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Monoamine Oxidase Inhibitors from Cinnamomi Cortex

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Abstract – Four compounds were isolated from the dichloromethane fraction of Cinnamomi Cortex through bioassay-guided isolation. Their structures were identified as coumarin (**1**), 3,3-dimethoxy-1-propenyl benzene (**2**), cinnamic acid (**3**) and *o*-methoxy cinnamaldehyde (**4**) on the basis of spectroscopic data. All four compounds showed inhibitory activities *in vitro* against monoamine oxidase (MAO) prepared by mouse brain. The IC_{50} values were 41.4 μM (**1**), 110.6 μM (**2**), 252.5 μM (**3**) and 83.1 μM (**4**), respectively.

Key words – Cinnamomi Cortex, Lauraceae, monoamine oxidase inhibition, coumarin, 3,3-dimethoxy-1-propenyl benzene, cinnamic acid, *o*-methoxy cinnamaldehyde

Introduction

Monoamine oxidase (MAO; EC 1.4.3.4), a flavin adenine dinucleotide (FAD) containing enzyme, occurs in the outer mitochondrial membrane. It catalyses the oxidative deamination of neurotransmitters such as catecholamines and serotonine, and dietary amines (Tipton *et al.*, 1987). Monoamine oxidase is classified into type A and type B according to substrate specificity and sensitivity to specific inhibitors. MAO-A has been known to be active with norepinephrine and serotonin as substrates, and sensitive to inhibition by a low concentration of clorgyline, whereas MAO-B has been known to be active with β-phenylethylamine and benzylamine, and sensitive to inhibition by a low concentration of deprenyl. The MAO-A inhibitors have been introduced into therapy for the treatment of depression, and MAO-B inhibitors have been used to overcome the lack of dopamine in Parkinsons disease. A number of inhibitors of MAO have been identified, including alkaloids (Rosazza *et al.*, 1992), xanthones (Schaufelberger *et al.*, 1987), azaphilones (Yoshida *et al.*, 1996), coumarins (Hossain *et al.*, 1996) and cinnamic acid derivatives (Noro *et al.*, 1983). During our screening on monoamine oxidase inhibitory constituents from natural products, the MeOH extract of Cinnamomi Cortex showed significant inhibitory activity against mouse brain MAO *in vitro*. The Cinnamomi Cortex (Lauraceae) is one of the most widely used crude drugs, the chemical composition has been investigated in detail. Cinnamaldehyde as the main component in the essential oil, coumarin, cinnamic acid, β-sitosterol, cholin, protocatechuic acid, vanillic acid, and small amounts of syringic acid were isolated and identified from the stem bark of *Cinnamomum cassia* (Tang *et al.*, 1992). A number of new closely related diterpenes named cinncassiols were isolated from the fraction exhibiting anti-allergic activity (Nohara *et al.*, 1982). In connection with the isolation of the diterpenes described above, several aromatic compounds and a number of flavone-3-ol derivatives, including procyanidins, were further isolated from the bark of *C. cassia* (Morimoto *et al.*, 1986). Most of the pharmacological studies on the chemical constituents of Cinnamomi Cortex have been limited to the effects of essential oil and its major component cinnamaldehyde, which exhibitied bactericidal activity, fungicidal activity, sedative activity, hypotensive effect, antimutagenic activity and so on. This paper describes the bioassay guided isolation and structure elucidation of the monoamine oxidase inhibitory components from Cinnamomi Cortex.

Experimental

Plant material – Cinnamomi Cortex was pur-

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chased at Hoechundang drug store, Taejon, Korea in 1998. A voucher specimen (CNU95125) is deposited in the herbarium of the College of Pharmacy, Chungnam National University.

Reagents and Instruments – Thin layer chromatography was carried out with pre-coated TLC plate Kieselgel 60F_{254} (Merck, 1.05715.) and RP-18 F_{254S} (Merck, 1.15685.). Kieselgel 60 (70-230 mesh and 230-400 mesh, Merck) and Sephadex LH-20 (Sigma) were used for the stationary phases of column chromatography. Kynuramine and zinc sulfate (Sigma) were used to measure the inhibitory activities of MAO. The fluorescence intensity of the reaction product, 4-hydroxyquinoline, was measured by Hitachi Model F-300 fluorophotometer. ^{1}H and ^{13}C NMR were recorded on a Bruker DRX 300 spectrometers. EI-MS spectra were measured with a Hewlett-Packard MS Engine 5989A mass spectrometer.

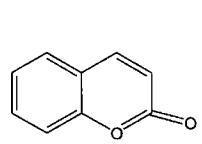
Extraction and isolation – Cinnamomi Cortex (770 g) was coarsely crushed and extracted with MeOH at room temperature two times. The MeOH filtrate was evaporated *in vacuo* to give MeOH extract (25 g) and was partitioned with CH_2Cl_2 and H_2O. The concentrated CH_2Cl_2 fraction (10 g) was subjected to silica gel column chromatography and gave eight fractions (Fr. 2A-2H). Fr. 2D was further chromatographed on a silica gel column using hexane-EtOAc (9:1-5:1) as eluents to give compound **1** (40 mg), compound **2** (88 mg) and compound **3** (13 mg). Compound **4** (7 mg) was obtained from Fr. 2E by repeated silica gel column chromatography.

Compound 1 (coumarin) – white powder, mp 68-69°C, EI-MS *m/z* (rel. int.): 146(M^+, 100), 118 (90), 90 (52), 63 (36); IR ν_{max} (KBr) cm^{-1}: 1710 (C=O), 1620, 1580 (aromatic, C=C), 1250 (aromatic, C-O); ^{1}H-NMR (300MHz, $CDCl_3$) δ : 6.22(1H, d, *J*=9.5Hz, H-3), 7.52(1H, d, *J*=9.5Hz, H-4), 7.28 - 7.36 (2H, m, H-5 and H-7), 7.06-7.14 (2H, m, H-6, and H-8); ^{13}C-NMR (75MHz, $CDCl_3$) δ : 160.6 (C-2), 116.3 (C-3), 143.8 (C-4), 127.4 (C-5), 124.3 (C-6), 131.3 (C-7), 116.1 (C-8), 154.0 (C-9), 118.8 (C-10).

Compound 2 (3,3-dimethoxy-1-propenyl benzene) – yellowish oil, EI-MS *m/z* (rel. int.): 178(M^+, 27), 147(M^+-OCH_3, 100), 131(31), 115(49), 103(28); IR ν_{max} (KBr) cm^{-1}: 1590, 1560 (aromatic, C=C), 1260 (C-O); ^{1}H-NMR(300MHz, $CDCl_3$) δ : 7.37-7.44 (2H, m, H-2 and H-6), 7.25-7.36 (3H, m, H-3, H-4 and H-5), 6.74 (1H, d, *J*=16.2 Hz, H-7), 6.17 (1H, dd, *J*= 16.2, 4.9 Hz, H-8), 4.97 (1H, d, *J*=4.9, H-9), 3.39 (6H, s, -OCH_3); ^{13}C-NMR (75MHz, $CDCl_3$) δ : 136.1 (C-1), 126.7 (C-2 and C-6), 128.6 (C-3 and C-5), 128.1 (C-4), 133.6 (C-7), 125.7 (C-8), 102.9 (C-9), 52.7 (-OCH_3).

Compound 3 (cinnamic acid) – white powder, mp 125-126°C, EI-MS *m/z* (rel. int.) : 148(M^+, 80), 147(100), 131(23), 103(58), 91(25); IR ν_{max} (KBr) cm^{-1}: 3400(-OH), 1680 (C=O), 1625, 1545 (aromatic, C=C); ^{1}H-NMR(300MHz, $CDCl_3$) δ : 7.52-7.60 (2H, m, H-2 and H-6), 7.40-7.47 (3H, m, H-3, H-4 and H-5), 7.81 (1H, d, *J*=16.0 Hz, H-7), 6.47 (1H, d, *J*=16.0 Hz, H-8); ^{13}C-NMR (75MHz, $CDCl_3$) δ : 134.1 (C-1), 128.4 (C-2 and C-6), 129.0 (C-3 and C-5), 130.7 (C-4), 147.1 (C-7), 117.3 (C-8).

Compound 4 (*o*-methoxy cinnamaldehyde) – white powder, EI-MS *m/z* (rel. int.) : 162(M^+, 26), 147(19), 131(100); IR ν_{max} (KBr) cm^{-1}: 1720 (C=O), 1620, 1590 (aromatic, C=C), 1240 (C-O); ^{1}H-NMR(300MHz, $CDCl_3$) δ : 6.88 (1H, d, *J*=7.9 Hz, H-3), 7.34 (1H, ddd, *J*=1.7, 7.6, 7.9 Hz, H-4), 6.93 (1H, dd, *J*=7.6, 7.6 Hz, H-5), 7.48 (1H, dd, *J*=7.6, 1.7 Hz, H-6), 7.77 (1H, d, *J*=16.1 Hz, H-7), 6.72 (1H, dd, *J*=16.1, 7.8 Hz, H-8), 9.62 (1H, *J*=7.8 Hz, H-9), 3.85 (3H, s, -OCH_3); ^{13}C-NMR (75MHz, $CDCl_3$) δ : 123.0 (C-1), 158.3 (C-2), 111.3 (C-3), 132.7 (C-4), 120.9 (C-5), 128.9 (C-6), 148.4 (C-7), 129.1 (C-8), 194.6 (C-9), 55.6 (-OCH_3).



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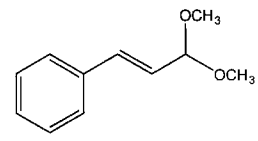

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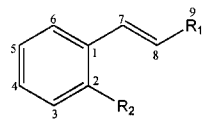

3 R_1= COOH R_2=H

4 R_1=CHO R_2=OCH_3

Enzyme preparation – Mice (male, ICR, 25-30 g) were purchased from Samyook Animal Center (Suwon, Korea). The animals were fed with laboratory chow and water *ad libitum* and killed by cervical dislocation. A crude mitochondrial fraction was prepared from mouse brain according to the reported method (Naoi *et al.*, 1989).

Assays – MAO activity was measured fluorometrically using kynuramine as an amine substrate according to the reported with slight modification (Kraml *et al.*, 1965; Naoi *et al.*, 1989). In brief, samples (2 μl) dissolved in DMSO were added to 0.2 M potassium phosphate buffer (74 μl, pH 7.4) which containing 4 μl of MAO suspension and 20 μl of

Table 1. Inhibitory activities of monoamine oxidase by isolated compounds from Cinnamomi Cortex.

Compound	IC_{50} (μM)
Coumarin (**1**)	41.4
3,3-Dimethoxy-1-propenyl benzene (**2**)	110.6
Cinnamic acid (**3**)	252.5
O-methoxy cinnamaldehyde (**4**)	83.1
Iproniazid	3.2
Clogyline	3.8

500 μM kynuramine. After incubation at 37°C for 30 min, reaction was stopped by addition of 25 μl of 10% $ZnSO_4$ and 5 μl of 1N NaOH, and then centrifuged at 3,000 g for 5 min. The 70 μl of the supernatent was transferred to fluoro 96-well plate and added 140 μl of 1N NaOH. After mixing the solution, the fluorescence intensity of the reaction product, 4-hydroxyquinoline, was measured at 380 nm (emission) with excitation at 315 nm. As a blank test, the reaction was carried out omitting the enzyme.

Results and Discussion

In the screening tests *in vitro* to find MAO inhibitors from medicinal plants, the barks of Cinnamomi Cortex were found to possess a strong inhibitory effect on this enzyme. The dried barks of Cinnamomi Cortex were extracted with MeOH. MeOH extract was partitioned with CH_2Cl_2 and H_2O. Four compounds (**1-4**) were isolated from CH_2Cl_2 fraction through bioassay-guided isolation. Compound **1** was obtained as white powder, mp 68-69°C, and identified as coumarin by NMR and EI-MS spectroscopic analysis. It inhibited MAO by 97.6% at a concentration of 100 μg/ml. The concentration of compound **1** in the reaction mixture to give 50% inhibition (IC_{50}) was 41.4 μM, though the activity was weaker than that of clorgyrine and iproniazid, selective inhibitors of MAO. Compound **2**, **3** and **4** showed similar spectral patterns in ^1H and ^{13}C NMR with cinnamyl aldehyde. Their structures were identified as 3,3-dimethoxy-1-propenyl benzene, cinnamic acid and *O*-methoxy cinnamaldehyde, respectiviely. Compound **2**, **3** and compound **4** showed mild inhibitory activities against MAO with IC_{50} values at 110.6 μM, 252.5 μM, and 83.2 μM, respectively. Some coumarin compounds and cinnamaldehyde derivatives showed potent inhibitory activities against MAO. For example, a series of coumarin derivatives was synthesized and demonstrated that ether derivatives were selective MAO-B inhibitors and sulfonic acid esters selective MAO-A inhibitors, respectively (Rendenbach-Müller *et al.*, 1994). Isocoumarin compound (e.g. 5-methymellein) showed similar potent activity comparable to that of clinically used MAO inhibitor such as clorgyline (Lee *et al.*, 1999). Also cinnamic acid derivative, ethyl *p*-methoxy-*trans*-cinnamate, was isolated from *Kaempferia galanga* as inhibitory constituent against MAO (Noro *et al.*, 1983). Much amounts of cinnamaldehyde can be isolated from Cinnamomi Cortex. Therefore, it will be a good resource for supplying cinnamaldehyde derivatives. Further extensive studies are in progress in our laboratory to verify MAO activities with coumarin compounds and cinnamic acid derivatives.

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