1-Methyl Substituent and Stereochemical Effects of 2-Phenylcyclopropylamines on the Inhibition of Rat Brain Mitochondrial Monoamine Oxidase A and B*

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Abstract □ (E)-2-Phenylcyclopropylamine ((E)-TCP), (Z)-2-phenylcyclopropylamine ((Z)-TCP), (E)-1-methyl-2-phenylcyclopropylamine ((E)-MTCP), and (Z)-1-methyl-2-phenylcyclopropylamine ((Z)-MTCP) were synthesized and used to determine to what extent 1-methyl substitution and stereochemistry of 2-phenylcyclopropylamines affect inhibition of monoamine oxidase(MAO). Inhibition of rat brain mitochondrial MAO-A and B by the compounds were measured using serotonin and benzylamine as the substrate, respectively and IC_{50} values obtained with 95% confidence limits by the method of computation. For the inhibition of MAO-A, (E)-MTCP ($IC_{50}=6.2\times10^{-8}$ M) was found to be 37 times more potent than (Z)-MTCP ($IC_{50}=2.3\times10^{-6}$ M), whereas the activity of (E)-TCP ($IC_{50}=2.9\times10^{-7}$ M) was slightly less than that of (Z)-TCP ($IC_{50}=2.3\times10^{-7}$ M). Similarly, for the inhibition of MAO-B, (E)-MTCP ($IC_{50}=6.3\times10^{-8}$ M) was 7 times more potent than (Z)-MTCP ($IC_{50}=4.7\times10^{-7}$ M) and (E)-TCP ($IC_{50}=7.8\times10^{-8}$ M), 0.6 times as potent as (Z)-TCP ($IC_{50}=4.4\times10^{-8}$ M). The results suggested that while without 1-methyl group, potency of a (Z)-isomer was comparable to that of (E)-isomer, the methyl group in its (Z)-position was very unfavorable to the inhibition of MAO and that in its (E)-position, the methyl group contributed positively to the potency as found by the fact that (E)-MTCP was 1-5 times more potent than (E)-TCP. In view of the selective inhibition of MAO-A or B, all compounds elicited 4-10 times higher preference for the inhibition of MAO-B over MAO-A and 1-methyl substitution as well as the stereochemical factors did not significantly influence the selectivity.

Keywords 2-Phenylcyclopropylamines, (E)-2-Phenylcyclopropylamine, (Z)-2-Phenylcyclopropylamine, (E)-1-Methyl-2-phenylcyclopropylamine, (Z)-1-Methyl-2-phenylcyclopropylamine, Synthesis, Rat brain mitochondrial MAO, MAO inhibition potency, MAO inhibition selectivity.

Monoamine oxidase (MAO, EC 1.4.3.4) is an enzyme involved in the oxidative degradation of biogenic amines and known to exist in multiple forms named MAO-A and B[1,2]. The chemical compounds which can block activities of the enzyme can be considered as potential therapeutic agents useful for treatment of either depressive disorders or Parkinsonism and as probes not only to evaluate physiological roles of the specific amines in the brain but also to apply to topographic investigations of MAO[3,4].

As such compounds, 2-phenylcyclopropylamines were first synthesized by Kaiser *et al.*[5] and their

MAO inhibitory properties reported by Zirkle *et al.*[6] in relation to their structure-activity relationships. Among 2-phenylcyclopropylamines, tranylcypromine (*trans-dl-2-phenylcyclopropylamine*, (E)-TCP, 1) is an antidepressant clinically available known as a nonselective inhibitor.

Regarding the mode of interaction between MAO and (E)-TCP, Belleau and Moran[7], primarily emphasizing the double bond-like electron density of cyclopropane ring, reported that (E)-TCP would show strong affinity to MAO because of its structural resemblance to the transition state of substrate formed during its oxidation by MAO and that in the enzyme-inhibitor complex, the N$-C_1-C_2$ atoms of the cyclopropylamine must be

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1 ((*E*)-TCP)

2 ((*Z*)-TCP)

3 ((*E*)-MTCP)

4 ((*Z*)-MTCP)
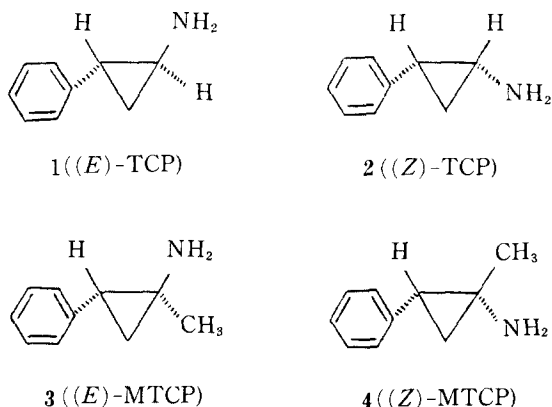
coplanar with the phenyl ring. On the other hand, Zirkle *et al.*[6] reported that even though the fact that (*Z*)-2-phenylcyclopropylamine((*Z*)-TCP, 2) is 2.5 times more potent than (*E*)-TCP as measured by *in vivo* tryptamine convulsion potentiation might support a coplanarity of N-C_1-C_2 with phenyl ring, the equipotency of (*E*)-1-methyl-2-phenylcyclopropylamine ((*E*)-MTCP, 3) with (*Z*)-1-methyl-2-phenylcyclopropylamine ((*Z*)-MTCP. 4) would not be explained with such a mode of interaction and proposed that phenyl ring must lie in the same plane as the C_2-C_3 atoms but amino group be in a different plane. As a supporting evidence, a rigid and active analog, 1-amino-cycloprop(a)indane was presented.

We found that in these earlier studies using *in vivo* potency,[6,7] direct interactions of 2-phenylcyclopropylamines with MAO could not be examined properly because *in vivo* inhibitions are to be heavily influenced, in addition to their affinity to MAO, by pharmacokinetic factors espeially such ones as absorption and metabolism. Besides, it appeared that since IC_{50} values will be variable depending upon substrates used, enzyme source and its purity, and experimental conditions, complete controlled data are needed in such analyes. Moreover, inhibition of MAO by tranylcyromine being established recently to be a suicide inhibition by Silverman[8], the analysis by Zirkle *et al.*[6] in which affinity data were directly derived from ED_{50} values were not based on sound assumptions.

Therefore, as a first attempt toward the analys regarding the mode of interaction between phenylcyclopropylamines and MAO only with finity terms and toward the assessment of strucral contributions with separate kinetic parameters of affinity (K_i) and inactivation rate (k_{inact}),we nthesized (*E*)-TCP, (*Z*)-TCP, (*E*)-MTCP,

and (*Z*)-MTCP in pure forms and accurate measurements were made to obtain IC_{50} values for the inhibition of MAO-A and B. For the purpose, rat brain mitochondrial MAO was used and assay methods were examined suitable to the experimental conditions.

EXPERIMENTAL METHODS

Materials and animals

Tranylcypromine sulfate was a kind gift of Dr. S. Crooke of Smith Kline & French Laboratories, U.S.A., from which authentic tranylcypromine base and hydrochloride salt were prepared. Serotonin-creatinin sulfate complex (H7752), benzylamine hydrochloride (B5136), Trizma base (T1503), and bovine albumin (A7906) were purchased from Sigma Chemical Co., St. Louis, U.S.A.. A 3% OV-17 80/100 mesh Chromosorb W (HP) was obtained from Supelco Inc., U.S.A. and Kieselgel 60G (Art 7731) and Kieselgel 60 (Art 7734, 70-230 mesh ASTM) of E. Merck, Germany were used for TLC and column chromatography, respectively. Methylsuccinic acid was purchased from Tokyo Kasei, Japan. All other chemicals and solvents were obtained from local market and were of reagent grade.

Male Sprague-Dawley rats (150-200g) were accommodated in a controlled animal room for at least 2 weeks prior to use and given food(Samyang animal food) and water *ad libitum*.

Instrumentation

Melting points were taken using a sybron thermolyne (Olympus, Tokyo) and are uncorrected. Perkin-Elmer model 710 infrared spectrophotometer was used to obtain all IR spectra. NMR spectra were recorded on a Bruker WP 60 CW or a Varian EM-360A 60 MHz spectrometer using tetramethylsilane as an internal standard. UV/VIS absorptions were recorded using Hitachi model 200-20 UV-VIS spectrophotometer. Hewlett Packard model HP 5985B GC/MS system at Natural Products Research Institute, Seoul National University was used to collect all direct probe mass spectral data. Electron ionization voltage was 70 eV. Gas chromatography was performed on a Hitachi model 163 gas chromatograph equipped with a hydrogen flame detector. A glass column (2.0 m×3 mm i.d.) packed with 3% OV-17 on 80/100 mesh Chromosorb W(HP) was used with carrier gas(N₂) at 50 m*l* min⁻¹. The standard operating conditions were: column temperature, 100℃ programmed to 250℃ at 10℃ min⁻¹; injector temperature, 250℃; detector temperature, 250℃. A Sorvall superspeed refrigerated

centrifuge, RC 2-B, Sorvall Inc., U.S.A. and a motor-driven glass homogenizer (Potter Elvehjem type) with a Teflon resin pestle were used. Elementary analyses were done by Analytical Research Section of Dong-A Pharm. Co., Ltd., Seoul.

Synthesis of (E)-2-phenylcyclopropylamine ((E)-TCP, 1) and (Z)-2-phenylcyclo-propylamine ((Z)-TCP, 2)

Ethyl diazoacetate was synthesized by direct diazotization[9] of ethyl glycinate HCl prepared from glycine. From 30g (0.215 mol) of ethyl glycinate HCl, 23.7g (96.6%) of ethyl diazoacetate was obtained. IR(neat) 2130 cm^{-1}(diazo $\overset{+}{N}=\overset{-}{N}=$), 1680(C=O). The ethyl diazoacetate (11.9 g, 0.104 mol) was condensed with styrene (8.44g, 0.081 mol) by the procedure of Burger and Yost[10] to yield 11.26g (73.2% from styrene) of ethyl 2-phenylcyclopropanecarboxylate (5). IR(neat) 1710 cm^{-1}(ester C=O). NMR(CDCl$_3$) δ 7.09(s, 5 H, arom. H), 4.10-3.60(m, 2H, CH$_2$ of ethyl), 2. 65-2.10(m, 1H, benzylic H), 1.90-0.73(m, 6H, 3 cyclopropyl H & CH$_3$ of ethyl). (E)/(Z) ratio of 5 was measured to be 3 : 2 by NMR. Two peaks were observed by GC at t_R 8.1 and 7.7 min(1.4 : 1).

9.5g(0.05mol) of 5 was selectively hydrolyzed by the method of Walborsky and Plonsker[11]. 3.4 g (41.8%) of (E)-2-phenylcyclopropanecarboxylic acid (6) was obtained. Recrystallization from hot water, mp 90-92℃ (lit.[11] 91-92℃). IR(KBr) 1680, 1700 cm^{-1}(acid C=O), 2900-3060(acid OH). NMR(CDCl$_3$) δ 10.7(bs, 1H, COOH), 7.21(s, 5H, arom. H), 2.75-2.35(m, 1H, benzylic H), 2.0-1.2 (m, 3H, cyclopropyl H). MS, m/z(relative intensity) 162(43, M$^+$), 144(16), 117(base peak), 107(24), 91(28), 77(10), 65(10), 51(13). GC, t_R 8.6min. 1.23g(15.3%) of (Z)-2-phenylcyclopropanecarboxylic acid (7) was obtained. Two recrystallizations from benzene/petroleum ether (30-60℃), mp 106-109℃(lit.[11] 106-107℃). IR(KBr) 1690 cm^{-1}(acid C=O), 3100-2800 (acid OH). NMR(CDCl$_3$) δ 11.35(s, 1H, COOH), 7.2(s, 5H, arom. H), 2.8-2.3(m, 1H, benzylic H), 2. 18-1.83(m, 1H, cyclopropyl H), 1.77-1.05(m, 2H, cyclopropyl H). GC, t_R 8.6min.

(E)-TCP HCl (1 HCl) was synthesized from 6(0.97g, 6m mol) via (E)-2-phenylcyclopropane-carbonyl chloride by the method of Kaiser et al.. Yield 430mg(42.6%). mp 151-155℃(lit.[12] 151-154℃). IR(KBr) 3200-2600 cm^{-1} (-$\overset{+}{N}$H$_3$, aryl C-H, aliphatic C-H), 1600, 1500 (-$\overset{+}{N}$H$_3$). NMR(DMSO-d$_6$/CDCl$_3$) δ 8.95(bs, 3H, -$\overset{+}{N}$H$_3$), 7.2(s, 5H, arom. H), 2.9-2.5(m, 2H, benzylic H & cyclopropyl H), 1.9-1.53(m, 1H, cyclo-

propyl H), 1.35-1.0(m, 1H, cyclopropyl H). MS, m/z (relative intensity) 133(82, M$^+$) 132(base peak), 115(48), 104(14), 91(19), 77(24), 56(42). GC of 1, t_R 4.7min. TLC of 1(CHCl$_3$/EtOAc/(C$_2$H$_5$)$_3$N(85 : 5 : 10), I$_2$ detection), R$_f$ 0.31. (Z)-TCP HCl (2 HCl) was synthesized according to modified Curtius reaction[13]. 7(0.8 g, 0.005mol) was converted to 310mg(36.9%) of 2 HCl. mp 164-165℃(lit.[10] 164-166℃). IR(KBr 3050, 2850 cm^{-1} (-$\overset{+}{N}$H$_3$, aryl C-H, aliphatic C-H) 1600, 1500 (-$\overset{+}{N}$H$_3$). NMR(DMSO-d$_6$/CDCl$_3$) δ 8.43(bs, 3H, -$\overset{+}{N}$H$_3$), 7.37(m, 5H, arom. H), 2.98 -2.7(m, 1H, benzylic H), 2.6-2.12(m, 1H cyclopropyl H), 1.55-1.15(m, 2H, cyclopropyl H) GC of 2, t_R 4.7min. TLC of 2(CHCl$_3$/EtOAc/(C$_2$ H$_5$)$_3$N(85 : 5 : 10), I$_2$ detection), R$_f$ 0.48.

Synthesis of (E)-1-methyl-2-phenylcyclo propylamine ((E)-MTCP, 3) and (Z)-1-methyl-2-phenylcyclopropylamine ((Z)-MTCP, 4)

Ethyl α-diazopropionate was synthesized from DL-alanine ethyl ester HCl prepared from DL-α-alanine by the procedure of White and Baumgarten[14]. DL-alanine ethyl ester HCl(30g 0.195 mol) was converted to 17.11g (46%) o ethyl N-carbethoxyalanate by reacting it wit ethyl chloroformate (31.8g, 0.293 mol) in the pres ence of triethylamine (59.2g, 0.585 mol). m 24-27℃(lit.[14] 24-25℃). IR(neat) 3350cm^{-1}(NH 1680-1720(amide I & ester C=O), 1520(amid II). Ethyl N-carbethoxyalanate (17.1g, 0.09mo was reacted with N$_2$O$_4$ which was prepared fror P$_2$O$_5$ and nitric acid (86%, d 1.50) to obtain 19 (96.8%) of ethyl N-carbethoxy-N-nitrosoalan ate. IR(neat) 1730cm^{-1}(ester C=O), 1500(N=O Ethyl N-carbethoxy-N-nitrosoalanate (19g, 0.08 mol) was pyrolyzed and 9.0g (80%) of ethyl α-diazopropionate was obtained. IR(neat) 208 cm^{-1}(diazo $\overset{+}{N}=\overset{-}{N}=$), 1680(C=O).

(E)-1-methyl-2-phenylcyclopropanecarboxyl acid (9) and (Z)-1-methyl-2-phenylcycl propanecarboxylic acid (10) were prepared l condensation of ethyl α-diazopropionate with st rene and selective hydrolysis of the product described for the synthesis of 6 and 7[5,10] Treatment of ethyl α-diazopropionate (8.97g, 07mol) with styrene (10.94g, 0.105mol) gave 8. g (59%) of ethyl 1-methyl-2-phenylcycl propanecarboxylate (8). IR(neat) 1720 cm (ester C=O). Two peaks(t_R 7.7, 6.8min (5 : 4 were observed on GC. From 8(8.48g, 0.04mol) 35g(18.4%) of 9 was first isolated. mp82-84 (lit.[5] 81-83℃). IR(KBr) 1670cm^{-1}(acid C=(3100-2800(acid OH). NMR(CDCl$_3$) Data shown Table I. MS, m/z (relative intensity) 176(4

M.), 158(21), 131(base peak), 115(34), 107(23), 91(53), 77(14), 63(7), 51(5). Further hydrolysis yielded 1.21g(16.5%) of **10**. Recrystallization from hexane, mp 103-104℃ (lit.[5] 104-105℃), IR(KBr) 1680 cm^{-1}(acid C=O), 3100-2800(acid OH). NMR(CDCl₃) Data shown in Table I. MS, m/z(relative intensity) 176(44, M.), 158(20), 131 (base peak), 115(35), 107(23), 91(57), 77(15), 63(8), 51(6). From mother liquor after the crystallization of **10**, an additional crop (0.9g, (E)/ (Z) ratio=2:1) was obtained.

A hydrochloride salt of (E)-MTCP, **3** HCl was synthesized from **9** (1.05g, 0.006 mol) via the intermediate, (E)-1-methyl-2-phenylcyclo-propanecarbonyl chloride as described by Kaiser et al.[5]. Yield 560 mg(51.4%). Three recrystallizations from MeOH/EtOAc/Et₂O, mp 192-193℃ lit[5]. 198-199℃). IR(KBr) 3200-2800 cm^{-1} (-NH₃, aryl C-H, aliphatic C-H), 1600, 1560, 1530, 1500 (-NH₃, CH₃). NMR(DMSO-d₆/ CDCl₃) Data shown in Table II. MS, m/z(relative intensity) 147(62, M.), 146(69), 132(base peak), 115(44), 103(21), 91(36), 77(29), 70(77), 63(11), 51(14). GC of **3**, t_R 4.4min. TLC of **3**(EtOAc/MeOH/NH₄OH (17:2:1), I₂ detection), R$_f$ 0.53. **10**(1.05g, 0.006 mol) was similarly treated as described for the synthesis of **3**. 540 mg of precipitate was obtained. mp 160-190℃. TLC of the base showed two spots at R$_f$ 0.73 and 0.53. (Z)/(E) ratio of the mixture of MTCP isomers was measured to be 3:1 by NMR. Fractionation of the mixture (400 mg) as free base by silica gel chromatography using ethyl acetate as an eluent resulted in the separation of (Z) isomer, **4** HCl(200 mg) and (E)-isomer(20 mg). **4** HCl was identified as follows: mp 189-190℃(lit[5]. 193-194℃). IR(KBr) 3200-2800cm^{-1} (-NH₃, aryl C-H, aliphatic C-H), 1600, 1570, 1520, 1500(-NH₃, CH₃). NMR(DMSO-d₆/CDCl₃) data shown in Table II. MS, m/z(relative intensity) 147(75, M.), 132(base peak), 115(48), 103(24), 91(58), 78(22), 70(81), 51(16). GC of t_A 4.4min. TLC of **4** (EtOAc/MeOH/NH₄OH (17:2:1), I₂ detection), R$_f$ 0.73.

In another experiment, synthesis of **8** was carried out by cyclization reaction of ethyl 4-chloro 2-methyl-4-phenylbutanoate in the presence of K t-butoxide as described by DePuy et al.[15] Thus, Friedel-Crafts reaction of methylsuccinic anhydride (22.8g, 0.2 mol) with benzene(120 m*l*, 1.325 mol) gave 24.9g(64.8%) of 3-benzoyl-2-methyl-propionic acid. mp 137-139.5℃ (lit.[16] 138-140℃). NaBH₄ reduction of 3-benzoyl-2-methyl-propionic acid(21.12g, 0.11 mol) and subsequent dehydration gave α-methyl-γ-phenyl-γ-butyr-olactone(15.16g, 78.3%). IR(neat) 1780 cm^{-1} (lactone C=O). NMR(CDCl₃) δ 1.30(d, 3H, CH₃), 2.30-3.06(m, 3H, -CH₂ & -CH), 5.13-5. 63(m, 1H, benzylic H), 7.30(s, 5H, arom. H). MS, m/z(relative intensity) 176(59, M.), 132(33), 117 (base peak), 105(92), 91(32), 77(48), 51(15). GC, t_R 9.7min. TLC(ether/cyclohexane(3:7), I₂ detection), R$_f$ 0.18. α-Methyl-γ-phenyl-γ -butyrolactone (7.44g, 0.043 mol) was treated with SOCl₂ followed by with HCl-saturated EtOH to obtain 7.7g of a mixture of ethyl 4-chloro -2-methyl-4-phenylbutanoate and unreacted lactone(7:3), which was further reacted with K-t -butoxide to yield 4.7g(53.6%) of **8**. IR(neat) 1720 cm^{-1}(ester C=O) no lactone C=O at 1780 cm^{-1}. Selective hydrolysis[5] yielded two hydrolyzed fractions(1.9 and 1.25g), all of which was found to be (E)-isomer, **9** by NMR. 1.5g(0.0085 mol) of **9** was converted to 500 mg of **3** HCl in 32% yield. Two recrystallizations from MeOH/ EtOAc/Et₂O, mp 189-190℃. IR, NMR, MS, GC, and TLC were identical with those of previously synthesized **9**. Anal. Calcd. for C₁₀H₁₄NCl: C, 65.41; H, 7.63; N, 7.63. Found: C, 65.32; H, 7.72; N, 7.78.

MAO inhibition studies

Activity of MAO-A was measured according to Sjoerdsma et al.[17] described for the metabolism of serotonin and by UV method of Udenfriend et al.[18] which was applied to the determination of serotonin. Specifically, determination of IC₅₀ values for the inhibition of MAO-A as well as the preparation of rat brain mitochondrial MAO, all suitable to the present laboratory conditions was carried out as previously reported[19]. Activity of MAO-B was measured using benzylamine as a substrate according to Tabor et al.[20] with modifications. Optimum conditions for the measurement of enzyme activity and IC₅₀ values were sought in the experiment. The mitochondria protein content was determined according to Lowry et al.[21] with bovine albumin as the standard. IC₅₀ values with 95% confidence limits were calculated by computation using a program of Tallarida and Murray[22].

1) Inhibition of MAO-A

A preliminary experiment was done to find a concentration range achieving 20-80% inhibitions so as to treat the data by computation. Eight to nine concentrations as can be found in Fig.3 were finally tested to obtain IC₅₀ values.
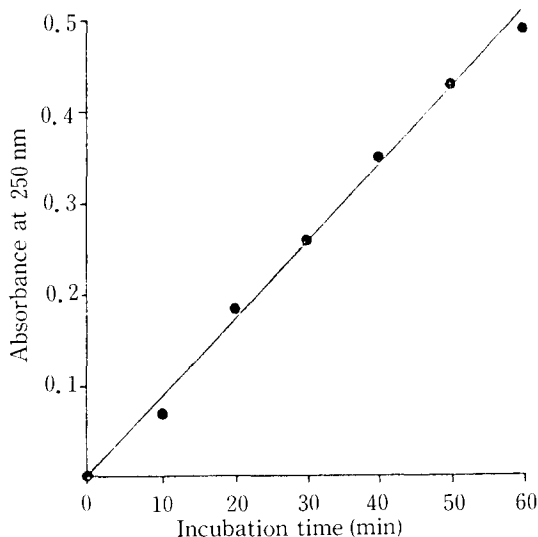
2) Inhibition of MAO-B

Measurements of MAO-B activity: Incubation mixture was made to contain 0.2 m*l* of 40 mM benzylamine(final concentration, 2 mM), 0.3 m*l* of enzyme source (4 mg protein/4 m*l*), and 50
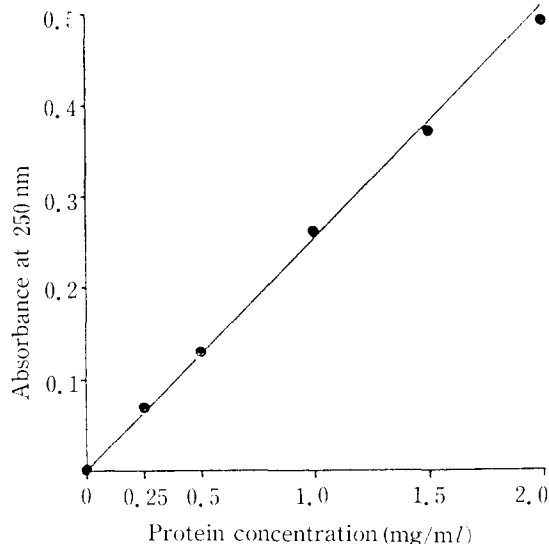
Fig. 1. Relationship between enzyme concentration and absorbance measured at 250 nm.

Each point represents the mean of the triplicate determinations.

Fig. 2. Relationship between incubation time and absorbance measured at 250 nm.

Each point represents the mean of triplicate determinations.

mM Tris-HCl buffer, pH 8.2 in a volume of 4 ml. The mixture was incubated at 37°C for 30 min and the reaction stopped by addition of 1 ml
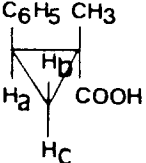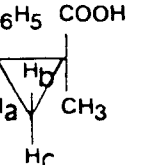
of 3% $ZnSO_4$. Subsequently it was vortex-mixed for 10 sec and centrifuged at 3000 rpm for 15 min. The supernatant was taken and the absorbance of benzaldehyde produced was measured at 250 nm using a blank which was prepared following the same procedures except for the use of enzyme treated in a boiling water bath for 15 min.

In order to determine linear relationships between the amount of enzyme and the benzaldehyde produced, concentration of benzylamine (2 mM) and incubation time (30 min) were fixed and varying amounts of enzyme were used corresponding to 1, 2, 4, 6, and 8 mg protein in 4 ml of an incubation mixture. In addition, a linearity of the incubation time with the measured absorbance of benzaldehyde was examined by varying incubation times over 0-60 min at an interval of 10 min with a fixed concentration of benzylamine (2 mM) and enzyme (4 mg/4 ml). Molar extinction coefficient of benzaldehyde at 250 nm was measured in an aqueous solution of 0.0792 mM benzaldehyde which was prepared similarly to the measurement of enzyme activity.

The molar extinction coefficient of benzaldehyde at 250 nm was determined to be 13,258 close to the value reported by Tabor et al.[20] In the experiment, absorbance of 2 mM benzylamine at 250 nm was ~0.28 after work-up which will strongly interfere with the measurement of benzaldehyde Since a small portion of 2 mM benzylamine (~1%) is oxidized to benzaldehyde, benzaldehyde itself by the metabolic process was measured by subtracting an absorbance value due to benzylamine using the blank. Tabor et al.[20] used a low concentration of benzylamine (10 μM) with highly active MAO, so that the benzaldehyde could be measured without an interference of benzylamine. The enzyme activity in our experiment was so low that we had to use, instead, high concentration of benzylamine (2 mM) to obtain measurable amount of benzaldehyde. Fig.1 shows a good linear relationships (r=0.998) between the amount of enzyme over 0.25-2.0 mg/ml and the benzaldehyde produced. When the amount of benzaldehyde was measured over 0-60 min every 1 min, a linearity was observed (r=0.997) as shown in Fig.2. Accordingly, in the experiment, 1.0 mg ml of enzyme and 30 min of incubation time were used, with which the absorbance of benzaldehyd without an inhibitor was approximately 0.26.

Inhibition of MAO-B: After obtaining concentration ranges possible to measure IC_{50} with 95% confidence limits by the method of computation six to nine final concentrations of inhibitors were set as illustrated in Fig.4 and tested.

Table I. NMR spectral data for 1-methyl-2-phenylcyclopropanecaboxylic acid (CDCl₃).

Compound	Chemical shift (δ)						Coupling constant (Hz)		
	$-CH_3$	$-C_6H_5$	$-COOH$	H_a	H_b	H_c	J_{ab}	J_{bc}	J_{ac}
C₆H₅ CH₃ (structure) Hb Ha COOH Hc	0.98	7.23	11.48	2.91	1.23	1.76	7.5	4.5	9.0
(E)-isomer (**9**)	s	s	bs	dd	dd	dd			
C₆H₅ COOH (structure) Hb Ha CH₃ Hc	1.40	7.15	10.53	2.35	1.85	1.13	7.5	4.5	8.2
(Z)-isomer (**10**)	s	s	bs	t	dd	dd			

Following preincubation of the mixture consisting of 0.4 ml of inhibitor, 0.3 ml (4 mg protein) of enzyme, and 3.1 ml of 50 mM Tris-HCl buffer, pH 8.2 at 37°C for 30 min, to it was added 0.2 ml of 40 mM benzylamine (2 mM in a volume of 4 ml), and the incubation continued for another 30 min. Absorbance resulting from benzaldehyde was obtained as described for measurements of MAO-B activity. Absorbance from the use of H₂O in place of the inhibitor was designated '0% inhibition'.
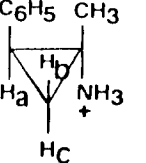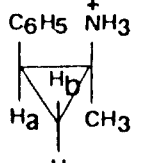
Blanks were the ones prepared by treating the incubation mixture in a boiling water bath after the preincubation.

RESULTS AND DISCUSSION

Synthesis of (E)-and (Z)-isomers of 2-phenylcyclopropylamine (TCP) and 1-methyl-2-phenylcyclopropylamine (MTCP)

Ratio of (E)/(Z)-isomers in the product, 5

Table II. NMR spectral data for 1-methyl-2-phenylcyclopropylamine HCl (DMSO-d₆/CDCl₃).

Compound	Chemical shift (δ)						Coupling constant (Hz)		
	$-CH_3$	$-C_6H_5$	$-N^+H_3$	H_a	H_b	H_c	J_{ab}	J_{bc}	J_{ac}
C₆H₅ CH₃ (structure) Hb Ha NH₃+ Hc	1.15	7.27	8.85	2.70	1.10	1.60	7.0	6.0	10.0
(E)-isomer (3HCl)	s	s	bs	dd	t	dd			
C₆H₅ NH₃+ (structure) Hb Ha CH₃ Hc	1.63	7.35	8.32	2.20	1.40	1.13	6.5	6.0	9.5
(Z)-isomer (4HCl)	s	s	bs	dd	t	dd			

formed by condensation of ethyl diazoacetate with styrene was found to be 3 : 2 by GC and NMR, which was similar to the reported values of 3-4 : 1[12] and 65 : 35[23] The mp of synthesized (E)-TCP HCl (1 HCl) was 151-155℃ by repeated recrystallizations. There are diverse values reported for 1 HCl; 164-166℃[24], 153.5-156.5℃[10], 151-154℃[12], and 151-155℃[25]. Purity of the synthesized 1 was tested by GC (t_R=4.7 min) and TLC(R_f=0.31) and proved to be pure. The mp, 164-165℃ of synthesized (Z)-TCP HCl (2 HCl) was consistent with a value reported by Burger and Yost[10], which however did not reach 169 -170℃[13]. It was possible to resolve (E)-and (Z) -isomers by TLC (2, R_f=0.48) and 2 HCl was found to be pure by TLC and GC.

Ethyl α-diazopropionate was prepared by pyrolyzing ethyl N-carbethoxy-N-nitrosoalanate which was prepared by treating carbamate of alanine ester with N_2O_4[26]. The condensation product, 8 of ethyl α-diazopropionate with styrene was found to be a mixture of $(E)/(Z)$-isomers in 5 : 4 ratio by GC and NMR. NMR data of methyl esters of 9 and 10[27] and ethyl ester of 10[28] have appeared in literatures. Compounds, 9 and 10 were converted *via* acid chlorides to 1-methyl-2-phenylcyclopropylamines. (E)-MTCP HCl (3 HCl) obtained in 51% yield showed an mp of 192-193℃, lower than 198-199℃[5]. The compund, 3 was however proved to be pure by GC(t_R=4.4 min) and TLC (R_f=0.53). NMR indicated absence of a contamination with (Z)-isomer. The resulting product from 10 by the same procedure was an isomerized mixture of $(Z)/(E)$-isomers in 3 : 1 ratio. (Z) -MTCP, 4 isolated by silica gel chromatography was found to be pure by GC(t_R=4.4 min), TLC (R_f=0.73), and NMR.

Table I represents NMR spectral analyses of (E)-1-methyl -2-phenylcyclopropanecarboxylic acid, 9 and its (Z)-isomer, 10. The same spectral analyses for (E)-and (Z)-MTCP HCl, 3 and 4 were shown in Table II. Although partial NMR data of 9 and 10 have been reported concerning chemical shifts of methyl, phenyl, and carboxyl protons[15], complete analyses like the ones presented here were considered to be new. C-1 methyl protons of (E)-isomers were found to be more shielded compared to those of (Z)-isomers. Deshielding effects due to carboxyl or ammonium group were evidently reflected on chemical shifts of cyclopropyl protons, H_b and H_c. The cyclopropyl protons were observed to be splitted into doublet of the doublet (dd) except for the protons recorded as a triplet because of incomplete resolution. The coupling constants between cyclopropyl protons all com-

plied with a generalization that the value between geminal protons were the smallest, on the other hand, with the largest value between cis protons.

We also examined synthesis of 3 and 4 *en route* cyclization of ethyl 4-chloro-2-methyl -4-phenylbutanoate in the presence of K-t-butoxide and isolated one (E)-isomer, 6, after hydrolysis. Optimum reaction conditions to accomplish a complete conversion of α-methyl-γ-phenyl-γ -butyrolactone to ethyl 4-chloro-2-methyl-4-phenylbutanoate were not found. Therefore, the yield of 3 HCl was low by this synthetic route.

Inhibition of MAO-A and MAO-B

Dose-inhibition curves for the inhibition of MAO -A and B by (E)-TCP, (Z)-TCP, (E)-MTCP, and (Z)-MTCP were shown in Fig. 3 and 4. Table III and IV represent IC_{50} values with 95% confidence limits.

In this study, (Z)-TCP was found to be 1.3 and 1.8 times as potent as (E)-TCP for the inhibition of MAO-A and MAO-B, respectively. Whereas, according to *in vivo* ED_{50}(p.o.)[6] measured by tryptamine convulsion potentiation, (E) -TCP(0.18 mg/kg) was 2.3 times more potent
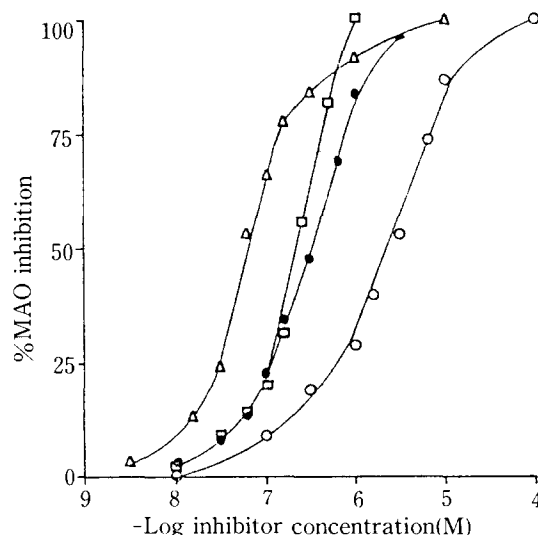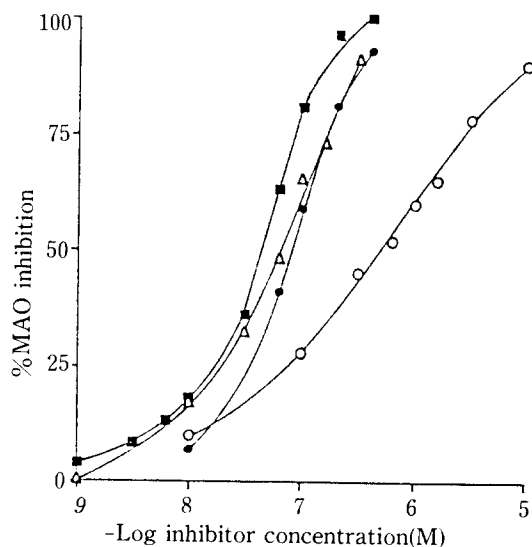

Fig.3. *In vitro* **inhibition of rat brain MAO-A by** (E)-2-phenylcyclopropylamine(- ● -), (Z)-2-phenylcyclopropylamine(-□ -), (E)-1-methyl-2-phenylcyclopropylamine(-△-), and (Z)-1-methyl -2-phenylcyclopropylamine(-○-).

Inhibitors were preincubated with enzyme for 30 min prior to substrate(serotonin) addition. Each point is the average of triplicate determinations.

Table III. Inhibition of the oxidation of serotonin by rat brain MAO (MAO-A).

Inhibitor[1]	IC_{50} (M)[2]	Relative potency[4]
(E)-TCP	$2.9 \times 10^{-7} (2.3 \times 10^{-7} - 3.6 \times 10^{-7})$[3]	1.0
(Z)-TCP	$2.3 \times 10^{-7} (1.9 \times 10^{-7} - 2.8 \times 10^{-7})$	1.3
(E)-MTCP	$6.2 \times 10^{-8} (4.8 \times 10^{-8} - 7.9 \times 10^{-8})$	4.7
(Z)-MTCP	$2.3 \times 10^{-6} (1.7 \times 10^{-6} - 3.1 \times 10^{-6})$	0.13

1) TCP, 2-phenylcyclopropylamine; MTCP, 1-methyl-2-phenylcyclopropylamine. 2) Measured by the method of Sjoerdsma *et al.*[17] and Udenfriend *et al.*[18]. 3) 95% Confidence limits. 4) Potency relative to that of tranylcypromine ((E) -TCP) which is assigned a value of 1.0.

Table IV. Inhibition of the oxidation of benzylamine by rat brain MAO (MAO-B).

Inhibitor[1]	IC_{50} (M)[2]	Relative potency[4]
(E)-TCP	$7.8 \times 10^{-8} (6.2 \times 10^{-8} - 9.7 \times 10^{-8})$[3]	1.0
(Z)-TCP	$4.4 \times 10^{-8} (3.5 \times 10^{-8} - 5.4 \times 10^{-8})$	1.8
(E)-MTCP	$6.3 \times 10^{-8} (5.1 \times 10^{-8} - 7.8 \times 10^{-8})$	1.2
(Z)-MTCP	$4.7 \times 10^{-7} (4.2 \times 10^{-7} - 5.4 \times 10^{-7})$	0.17

1),3),4) See Table III. 2) Measured by the method of Tabor *et al.*[20] with modifications.

Fig.4. *In vitro* inhibition of rat brain MAO-B by (E)-2-phenylcyclopropylamine(-●-), (Z)-2-phenylcyclopropylamine(-□-), (E)-1-methyl-2-phenylcyclopropylamine(-△-), and (Z)-1-methyl-2-phenylcyclopropylamine(-○-).

Inhibitors were preincubated with enzyme for 30 min prior to substrate(benzylamine) addition. Each point is the average of triplicate determinations.

-B, in which reported IC_{50} values of (E)-TCP and (Z)-TCP were 1.6×10^{-6} and 8.9×10^{-7} M for the inhibition of MAO-A and 1.4×10^{-7} and 8.9×10^{-8} M for the inhibition of MAO-B. respectively.

When the potencies of (E)-MTCP and (Z)-MTCP were compared with those of (E)-TCP, as shown in Table III and IV, (E)-MTCP showed 4.7 and 1.2 times higher potencies than (E)-TCP for the inhibition of MAO-A and MAO-B, whereas the potencies of (Z)-MTCP were much lower to be 0.13 and 0.17 times of those of (E)-TCP for MAO-A and MAO-B. There has not been a report concerning *in vitro* IC_{50} of (Z)-MTCP. It was found that the result on (E)-MTCP for the inhibition of MAO-A was consistent with the report[30] that in the experiment using whole brain homogenate of the rat, (E)-MTCP($IC_{50} = 2.5-5.0 \times 10^{-7}$ M) was 4-5 times more active than (E)-TCP($IC_{50} = 9.7 \times 10^{-7}$ M). The difference in specific IC_{50} values obviously resulted from different enzyme sources and the experimental conditions, whereby, for example, the previous workers[30] simply connected three points to obtain one IC_{50} value. As for the TCP compounds, *in vitro* IC_{50} values we obtained for MTCP compounds were found to differ considerably from *in vivo* ED_{50} values[5], that is, (Z)-MTCP(0.13 mg/kg) was 2 times more potent than (E)-MTCP(0.25 mg/kg) and (E)-MTCP 0.7 times as potent as (E)-TCP.

The results we obtained in the present study suggested that (E)-MTCP is more potent than either (E)-or (Z)-TCP, whereas (Z)-isomer of MTCP was far inferior in its potency compared to all others. Assuming that (E)-MTCP, in comparison with (Z)-MTCP, only differs in its binding affinity in which the methyl group is involved, it may be said that the methyl group in (Z)-position is very unfavorable for the binding but in (E)-posi-

than (Z)-TCP(0.42 mg/kg), indicating obvious discrepancies between *in vitro* and *in vivo* data. Burger and Nara[29] also reported in their *in vitro* study using highly purified beef liver MAO that (Z)-TCP was 1.79 and 1.12 times as potent as (E)-TCP for the inhibition of MAO-A and MAO

tion, it contributes positively to the binding and others, if any. According to Tullman and Hanzlik[31] in their experiment using rat liver MAO, the methyl group of N-(1-methyl) cyclopropylbenzylamine(N-(1-Me)CBA; $K_i=1050$ μM, $k_{inact}=0.041$ min^{-1}) was not favorable in potency compared to N-cyclopropylbenzylamine (N-CBA; $K_i=55$ μM, $k_{inact}=0.111$ min^{-1}) in terms of both affinity and inactivation rate. Similarly, Silverman and Hoffman[32] reported in their experiment using pig liver MAO that the 1-methyl group of N-(1-Me)CBA ($K_i=180$ μM, $k_{inact}=0.06$ min^{-1}) was not favorable compared to N-CBA($K_i=280\mu$M, $k_{inact}=1.89$ min^{-1}) with respect to inactivation rate, causing an overall low potency. Since (E)-MTCP turned out to be 1.2-4.7 times more potent than (E)-TCP in our study, the role of 1-methyl group may differ from that of N-cyclopropylbenzylamines. In order to seek rational explanations for this, it may require kinetic analysis using cyclopropane methyl-substituted 2-phenylcyclopylamines to derive K_i and k_{inact} possibly by the method of Kitz and Wilson[33].

In the experiment using purified beef liver MAO[20], both (E)-and(Z)-TCP were 11-10 times more selective for the inhibition of MAO-B than MAO-A. In our study, the ratios between the potencies in inhibition of MAO-B and MAO-A (B/A ratios) were found to be as follows: (E)-TCP, 4; (Z)-TCP, 5; (E)-MTCP, 10; and (Z)-MTCP, 5. The results clearly suggested that while the compounds elicited a little higher preference for the inhibition of MAO-B, 1-methyl substitution or stereochemical factors did not significantly influence the selectivity. This implies that when structures of 2-phenylcyclopropylamines are to be modified aiming to find highly selective inhibitors, our attention ought to be focussed on the phenyl ring. Similar examples can be found in amphetamine derivatives where substituted-ring analogs have been proved highly selective to the inhibition of MAO-A [34,35] while $(+)$-amphetamine is only 5 times more selective for MAO-B[36].

ACKNOWLEDGEMENTS

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