1-Methyl-2-undecyl-4(1*H*)-quinolone as an Irreversible and Selective Inhibitor of Type B Monoamine Oxidase

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The inhibitory compound of monoamine oxidase (MAO) activity was isolated from the CH_2Cl_2 fraction of the fructus of *Evodia rutaecarpa* and identified as 1-methyl-2-undecyl-4(1*H*)-quinolone (1). Compound 1 showed a selective inhibition of type B MAO (MAO-B) activity with the IC_{50} value of 15.3 μM using a substrate kynuramine, but did not inhibit type A MAO (MAO-A) activity. The kinetic analysis using Lineweaver-Burk plots indicated that compound 1 competitively inhibited MAO-B activity with the K_i value of 9.91 μM. The inhibition of MAO-B by compound 1 was found to be irreversible by dialysis of the incubation mixture. These results suggest that compound 1 is a potent irreversible inhibitor of MAO-B, and may regulate catecholamine content in the neurons.

Key words 1-methyl-2-undecyl-4(1*H*)-quinolone; *Evodia rutaecarpa*; monoamine oxidase inhibitor

Monoamine oxidase (MAO; EC 1.4.3.4) is a flavin-containing enzyme that catalyzes the oxidation of a variety of amine-containing neurotransmitters such as catecholamines and serotonin to yield the corresponding aldehyde.[1—3)]

MAO exists in two isoforms, namely type A and type B (MAO-A and MAO-B), which are characterized by their sensitivity towards specific substrates and inhibitors. MAO-A preferentially deaminates serotonin and norepinephrine, and is selectively inhibited by clorgyline. However, MAO-B preferentially deaminates β-phenylethylamine and benzylamine, and is selectively inhibited by *l*-deprenyl and pargyline.[4,5)]

The inhibitors of MAO-A are expected to be clinically useful to treat depression and anxiety, while those of MAO-B appear to be helpful in the prevention and adjunct treatment of Parkinson's disease.[6,7)] A number of inhibitors of MAO activity such as quinolines,[8,9)] isoquinolines,[10—12)] coumarins,[13,14)] and xanthones[15,16)] have been reported.

As a part of our ongoing research for MAO inhibitors from natural resources, we found that a MeOH extract from the fruits of *Evodia rutaecarpa* Bentham (Rutaceae) strongly inhibited the MAO activity in mouse brain. The MeOH extract was, therefore, subjected to the bioactivity-guided fractionations to isolate the active compound. The finally purified bioactive substance, compound **1** (Fig. 1), was identified by comparison of its spectral data as 1-methyl-2-undecyl-4(1*H*)-quinolone and was shown to be a selective inhibitor on MAO-B.

Results and Discussion

In the search for MAO inhibitors from the medicinal plants, we found that CH_2Cl_2 fraction of the dried unripe fruits of *E. rutaecarpa* showed the potent inhibitory effects on MAO in mouse brain. A CH_2Cl_2 fraction of *E. rutaecarpa* at a concentration of 200 μg/ml (89.1% inhibition) showed a potent inhibitory activity against MAO in mouse brain. Activity-guided separation and purification of the extract yielded a known quinolone alkaloid, 1-methyl-2-undecyl-4(1*H*)-quinolone (**1**) as an active compound (Fig. 1). The structure was identified by comparison with the physical properties and spectral data (UV, IR, MS, ^{1}H- and ^{13}C-NMR) with literature values.[17,18)]

Compound **1** inhibited MAO activity in a concentration-dependent manner and the IC_{50} value was 35.2 μM: compound **1** exhibited a less inhibitory effect than iproniazid (IC_{50} value: 12.5 μM) which was used as a positive control (data not shown).

The inhibitory activity of compound **1** against MAO-A and MAO-B activities in mouse brain were investigated. *l*-Deprenyl-treated MAO preparation was used for the measurement of MAO-A activity, and a clorgyline-treated one was for MAO-B. MAO-A activity in our preparations was about 32% of the total MAO activity and MAO-B activity was about 61% (Table 1).

Compound **1** preferentially inhibited the MAO-B activity than MAO-A activity in a concentration-dependent manner with the IC_{50} values of 15.3 and 338.2 μM, respectively (Table 1). These data indicate that compound **1** is a potent inhibitor for MAO-B activity.

According to the kinetic properties of MAO-B from mouse brain, the values of K_m and V_{max} by using kynuramine were 92.5±0.5 μM and 4.28±0.04 nmol/min/mg protein, respectively (n=5) (Fig. 2). Kinetic study was performed on MAO-B at different concentrations of the substrate in the absence or presence of compound **1**. Lineweaver–Burk reciprocal plot analyses clearly demonstrated that the inhibition of MAO-B activity with compound **1** was competitive with the K_i value of 9.91±0.2 μM (n=4) (Fig. 2).

MAO-B from mouse brain mitochondrial fraction (0.1 mg protein) was incubated with 15 μM compound **1** or 40 μM

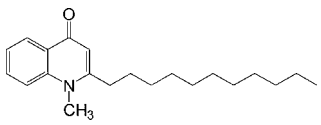

Fig. 1. Chemical Structure of 1-Methyl-2-undecyl-4(1*H*)-quinolone (Compound **1**) from *Evodia rutaecarpa*

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Table 1. Inhibitory Effects of Compound **1** on MAO-A and MAO-B in Mouse Brain

Types	Concentration (μM)	MAO activity (nmol/min/mg protein) (% of control)	IC_{50} value (μM)
Control		2.305±0.11	
MAO-A (*l*-deprenyl-treated)			338.2
(Control+*l*-deprenyl)		0.738±0.08 (100)	
Compound **1**	50	0.595±0.03 (80.7)*	
	100	0.510±0.04 (69.1)**	
	200	0.416±0.05 (56.4)**	
	400	0.366±0.01 (49.6)**	
MAO-B (clorgyline-treated)			15.3
(Control+clorgyline)		1.406±0.10 (100)	
Compound **1**	3	1.102±0.07 (78.4)*	
	6	0.938±0.06 (66.7)**	
	15	0.687±0.03 (48.9)***	
	30	0.522±0.06 (37.1)***	

The activities of MAO-A and MAO-B in mouse brain were measured in the presence of 1 μM *l*-deprenyl or clorgyline, respectively. Data represent the means±S.E.M. of three independent experiments performed in triplicate. Significantly different from the control value: * $p<0.05$; ** $p<0.01$; *** $p<0.001$ (Student's *t*-test).

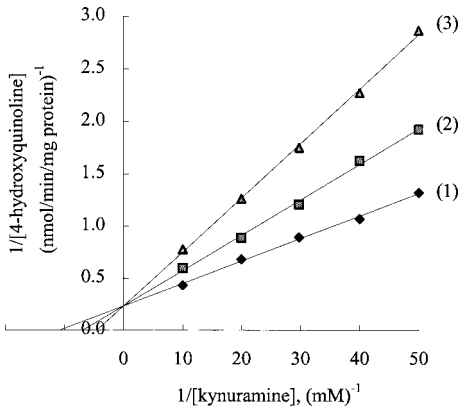

Fig. 2. Inhibition of Type B Monoamine Oxidase (MAO-B) by Compound **1** Added in the Enzyme Reaction Mixture

The reciprocal of MAO-B activities was plotted against the reciprocal of substrate concentrations ($n=5$). Concentrations of compound **1**: (1) 0 μM; (2) 6 μM; (3) 15 μM.

Table 2. Effects of Dialysis on the Inhibition of MAO-B by Compound **1**

		MAO-B activity (nmol/min/mg protein) (% of control)
Before dialysis		
MAO-B (clorgyline-treated)		0.694±0.06 (100)
Compound **1**	15 μM	0.398±0.04 (57.4)**
Amitriptyline	40 μM	0.383±0.05 (55.7)**
After dialysis		
Dialysate (compound **1**)		0.363±0.03 (52.2)**
Dialysate (amitriptyline)		0.642±0.03 (92.5)
Amitriptyline	40 μM	0.381±0.02 (59.3)**

MAO-B (clorgyline-treated) were incubated with 15 μM compound **1** or 40 μM amitriptyline for 30 min at 37 °C, and then dialyzed against 10 mM potassium phosphate buffer, pH 7.4 at 4 °C over night. MAO-B activity in the dialysate was measured using 100 μM kynuramine in the absence of and in the presence of compound **1** or amitriptyline. Amitriptyline, a reversible MAO-B inhibitor, was used for positive control. Results represent the means±S.E.M. ($n=3$). Significantly different from the control value: ** $p<0.01$ (Student's *t*-test).

amitriptyline for 30 min at 37 °C, and then the reaction mixture was dialyzed against 10 mM potassium phosphate buffer pH 7.4 at 4 °C overnight. MAO-B activity in the dialysate was examined using 100 μM kynuramine. MAO-B activity by compound **1** was not recovered, whereas the activity by amitriptyline, a reversible inhibitor, was recovered (Table 2).[19,20] These results indicated that compound **1** is an irreversible inhibitor.

Irreversible MAO inhibitors such as phenelzine and tranylcypromine have proven efficacy, not only in depression, such as geriatric depression, but also in anxiety or stress-related conditions associated with panic or phobias and in appetite disorders.[21—23] Indeed, an increase of MAO-B activity has been observed in some neurological disorders such as Parkinson's disease, Alzheimer's disease and Huntington's chorea.[6,7]

Compounds that selectively inhibit the MAO-B isozyme are used in the treatment of Parkinson's disease. Selegiline, a MAO-B inactivator having a selectivity for inhibition of MAO-B/MAO-A of 39, is used to treat Parkinson's disease.[24] In this study, compound **1** as an irreversible inhibitor of MAO-B has a selectivity for inhibition of MAO-B/MAO-A of 22, and so it might be effective in Parkinson's disease.

In conclusion, this study described herein suggests that compound **1**, a quinolone alkaloid, from the fructus of *E. rutaecarpa* selectively inhibits the mouse brain MAO-B activity and that the inhibitory pattern of compound **1** is irreversible and competitive. These results also suggest that compound **1** may contribute to the treatment of Parkinson's disease or Alzheimer's disease. The *in vivo* pharmacological investigations of compound **1** need to be studied further.

Experimental

General Experimental Procedures Melting points were measured without correction on Electrothermal model 9100. IR spectra were taken on a JASCO FT/IR 300E spectrometer. UV spectra were obtained on a Milton Roy 3000 spectrometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained on a Varian Unity NMR spectrometer using $CDCl_3$ as a solvent. The low- and high-resolution FAB-MS were measured on a JEOL JMS-HX110/110A mass spectrometer. Elemental analysis was obtained on a flash EA 1112series/CE instruments. Sephadex LH-20 (Pharmacia Fine Chemical Industries Co.) and silica gel (70—230 mesh) (Merck) were used for column chromatography and silica gel 60 F_{254} (Merck) for TLC. Fluorescence was measured on a Perkin Elmer Model LS50B luminescence (U.S.A.).

Kynuramine, dihydrobromide, amitriptyline, 4-hydroxyquinoline, clorgyline, deprenyle and zinc sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals were used of reagent grade.

Materials and Methods The dried unripe fruits of *E. rutaecarpa*

Bentham (Rutaceae) were purchased at an herbal drug store in Cheongju, Korea in April 2000 and identified by Prof. K. S. Lee, a plant taxonomist at Chungbuk National University. A voucher specimen (No. CBNU00125) was deposited at College of Pharmacy, Chungbuk National University.

Extraction and Activity-Guided Isolation The dried fruits of *E. rutaecarpa* (3.0 kg) were extracted three times with MeOH at room temperature. The MeOH extracts were combined and concentrated *in vacuo* at 40 °C. The resulting extract (220 g) was suspended in 1% HCl and partitioned with *n*-hexane. The water layer was made alkaline with NaOH and partitioned with CH_2Cl_2. The CH_2Cl_2 layer was concentrated to afford the alkaloid fraction. This concentrated alkaloid fraction (20 g) was subjected to a silica gel column chromatography using a CH_2Cl_2–MeOH step gradient as eluents to give seven fractions (A1—A7). The each fractions were tested for MAO activity, the higher inhibitory activities on MAO were found in the Fr. A2 (82.6% inhibition at 150 μg/ml). Compound **1** (yield 1.1 g) was obtained from Fr. A2, and identified as 1-methyl-2-undecyl-4(1*H*)-quinolone. The purity of compound **1** on HPLC was measured 97.6% (area percent).[25]

1-Methyl-2-undecyl-4(1*H*)-quinolone (**1**): White powder; mp 68—70 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.44 (1H, d, J=8.0 Hz), 7.65 (1H, t, J=7.5 Hz), 7.50 (1H, d, J=8.5 Hz), 7.36 (1H, t, J=7.5 Hz), 6.23 (1H, s), 3.73 (3H, s), 2.70 (2H, t, J=8.0 Hz), 1.67 (2H, m), 1.42 (2H, m), 1.27 (14H, br s), 0.88 (3H, t, J=6.5 Hz). ^{13}C-NMR ($CDCl_3$) δ: 154.8, 111.2, 177.8, 126.6, 126.7, 123.3, 132.0, 115.3, 142.0, 34.1, 34.8, 28.6, 29.3, 29.5, 29.6, 31.9, 22.7, 14.1. IR (KBr) cm^{-1}: 2960, 2920, 2855, 1640, 1605, 1575, 1306, 1180, 775, 760. UV λ_{max} (MeOH) nm (log ε): 215 (4.2), 240 (4.3), 320 (4.1), 335 (4.1). HR-FAB-MS *m/z*: 314.2484 $[M+H]^+$ (Calcd for $C_{21}H_{32}NO$: 314.2484). *Anal.* Calcd for $C_{21}H_{31}NO$: C, 80.46; H, 9.97; N, 4.47; O, 5.10. Found: C, 79.59; H, 10.84; N, 4.49; O, 5.34. Spectral data and physical constants were identified with the previous reports.[17,18]

MAO Preparation and Assay for MAO Activity The crude MAO was prepared by Naoi's method[26] with minor modification[12] and kept at −70 °C until use. The MAO activity with kynuramine as a substrate was assayed by a modification of the fluorometric method of Kraml.[27] The fluorescence intensity of 4-hydroxyquinoline formed from kynuramine by MAO was measured at 380 nm (emission) with excitation at 315 nm in a fluorophotometer. The activities of MAO-A and MAO-B in mouse brain were measured in the presence of 1 M *l*-deprenyl (type B inhibitor) or clorgyline (type A inhibitor) with a pre-incubation time of 15 min, respectively. The test solutions were dissolved in dimethylsulfoxide, which was confirmed to have no effect on MAO activity below 2.8% concentration.

Statistical Methods The values of the Michaelis constant (K_m) and the maximum velocity (V_{max}) were obtained by Lineweaver–Burk's plot using various concentrations of kynuramine. The amount of protein was determined by the method of Lowry *et al.*[28] using bovine serum albumin as a standard. The results were expressed as the means±S.E.M. of three independent experiments performed in triplicate. The significance of the difference between the control and the drug treated groups was analyzed by Student's *t*-test.

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References

1) Nagatsu T., Yamamoto T., Harada M., *Enzymologia*, **39**, 15—25 (1970).
2) Tipton K. F., *Cell Biochem. Funct.*, **4**, 79—87 (1986).
3) Tipton K. F., O'Carrol A. M., McCrodden J. M., *J. Neural Transm.* (Suppl.), **23**, 25—35 (1987).
4) Bach A. W., Lan N. C., Johnson D. L., Abell C. W., Bembenek M. E., Kwan S. W., Seeburg P. H., Shin J. C., *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 4934—4938 (1988).
5) Kalgutkar A. S., Castagnoli N. J., Testa B., *Med. Res. Rev.*, **15**, 325—388 (1995).
6) Fowler C. J., Ross S. B., *Med. Res. Rev.*, **4**, 323—358 (1984).
7) Thomas T., *Neurobiol. Aging*, **21**, 343—348 (2000).
8) Naoi M., Nagatsu T., *Life Sci.*, **40**, 1075—1082 (1987).
9) Mitsui N., Noro T., Kuroyanagi M., Miyase T., Umehara K., Ueno A., *Chem. Pharm. Bull.*, **37**, 363—366 (1989).
10) Thull U., Kneubuhler S., Gaillard P., Carrupt P. A., Testa B., Altomare C., Carotti A., Jenner P., McNaught K. St. P., *Biochem. Pharmacol.*, **50**, 869—877 (1995).
11) Bembenek M. E., Abell C. W., Chrisey L. A., Rozwadowska M. D., Gessner W., Brossi A., *J. Med. Chem.*, **33**, 147—152 (1990).
12) Ro J. S., Lee S. S., Lee K. S., Lee M. K., *Life Sci.*, **70**, 639—645 (2001).
13) Gnerre C., Catto M., Leonetti F., Weber P., Carrupt P. A., Altomare C., Carotti A., Testa B., *J. Med. Chem.*, **43**, 4747—4758 (2000).
14) Huong D. T., Choi H. C., Rho T. C., Lee H. S., Lee M. K., Kim Y. H., *Arch. Pharm. Res.*, **22**, 324—326 (1999).
15) Suzuki O., Katsumata Y., Oya M., Chari V. M., Vermes B., Wagner H., Hostettmann K., *Planta Med.*, **42**, 17—21 (1981).
16) Ohishi N., Suzuki T., Ogasawara T., Yagi K., *J. Mol. Catal. B-Enzym.*, **10**, 291—294 (2000).
17) Tang Y. Q., Feng X. Z., Huang L., *Phytochem.*, **43**, 719—722 (1996).
18) Sugimoto T., Miyase T., Kuroyanagi M., Ueno A., *Chem. Pharm. Bull.*, **36**, 4453—4461 (1988).
19) Giller E., Jatlow P., Bialos D., Harkness L., Docherty J., *Psychiatry Res.*, **2**, 259—265 (1980).
20) Roth J. A., *J. Neurochem.*, **27**, 1107—1112 (1976).
21) Sheehan D. V., *J. Clin. Psychiat.*, **45**, 29—36 (1984).
22) Walsh B. T., Gladis M., Roose S. P., Stewart J. W., Stetner F., Glassman A. H., *Arch. Gen. Psychiat.*, **45**, 471—475 (1988).
23) Liebowitz M. R., Schneier F. R., Hollander E., Welkowitz L. A., Saoud J. B., Feerick J., Campeas R., Fallon B. A., Street L., Gitow A., *J. Clin. Psychiat.*, **52**, 10—15 (1991).
24) Tetrud J. W., Langston J. W., *Science*, **245**, 519—522 (1989).
25) Sugimoto T., Miyase T., Kuroyanagi M., Ueno A., *Chem. Pharm. Bull.*, **36**, 4453—4461 (1988).
26) Naoi M., Nagatsu T., *J. Neurochem.*, **46**, 655—657 (1986).
27) Kraml M., *Biochem. Pharmacol.*, **14**, 1684—1686 (1965).
28) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 265—275 (1951).