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Acetylcholinesterase Inhibitors from the Stem of *Zea mays*

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Abstract – Five compounds were isolated from the stem of *Zea mays*. Based on spectral data, they were identified as 4-hydroxybenzaldehyde (**1**), *N-trans-p*-coumaryl tyramine (**2**), *N-trans*-ferulyl tryptamine (**3**), *N*-(*p*-coumaryl) serotonine (**4**), and *N*-(*p*-coumaryl)-tryptamine (**5**). All isolates were evaluated *in vitro* for their inhibitory activity on acetylcholinesterase. Among tested compounds, compounds **2** - **5** exhibited acetylcholinesterase inhibitory activity, with IC_{50} values of 125, 60.4, 183.5 and 53.3 μM, respectively. Compound **1** did not show acetylcholinesterase inhibitory activity in the present study.
Keywords – *Zea mays*, Stem, Phenolic amides, Acetylcholinesterase inhibitory activity

Introduction

Cholinergic hypothesis is widely accepted one of the major causes of Alzheimer's disease, which is a serious loss of cholinergic function in the brain.[1-3] Acetylcholinesterase is the key enzyme in the hydrolysis of acetylcholine, so acetylcholinesterase inhibitors have been studied as therapeutic agents for Alzheimer's disease.[4-6] Now, donepezil, galanthamine, and rivastigmine are available for treatment of patients with mild-to-moderate Alzheimer's disease,[7] though these compounds have been reported to have substantial side effects and low bioavailability.[8,9] To solve these problems, many researchers have focused on acetylcholinesterase inhibitors from plant sources.

As part of continuous investigations to find biologically active compounds from plants, we found that the chloroform soluble fraction of *Zea mays* showed inhibitory activity against acetylcholinesterase. *Z. mays* is widely cultivated in the world as a grain and feed, and its seed, seeds oil, stigma, spike, leaf, and root have been used in Chinese traditional medicines.[10] Phenolic compounds,[11-13] carotenoids,[14] sterols,[15,16] and phenolic amides[17] have been reported from this plant. The present study deals with the isolation of bioactive constituents of *Z. mays* stem and their acetylcholinesterase inhibitory activities.

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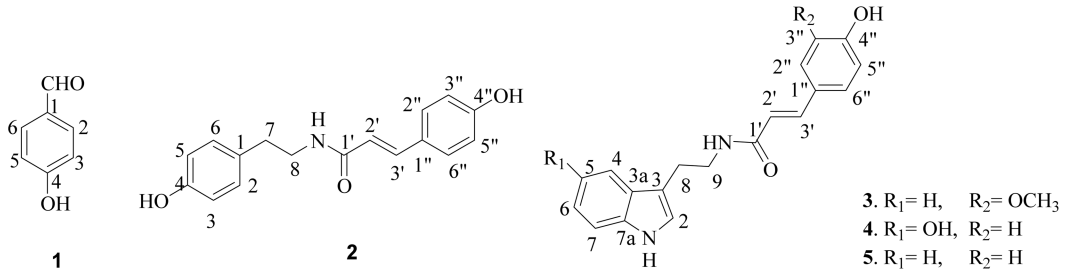
Experimental

General experimental procedures – UV/Vis determinations were carried out using a V-530 spectrophotometer (JASCO, Tokyo, Japan). The MS spectrum was measured using a JMS-700 (JEOL, Japan). NMR spectra were recorded on an AVANCE 600 (Bruker, Rheinstetten, Germany). The chemical shifts were represented as parts per million (ppm) referenced to the residual solvent signal. Column chromatography was carried out using a Kieselgel 60, 63 - 200 μm and 40 - 63 μm (Merck, Darmstadt, Germany) and YMC gel ODS-A, 150 μm (YMC, Kyoto, Japan). TLC was performed on a glass backed Kieselgel 60 F254 and RP F254s plates. All other chemicals and reagents used were of analytical grade.

Plant material – The stem of *Zea mays* was collected from a cultivation area in Yanggu (Kangwon Province, Korea). A voucher specimen (KNUH-S-1008-01) was deposited in the Herbarium of College of Pharmacy, Kangwon National University, Korea.

Extraction and isolation – The air dried stem of *Z. mays* was ground and extracted with MeOH (5.2 kg, 36 L × 2) for one week at room temperature. All extracts were combined and concentrated *in vacuo* at 40 °C. The MeOH extract (890 g) was suspended in water and then successively partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, leaving a residual water soluble fraction. Each fraction was evaporated *in vacuo* to yield the residues of *n*-hexane fraction (fr.) (120 g), $CHCl_3$ fr. (100 g), and *n*-BuOH fr. (70 g). Among the solvent fractions, $CHCl_3$



1 2

Fig. 1. The structures of compounds **1** - **5** of the stem of *Z. mays*..

fraction showed a 66.1% inhibition rate against acetylcholinesterase at 100 μg/ml. To isolate active compounds from the $CHCl_3$ soluble fraction, various column chromatography were performed. The $CHCl_3$ soluble fractions (90 g) were applied to silica gel chromatography (1.5 kg, 63 - 200 μm, 15 × 50 cm) using isocratic elution with $CHCl_3$: MeOH (9 : 1), in order to divide the fraction into six fractions (Fr. 1 - Fr. 6). Fr. 2 (9 g) and Fr. 3 (60 g) were re-chromatographed on silica gel (1 kg, 63 - 200 μm, 15 × 50 cm) using stepwise gradient elution with benzene : EtOAc : MeOH (10 : 1 : 0.1 → 7 : 2 : 1) to yield eight sub-fractios (Fr. 2-1 - Fr. 2-8). Fraction 2-2 (4.5 g) was applied to further chromatography on a flash column (Redisep, 130 g, 70% MeOH) and semi preparative HPLC (Econo-prep C-18, Phenomenex, 10 × 300 mm, 40 % MeOH) using isocratic elution with MeOH : H_2O (70 : 30) and MeOH : H_2O (40 : 60), respectively, to give compound **1** (19 mg). Compound **2** (23 mg) was obtained from Fr. 2-4 by filtration. Fr. 2-3 (11 g) was applied to further chromatography on silica gel (500 g, 63 - 200 μm, 8 × 50 cm) using isocratic elution with $CHCl_3$: MeOH (29 : 1) to give four subfractions (Fr. 2-3-1 - Fr. 2-3-4). Fr. 2-3-2 (1.2 g) was then purified by Sephadex LH 20 (70 g, Pharmacia, 5 × 50 cm, 50% MeOH) to give compound **3** (218 mg). Fr. 2-3-1 (0.5 g) was applied to further chromatography on semi preparative HPLC (μ-Bondapak C-18, 19 × 150 mm) using gradient elution with MeOH : H_2O (30 : 70 → 45 : 55) to afford compounds **4** (17 mg) and **5** (11 mg), respectively.

Compound (1) – HREI-MS *m/z* 122.0347 (cacld. for $C_7H_6O_2$, 122.0368); ^{1}H-NMR (CD_3OD, 600 MHz) δ_H : 9.75 (1H, s, CHO), 7.76 (2H, d, *J* = 8.5 Hz, H-3 and H-5), 6.90 (2H, d, *J* = 8.5 Hz, H-2 and H-6); ^{13}C-NMR (CD_3OD, 150MHz) δ_C : 192.9 (CHO), 165.2 (C-4), 133.5 (C-3, C-5), 130.3 (C-1), 116.9 (C-2, C-6); EI-MS *m/z* 122 $[M]^+$ (100), 121, 93, 65.

Compound (2) – HREI-MS *m/z* 283.1205 (calcd. for $C_{17}H_{17}NO_3$, 283.1208)); ^{1}H-NMR (CD_3OD, 600 MHz) δ_H : 8.00 (1H, t, *J* = 5.6 Hz, NH), 7.38 (2H, d, *J* = 8.7 Hz, H-2" and H-6"), 7.32 (1H, d, *J* = 15.7 Hz, H-3'), 7.01 (2H, d, *J* = 8.4 Hz, H-2 and H-6), 6.79 (2H, d, *J* = 8.7 Hz, H-3" and H-5"), 6.68 (2H, d, *J* = 8.4 Hz, H-3 and H-5), 6.40 (1H, d, *J* = 15.7 Hz, H-2'), 3.33 (2H, q, *J* = 7.5 Hz, H-7), 2.64 (2H, t, *J* = 7.5 Hz, H-8); ^{13}C-NMR (CD_3OD, 150MHz) δ_C : 165.76 (C-1'), 159.25 (C-4"), 156.09 (C-4), 139.03 (C-3'), 129.99 (C-1), 129.93 (C-2" and C-6"), 129.64 (C-2 and C-6), 126.38 (C-1"), 119.20 (C-2'), 116.19 (C-3 and C-5), 115.57 (C-3" and C-5"), 41.14 (C-8), 34.91 (C-7); EI-MS *m/z* : 283 $[M]^+$, 164, 147, 120 (100), 107, 91.

Compound (3) – HREI-MS m/z 336.1486 (cacld. for $C_{20}H_{20}N_2O_3$, 336.1474); ^{1}H- and ^{13}C-NMR (CD_3OD, 600 MHz): see Table 1; EI-MS *m/z* 336 $[M]^+$, 194, 177, 145 (100), 131, 117.

Compound (4) – HREI-MS *m/z* 322.1333 (cacld. for $C_{19}H_{18}N_2O_3$, 322.1317); ^{1}H- and ^{13}C-NMR (CD_3OD, 600 MHz): see Table 1; EI-MS *m/z* 322 $[M]^+$, 293, 186, 176 (100), 159, 147, 130, 117.

Compound (5) – HREI-MS m/z 306.1370 (cacld. for $C_{19}H_{18}N_2O_2$, 306.1368); ^{1}H- and ^{13}C-NMR (CD_3OD, 600 MHz): see Table 1; EI-MS *m/z* 306 $[M]^+$, 147 (100), 130, 119, 91.

Determination of acetylcholinesterase inhibitory activity – The acetylcholinesterase inhibition assay was measured according to the method of Ellman *et. al*.[18] with slight modification. Briefly, tested compounds were dissolved in DMSO. The reaction mixture had a final volume of 1 mL and contained sodium phosphate buffer (100 mM, pH 8.0), up to 10 μL of the tested sample solution, and 20 μL of acetylcholinesterase (5 U/mL), which were mixed and incubated for 10 min at 37 °C. The reactions were started with the addition of 40 μL of 10 mM dithionitrobenzoic acid (DTNB) and 10 μL of 75 mM acetylthiocholine iodide (ATCI) as a substrate. The hydrolysis was monitored by following the formation of the yellow 5-thio-2-nitrobenzoate anion at 412 nm for 6 min using a spectrophotometer.[19]

Table 1. ^{1}H- and ^{13}C-NMR data of compounds 3-5 (500 MHz)

No.	3		4		5	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	7.07 (s)	120.95	7.02 (s)	122.88	7.06 (s)	122.05
3		111.91		111.00		111.93
3a		127.40		128.05		127.40
4	7.57 (d, 8.0)	117.93	6.96 (d, 2.3)	102.13	7.57 (d, 7.8)	117.95
5	7.06 (m)	122.05		149.74	7.07 (t, 7.8)	120.97
6	6.99 (m)	118.23	6.66 (dd, 2.3, 8.6)	111.13	6.99 (t, 7.8)	118.25
7	7.32 (d, 8.1)	110.84	7.15 (d, 8.6)	111.27	7.32 (d, 8.2)	110.86
7a		138.79		131.74		136.78
8	2.99 (t, 7.2)	25.01	2.92 (t, 7.3)	25.07	2.99 (t, 7.2)	25.02
9	3.59 (t, 7.2)	40.23	3.56 (t, 7.3)	40.08	3.58 (t, 7.2)	40.25
1'		167.84		167.89		167.92
2'	6.40 (d, 15.7)	117.51	6.39 (d, 15.7)	117.18	6.38 (d, 15.7)	117.19
3'	7.44 (d, 15.7)	140.58	7.45 (d, 15.7)	140.32	7.46 (d, 15.7)	140.37
1"		126.92		126.37		126.37
2"	7.09 (d, 1.6)	110.18	7.38 (d, 8.7)	129.16	7.37 (d, 8.5)	129.19
3"		147.87	6.78 (d, 8.7)	115.31	6.78 (d, 8.5)	115.35
4"		148.40		159.09		159.08
5"	6.78 (d, 8.1)	115.08	6.78 (8.7)	115.31	6.78 (8.5)	115.35
6"	7.00 (dd, 8.1, 1.6)	121.80	7.38 (d, 8.7)	129.16	7.37 (8.5)	129.19
OCH_3	3.85 (s)	53.98				

Chemical shifts are reported in parts per million (δ) and coupling constants (*J*) are expressed in Hertz.

Results and Discussion

Compounds **1, 2, 3,** and **5** were identified as 4-hydroxybenzaldehye, *N-trans-p*-coumaryl tyramine, *N-trans*-ferulyl tryptamine, and *N*-(*p*-coumaryl)-tryptamine, [17,20,21] respectively. Compound **4** was obtained as a white powder and produced a molecular ion $[M]^+$ at *m/z* 322.1333 by HR-EI-MS, consistent with an molecular formula of $C_{19}H_{18}N_2O_3$ (cacld for 322.1317). ^{1}H-NMR spectrum signals at δ 7.15 (1H, d, *J* = 8.6 Hz, H-7), 7.02 (1H, s, H-2), 6.96 (1H, d, *J* = 2.3Hz, H-4), 6.66 (1H, dd, *J* = 2.3, 8.6 Hz, H-6), 3.56 (2H, t, *J* = 7.3 Hz, H-9), and 2.92 (2H, t, *J* = 7.3 Hz, H-8) showed **4** has a serotonin moiety.[22] Mass fragmentation ions at *m/z* 176 supported the presence of serotonin moiety.[23-25] Another ^{1}H-NMR spectrum exhibited aromatic signals at δ 7.38 (2H, d, *J* = 8.7 Hz, H-2" and -6") and 7.37 (2H, d, *J* = 8.5 Hz, H-3" and -5"), two olefinic protons at δ 7.45 (1H, d, *J* = 15.7 Hz, H-3') and 6.39 (1H, d, *J* = 15.7 Hz, H-2'), respectively, which suggested a coumaryl moiety attached to a serotonin moiety in this compound (Table 1). These data allowed us to identify the structure of **4** as *N*-(*p*-coumaryl) serotonine.[26] All isolates were tested for their inhibitory activity against acetylcholinesterase (Table 2).

Table 2. Acetylcholinesterase inhibitory activity of compounds **1-5** of the stem of *Z. mays*.

Tested compounds	IC_{50}[1)] (μg/μl)	IC_{50} (μM)
1	–[2)]	–
2	35.5	125
3	20.3	60.4
4	59.1	183.5
5	16.3	53.3
Berberine[3)]	1.5	4.5

1) The inhibitory activity dose that reduced 50% of acetylcholinesterase activity.
2) '–' means that did not show inhibitory activity.
3) A positive control

Among them, *N-trans-p*-coumaryl tyramine (**2**), *N-trans*-ferulyl tryptamine (**3**), *N*-(*p*-coumaryl) serotonine (**4**), and *N*-(*p*-coumaryl)-tryptamine (**5**) exhibited acetylcholinesterase inhibitory activity, with IC_{50} values of 125, 60.4, 183.5 and 53.3 μM, respectively. *N-trans-p*-coumaryl tyramine (**2**) was previously reported as an acetylcholinesterase inhibitor with almost same IC_{50} value.[27] Among tryptamine derivatives (**3-5**), *N*-(*p*-coumaryl)-tryptamine (**5**) exhibited relatively high inhibitory activity, though it's inhibitory activity was lower than that of the positive control. In the

present study, tryptamine derivatives showed more inhibitory potent than the tyramine derivatives and non-substituted compounds, with bare indole skeletons being more potent than hydroxylated indole skeleton.

These results suggested that extracts from the stem of *Z. mays* are potential candidates as therapeutic or preventive agents for Alzheimer's disease.

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