-5'), 150.5 (C-6'), 39.6 (C-7'), 137.1 (C-8'), 116.2 (C-9'), 156.0 (C-1''), 117.5 (C-2'', 6''), 129.8 (C-3'', 5''), 134.3 (C-4''), 39.9 (C-7''), 137.7 (C-8''), 115.9 (C-9'').

Coumaric acid (7) – White powder; mp: 145 - 147 °C; UV (MeOH) λ_{max} (log ε) nm: 236 (3.4), 320 (3.3); EIMS *m/z*: 164 [M]$^+$; IR ν_{max} cm^{-1} (KBr): 3250, 1605 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.42 (1H, d, J = 8.4 Hz, H-2), 6.79 (1H, d, J = 8.4 Hz, H-3), 6.79 (1H, d, J = 8.4 Hz, H-5), 7.42 (1H, d, J = 8.4, H-6), 7.57 (1H, d, J = 16.2, H-7), 6.25 (1H, d, J = 16.2 Hz, H-8); ^{13}C-NMR (75 MHz, CD_3OD): δ 127.3 (C-1), 131.2 (C-2), 116.9 (C-3), 161.2 (C-4), 116.9 (C-5), 131.2 (C-6), 146.8 (C-7), 115.7 (C-8), 171.1 (C-9).

Magaldehyde B (8) – yellowish powder; mp, 156 - 158 °C; UV (MeOH) λ_{max} (log ε) nm: 284 (3.40), 324 (3.30); IR ν_{max} cm^{-1} (KBr): 3500, 1670, 1625, 1600; EIMS *m/z*: 280 [M]$^+$; ^{1}H-NMR (300 MHz, CD_3OD): δ 6.80 (1H, d, J = 8.1 Hz, H-3), 7.22 (1H, dd, J = 8.1, 2.1 Hz, H-4), 7.26 (1H, d, J = 2.1 Hz, H-6), 7.51 (1H, d, J = 15.9, H-7), 6.01 (1H, dd, J = 7.8, 15.9 Hz, H-8), 9.50 (1H, d, J = 7.8 Hz, H-9), 7.46 (1H, d, J = 2.4, H-2'), 6.89 (1H, d, J = 8.4 Hz, H-5'), 7.42 (1H, dd, J = 8.4, 2.4 Hz, H-6'), 3.37 (1H, d, J = 6.6, H-7'), 6.01 (1H, m, H-8'), 5.01 (1H, m, H-9'); ^{13}C-NMR (75 MHz, CD_3OD): δ 127.6 (C-

1), 159.1 (C-2), 117.7 (C-3), 133.0 (C-4), 127.5 (C-5), 132.1 (C-6), 156.3 (C-7), 126.5 (C-8), 196.3 (C-9), 131.0 (C-1'), 129.3 (C-2'), 130.3 (C-3'), 155.6 (C-4'), 115.6 (C-5'), 129.8 (C-6'), 35.4 (C-7'), 138.5 (C-8'), 115.7 (C-9').

Syringin (9) – White powder; mp: 145 - 147 °C; UV (MeOH) λ_{max} (log ε) nm: 236 (3.4), 320 (3.3); EIMS *m/z*: 164 $[M]^+$; IR (KBr) 3250, 1605 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD): δ 6.76 (2H, s, H-2,6), 6.56 (1H, d, J = 15.9, H-7), 6.34 (1H, dd, J = 15.9, 5.4 Hz, H-8), 4.24 (1H, dd, J = 5.4, 1.2 Hz, H-9), 4.88 (1H, d, J = 7.2 Hz, H-1'), 3.20 - 3.40 (4H, m, H-2',3',4',5'), 3.67 (1H, dd, J = 12.0, 4.0 Hz, H-6'a), 3.80 (1H, dd, J = 12.0, 2.0 Hz, H-6'b), 3.87 (6H, s, OCH_3-3',7'); ^{13}C-NMR (75 MHz, CD_3OD): δ 136.0 (C-1), 105.5 (C-2), 154.5 (C-3), 135.3 (C-4), 154.5 (C-5), 105.5 (C-6), 131.3 (C-7), 130.2 (C-8), 63.7 (C-9), 105.6 (C-1'), 75.9 (C-2'), 78.5 (C-3'), 71.5 (C-4'), 78.0 (C-5'), 62.8 (C-6'), 57.1 (3',5'-OCH_3).

Classical pathway assay – Anti-complement activity was determined by modified method of Mayer as described previously (Min *et al*., 2003). For the classical pathway assay, a diluted solution of normal human serum (80 μL) collected from a healthy volunteer (Man) was mixed with gelatin veronal buffer (80 μL) with or without sample. Each sample was dissolved in DMSO, and used as a negative control. The mixture was pre-incubated at 37 °C for 30 min, and sensitized erythrocytes (sheep red blood cells, 40 μL) were added. After incubation under the same conditions, the mixture was centrifuged (4 °C, 1500 rpm) and the optical density of the supernatant (100 μL) measured at 405 nm. For the AP assay, rabbit erythrocytes (3.0×10^8 cells/mL) and optimally diluted serum (C/7.5 - C/5) in GVB containing 4 nM EGTA and 5 mM Mg^{++} (Mg-EGTA-GVB) were used for optimal hemolysis (APH_{50}). The overall procedure was identical with that of CP assay. Tiliroside was employed as positive control (Jung *et al*., 1998). The purities of compounds used for the assay were above 95% checked by HPLC.

Results and Discussion

Column chromatography of the hexane- and EtOAc-soluble fractions of the MeOH extract from the stem bark of *M. obovata* on silica gel and RP-C_{18} columns led to the isolation of nine compounds (**1** - **9**). The isolated compounds were identified as obovatol (**2**) (Kazuo *et al*., 1982), magnolol (**3**) (Shoji *et al*., 1991), honokiol (**4**) (Shoji *et al*., 1991), *p*-coumaric acid (**7**) (Iiyama *et al*., 1990), and syringin (**9**) (Shoji *et al*., 1991).

Compound **1** was obtained as colorless oil and its molecular formula of $C_{19}H_{20}O_2$ was established by the molecular ion peak at *m/z* 280 $[M]^+$ in the EIMS. The IR spectrum showed the presence of a hydroxyl group at 3550 cm^{-1}, an olefinic double bond at 1645 cm^{-1}, and an aromatic group at 1610 cm^{-1}. The ^{1}H-NMR spectrum of **1** showed two sets of ABX-type aromatic signals at δ 6.91 (1H, d, J = 8.0 Hz), 7.26 (1H, d, J = 2.5 Hz), and 7.08 (1H, d, J = 2.1 Hz), and at δ 6.96 (1H, d, J = 8.2 Hz), 7.26 (1H, d, J = 2.5 Hz) and 7.30 (1H, dd, J = 8.2, 2.5 Hz), two allyl group signals at [δ 3.35 (2H, d, J = 6.0 Hz), 5.09 (2H, m), and 6.01 (1H, m) and δ 3.45 (2H, d, J = 6.0 Hz), 5.15 (2H, m), and 6.01 (1H, m)], and a methoxy group at δ 3.90 (3H, s). The ^{13}C-NMR spectrum of **1** exhibited the presence of 19 carbons with a methoxy group at δ 55.7, two oxygenated aromatic carbons at δ 151.0 and 157.2, two benzylic methylene signals at δ 34.8 and 39.6, and two exo-methylene carbons at δ 115.4 and 115.7. Accordingly, compound **1** was identified as 4-methoxy-honokiol (3,5-dially-2'-hydroxy-4-methoxybiphenyl) by comparison of their spectral data with those previously reported data (Nitao *et al*., 1991).

Compound **5** was obtained as colorless oil and its molecular formula of $C_{33}H_{44}O_3$ was established by the molecular ion peak at *m/z* 488 $[M]^+$ in the EIMS. The IR spectrum showed the presence of hydroxyl group at 3500 cm^{-1} and aromatic ring at 1600 cm^{-1}. The ^{1}H-NMR spectrum of **5** revealed the presence of two 1,2,4-trisubstituted benzene rings at δ 6.94 (1H, dd, J = 9.0, 2.4 Hz), 6.99 (1H, d, J = 9.0 Hz), 7.11 (1H, d, J = 2.4 Hz), 7.09 (1H, d, J = 6.9 Hz), 7.14 (1H, dd, J = 6.9, 2.1 Hz) and 7.16 (1H, d, J = 2.1 Hz), and two allyl groups at δ 3.38 (2H, d, J = 6.0 Hz), 5.02 (2H, m), 5.92 (1H, m), 3.48 (2H, d, J = 6.0 Hz), 5.02 (2H, m) and 5.92 (1H, m). The ^{13}C-NMR and DEPT spectra of **5** showed the twelve aromatics and two allyl group carbons, which were assignable to a *neo*-lignan moiety, as compared with those of honokiol (**4**). Moreover, the remaining fifteen carbon signals indicated the occurrence of four quaternary methyls at δ_C 19.2, 21.0, 27.2 and 31.0, six methylenes at δ_C 20.0, 22.1, 22.7, 37.7, 40.5 and 44.9, two methins at δ_C 50.0 and 51.1, a quaternary carbon at δ_C 35.1, and two oxygenate quaternary carbons at δ_C 88.3 and 73.0. In addition, the ^{1}H-NMR spectrum showed signals for four methyl groups at δ_H 0.84, 1.02, 1.32, and 1.24. These signals were almost the same as those of a eudesman-type sesquiterpene isolated from *Laggera pterodonta* (Zhao *et al*., 1997). Long-range correlations between δ_H 1.02 (H-12) and δ_C 88.3 (C-4), 6.94 (H-4") and 148.9 (C-2") confirmed that the hydroxyl group at C-2" on the honokiol ring was linked to the C-4 position in eudesmol moiety associated with the ether bond. On the basis of the

above evidence, compound **5** was identified as eudeshonokiol B by comparison of several physical and spectral data with those reported in the literature (Fukuyama *et al*., 1992).

Compound **6** was obtained as colorless oil and its molecular formula of $C_{33}H_{44}O_4$ was established by the molecular ion peak at *m/z* 504 $[M]^+$ in the EIMS. The IR spectrum indicated the presence of hydroxyl group at 3550 cm^{-1} and aromatic ring at 1600 cm^{-1}. The ^{1}H-NMR spectrum of **6** showed the presence of two AB type aromatic protons at δ 6.79 (2H, d, J = 8.4 Hz) and 7.06 (2H, d, J = 8.4 Hz), *meta*-coupled aromatic protons at δ 6.27 (1H, d, J = 2.0 Hz) and 6.57 (1H, d, J = 2.0 Hz), and two allyl groups at δ 3.19 (2H, d, J = 6.8 Hz), 4.98 (2H, m), and 5.82 (1H, m) and δ 3.31 (2H, d, J = 6.8 Hz), 4.98 (2H, m), and 5.82 (1H, m), which were assignable to be an obovatol moiety (**2**). In addition, ^{1}H-NMR spectrum of **6** showed signals of four methyl groups at δ 0.85, 1.00, 1.02, and 1.32, which indicated the presence of eudesmol moiety, as compared with that of **5**. The ^{13}C-NMR spectrum of **6** also indicated the presence of an obovatol moiety and a sesquiterpene moiety, the latter of which consisted of four quarternary methyls (δ_C 19.3, 21.2, 26.6 and 27.3), six methylenes (δ_C 20.4, 22.9, 22.6, 38.6, 40.7 and 45.1), two methins (δ_C 49.7 and 53.1), a quarternary carbon (δ_C 35.2), and two oxygenate quarternary carbons (δ_C 87.7 and 72.9). Furthermore, the long-range correlations between δ_H 1.32 (H-12) and δ_C 87.7 (C-4), δ_H 6.27 (H-3')/6.57 (H-5') and δ_C 132.6 (C-1'), estimated an ether linkage through the –OH group at C-1' in obovatol and C-4 in eudesmol. Therefore, compound **6** was identified as eudesobovatol B, which had previously been isolated from *M. obovata* (Fukuyama *et al*., 1992).

Compound **8** was obtained as pale yellow needle, mp 156 - 158 °C, and its molecular formula of $C_{18}H_{16}O_3$ was established by the molecular ion peak at *m/z* 280 $[M]^+$ in the EIMS. The ^{1}H-NMR spectrum of **8** showed two sets of ABX-type aromatic signals at [δ 6.80 (1H, d, J = 8.1 Hz), 7.22 (1H, dd, J = 8.1, 2.1 Hz) and 7.26 (1H, d, J = 2.1 Hz), and 6.89 (1H, d, J = 8.4 Hz), 7.42 (1H, dd, J = 2.4, 8.4 Hz) and 7.46 (1H, d, J = 2.4 Hz)], an allyl group signals at δ 3.37 (2H, d, J = 6.6 Hz), 5.01 (2H, m), and 6.01 (1H, m), and resonances for an α,β-unsaturated aldehyde group at δ 6.58 (1H, dd, J = 7.8, 15.9 Hz), 7.51 (1H, d, J = 15.0 Hz) and 9.50 (1H, d, J = 7.8 Hz). The ^{13}C-NMR spectrum of **8** exhibited the presence of 18 carbons with a benzylic methylene signal at δ 35.4, two olefinic carbons at δ 126.5 and 156.3, and an aldehyde carbon at δ 196.3. These ^{1}H- and ^{13}C-NMR spectroscopic features were similar to those of randainal, which was isolated from *Magnolia officinalis* (Wang *et al*., 1995). Therefore, **8** was identified to be as magnaldehyde B (3'-allyl-4'-hydroxyphenyl-2-hydroxy-5-cinnamic aldehyde) by comparison of their spectral data with those previously reported data (Shoji *et al*., 1991).

Table 1. Anti-complement effects of compounds **1 - 9** on the complement system

Compound	Classical pathway IC_{50} (μM)[a]
4-Methoxyhonokiol (**1**)	> 600
Obovatol (**2**)	> 200
Magnolol (**3**)	> 200
Honokiol (**4**)	> 200
Eudeshonoliol B (**5**)	> 200
Eudesobovatol B (**6**)	> 200
p-coumaric acid (**7**)	> 200
Magnaldehyde B (**8**)	102.7 ± 8.9
Syringin (**9**)	> 200
Tiliroside[b]	76.6 ± 2.2

[a] The values represent the mean ± S.D. of three experiments.
[b] Tiliroside and rosmarinic acid were used as positive controls (Jung *et al*., 1998).

In previous studies for the anti-complement activities from natural sources, we reported that the oleanane-type triterpene saponins, which include ginseng saponins from Korean ginseng, hederagenin saponins from *Dipsacus asper*, kaikasaponins and soyasaponins from *Pueraria lobata* and bisdesmosidic saponins from *Tiarella polyphylla*, and flavonoids from *Persicaria lapathifolia*, have significant anti-complement activities (Min *et al*., 2008; Park *et al*., 1999). Furthermore, lanostane-type triterpenes, prostane-type triterpenes, ursane-type triterpenoids, and dammarane-type saponins inhibited the hemolytic activity of the complement system (Thuong *et al*., 2006). However, there has been no investigation of the activities of the noelignans and phenylpropanoids. To facilitate the understanding of the anti-complement activities, those of isolated neolignans and phenylpropanoids (**1 - 9**) were evaluated using the classical pathway of the complement system and the results (IC_{50} values) are presented in Table 1. Among the compounds tested, magnaldehyde B (**8**) showed inhibitory activity against classical pathway of the complement system, with IC_{50} value of 102.7 μM. However, compounds **1 - 7** and **9** did not inhibit the hemolytic activity of the complement but rather increased hemolysis. The inhibitory effect of magnaldehyde (**8**) on the complement system was comparable to that of that of tiliroside (IC_{50} value, 76.6 μM), which was isolated from *Magnolia fargesii* (Jung *et al*., 1998) and used as a positive control. Compound **8** substituted an aldehyde group at C-9 on neolignan moiety. On the other hand, compounds **1 - 6** with a methylene group at C-9 resulted

in a loss of *in vitro* anti-complement activity against the classical pathway. This result indicated that an aldehyde group in the neolignan is important for the anti-complement activity.

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