Coumarins Isolated from *Angelica gigas* Inhibit Acetylcholinesterase: Structure−Activity Relationships

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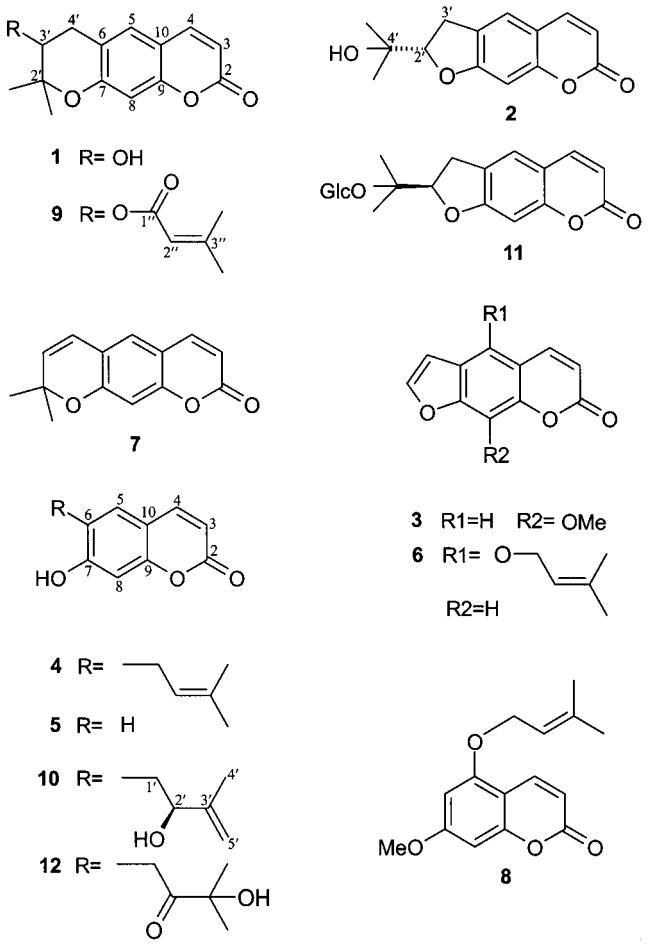
Acetylcholinesterase (AChE) inhibitory activity−guided fractionation of *Angelica gigas* led to isolation and identification of a new coumarin, peucedanone (**12**), and isolation of 11 known coumarins. Among them, decursinol (**1**) represented the highest inhibitory activity toward AChE in vitro. The correlation of the inhibitory activities of the coumarins toward AChE with their chemical structures was studied.

According to the cholinergic hypothesis of the pathogenesis of Alzheimer's disease (AD), memory impairments in AD patients result from a deficit of cholinergic functions in the brain. One promising therapeutic strategy for activating central cholinergic functions has been the use of inhibitors of AChE, the enzyme responsible for the metabolic hydrolysis of ACh. Hypothetically, inhibitors of AChE should increase the efficiency of cholinergic transmissions by preventing the hydrolysis of released ACh, thus making more ACh available at the cholinergic synapse.[1−3]

As part of our continuing research seeking AChE inhibitory components from natural resources,[4,5] we recently found that the methanolic extract of the underground part of *Angelica gigas* Nakai (Umbelliferae) significantly inhibited AChE activity. This plant has been used traditionally in Korean herbal medicine under the Korean names "Zam Dang Gui" not only for the treatment of anemia but also as a sedative, an anodyne, or a tonic agent.[6,7] To date, only a few reports on the biological activities of coumarins from *A. gigas*[8−10] are available. In the present study, we attempted to isolate AChE inhibitory components of *A. gigas* by subsequent bioactivity-guided fractionations. As a result, we isolated and identified a new coumarin, peucedanone (**12**), and 11 known coumarins (**1**−**11**) from the methanolic extract of the underground part of *A. gigas*. In addition, we studied the structure−activity relationships of isolated coumarins as AChE inhibitors.

The methanolic extract of the underground part of *A. gigas* was found to exhibit a significant inhibitory activity against AChE (42.1% inhibition at a concentration of 100 μg/mL; $P < 0.001$). The methanolic extract was suspended in H_2O and partitioned with CH_2Cl_2. At a concentration of 100 μg/mL, AChE inhibition of the two fractions was 19.7% for the aqueous fraction ($P < 0.01$) and 49.3% for the CH_2Cl_2 fraction ($P < 0.001$). Subsequent activity-guided fractionation of the CH_2Cl_2 fraction by silica gel column chromatography followed by HPLC using an RP-18 column yielded compounds **1**, **2**, and **3**. The MS, UV, IR, and ^{1}H and ^{13}C NMR spectra of **1**, **2**, and **3** corresponded closely with those data already reported for decursinol (**1**),[11,12] marmesin (**2**),[13] and xanthotoxin (**3**),[14,15] respectively. AChE inhibitory assays gave IC_{50} values for compounds **1**, **2**, and **3** of 2.8×10^{-5}, 6.7×10^{-5}, and 5.4×10^{-5} M, respectively. Since all of these active compounds were coumarin derivatives, we promptly attempted to search for other structurally diverse coumarins that had been reported in this plant[7,11,12] in order to clarify the relationship between these coumarin derivatives and AChE inhibitory activity. Compounds **4**−**12** were isolated from the fractions derived from the initial CH_2Cl_2 fraction by silica gel column chromatographies. Spectral data of compounds **4**−**11** matched those of 7-demethylsuberosine (**4**),[15,16] umbelliferone (**5**),[15] isoimperatorin (**6**),[17] xanthyletin (**7**),[15,18] 7-methoxy-5-prenyloxycoumarin (**8**),[19] decursin (**9**),[11,12] 7-hydroxy-6-(2-(*R*)-hydroxy-3-methylbut-3-enyl)coumarin (**10**),[20] and nodakenin (**11**).[21] This is the first report of compounds **2**, **3**, **6**−**8**, and **10** from *A. gigas*.



Compound **12** was obtained as a pale yellowish prism. The molecular formula $C_{14}H_{14}O_5$ was established by HREIMS, *m/z* 262.0834 $[M]^+$ (calcd for $C_{14}H_{14}O_5$, *m/z* 262.0837). The presence of 7-hydroxy-6-substituted cou-

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Table 1. AChE Inhibition by Coumarins Isolated from *A. gigas*

compound	IC_{50} (M)
1	2.8×10^{-5}
2	6.7×10^{-5}
3	5.4×10^{-5}
4	2.4×10^{-3}
5	2.9×10^{-2}
6	6.9×10^{-5}
7	1.5×10^{-4}
8	2.4×10^{-4}
9	3.9×10^{-4}
10	1.3×10^{-4}
11	6.8×10^{-5}
12	1.8×10^{-4}

marin was indicated from the UV absorptions at 224, 316, and 339 nm,[22] IR bands at 1713 and 1619 cm^{-1}, and the typical AB-type signals at δ 6.18 and 7.84 (each 1H, d, J = 9.51 Hz) for H-3 and H-4, respectively, and two singlet aromatic protons at δ 7.30 and 6.71 in the ^1H NMR spectrum. All carbon resonances were fully assigned by ^{13}C NMR, $^1H-^1H$ COSY, $^{13}C-^1H$ COSY, and HMBC spectra. In the HMBC spectrum, the C-1′ methylene protons (δ 4.01) showed long-range heteronuclear interactions with the C-5, C-6, C-7, and C-2′ carbons (δ 132.3, 122.4, 161.3, and 215.0). The C-2′ carbonyl carbon (δ 215.0) showed correlation with the C-1′ methylene proton (δ 4.01) and two methyl protons (δ 1.38). From the spectroscopic data above, **12** was identified as 7-hydroxy-6-(3-hydroxy-3-methyl-2-oxobutyl)-coumarin, a new 7-oxocoumarin that we have designated peucedanone.[23]

Compounds **4**−**12** were tested for inhibitory activity against AChE. The IC_{50} values of the 12 coumarins including decursinol (**1**), marmesin (**2**), and xanthotoxin (**3**) are compared in Table 1. As shown in Table 1, among the 12 isolated coumarins, decursinol (**1**) was the most potent in inhibiting AChE, and both isoimperatorin (**6**) and nodakenin (**11**) were also found to be active in inhibiting AChE. The degree to which coumarins inhibited AChE differed depending on the characteristics of cyclization of the isoprenyl (IP) unit at C-6 and the functional groups attached to the coumarin nucleus. While decursinol (**1**), a dihydropyranocoumarin, was a potent inhibitor of AChE ($IC_{50} = 2.8 \times 10^{-5}$ M), the three furanocoumarins, marmesin (**2**), xanthotoxin (**3**), and isoimperatorin (**6**), which differ structurally from decursinol (**1**) in the form of cyclization of the IP unit at C-6, were less potent inhibitors of AChE ($IC_{50} = 6.7 \times 10^{-5}$, 5.4×10^{-5}, and 6.9×10^{-5} M, respectively). Also, nodakenin (**11**), a furanocoumarin glycoside, showed inhibitory activity ($IC_{50} = 6.8 \times 10^{-5}$ M) similar to the furanocoumarins noted above. This suggests that the glucose moiety of nodakenin had little affect in inhibiting AChE. Interestingly, decursin (**9**), which is structurally analogous to decursinol (with an oxygenated IP unit instead of a free hydroxyl group at C-3′), was a poor inhibitor ($IC_{50} = 3.9 \times 10^{-4}$ M). Xanthyletin (**7**), a pyranocoumarin without a free hydroxy group at C-3′, was also inactive ($IC_{50} = 1.5 \times 10^{-4}$ M). From these results, we postulate that the existence of a free hydroxyl group at C-3′ is important in the inhibition of AChE by pyranocoumarins. It appears that most simple coumarins (without a cyclized IP unit at C-6, e.g., **4**, **5**, **8**, **10**, and **12**) are much less active inhibitors of AChE than coumarins with a cyclized IP unit. However, as shown in the cases of 7-hydroxy-6-(2-(*R*)-hydroxy-3-methylbut-3-enyl)coumarin (**10**) and peucedanone (**12**), oxygenated IP units could enhance the AChE inhibitory activity. As most of the coumarins tested have only modest to slight inhibitory activity against AChE, we suggest that the coumarin skeleton containing a pyrone moiety plays an important role in the inhibitory activity against AChE. This finding is consistent with previous independent work that showed that (i) a simple coumarin derivative, 3-choloro-7-hydroxy-4-methylcoumarin, bound to the peripheral site of AChE and inhibited AChE activity[24,25] and that (ii) an intact pyrone is necessary for inhibiting AChE activity.[26]

We have shown that the methanolic extract of the underground part of *A. gigas* significantly inhibits AChE activity. Various coumarins isolated from the extract are responsible for the significant AChE inhibitory activity. A dihydropyranocoumarin, decursinol (**1**), was found to be the most potent inhibitor of AChE. Furthermore, the structure−activity relationships among the coumarins can provide useful information on the interaction between AChE and its ligand. The mechanism responsible for the inhibitory effect of decursinol (**1**) on AChE in vitro and whether this compound has an anti-amnestic effect are now being studied in our laboratory.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were run on a JEOL GSX 400 spectrometer at 400 and 100 MHz, respectively, with TMS as internal standard. FT-IR spectra were recorded on a Perkin-Elmer 1710 spectrophotometer. UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer. EIMS spectra were obtained on a VG Trio II spectrometer, and FABMS spectra were obtained on a VG 70-VSEQ mass spectrometer with direct inlet system using PEG 600/glycerol as a matrix. High-resolution mass spectral analyses were obtained on a JEOL JMS AX 505 WA spectrometer. Column chromatography was performed on Merck (9025) silica gel 60 (0.04−0.063 mm). Analytical TLC was performed on precoated Merck F_{254} silica gel plates and visualized by spraying with anisaldehyde−H_2SO_4. An HPLC system (Hitachi L-6200, Japan) equipped with a UV−visible detector and Microsorb C_{18} semipreparative column (Rainin Inst. Co.) was used for isolation.

Plant Material. The underground parts of *A. gigas* were purchased in a local market for Oriental medicine in Chechon, Chung-Buk, Korea, in 1998. The plant was authenticated by Dr. Dae S. Han, Professor Emeritus, College of Pharmacy, Seoul National University. Voucher specimens (SNUPH-0415) have been deposited in the Herbarium of the College of Pharmacy, Seoul National University.

Extraction and Isolation. The dried underground part (5 kg) of *A. gigas* was extracted with methanol in an ultrasonic apparatus. Upon removal of solvent in vacuo, the methanolic extract yielded 350 g. This methanolic extract was then suspended in H_2O and partitioned successively with CH_2Cl_2. Silica gel column chromatography of the CH_2Cl_2 fraction (220 g) with a mixture of *n*-hexane−$CHCl_3$−MeOH as eluent afforded seven fractions (fractions 1−7). Fraction 4 (9.3 g), the fraction with the highest anti-AChE activity, was subjected to silica gel column chromatography with an *n*-hexanes−EtOAc−MeOH mixture and yielded 10 subfractions (fractions 4-1−4-10). Among the 10 subfractions, the most active subfraction, fraction 4-9 (700 mg), yielded compounds **1** (120 mg), **2** (170 mg), and **3** (10 mg) by additional purification steps on RP-18 HPLC (H_2O−AcCN, 80:20). Compounds **4** (710 mg) and **5** (42 mg) were precipitated from fractions 4-3 and 4-5 upon standing, respectively. After silica gel column chromatography of fraction 2 (8.6 g) with an *n*-hexanes−EtOAc mixture, compound **6** (85 mg) was obtained. Compounds **7** (8 mg) and **8** (6 mg) were isolated through additional silica gel column chromatography of fraction 2 with *n*-hexanes−EtOAc and RP-18 HPLC (H_2O−MeOH−AcCN, 5:70:25 and 15:65:20, respectively). About 45 g of compound **9** was obtained in a crude state from fraction 3 and purified by recrystallization with EtOH. Compound **10** (3 mg) was isolated through silica gel column chromatography of fraction 5 (1.5 g) with an *n*-hexanes−

EtOAc−MeOH mixture and then RP-18 HPLC (H_2O−MeOH−AcCN, 35:60:5). The silica gel column chromatography of fraction 6 (15.0 g) with a $CHCl_3$−acetone−MeOH mixture yielded compounds **11** (914 mg) and **12** (22 mg).

Compound 12: pale yellowish prism (from $CHCl_3$); $[\alpha]_D$ +4.8° ($CHCl_3$−MeOH, 1:4, 0.5); UV λ_{max} (log ϵ) 339 (5.10), 316 (5.11), and 224 (5.12) nm; IR ν_{max} (KBr) 3340, 1713, 1618, 1394, 1145, 1044, 827 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 6.18 (1H, d, J = 9.51 Hz, H-3), 7.84 (1H, d, J = 9.51 Hz, H-4), 7.30 (1H, s, H-5), 6.71 (1H, s, H-8), 4.01 (2H, s, H-1′), 1.38 (6H, s, H-4′N and 5′); ^{13}C NMR (75 MHz, CD_3OD) 164.4 (C-2), 112.8 (C-3), 146.5 (C-4), 132.3 (C-5), 122.4 (C-6), 161.3 (C-7), 103.5 (C-8), 156.5 (C-9), 113.3 (C-10), 38.8 (C-1′), 215.0 (C-2′), 78.7 (C-3′), 27.6 (C-4′ and C-5′) ppm; EIMS *m*/*z* (rel int) 262 $[M]^+$ (30), 219 (26), 204 (27), 201 (11), 176 (100), 175 (82), 147 (19); HREIMS, *m*/*z* 262.0834 $[M]^+$ (calcd for $C_{14}H_{14}O_5$, *m*/*z* 262.0837).

Determination of AChE Activity. AChE activity was determined by the modified method of Ellman et al.[4,5,27]

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