Monoamine Oxidase Inhibitor from the Rhizomes of
Kaempferia galanga L.[1]

Tadataka Noro,* Toshio Miyase, Masanori Kuroyanagi,
Akira Ueno and Seigo Fukushima

Shizuoka College of Pharmacy, 2-2-1, Oshika, Shizuoka 422, Japan

Monoamine oxidase (MAO) inhibitor contained in the rhizomes of Kaempferia galanga L. (Zingiberaceae) was isolated and identified as ethyl p-methoxy-trans-cinnamate (I). The type of inhibition with respect to benzylamine by I was competitive.

Keywords——monoamine oxidase; inhibitor; competitive inhibitor; benzylamine; Kaempferia galanga; Zingiberaceae; ethyl p-methoxy-trans-cinnamate; p-methoxy-trans-cinnamic acid

The purpose of this study was not only the development of new medicinal drugs but also to examine the effects of traditional oriental drugs. Inhibitors of monoamine oxidase (MAO, EC 1.4.3.4) are candidates as drugs for the treatment of depression.

In screening tests *in vitro* aiming to find MAO inhibitors from many crude oriental drugs and plant materials, the rhizomes of Kaempferia galanga L. (Japanese name, "sanna", Zingiberaceae) were found to possess a strong inhibitory effect on this enzyme. The rhizomes of Kaempferia galanga have been used as an aromatic stomachic and an incense. The constituents of the rhizomes hitherto reported are ethyl p-methoxycinnamate, ethyl cinnamate, cinnamaldehyde, camphene, l-Δ^3-carene, borneol, p-methoxystyrene and pentadecane.[2] In this paper, we describe the isolation of a MAO inhibitor from the rhizomes of Kaempferia galanga.

Results and Discussion

The activity of MAO towards benzylamine as a substrate was assayed spectrophotometrically at 250 nm by the method of Tabor *et al.*[3] with a slight modification. The rhizomes of Kaempheria galanga were extracted with benzene, chloroform, acetone, methanol and water under reflux as shown in Chart 1. The inhibitory activity of each extract against MAO and the yields are shown in Chart 1. The most active (benzene) extract was fractionated by silica gel column chromatography with a hexane–benzene–acetone gradient system, and the fractions were monitored by thin layer chromatography (TLC) and also by measurement of inhibitory activity against MAO. The most active fraction of the benzene extract was recrystallized from hexane to give an active compound as colorless prisms (I), mp 47 °C. This was identical with synthetic ethyl p-methoxy-trans-cinnamate on the basis of melting point, spectral comparisons and elemental analysis. Compound I is a major constituent of the rhizomes. The concentration of I in the reaction mixture required to give 50% inhibition (IC_{50}) was 6.8×10^{-5} M.

Compound I was also obtained as the most active inhibitory principle against MAO from other extracts (chloroform ext., acetone ext. and methanol ext.). p-Methoxy-trans-cinnamic acid (II), which was obtained from the rhizomes at the same time, had essentially no effect on MAO at the same concentration.

Kinetic studies were done on the effect of **I** on the oxidation of benzylamine by MAO. The results are shown as Lineweaver–Burk plots[4] in Fig. 1. The type of inhibition by **I** was competitive. K_m of MAO for benzylamine was 1.39×10^{-3} M and the K_i value of **I** for oxidation of benzylamine by MAO was 3.04×10^{-5} M.

The types of inhibitor of MAO hitherto reported include hydrazines,[5] xanthones,[6] chalcones,[7] amines,[8] alkaloids,[8] cinnamamide[8] and so on. The result obtained in this study shows that **I** is an inhibitor of a new structural type. We are synthesizing various cinnamic acid derivatives in addition to the above compounds, and extensive studies on their MAO inhibitory activities are in progress in our laboratory.
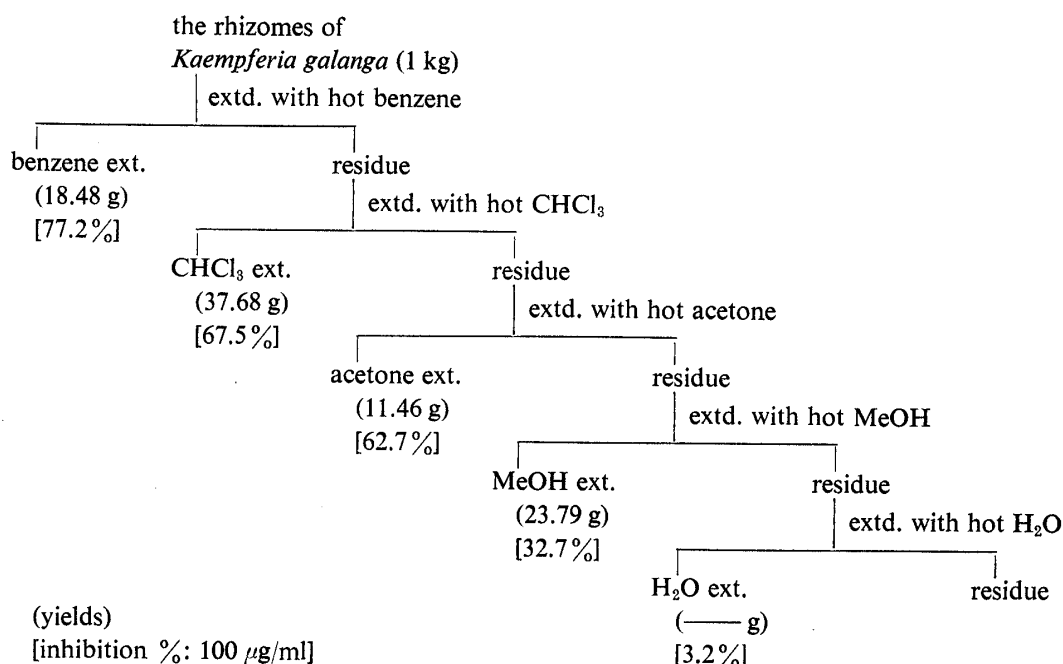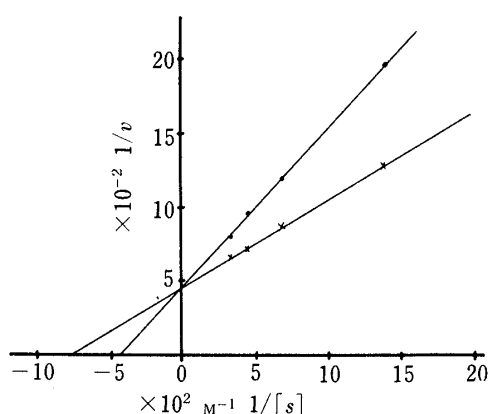
the rhizomes of
Kaempferia galanga (1 kg)
    | extd. with hot benzene

benzene ext.　　　　　residue
(18.48 g)　　　　　　　| extd. with hot $CHCl_3$
[77.2%]

$CHCl_3$ ext.　　　　　residue
(37.68 g)　　　　　　　| extd. with hot acetone
[67.5%]

acetone ext.　　　　　residue
(11.46 g)　　　　　　　| extd. with hot MeOH
[62.7%]

MeOH ext.　　　　　　residue
(23.79 g)　　　　　　　| extd. with hot H_2O
[32.7%]

H_2O ext.　　　　　　residue
(yields)　　　　　　　(——— g)
[inhibition %: 100 μg/ml]　[3.2%]

Chart 1

Fig. 1. Inhibitory Action of Ethyl *p*-Methoxy-*trans*-cinnamate (**I**) on MAO

Lineweaver–Burk plots in the absence (0 M, ×——×) and in the presence (2.42×10^{-5} M, ●——●) of **I** with benzylamine as a substrate. v, μmol substrate metabolized $\cdot g^{-1}$ enzyme $\cdot \min^{-1}$; s, substrate.

Experimental

The following instruments were used to obtain physical data. Melting points were determined on a Yanagimoto melting point apparatus, and are uncorrected. The infrared (IR) spectra were recorded on a JASCO IR A-202 infared spectrophotometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a JEOL JNM-FX 90Q FT NMR spectrometer (90 MHz) with tetramethylsilane as an internal standard (δ value; s, singlet; d, doublet; t, triplet; q, quartet; br, broad). Mass spectra (MS) were recorded on a JEOL JMS-01SG-2 mass spectrometer. Silica gel 60 GF_{254} (Merck) was used for TLC and detection was achieved by illumination with an ultraviolet lamp or by spraying 20% H_2SO_4 followed by

heating. For column chromatography, Silica gel 60 (Merck) was used. The spectrophotometric measurements were carried out with a Hitachi model 101 spectrophotometer.

Enzyme and Chemicals——Monoamine oxidase (EC 1.4.3.4) from bovine plasma was obtained from Sigma Chemical Co. Benzylamine and Tween 80 were obtained from Wako Pure Chemical Industries, Ltd. Sodium phosphate dibasic 12 hydrate and potassium phosphate monobasic were obtained from Kanto Chemical Co. Buffer was Sörensen's 0.2 M potassium phosphate–sodium phosphate buffer of pH 7.4. The substrate, 0.1 M benzylamine sulfate, was prepared immediately before use as follows. Benzylamine (1.07 g), which had been purified by redistillation, and 2 N H_2SO_4 (5 ml) were dissolved in H_2O to make 100 ml of solution. Enzyme solution containing about 0.02 unit per ml in 0.2 M potassium phosphate buffer of pH 7.4 was prepared immediately before use.

Test Solution——When the test samples were not easily soluble in water, they were dissolved in ethanol and Tween 80, and diluted with water to give final concentrations of 3% (v/v) for ethanol and 1.5% (w/v) for Tween 80 in the assay mixtures. At these concentrations of ethanol and Tween 80, MAO activities were little affected.

Assay of Monoamine Oxidase Activity——The MAO activities with benzylamine as a substrate were measured spectrophotometrically by the method of Tabor et al.[3] with a slight modification. Test solution (1.0 ml), 0.2 M potassium phosphate buffer (4.8 ml) and enzyme solution (0.1 ml) were mixed and preincubated at 25 °C for 15 min. After this, the substrate (0.1 ml) was added to the assay mixture and the whole was incubated at 25 °C for 30 min. Then, 1 N HCl (1.0 ml) was added to stop the reaction. The absorbance of the reaction mixture was measured spectrophotometrically at 250 nm. A blank was prepared in the same way, but the enzyme solution was added to the assay mixture after adding 1 N HCl.

Estimation of Percent Inhibition——The percent inhibition of the oxidative reaction was calculated by the method of Aoyagi et al.[9]

Extraction and Separation——The dried rhizomes (1 kg, commercial product) of *Kaempferia galanga* were extracted three times with 3 l of benzene under reflux for 3 h, then with $CHCl_3$, acetone, methanol and H_2O in the same way, and the extracts were each concentrated *in vacuo*. These extracts were assayed for inhibitory effects on MAO. The yields and inhibitory activities are shown in Chart 1. These extracts were each fractionated by silica gel column chromatography with a hexane–benzene–acetone gradient system. The eluates were monitored by TLC and also by measurement of inhibitory activity against MAO. The most active fraction (eluted with hexane : benzene = 1 : 1, v/v) of the benzene extract was purified by recrystallization from hexane to give the active compound as colorless prisms (**I**). The eluate with benzene : acetone (1 : 1, v/v) of the benzene extract gave pale yellow prisms (**II**) from EtOH. Compound **I** was also obtained as the most active principle against MAO from other extracts ($CHCl_3$ ext., acetone ext. and MeOH ext.).

Ethyl *p*-Methoxy-*trans*-cinnamate (I)——Colorless prisms from hexane, mp 47 °C (lit. mp 48—50 °C).[2] IR (KBr) cm^{-1}: 1715, 1635, 1610, 1180, 838. MS m/z (rel. int. %): 206 (M$^+$, 55), 161 (100), 134 (62), 133 (53), 118 (26), 90 (22), 89 (35), 77 (22), 63 (24). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, $J = 7.2$ Hz), 3.74 (3H, s), 4.22 (2H, q, $J = 7.2$ Hz), 6.26 (1H, d, $J = 15.9$ Hz), 6.83 (2H, d, $J = 8.6$ Hz), 7.40 (2H, d, $J = 8.6$ Hz), 7.61 (1H, d, $J = 15.9$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.34 (q), 55.21 (q), 60.20 (t), 114.37 (d), 115.89 (d), 127.27 (s), 129.65 (d), 144.17 (d), 161.49 (s), 167.07 (s). *Anal.* Calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.99; H, 6.71. This was identical with synthetic ethyl *p*-methoxy-*trans*-cinnamate on the basis of mp, spectral comparisons and elemental analysis. Compound **I** is the major constituent of the rhizomes.

***p*-Methoxy-*trans*-cinnamic Acid (II)**——Pale yellow prisms from EtOH, mp 173 °C (lit. mp 171—172 °C).[2] *Anal.* Calcd for $C_{10}H_{10}O_3$: C, 67.40; H, 5.16. Found: C, 67.51; H, 5.14. This was identical with synthetic *p*-methoxy-*trans*-cinnamic acid on the basis of mp, spectral comparisons and elemental analysis.

Lineweaver–Burk Plots[4]——Lineweaver–Burk plots for MAO under our assay conditions were obtained in the absence and in the presence of **I** using benzylamine as a substrate, as shown in Fig. 1.

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