Studies on the Monoamine Oxidase (MAO) Inhibitory Potency of TL-1, Isolated from a Fungus, *Talaromyces luteus*

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The compound tentatively named TL-1 was isolated from *Talaromyces luteus* as a metabolite having monoamine oxidase (MAO) inhibitory potency. TL-1 showed mixed-type inhibition of MAO in mouse liver when kynuramine was used as a substrate, and the IC_{50} was 6.6 μM. The inhibition constants (K_i) for MAO-A and -B in mouse liver were 39.9 and 7.85 μM, respectively. On the other hand, the K_i values for MAO-A and -B in mouse brain were 74.0 and 0.71 μM, respectively. Despite the marked structural resemblance between TL-1 and 7-episclerotiorin, the latter compound had little inhibitory effect on MAO.

Keywords *Talaromyces luteus*; fungal metabolite; monoamine oxidase; inhibitor; sclerotiorin; azaphilone; derivative; kinetic study

Introduction

During a survey of inhibitory potency towards monoamine oxidase (MAO: EC 1.4.3.4.) in 122 species of Ascomycetes,[1] some species having an MAO-inhibitory effect were found. A compound tentatively named ENA-1 was isolated as an MAO inhibitor from *Emericella navahoensis* among those species, and the results on MAO inhibitory potency of this compound have already been reported.[2]

In this paper, the characterization and kinetic studies on the MAO inhibitory potency of TL-1, which had been isolated from *Talaromyces luteus* at the same time as ENA-1, are described. The inhibitory potencies of some TL-1 related compounds were also examined. TL-1 was found to be a new compound related to 7-episclerotiorin (see Table I). Details on the isolation and chemical characterization of this compound will be reported in a separate paper.

Materials and Methods

Isolation of TL-1 from *Talaromyces luteus* *T. luteus* strain IFM 42239 was grown in stationary culture at 25 °C for 21 d on sterilized polished rice (20 kg) using Roux flasks containing 200 g of the rice in each flask. After cultivation, the moldy rice was extracted with ethyl acetate. The solvent was evaporated off and the residue (120.8 g) was repeatedly chromatographed on silica gel to obtain TL-1 (24.9 mg).

Preparation of Crude MAO Mouse liver was homogenized with 4 volumes of 0.15 M KCl and mouse brain was homogenized with 10 volumes of 0.25 M sucrose in a Teflon homogenizer on ice. The homogenates were centrifuged at 1000 × *g* for 10 min to remove cell debris, and 0.5 ml of the supernatant was usually used for assays.

Assay of MAO Activity The MAO activity with kynuramine as a substrate was assayed by a modification of the fluorometric method of Kraml.[3] Fluorescence of 4-hydroxyquinoline, which was formed from kynuramine by MAO, was measured at 380 nm with excitation at 315 nm. The activities of MAO-A and -B in mouse liver or brain were measured in the presence of 1 μM *l*-deprenyl (MAO-B inhibitor) and clorgyline (MAO-A inhibitor), respectively.[4] The test solutions were dissolved in dimethylsulfoxide, which was confirmed to have no effect on MAO activity below 2.8% concentration. Kinetic data were calculated from MAO activity in the absence and presence of TL-1, with kynuramine at various concentrations. Protein was measured by the biuret method.

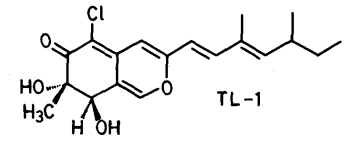
Results and Discussion

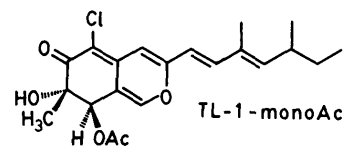
TL-1 inhibited MAO in mouse liver, with an IC_{50} of 6.6 μM. Among the TL-1 related compounds, *i.e.*, tetrahydro-TL-1, TL-1-monoactate, TL-1-diacetate, TL-1-methylamine adduct, sclerotiorin and 7-episclerotiorin, TL-1-monoacetate and -diacetate inhibited the enzyme effi-

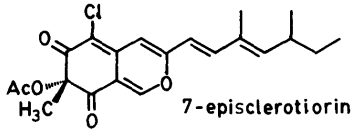
TABLE I. Inhibition of MAO by TL-1 Derivatives

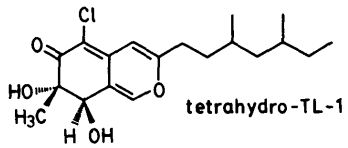
Compounds	IC_{50} (μM)
TL-1	6.6
Tetrahydro-TL-1	—
TL-1-methylamine-adduct	68.7
TL-1-monoAc	1.9
TL-1-diAc	7.4
Sclerotiorin	—
7-Episclerotiorin	—

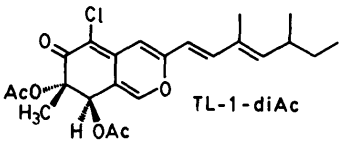
—: IC_{50} not determined.

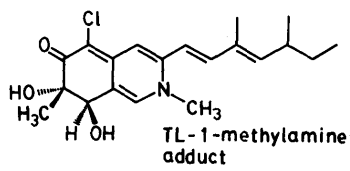


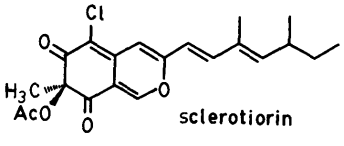












ciently as shown in Table I.

On the other hand, sclerotiorin and 7-episclerotiorin had no effect. It is interesting that the MAO inhibitory potency disappears on conversion of the steric configuration of the hydroxyl group at the 8-position and hydrogenation of the double bonds in the side chain.

When the MAO activity in mouse liver homogenate was measured in the presence of TL-1, mixed-type inhibition was observed, with a K_i value of 3.6 μM. When the homogenate was preincubated with TL-1 for various times before adding kynuramine, no time-dependent change of the inhibitory effect was observed, suggesting that this compound inhibited MAO reversibly.

The effect of TL-1 on MAO-A was then examined by treating mouse liver homogenate with *l*-deprenyl (MAO-B inhibitor) and using kynuramine as a substrate. The inhibition pattern was of mixed-type and the K_i value was 39.9 μM as determined from the Lineweaver–Burk plot and Dixon plot, respectively. On the other hand, TL-1 more potently inhibited the mouse liver MAO-B when the liver homogenate was treated with clorgyline (MAO-A inhibitor): its inhibition pattern of mixed-type and the K_i value was 7.9 μM. It was thus demonstrated that TL-1 is a more potent inhibitor of MAO-B than MAO-A in mouse liver (Table II).

TL-1 also inhibited MAO in mouse brain homogenate; its IC_{50} was 5.1 μM, and the K_i value was 1.4 μM. While MAO-A (*l*-deprenyl-treated) in mouse brain was slightly inhibited by TL-1, the inhibitory potency towards MAO-B (clorgyline-treated) in mouse brain was significantly higher. The K_i values towards MAO-A and MAO-B were 74.0 and 0.71 μM, respectively (Table II). The inhibition pattern of TL-1 towards MAO-B in mouse brain was of mixed-type. It is thus demonstrated that TL-1 inhibited MAO-B more potently than MAO-A also in brain homogenate.

MAO is known to play an important physiological role by regulating the levels of norepinephrine, dopamine, serotonin, *etc.* in various organs.[5] Since isonicotinic acid hydrazin (INH) was found to have MAO inhibitory potency and antidepressant activity,[6] some MAO inhibitors had been used as antidepressants. However, side effects such as hepatotoxicity and cheese effect were found.[7] Only one such agent, safrazine, is still in use as an antidepressant at present in Japan.

It has recently been reported that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is oxidized to 1-methyl-4-phenylpyridinium ion (MPP^+) by MAO-B and MPP^+ causes Parkinson-like symptoms.[8]

It is currently expected that MAO-B selective inhibitors will be important as therapeutic agents in Parkinson's disease,[9,10] and the discovery of safe MAO-B inhibitors may therefore be valuable for the development of psychiatic treatment. TL-1, which is a selective MAO-B inhibitor, may be useful.

TABLE II. Kinetic Data on the Inhibition of MAO by TL-1

Enzyme source	K_i (μM)	IC_{50} (μM)
Mouse brain homogenate		
Untreated	1.43	5.1
MAO-A (deprenyl-treated)	74.0	600.0
MAO-B (clorgyline-treated)	0.71	4.0
Mouse liver homogenate		
Untreated	3.57	5.3
MAO-A (deprenyl-treated)	39.9	43.0
MAO-B (clorgyline-treated)	7.85	12.0

K_i values were calculated from the results of assays in the absence and presence of TL-1 (2.85, 5.70, 11.4, 22.8, 28.5, 57.0, 114.0, 285.0 and 570 μM). Assays were performed at three concentrations of kynuramine (0.125—0.5 mM) as a substrate.

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