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Biotransformation of Tranylcypromine in Rat Liver Microsomes

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Abstract□Metabolism of tranylcypromine (TCP) in rat liver microsomes was studied *in vitro* using fortified microsomal preparations. As well as unlabeled TCP, two deuterium labeled analogs, TCP-phenyl-d_5 and TCP-cyclopropyl-d_2 were used and GC/MS employed for the analysis. It was found that TCP was converted chemically to hydrocinnamaldehyde which was then metabolized to cinnamaldehyde and hydrocinnamyl alcohol. Schiff bases of TCP with hydrocinnamaldehyde and acetaldehyde were detected and possibility of the metabolic formation of N-ethylideneTCP was proposed. In addition, acetophenone (benzoylacetic acid), benzaldehyde, benzoic acid, and benzyl alcohol were detected as the metabolites. Chemical decomposition studies suggested that parts of the oxidized products might be derived by air oxidation processes. A potential metabolite assumed to be N-ethylidene-1,2-dihydroxy-3-phenylpropanamine oxide was also detected.

Keywords□Tranylcypromine, deuterium labeled tranylcypromines, metabolism, rat liver microsomes.

Tranylcypromine (*trans-dl*-2-phenylcyclopropylamine) is an antidepressant clinically available and known to exert its effects by inhibiting monoamine oxidase (EC 1.4.3.4), thus elevating levels of the biogenic amines such as dopamine, serotonin and norepinephrine in brain.[1] A mechanistic process involved in the suicidal inhibition of MAO by tranylcypromine has been proposed by Silverman.[2]

The drug has been favorably used for the treatment of atypical depressions and depression associated with anxiety and phobias although toxic effect of a hypertensive crisis related to drug interaction has limited its wide use.[3] However, there are only scanty informations regarding metabolic fates of tranylcypromine which are essential to the predictions of safety and efficacy of the drug. According to *in vivo* biotransformation pathways so far established by three laboratories, tranylcypromine is N-acetylated[4,5] and ring hydroxylated[6] in rats. The combined N-acetylation and ring-hydroxylation leading to glucuronide conjugate of phenyl-hydroxylated N-acetyltranylcypromine occurs in rats.[5] Hippuric acid was detected in rat urine.[7] Once detection of amphetamine and methamphetamine in the plasma of a patient overdosed with tranylcypromine was reported[8] but refuted afterwards.[9]

As suggested by *in vivo* results, formation of hippuric acid was indicative of the involvement of ring opening and oxidative processes leading to benzoic acid in the metabolism of tranylcypromine. The oxidative metabolism was also implicated in the formation of *p*-hydroxy metabolite. It appeared therefore necessary to investigate the oxidative pathways more precisely by *in vitro* metabolic studies using rat liver microsomes. In this regard, there has not been a report concerning *in vitro* metabolism of tranylcypromine.

For the purpose, we synthesized highly-enriched tranylcypromine-phenyl-d_5 and used it with previously-synthesized tranylcypromine-cyclopropyl-d_2[10] to trace and identify metabolites by means of GC/MS.

EXPERIMENTAL METHODS

Chemicals and animals

Tranylcypromine sulfate was a gift of Smith Kline & French Laboratories, U.S.A. from which authentic tranylcypromine HCl was prepared. Benzene-d_6 (>99.5 atom % D) was purchased from Fluka AG, Switzerland and CO gas and Diazald from Aldrich Chemical Co., Inc., U.S.A.. Bovine albumin, NADP · Na, glucose-6-phosphate · 2Na, and glucose-6-phosphate dehydrogenase were obtained from Sigma Chemical Co., St. Louis,

U.S.A.. Imipramine HCl was donated by Dr. K.H. Ko of College of Pharmacy, Seoul National University and desipramine HCl by Mr. K.R. Kim of Hwanin Pharm. Co., Ltd.. Hydrocinnamaldehyde and hydrocinnamyl alcohol were purchased from Tokyo Kasei, Japan. Cinnamaldehyde (Wako), benzaldehyde (Junsei), benzyl alcohol (Junsei), and acetophenone (Wako) were obtained from a local market. All other chemicals used for metabolic studies were of the highest grade commercially available.

Male Sprague-Dawley rats weighing 200-250g were housed in a controlled animal room at least for 2 weeks before use and given food (Samyang animal food) and water *ad libitum*. Each of the rats was treated with phenobarbital (75 mg/kg, i.p.) over three consecutive days and after the final dose fasted for a day before excising livers.

Instrumentation

Melting points were taken with sybron thermolyne (Olympus, Tokyo) and are uncorrected. Shimadzu model 435 infrared spectrometer was used to obtain IR spectra. NMR spectra were taken on Varian EM-360L 60 MHz spectrometer. UV/VIS absorptions were determined using Hitachi model 200-20 UV/VIS spectrophotometer. Hitachi model 163 gas chromatograph equipped with a hydrogen flame detector was employed with a glass column (2.0m × 3mm i.d.) packed with 3% OV-17 on 80/100 mesh Chromosorb W(HP). Analytical conditions were: carrier gas (N_2) flow rate, 50 m*l* min^{-1}; injector and detector temperatures, 250 °C for synthesized compounds and 280 °C for imipramine metabolism; and column temperature, programmed from 100 °C to 250 °C at 10 °C min^{-1} for synthesized compounds, from 200 °C to 280 °C at 10 °C min^{-1} for imipramine metabolism. Sorvall superspeed refrigerated centrifuge (RC 2-B, Sorvall Inc., U.S.A.) and Beckman L7-55 ultracentrifuge were used. Motor-driven glass homogenizer of a Potter Elvehjem type with a Teflon resin pestle was used.

Synthesis of tranylcypromine-phenyl-d_5 and authentic compounds

Tranylcypromine-phenyl-d_5: (*E*)-2-phenylcyclopropanecarboxylic acid-d_5 (1.67g, 0.01 mol) was prepared and converted to (*E*)-2-phenylcyclopropylisocyanate-d_5 *via* (*E*)-2-phenylcyclopropanecarbonyl chloride-d_5 as previously described.[5] The isocyanate was dissolved in benzene (10 m*l*) and refluxed with 10N KOH (4 m*l*) for 1hr. The solvent

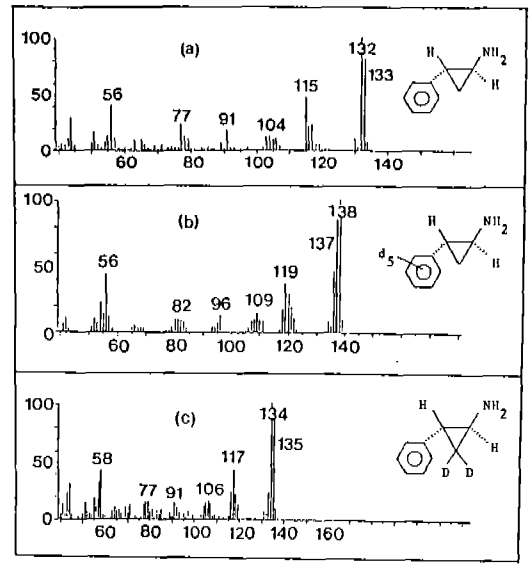


Fig. 1. Mass spectra of (a) tranylcypromine, (b) tranylcypromine-d_5, and (c) tranylcypromine-d_2.

was removed and following the addition of H_2O (10 m*l*), (*E*)-2-phenylcyclopropylamine-d_5 was taken up into ether to which was added HCl-ether to obtain a hydrochloride salt. Two recrystallizations from MeOH/EtOAc/ether gave 850 mg of (*E*)-2-phenylcyclopropylamine-d_5 HCl (49% from (*E*)-2-phenylcyclopropanecarboxylic acid-d_5). mp 153-155 °C (lit.[11] 151-154 °C). IR(KBr) 2280 cm^{-1} (aryl C-D). NMR(D_2O) δ 3.2-2.8 (m, 1H, benzylic H), 2.8-2.35 (m, 1H, cyclopropyl H), 1.85-1.3 (m, 2H, cyclopropyl H), phenyl protons (absent). GC(base) one peak at t_R 4.7 min. Labelling percent was over 99% by NMR. Mass spectrum is shown in Fig. 1 with tranylcypromine and tranylcypromine-d_2 used for the experiments.

Benzoylacetic acid: Methyl benzoylacetate was prepared by the method of Dorsch and McElvain[12] and the ester (0.2g, 1.11 mmol) was stirred in concentrated HCl at room temperature for 3 hr to obtain benzoylacetic acid as a precipitate. The precipitate was filtered and washed with cold water thoroughly. Yield 80 mg (45%). mp 100-101 °C (lit.[13] 99-100 °C). IR(KBr) 2800-3100 cm^{-1} (acid OH), 1640 (C = O). NMR($CDCl_3$) δ 8.2-7.8 (m, 2H, arom. H), 7.8-7.4 (m, 3H, arom. H), 4.1 (s, 2H, CH_2).

3-Phenylpropanal oxime: NH_2OH·HCl (0.5g, 7.2 mmol) was dissolved in EtOH (7 m*l*) to which were added pyridine (1 m*l*) and hydrocinnamaldehyde (0.5g, 3.7 mmol). The solution was refluxed

for 1 hr. Following evaporation of the solvent, crystals were obtained on cooling the residue in H_2O. Two recrystallizations from EtOH/H_2O (1:1) gave 0.34g (45.6%) of oxime. mp 92-95 °C (lit.[14] 93-94.5 °C). IR(KBr) 1660 cm^{-1} (C = N), 950 (N − O), 1720 (C = O, absent). NMR ($CDCl_3$) δ 7.25 (m, 5H, arom. H), 3.0-2.5 (m, 4H, CH_2CH_2), 6.9-6.65 (m, 1H, CH = N), 9.25 (bs, 1H, OH).

Tranylcypromine Schiff bases: A solution of tranylcypromine (26.6 mg, 0.2 mmol) and each of benzaldehyde, hydrocinnamaldehyde, and acetophenone (0.6 mmol) was heated in EtOH (5 m*l*) at 80 °C for 12 hr. The same solution with acetaldehyde was stirred at room temperature for 24 hr. Solvent was evaporated and the residue was analysed in $CHCl_3$ using GC/MS to obtain t_R and mass spectrum of a Schiff base. The conditions for GC/MS analysis were the same as those for the analysis of microsomal metabolites. Retention times and major fragment ions were as follows: acetaldehyde-TCP; t_R 10.8 min, *m/z* 159 (M^+), 144, 132, 117, 55 (base peak). hydrocinnamaldehyde-TCP; t_R 22.6 min, *m/z* 249 (M^+), 247, 232, 143, 115 (base peak), 105, 91. benzaldehyde-TCP; t_R 20.7 min, *m/z* 221 (M^+), 194, 130, 117 (base peak), 90. acetophenone-TCP; t_R 21.3 min, *m/z* 235 (M^+), 130, 115, 104 (base peak), 78.

Preparation of liver microsomes

Livers obtained from phenobarbital-treated rats were washed with 0.05M phosphate buffer, pH 7.4 containing 1.15% KCl and 75g of the liver was homogenized with 300 m*l* of the same buffer. The homogenates were centrifuged at 1,500g for 5 min and the supernatant was recentrifuged at 10,000g for 20 min. One third of the supernatant was used for a 10,000g supernatant and the remaining portion was centrifuged at 100,000g for 1 hr to obtain a microsomal pellet. The pellet was washed with 200 m*l* of 0.1M pyrophosphate buffer, pH 7.4 and the suspension centrifuged at 100,000g for 1 hr to obtain a washed pellet. Microsomal enzyme preparations were made by resuspending it in 40 m*l* of 0.05M phosphate buffer, pH 7.4 containing 1.15% KCl.

Protein content was determined by Lowry *et al.*[15] using bovine albumin as a standard. Cytochrome p-450 content in 10,000g supernatant was determined by dithionite-difference spectroscopy[16] and CO-difference spectroscopy[17] was used to determine that in microsomes. It was found that cytochrome p-450 content in 10,000g supernatant was 0.58 nmole/mg protein and that in microsome, 1.97 nmole/mg protein.

Incubation, extraction, and analysis

Incubation: An incubation mixture was made to contain microsomal preparation (2 m*l*, protein 44 mg), NADPH-generating system prepared in 0.5M phosphate buffer, pH 7.6 (6 m*l*; NADP 15 μmol, G-6-P 150 μmol, G-7-P dehydrogenase 30 units, $MgCl_2$ 150 μmol), and tranylcypromine solution in H_2O (1 m*l*, 1mg). Six incubation mixtures were prepared for each of the tranylcypromine and labeled analogs. Following the incubation at 37 °C for 1 hr in a shaking water bath, the 6 mixtures were combined and frozen and extractions were made within 24 hr. Blanks were the same ones except the use of microsomal preparations boiled at 100 °C for 10 min.

Extraction: Combined incubation mixture (total 54 m*l*) was adjusted to pH 3.5 using 1M HCl and the mixture extracted with two volumes of CH_2Cl_2 twice. The combined CH_2Cl_2 solution was dried over anhydrous Na_2SO_4 and the volume reduced at ambient temperature *in vacuo*. The solution was then concentrated under a stream of dry nitrogen to a residue which was dissolved in 0.4 m*l* of $CHCl_3$ (acidic extracts). The extracted aqueous layer was made to pH 10.0 using 2M NaOH and the solution extracted with two volumes of CH_2Cl_2 twice. The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4 and the solvent evaporated to obtain a residue which was dissolved in 0.4 m*l* of $CHCl_3$ (alkaline extracts).

Analysis: GC/MS was performed using Shimadzu GCMS-QP 1000A with a Shimadzu CBP-10 (OV-1701) fused silica capillary column (25m × 0.2 mm i.d.). Electron energy was 70 eV. Analysis conditions were as follows: injector temperature, 270 °C; separator temperature, 250 °C; ion source temperature, 250 °C; and column temperature maintained at 100 °C for 4 min followed by programming to 250 °C at 8 °C min^{-1}.

Chemical decomposition studies

Tranylcypromine HCl (17 mg, 0.1 mmol) was dissolved in H_2O (0.2 m*l*) and diluted with 0.5 M phosphate buffer, pH 7.6 (10 m*l*). The solution was heated at 60 °C for 12 hr. Following the adjustment of pH to 3.5, it was extracted with CH_2Cl_2 (30m*l*) and CH_2Cl_2 solution was dried over anhydrous Na_2SO_4. The solvent was evaporated to obtain a residue which was dissolved in 0.1 m*l* of $CHCl_3$ (acidic extract). The aqueous layer was made to pH 10.0 and extracted with CH_2Cl_2 (30 m*l*). CH_2Cl_2 solution was dried, evaporated and diluted with $CHCl_3$ (0.1 m*l*) (alkaline extract). The acidic and alkaline extracts were analyzed using GC/MS under the same condi-

tions as for the analysis of microsomal metabolites.

RESULTS AND DISCUSSION

Analysis of acidic extracts

One of the TIC profiles of acidic extracts of microsomal incubations is shown in Fig. 2 with numbers of deuterium-carrying peaks. The results are summarized in Table I and Scheme 1.

1) Characterization of M-I, II, IV and IX

Peaks, M-I, II, and IV were identified to be benzaldehyde, acetophenone, and hydrocinnamaldehyde respectively. The same peaks were also observed in blanks, implying that besides metabolic processes, chemical decompositions of tranylcypromine during the incubation and work-up procedures might be responsible, in parts, for the formation of the products. This was verified by chemical decomposition studies at elevated temperatures which gave the same three products with hydrocinnamaldehyde as a major peak. Acetophenone was obviously due to the decomposition of benzoylacetic acid because it was found by NMR analysis that authentic benzoylacetic acid was easily decarboxylated to acetophenone in $CHCl_3$ at room temperature. It appears therefore that in the present microsomal incubations, hydrocinnamaldehyde from chemical cleavage of cyclopropyl ring of tranylcypromine underwent oxidations to benzaldehyde and benzoylacetic acid possibly by both metabolic and chemical processes.

Cyclopropyl-ring cleavage has been reported for 1-methyl-2,2-diphenylcyclopropylamine,[18] 76% of which was found to decompose to 4,4-diphenyl-2-butanone in saturated $NaHCO_3$ for 24 hr at room temperature. Depuy *et al.*[19] also reported similar cleavages of 1-methyl-2-phenylcyclopropanol in acidic and alkaline conditions. In case of tranylcypromine which has a pKa of 8.13,[20] ring opening by the base mechanism may not occur in microsomal incubations because pH of the incubation medium is 7.6. Further studies are necessary to clarify the mechanism governing cyclopropyl cleavage on tranylcypromine.

Benzoic acid listed as M-IX was detected after the CH_2N_2 treatment from microsomal incubations, blanks, and chemical decompositions. Although benzoic acid can be formed by air oxidation of the structures such as benzaldehyde, it is apparent that the product can be formed as a microsomal metabolite of tranylcypromine. Previously, Alleva[7] has reported detection of glycine conjugate

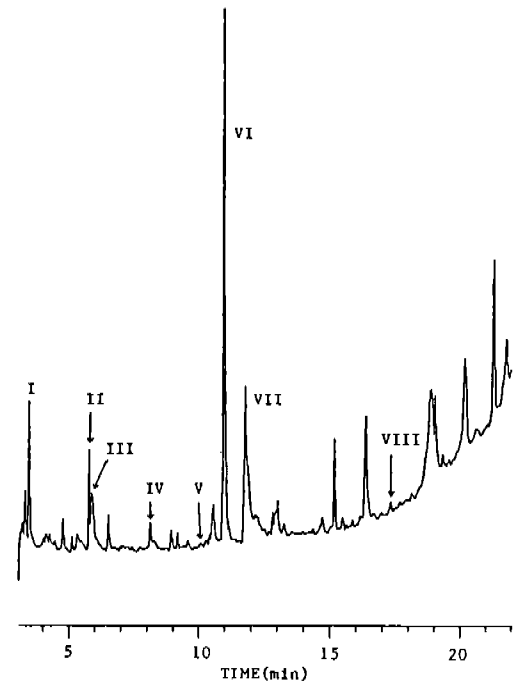


Fig. 2. TIC profile of acidic extracts of microsomal incubations of tranylcypromine and labeled analogs.

of benzoic acid, hippuric acid, as an *in vivo* metabolite of tranylcypromine in rats.

2) Characterization of M-III, V, and VI

M-III and V were deduced to be benzyl alcohol and hydrocinnamyl alcohol, respectively, by mass spectra and the structures were authenticated. The metabolites were, with no doubt, derived from enzymatic reduction of benzaldehyde (M-I) and hydrocinnamaldehyde (M-IV). A similar example can be found in a recent study by Kammerer *et al.*,[21] who identified benzyl alcohol and 1-phenyl-2-propanol as continuing metabolic products of benzaldehyde and 1-phenyl-2-propanone formed from 2-nitro-1-phenylpropane, an amphetamine metabolite, by rabbit liver microsomes.

M-VI was cinnamaldehyde and presumably formed by microsomal benzylic oxidation of hydrocinnamaldehyde and subsequent dehydration. It is also likely that suicidal inhibition product of cytochrome p-450 by tranylcypromine might be cinnamaldehyde. There is no evidence whether the same mechanism holds for tranylcypromine but cyclopropylamines[22,23] have been reported to be suicidal inhibitors of cytochrome p-450 with the

same mechanistic pathways as for the inhibition of MAO by tranylcypromine.[2] Traces of cinnamaldehyde found in blanks in contrast to its major presence in microsomal incubation mixtures are presumably due to air-induced benzylic oxidation of hydrocinnamaldehyde.

3) Characterization of M-VII and VIII

M-VII was observed as a relatively large peak in both acidic and alkaline extracts of microsomal incubations but not in blanks. N-Ethylidene-1,2-dihydroxy-3-phenylpropanamine oxide was proposed for the structure of M-VII. The proposal was based on the possible interpretation of observed masses and feasible metabolic pathways and was to encompass observed extraction characteristics.

It would be possible for such a structure of hydroxyl and imine groups to be extracted incompletely in acidic pH, thus allowing further extraction in basic pH. Molecular ion was *m/z* 209 (d_2 210, d_5 214) which is consistent with one nitrogen molecule of the postulated M-VII. Abstraction of methyl radical will give *m/z* 194 (d_2 195, d_5 199) and subsequent oxygen expulsion produce the ion, *m/z* 178 (d_2 179, d_5 183). *M/z* 117 (d_2 118, d_5 122) was proposed to be derived from *m/z* 178 by abstraction of two hydroxyl radicals and a hydrogen cyanide. The other fragment ions which could be interpreted similarly for the postulated structure, M-VII were; *m/z* 164 (d_2 164, d_5 169), 149 (d_2 149, d_5 154), 59 (d_2, d_5 59), and 75 (d_2, d_5 75).

As shown in Scheme 1, the potential metabolite was proposed to be formed by chemical ring cleavage of tranylcypromine to phenylpropanalimine followed by the oxidation in its tautomeric form of enamine to dihydroxy-hydroxylamine and subsequent adduct formation with acetaldehyde. There are precedented examples of the adduct formation of hydroxylamine with acetaldehyde such as acetaldehyde adducts of N-hydroxyamphetamine[24] and hydroxylamine metabolites of chlorpromazine and promazine.[25] More detailed description on the acetaldehyde adduct will be made in relation to Schiff base products, M-X and M-XI detected in alkaline extracts.

M-VIII was initially postulated to be oxime metabolite, 3-phenylpropanal oxime by mass fragment ions such as *m/z* 91 (base peak), 149 (d_5 154), 132 (d_5 137), 105 (d_5 110), and 77 (d_5 82). However, synthesized authentic oxime gave different retention time (t_R 13.0 min) but with the same fragment ions, *m/z* 77, 91 (base peak), 105, 132, and 149 ($M^{+\cdot}$) except *m/z* 117 which was shown as a high intense ion (68%). Therefore, final proof of the structure for M-VIII was not made.

Analysis of alkaline extracts

TIC profile of alkaline extracts is shown in Fig. 3 and the results are summarized in Table I and Scheme 1. As well as an intact tranylcypromine (t_R 9.1 min) and postulated nitrone metabolite, M-VII found also in acidic extracts, two species, M-X and M-XI were detected.

Mass spectrum of M-X showed fragment ions such as *m/z* 159 (d_5 164, d_2 161), 144 (d_5 149, d_2 146), 132 (d_5 137, d_2 134), 117 (d_5 122, d_2 119) and 55 (d_5, d_2 55) and it was identified to be acetaldehyde adduct of tranylcypromine, N-ethylidene-tranylcypromine. Mass fragment ions such as *m/z* 249 (d_5 259, d_2 253), 247 (d_5 257, d_2 251), 232 (d_5 242, d_2 234), 143 (d_5 148, d_2 145), 115 (d_5 120, d_2 117), and 105 (d_5 110, d_2 107) were observed for M-XI, which were consistent with those of hydrocinnamaldehyde adduct of tranylcypromine. The hydrocinnamaldehyde adduct for M-XI was authenticated and it was obvious that chemically-formed hydrocinnamaldehyde (M-IV) reacted with tranylcypromine to yield a Schiff base product. Comparable sized product was also detected in blanks and in chemical decompositions at elevated

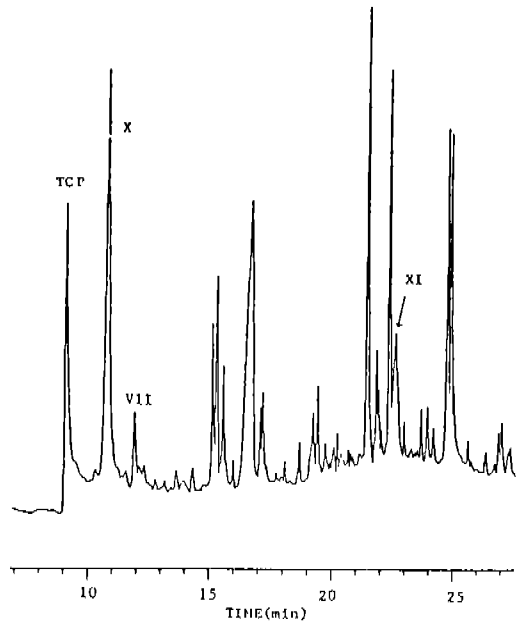


Fig. 3. TIC profile of alkaline extracts of microsomal incubations of tranylcypromine and labeled analogs.

temperatures.

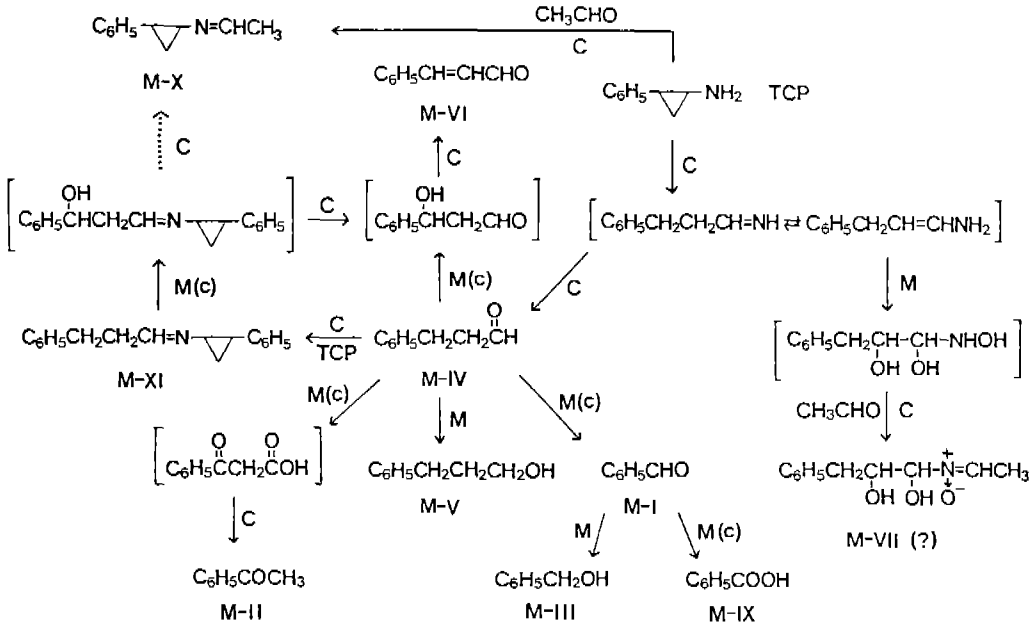
Detection of N-ethylidenetranylcypromine was implicative of the presence of acetaldehyde either in an incubation medium or in a process of sample work-up. When we postulated a structure for M-VII, presence of acetaldehyde was assumed. In literatures, a few cases of involvements of acetaldehyde were found in *in vitro* metabolic studies. Baba *et al.*[24] reported detection of hydroxylamine adduct with endogenous acetaldehyde in the metabolic study of amphetamine using 9,000g supernatant, the concentration of which was measured to be 19.7 nmole/g wet liver. On the other hand, Beckett *et al.*[25] described in their metabolic studies of chlorpromazine and promazine using 9,000g supernatant that acetaldehyde from an impure ether formed artifacts with hydroxylamine metabolites. However, it is not clear in the present study whether the source of the acetaldehyde was endogenous or from contaminations. Differing from previous experiments[24,25] using ether as a solvent and 9,000g supernatant as an enzyme source, CH_2Cl_2 and microsomes were used in our experiments. In blanks, traces of N-ethylidenetranylcypromine were detected although this does not provide complete evidence for the metabolic formation of acetaldehyde adduct because acetaldehyde may be able to be partly removed by boiling the enzyme. Sources of the contamination, if any, were not found so far.

Therefore, alternative possibility regarding formation of N-ethylidenetranylcypromine was sought in relation to metabolic processes as shown in Scheme 1. Hydrocinnamaldehyde adduct of tranylcypromine could undergo metabolic benzylic oxidation forming N-(3-hydroxy-3-phenylpropylidene) tranylcypromine. Retro-aldol degradation of the 3-hydroxy Schiff base will yield M-X, N-ethylidenetranylcypromine. When tranylcypromine-d_2 was used in the experiment, mass spectrum revealed two-mass shift on the contrary to the expected four-mass shift. This could be, however, explainable by assuming that equilibration between N-(3-hydroxy-

Table 1. Deuterium-carrying species from the metabolic incubations of tranylcypromine and deuterated analogs with rat liver microsomes.

Peak number	t_R (min)	Structure
Acidic Extracts		
M-I	3.5	benzaldehyde[3)4)]
M-II	5.8	acetophenone[3)4)]
M-III	5.9	benzyl alcohol
M-IV	8.1	hydrocinnamaldehyde[3)4)]
M-V	10.1	hydrocinnamyl alcohol
M-VI	11.0	cinnamaldehyde[3)]
M-VII	11.8	N-ethylidene-1,2-dihydroxy-3-phenylpropanamine oxide[1)]
M-VIII	17.3	unknown[5)]
M-IX		benzoic acid[2)3)4)]
Alkaline Extracts		
tranylcypromine	9.1	
M-X	10.8	N-ethylidenetranylcypromine[3)]
M-VII	11.8	N-ethylidene-1,2-dihydroxy-3-phenylpropanamine oxide[1)]
M-XI	22.6	N-(3-phenylpropylidene)tranylcypromine[3)4)]

[1)] Postulated structure. The other structures were confirmed with authentic compounds.
[2)] Detected as methyl benzoate (t_R 5.9 min) after the CH_2N_2 treatment.
[3)] Trace peaks were observed in blanks except for relatively large M-XI.
[4)] Detected from the mixture of tranylcypromine and buffer, pH 7.6 at 60 °C for 12 hrs.
[5)] Oxime structure was initially postulated, but disproved by authetic synthesized compound.



Scheme 1. Proposed *in vitro* microsomal metabolic pathways of tranylcypromine. M, Metabolic process; C, Chemical process; M(c), Major metabolic and minor chemical processes.

3-phenylpropylidene)tranylcypromine and its dehydrated product will eventually lead to labelling losses at hydrocinnamaldehyde portion. Retro-aldol formation of acetaldehyde from cinnamaldehyde has been reported.[26]

Future studies

Besides microsomal incubations, we also carried out experiments using 10,000g supernatant (50 mg protein) with fortified NADPH-generating system (NADP 10 μmol, G-6-P 100 μmol, $MgCl_2$ 100 μmol) and similar results were found. Metabolic activity of present microsomes with formulated NADPH-generating system was assessed by applying them to the known microsomal metabolism of imipramine to desipramine. Analysis of the alkaline extract of an incubation mixture initially consisted of 2 mg of imipramine and the same enzymatic components as those for tranylcypromine gave a peak of desipramine (t_R 6.8 min) with one third intensity relative to intact imipramine (t_R 6.3 min), which was demonstrating significant conversions of imipramine to the demethylated metabolite.

In spite of the fair microsomal activity, difficulty in interpreting present results of the detection of oxidized metabolites arose primarily from overlapping of metabolic pathways with chemical oxidations. Such oxidations were likely due to the chemical sensitivity of hydrocinnmaldehyde-like compounds and will occur during incubation, extraction, and solvent-evaporation procedures. Therefore, we found a need to quantitate metabolic products under varying enzymatic conditions so as to estimate metabolic contributions. As well as for such species as benzaldehyde, acetophenone, and benzoic acid, this will be even more important to find a convincing evidence for the metabolic formation of the postulated metabolites such as nitrone, M-VII and acetaldehyde Schiff base, M-X. According to our preliminary experiments, Schiff bases were, however, found easily convertible in solutions to aldehydes and tranylcypromine, so that final conclusion on metabolic formation by such quantitations might not be easily made.

In the present study, ring-hydroxylated metabolite of tranylcypromine was not detected. The metabolite was not found either in our earlier *in vivo* studies in rats,[5] while Baker *et al.*[6] reported detection of the metabolite. After the completion of microsomal metabolic studies, we synthesized authentic *p*-hydroxytranylcypromine by BBr_3 demethylation of *p*-methoxytranylcypromine which was obtained by condensing *p*-methoxystyrene and ethyl diazoacetate followed by taking similar procedures to the synthesis of tranylcypromine analogs[27] and found low extractability of the

p-hydroxytranylcypromine by present extraction procedures (unpublished results). Therefore, in order to prove whether *p*-hydroxy metabolite was formed by microsomes *in vitro,* we are investigating methods to improve extractability of the metabolite by choosing appropriate solvents and possibly adopting ion pair extractions.

In addition, a work is in progress to improve detection limits by derivatizing samples to detect hindered metabolites and to obtain additional supporting mass spectral data of M-VII for the postulated nitrone structure before synthesizing authentic compound.

ACKNOWLEDGEMENT

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