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# Monoamine Oxidase Inhibitors from *Aquilaria agallocha*

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**Abstract** – From the bioassay-directed fractionation and isolation of dichloromethane fraction of *Aquilaria agallocha*, four compounds having MAO inhibitory effect were isolated by repeated silica gel column chromatography. Their chemical structures were established as psoralen (**1**), bergapten (**2**), α-amyrin acetate (**3**) and 5-hydroxymethylfurfural (**4**) on the basis of their physicochemical and spectral data. Among these compounds, psoralen and bergapten showed high inhibitory activities *in vitro* against mouse brain MAO with $IC_{50}$ values 21.3 μM and 13.8 μM, respectively.

**Key words** – *Aquilaria agallocha*, monoamine oxidase inhibition, psoralen, bergapten, α-amyrin acetate, 5-hydroxymethylfurfural

## Introduction

Monoamine oxidase (MAO) is a flavin adenine dinucleotide (FAD)-containing enzyme of the outer mitochondrial membrane. MAO catalyzes the oxidative deamination of neurotransmitters such as catecholamines, serotonin and dietary amines (Tipton *et al.*, 1987). MAO exists as two isozymes referred to as type-A and B (MAO-A and MAO-B) according to substrate specificity and sensitivity to specific inhibitors. MAO-A preferentially oxidizes serotonin and norepinephrine, and is selectively inhibited by clorgyline, whereas MAO-B oxidizes benzylamine and β-phenylethylamine, and is inhibited by deprenyl. The MAO-A inhibitors have been introduced as an antidepressant and MAO-B inhibitors used to overcome the lack of dopamine in Parkinsons disease (Silverman, 1995). A number of MAO inhibitors have been identified, including alkaloids (Rosazza *et al.*, 1992), xanthones (Schaufelberger *et al.*, 1987) and coumarins (Hossain *et al.*, 1996). In our continuing search for MAO inhibitors from natural products, fifteen Vietnamese medicinal plants were screened for their inhibitory activities against MAO. Among these Vietnamese medicinal plants, the MeOH extract of *Aquilaria agallocha* stems showed high inhibitory activity against mouse brain MAO *in vitro*. The *A. agallocha* Roxb. (Thymelacaceae) growing in middle and south of Vietnam, has long been used as a sedative in the Oriental medicines and also widely used as an incense in the Orient (Loi, 1995). In this paper, we describe the bioassay-directed isolation and structure determination of MAO inhibitory components from *A. agallocha* stems.

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## Materials and Methods

**Plant material** – The *A. agallocha* stems were purchased at traditional drug market in Hanoi-Vietnam, 1998 and identified by Dr. Chau Van Minh, Institute of Natural Products Chemistry, National Center for Natural Science and Technology. The voucher speciemens (CNU-VN015) were deposited at the herbarium in the College of Pharmacy, Chungnam National University.

**Reagents and Instruments** – The melting points were measured using an Electro-thermal melting point apparatus and were uncorrected. The IR spectra were recorded form KBr pellets on a JASCO Report-100 IR spectrophotometer. Both the $^1$H- and $^{13}$C-NMR spectra was obtained from a Bruker DRX 300 or Jeol-LAA 40 WB-FT ($^1$H, 400 MHz; $^{13}$C, 100 MHz) spectrometer. The EI-MS spectra were taken on a Hewlett-Packard MS Engine 5989A mass spectrometer. The fluorescence intensities of the reaction product, 4-hydroxyquinoline, were measured on a fluorospectrophotometer (Model F-300, Hitachi, Tokyo, Japan).

**Extraction and isolation** – The *A. agallocha* dried stems (2 kg) were extracted 3 times with methanol to yield 190.0 g of a dried extract upon solvent removal under vacuum. The resulting extract was then suspended in distilled water and separated into two fractions using dichloromethane as the non-aqueous phase. The concentrated dichloromethane fraction (30.0 g) was chromatographed on silica gel column and eluted with $CH_2Cl_2$-MeOH

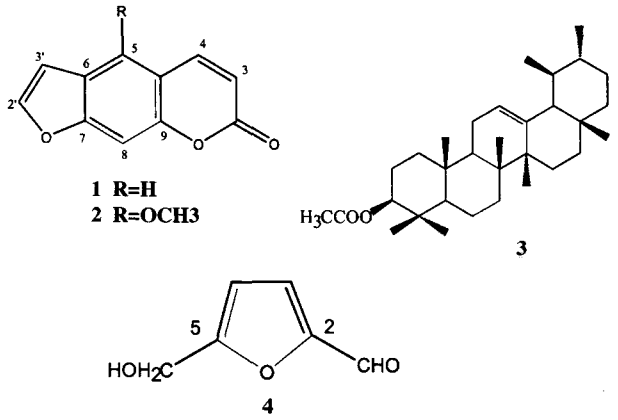


**Fig. 1.** Structures of isolated compounds from *A. agalocha*.

(50:1→10:1) to yield 12 fractions (Fr. 2A-2M). Fr. 2B and Fr. 2D were purified by recrystallization from dichloromethane to give compound **1** (1.2 g) and compound **2** (1.1 g), respectively. Fr. 2A was further chromatographed on a silica gel column with hexane-EtOAc (20:1 to 2:1) as gradient system to give compound **3** (32.6 mg). Compound **4** (180.2 mg) was obtained from Fr. 2F by repeated silica gel column chromatography with hexane-EtOAc (3:2).

**Compound 1 (psoralen)** – colorless needles, mp: 165-166 °C; EI-MS *m/z*: 186 ($M^+$, $C_{11}H_6O_3$); IR $\nu_{max}$ (KBr) $cm^{-1}$: 1710 (C=O); $^1$H-NMR (400 MHz, $CDCl_3$) : 6.39 (1H, d, *J*=9.62 Hz, H-3), 7.80 (1H, d, *J*=9.62 Hz, H-4), 7.68 (1H, s, H-5), 7.46 (1H, s, H-8), 7.70 (1H, d, *J*=2.32 Hz, H-2'), 6.83 (1H, d, *J*=2.32 Hz, H-3'); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ: 161.0 (C-2), 114.6 (C-3), 144.1 (C-4), 119.9 (C-5), 124.9 (C-6), 156.4 (C-7), 99.8 (C-8), 152.0 (C-9), 115.4 (C-10), 146.9 (C-2), 106.4 (C-3').

**Compound 2 (bergapten)** – colorless needles, mp: 185-187 °C; EI-MS *m/z*: 216 ($M^+$, $C_{12}H_8O_4$); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3190 (C-H), 1720 (C=O), 1620, 1580 (C=C); $^1$H-NMR (300 MHz, $CDCl_3$) δ: 6.19 (1H,d, J=9.78 Hz, H-3), 8.08 (1H, d, J=9.78 Hz, H- 4), 7.18 (1H, s, H-8), 7.51 (1H, d, J=2.39 Hz, H-2'), 6.93 (1H, d, J=2.39 Hz, H-3'), 4.19 (3H, s, -$OCH_3$); $^{13}$C-NMR (75MHz, $CDCl_3$) δ: 161.2 (C-2), 112.5 (C-3), 139.2 (C-4), 149.5 (C-5), 112.6 (C-6), 158.3 (C-7), 93.8 (C-8), 152.7 (C-9), 106.3 (C-10), 144.8 (C-2), 105.0 (C-3), 60.0 (-$OCH_3$).

**Compound 3 (α-amyrin acetate)** – white powder, mp: 180-182°C; EI-MS *m/z*: 468 ($M^+$, $C_{32}H_{52}O_2$); IR $\nu_{max}$ (KBr) $cm^{-1}$: 1722 (C=O); $^1$H-NMR (300 MHz, $CDCl_3$): δ: 5.12 (1H, t, *J*=3.6 Hz, H-12), 4.51 (1H, dd, *J*=9.1, 7.0 Hz, H-3α), 2.05 (3H, s, $OCOCH_3$-3), 0.80-1.07 (24H, 8 x $CH_3$); $^{13}$C-NMR (75MHz, $CDCl_3$) δ: 38.5 (C-1), 23.6 (C-2), 80.9 (C-3), 37.6 (C-4), 55.3 (C-5), 18.2 (C-6), 32.9 (C-7), 40.0 (C-8), 47.6 (C-9), 36.8 (C-10), 23.4 (C-11), 124.3 (C-12), 139.6 (C-13), 42.1 (C-14), 26.6 (C-15), 29.1 (C-16), 33.7 (C-17), 59.1 (C-18), 39.6 (C-19), 39.6 (C-20), 31.3 (C-21), 41.6 (C-22), 28.0 (C-23), 16.7 (C-24), 15.7 (C-25), 16.9 (C-26), 23.2 (C-27), 28.7 (C-28), 17.5 (C-29), 21.4 (C-30).

**Compound 4 (5-hydroxymethylfurfural)** – pale yellow oil, EI-MS *m/z*: 126 ($M^+$, $C_6H_6O_3$); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3400 (OH), 1680 (C=O), 1400 ($CH_2$), 1195 (C-O); $^1$H-NMR (300MHz, $CDCl_3$) δ: 7.23 (1H,d, *J*=3.53 Hz, H-3), 6.52 (1H, d, *J*=3.53 Hz, H-4), 9.61 (1H, s, CHO), 4.72 (2H, s, $CH_2OH$); $^{13}$C-NMR (75MHz, $CDCl_3$) δ: 152.1 (C-2), 123.2 (C-3), 109.9 (C-4), 160.9 (C-5), 177.7 (C-1'), 57.3 (C-2').

**Enzyme preparation** – Mice (ICR, male, 25-30 g) were obtained from the Samyook Animal Center (Soowon, Korea) and maintained under laboratory conditions on food and water *ad libitum* and were sacrificed by cervical dislocation. For MAO enzyme preparation, a crude mitochondrial fraction was isolated from the mouse brain according to the reported method of Naoi (Naoi *et al.*, 1989).

**Assays** – The MAO activity was measured fluorimetrically using kynuramine as a substrate according to the method reported by Kraml (Kraml, 1965) with a slight modification (Lee *et al*, 1999). Samples (2 μl) dissolved in dimethylsulfoxide (DMSO) were added to 0.2 M potassium phosphate buffer (73 μl, pH 7.4) which contained 5 μl of MAO suspension, and 20 μl of 500 μM kynuramine as a substrate. After incubation at 37°C for 30 min in water bath, the reaction was stopped by addition 25 μl of 10% $ZnSO_4$ and 5 μl of 1N NaOH and then centrifuged at 3000 g for 5 min. The 70μl of supernatant 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-hydroxyquinolone, was measured at 380 nm (emission) with 315 nm (excitation). As a blank test, the reaction was carried out omitting the enzyme.

## Results and discussion

As a result of our screening test *in vitro* of 15 Vietnamese medicinal plants to find MAO inhibitors, the methanol extract of *A. agallocha* stems and *Kaempferia galanga* rhizomes showed strong inhibitory effects on MAO with 93.6% and 95.7% inhibition at a concentration of 250 μg/ml, respectively. Three cinnamate derivatives such as ethyl *p*-methoxy-*trans*-cinnamate, ethyl *trans*-cinnamate and 3,6-dimethoxy-*trans*-cinnamate were isolated from the rhizomes of *K. galanga* and their $IC_{50}$ values *in vitro* against MAO mouse brain were 5.0 μM, 92.0 μM and 20.2 μM respectively (Huong *et al.*, 2000). In order to

**Table 1.** Inhibitory activities of Vietnamese medicinal plants on MAO

| No | Scientific name | Family name | Used part | Inhibition (%) |
|---|---|---|---|---|
| 1 | *Hibiscus sinensis* | *Malvaceae* | root | 32.8 |
| 2 | *Chrysanthemum sinense* | *Asteraceae* | flower | 49.3 |
| 3 | *Aquilaria agallocha* | *Thymelacaceae* | stem | 93.6 |
| 4 | *Dioscorea tokoro* | *Dioscoreaceae* | stem | 38.6 |
| 5 | *Pleomele cochinchinensis* | *Liliaceae* | stem | 34.9 |
| 6 | *Polygonum multiflorum* | *Polygonaceae* | stem | 26.9 |
| 7 | *Tinospora sinensis* | *Menispermaceae* | stem | 39.3 |
| 8 | *Smilax glabra* | *Liliaceae* | stem | 32.5 |
| 9 | *Acanthopanax aculeatus* | *Araliaceae* | stem | 32.6 |
| 10 | *Drynaria fortunei* | *Polypodiaceae* | stem | 43.1 |
| 11 | *Aristolochia balansae* | *Aristolochiaceae* | stem | 19.5 |
| 12 | *Ophiopogon japonicus* | *Liliaceae* | stem | 20.5 |
| 13 | *Lactuca indica* | *Asteraceae* | stem | 48.5 |
| 14 | *Adenosma caeruleum* | *Crophulariaceae* | stem | 39.4 |
| 15 | *Kaempferia galanga* | *Zingiberaceae* | rhizoma | 95.7 |

isolate MAO inhibitory components from Vietnamese medicinal plants, the methanol extract of *A. agallocha* was fractionated with dichloromethane and water. From the bioassay-directed fractionation and isolation of dichloromethane fraction, four compounds (**1-4**) having MAO inhibitory activities were isolated. Their chemical structures were established as psoralen (**1**), bergapten (**2**), α-amyrin acetate (**3**) and 5-hydroxymethylfurfural (**4**) on the basis of their physicochemical and spectral data .The $IC_{50}$ values of these compounds against mouse brain MAO were shown in Table 1. Among four isolated compounds, psoralen (**1**) and bergapten (**2**) showed potent inhibitory activities in mouse brain MAO with the $IC_{50}$ value of 21.3 μM and 13.8 μM, respectively, but weaker than that of clorgyline and iproniazid, a selective inhibitor of type A and type B MAO. α-Amyrin acetate (**3**) and 5-hydroxymethylfurfural (**4**) showed a lower activity on mouse brain MAO with the inhibitory percentage of 30.9% and 50.8% at concentration 100 μg/ml, respectively. Several coumarin compounds were reported to have strong MAO inhibitory activities (Yun *et al.*, 2001; Jo *et al.*, 2002) and a series of coumarin derivatives was synthesized to develope antidepressant and antiparkinsonian drugs (Rendenbach-Muller *et al.*, 1994). Coumarin type components are major constituent of *A. agarocha* and they showed strong inhibitory activities on MAO activities. As far as the sedative action of *A. agarocha*, coumarin components such as psoralen and bergapten might be responsible for its activity. Further investigations are needed to establish the pharmacological actions of naturally occurring coumarins.

**Table 2.** Inhibitory activities of MAO by isolated compounds from *A. agallocha.*

| Compound | $IC_{50}$ (μM) |
|---|---|
| Psoralen (**1**) | 21.3 |
| Bergapten (**2**) | 13.8 |
| α-Amyrin acetate (**3**) | >100 |
| 5-Hydroxymethyl furfural (**4**) | >100 |
| Iproniazid | 3.2 |
| Clorgyline | 3.8 |

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## References

Hossain, C.F., Okuyama, E., and Yamazaki, M., A new series of coumarin derivatives having monoamine oxidase inhibitory activity from *Monascus anka. Chem. Pharm. Bull.* **44**, 1535-1539 (1996).

Huong, D.T.H, Lee, M.K., Bae, K., and Kim Y.H., Monoamine oxidase inhibitors from *Kaempferia galanga. J. Pharm. Sci. (C.N.U.)* **16**, 52-56 (2000).

Jo, Y.S., Huong, D.T.L., Bae, K. Lee, M.K., and Kim, Y.H., Monoamine oxidase inhibitory coumarin from *Zanthoxylum schinifolium. Planta Med.* **68**, 84-85 (2002).

Kraml M., A rapid microfluorometric determination of monoamine oxidase. *Biochem. Pharmacol.* **14**, 1683-1685, 1965.

Lee, S.S., Kai, M., Lee, M.K., Effects of natural isoquinoline alkaloids on monoamine oxidase activity in mouse brain : inhibition by berberine and palmatine. *Med. Sci. Res.* **27**: 749-751 (1999).

Loi, D.T., *Vietnamese medicinal plants and medicine.* Science and Technology Pub. House, Hanoi, 1995.

Naoi, M., Takahashi, T., Darrez, H., Kabeya, R., Taguchi, E., N-Methyl isoquinolinim ion as an inhibitor of tyrosine hydroxylase, aromatic L-amino acid decarboxylase and monoamine oxidase. *Neurochem. Int.* **15**, 315-320 (1989).

Rendenbach-Müller, B., Schlecker, R., Traut, M., and Weifenbach, H., Synthesis of coumarins as subtype-selective inhibitors of monoamine oxidase. *Bioorg. Med. Chem. Lett.* **4**, 1195-1198

(1994).
Rosazza, J.P.N., Duffel, M.W., El-Marakby, S., and Ahn, S.H., Metabolism of the *Catharanthus* alkaloids: From *Streptomyces griseus* to monoamine oxidase B. *J. Nat. Prod.* **55**, 269-284 (1992).
Schaufelberger, D., and Hostettmann, K., Chemistry and pharmacology of *Gentiana lactea. Planta Med.* **49**, 219-221 (1987).
Silverman, R.B., Radical ideas about monoamine oxidase. *Acc. Chem. Res.* **28**, 335-342 (1995).
Tipton, K.F., OCarroll, A.M., McCrodden, J.M., The catalytic behavior of monoamine oxidase. *J. Neural Transm. (Suppl.)* **23**, 25-35 (1987).
Yun, B.S., Lee, I.K., Ryoo, I.J., and Yoo, I.D., Coumarins with monoamine oxidase inhibitory activity and antioxydative coumarino-lignans from *Hibiscus syriacus. J. Nat. Prod.* **64**, 1238-1240 (2001).

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