Arch. Pharm. Res. 8(2), 77~84 (1985)

Synthesis and Mass Spectrometry of Deuterium Labeled Tranylcypromine Hydrochloride*

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(Received June 13, 1985)

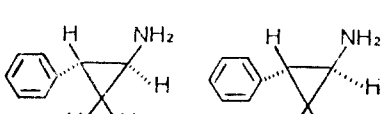
Abstract □ [$^2H_2$]Tranylcypromine hydrochloride (trans-3, 3-dideuterio-2-phenylcyclopropylamine HCl) was synthesized for application to the metabolic studies. Mass fragmentation processes for the tranylcypromine and its two synthetic intermediates, γ-phenyl -γ-butyrolactone and trans-2-phenylcyclopropanecarboxylic acid were described based upon comparisons between labeled and unlabeled compounds.

Keywords □ [$^2H_2$]Tranylcypromine, trans-3, 3-Dideuterio-2-phenylcyclopropylamine, β, β-Dideuterio-γ-phenyl-γ-butyrolactone, trans-3, 3-Dideuterio-2-phenylcyclopropanecarboxylic acid, Mass fragmentation processes, Metabolic studies.

Tranylcypromine (**1**, trans-*dl*-2-phenylcyclopropylamine) has been known as a competitive inhibitor of monoamine oxidase (MAO, EC 1.4.3.4) and the activity assumed to be due to the formation of charge transfer complex with the flavin moiety of the enzyme through its cyclopropane ring.[1] Paech et al.[2] recently reported a new finding that tranylcypromine might inhibit the MAO irreversively through an imine or ketone metabolic intermediate which can combine covalently with MAO protein.

In order to find an evidence supporting the metabolic formation of reactive species acting directly on MAO, it appeared for us to be first essential to determine general metabolic pathways of tranylcypromine on both *in vitro* and *in vivo* levels. In relation to this attempt, we have performed *in vivo* metabolic studies using an unlabeled tranylcypromine and reported a GC/MS evidence for the formation of N-acetyl and arylhydroxy N-acetyltranylcypromine.[3]

In the studies using an unlabeled drug, we found a difficulty in assigning metabolite peaks among the multicomponents from the data obtained as a form of GC/MS total ion current profile. Since recent trends are to use stable isotope labeled drugs for metabolic studies to help trace metabolites and interpret mass spectra to obtain a structural information,[4] we decided to synthesize deuterium labeled tranylcypromine. In this report we describe a synthesis of [$^2H_2$] tranylcypromine, **2** which contains two deuterium atoms incorporated into the cyclopropyl ring.



1 **2**

*Mechanism of the MAO Inhibition by 2-Phenylcyclopropylamines IV

EXPERIMENTAL METHODS

Materials

Deuterium oxide (>99.8 atom% D) and NaOD (40% in D_2O, >99.5 atom% D) were obtained from Fluka AG, Switzerland and heptafluorobutyric anhydride from Sigma Chemical Co. 3-Benzoylpropionic acid was purchased from Tokyo Kasei. Authentic hydrochloride salt of tranylcypromine was prepared using its sulfate salt (gift of Smith Kline & French Laboratories, U.S.A.).

Instrumentation

The melting points were determined using a Sybron Thermolyne, Olympus, Tokyo. Infrared spectra were recorded on a Perkin-Elmer Model 710 infrared spectrophotometer. Nuclear magnetic resonance spectra were taken on a Varian EM-360L or Varian EM-360A 60 MHz spectrometer using tetramethylsilane as internal standard (s=singlet, d=doublet, m=multiplet). Hewlett Packard Model HP 5985B GC/MS System was used to collect direct probe mass spectral data. The electron ionization voltage was 70 eV. Gas chromatographic data were obtained using Hitachi Model 163 gas chromatograph. A glass column (2.0m×3mm i.d.) packed with 3% OV-17 on 80/100 Chromosorb W(HP) was used with carrier gas(N_2) at 50 ml min^{-1}. The standard operating conditions were: column temperature 100°C, programmed to 250°C at 10°C min^{-1}; injector temperature 250°C; and detector temperature 250°C. GC/MS was carried out by the method described in the previous report[3].

Synthesis of β,β-Dideuterio-γ-phenyl-γ-butyrolactone (**6**)

The 3-benzoylpropionic acid (3.56g, 0.02 mol) was dissolved in D_2O(30 ml) and 2 ml of 40% NaOD in D_2O (0.8g, 0.02 mol). The mixture was stirred at 80°C for 24 h. After the solution was cooled to 0°C, $NaBH_4$ (832mg, 0.022 mol) was added slowly and the mixture stirred at room temperature for 24 h. The mixture was diluted with 30 ml of H_2O, acidified with HCl, and extracted with Et_2O (100 ml×2). The ether extracts were dried (Na_2SO_4), filtered, and evaporated. The residue was heated at 140°C under 20 mmHg for 2.5 h to give the labeled lactone **6** as a solid (3g, 91.4%), mp 33~34°C, lit.[5] 36~37°C. IR(KBr) 1770 cm^{-1} (lactone C=O). NMR($CDCl_3$)δ2.63 (s, 2H, CH_2), 5.45 (s, 1H, CH), 7.35 (m, 5H, aromatic H). Mass spectrum was given in Fig. 1 with that of an unlabeled lactone. The GC retention time (9.7 min) was identical with that of an authentic unlabeled compound.

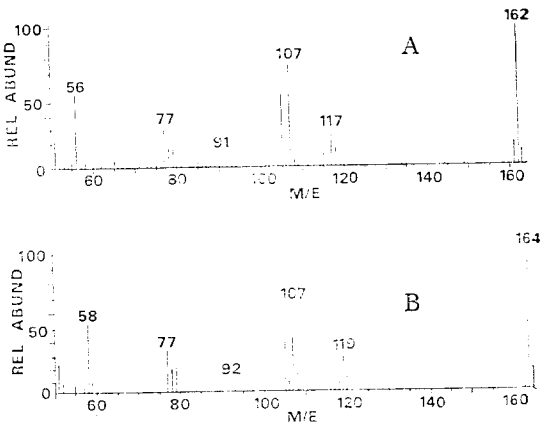


Fig. 1: Direct probe mass spectra of γ-phenyl-γ-butyrolactone (A) and [$^2H_2$]analog (B).

Synthesis of trans-3,3-Dideuterio-2-phenylcyclopropanecarboxylic acid (**9**)

To a suspension of **6** (2.46g, 0.015 mol) in 25 ml of benzene was added freshly-distilled $SOCl_2$ (0.045 mol). After the mixture was refluxed for 8 h, it was concentrated *in vacuo*. The residual oil was cooled to 0°C and to it was added 10 ml of EtOH saturated with HCl gas. The mixture was stirred at room temperature overnight and refluxed for 2 h. The solution was concentrated *in vacuo* and the residue dissolved in 100 ml of Et_2O. The ether solution was washed with 10 ml of 5% $NaHCO_3$, dried (Na_2SO_4), and concentrated to yield 3.07 g of **7**(89.7%). IR(neat) 1730 cm^{-1}(ester C=O). A potassium t-butoxide solution was prepared by adding metal potassium (0.5g, 0.013 mol) to 10 ml of t-butyl alcohol in N_2 atmosphere followed by dilution with 30 ml of benzene. The compound **7** (3 g, 0.013 mol) was added to the potassium t-butoxide solution prepared above and the mixture refluxed for 5 h. The mixture was cooled and to it was added 25 ml of benzene and

25 ml of H_2O. After shaking the mixture, the benzene layer was separated. The aqueous layer was extracted with 30 ml of benzene. The combined benzene extracts were washed with H_2O to the neutral pH, dried (Na_2SO_4), and evaporated to yield 2.07g of **8** (82.9%). IR(neat) 1720 cm^{-1} (ester C=O). The compound **8** (1.92 g, 0.01 mol) was dissolved in 10 ml of 85% EtOH which contained 2.24 g of KOH (0.04 mol). The mixture was refluxed for 10 h and concentrated under reduced pressure. After the addition of H_2O (10 ml) to the residual mixture, the solution was washed with Et_2O (50 ml×2), made acidic with HCl and extracted with Et_2O (100 ml×2). The ether solution was dried (Na_2SO_4) and evaporated to yield a mixture of cis and trans isomers of 2-phenylcyclopropanecarboxylic acid as a oily residue (1.25 g, 76.2%). To the residue was added H_2O and the mixture heated to 80°C. The hot H_2O solution was cooled to provide whited precipitates of labeled trans-2-phenylcyclopropanecarboxylic acid **9** (0.52g, 31.7% from **8**). mp 87~89°C, lit.[6] 86~88°C. IR(KBr) 1680, 1700 cm^{-1} (acid C=O). NMR($CDCl_3$) δ 1.87 (d, 1H, cyclopropyl CH; J=4 Hz), 2.6 (d, 1H, cyclopropyl CH; J=4 Hz), 7.25 (m, 5H, aromatic H), 10.7 (broad s, 1H, OH; exchanged with D_2O). Mass spectrum was shown in Fig. 2 with that of an unlabeled compound. The GC retention time (8.6 min) was consistent with that of an authentic unlabeled compound.

Synthesis of trans-3, 3-Dideuterio-2-phenylcyclopropylamine (2) Hydrochloride ([$^2H_2$] Tranylcypromine HCl)

To the acid **9** (0.32g, 0.0019 mol) was added 1.13 g of $SOCl_2$ (0.0095 mol). The solution was stirred at room temperature for 24 h and the $SOCl_2$ removed *in vacuo*. To the oily residue of acid chloride was added 6 ml of acetone and 0.43 g of NaN_3 (0.0066 mol) dissolved in H_2O (1.0 ml) at 0°C. The mixture was stirred at 0°C for 40 min, diluted with ice-cold H_2O (20 ml), and extracted with cold toluene (100 ml×2). The toluene solution was dried over Na_2SO_4 at 0°C. The filtered solution was refluxed for 1 h and evaporated to dryness at 80°C. To the residue of the isocyanate was added 7N HCl (15 ml) and the mixture refluxed for 3 h. The cooled solution was diluted with H_2O (20 ml), washed with Et_2O (100 ml×2) and made alkaline with NaOH. The solution was extracted with Et_2O (100 ml×2) and dried over Na_2SO_4. To the filtered ether extracts was added HCl gas-

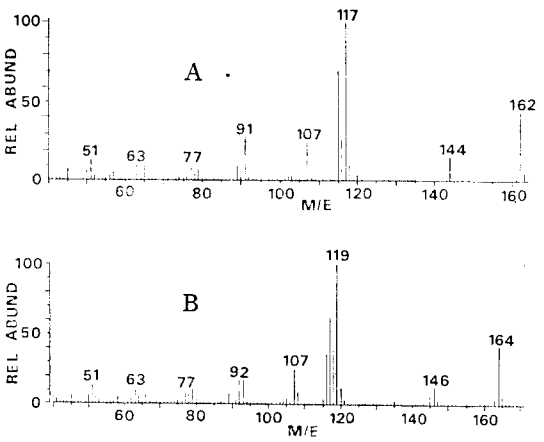


Fig. 2: Direct probe mass spectra of trans-2-phenylcyclopropanecarboxylic acid(A) and [$^2H_2$] analog(B).

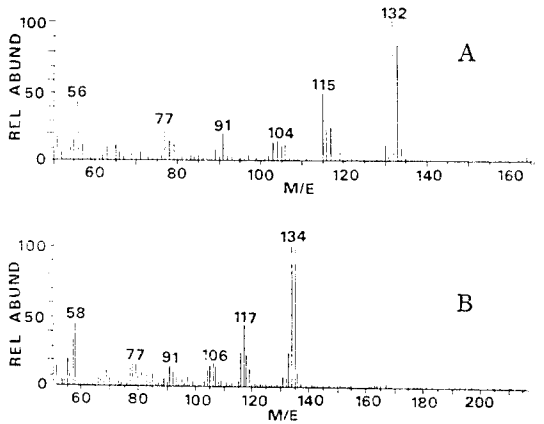


Fig. 3: Direct probe mass spectra of tranylcypromine (A) and [$^2H_2$]analog (B).

saturated ether and the ether was removed *in vacuo*. The residue was dissolved in a small amount of MeOH and EtOAC to which was added Na-dried Et_2O to yield a precipitate of hydrochloride salt of labeled tranylcypromine **2** (0.13g, 41.2%). mp 152~154°C, lit.[7] 151~154°C. IR(KBr) 2900, 1600, 1500 cm^{-1} ($-NH_3^+$). NMR ($CDCl_3$/DMSO-d_6)δ2.46 (d, 1H, cyclopropyl CH; J=4 Hz), 2.74(d, 1H, cyclopropyl CH; J=4 Hz), 7.25 (m, 5H, aromatic H), 8.8 (broad s, 3H, NH_3^+; exchanged with D_2O). Mass spectrum was given in Fig. 3 with that of an authentic unlabeled compound. The GC retention time (4.4 min) was consistent with that of an authentic unlabeled compound.

Deuterium Exchange Reactions of Aromatic Hydrogens of Tranylcypromine in D_2O and Heptafluorobutyric acid-d

To 1ml of heptafluorobutyric anhydride(1.653 g, 4 mmol) was added 0.5 ml of D_2O (0.025 mol) at 0°C. The mixture was allowed to warm to room temperature and maintained at 50°C for 5 min. To the acid solution prepared above was added tranylcypromine (52mg, 0.4 mmol) or tranylcypromine sulfate (72 mg, 0.4 mmol) and the mixture heated in a sealed ampoule for 72 h at two different temperatures, 150°C and 180°C. After cooling the mixture, it was made alkaline and extracted with $CHCl_3$. The tranylcypromine base was found to be completely decomposed at 180°C for 72h as determined by TLC on Kieselgel 60G (E. Merck, Darmstadt) with EtOAC/MeOH/NH_4OH (17:2:1) (R_f of the base, 0.52). GC/MS of the sample obtained from tranylcypromine sulfate at 180°C and its base at 150°C did not indicate any labelling on the phenyl hydrogens.

RESULTS AND DISCUSSION

An acid-catalyzed direct enrichment of aromatic hydrogens in tranylcypromine was initially chosen primarily in consideration of the economy of synthesis. Heptafluorobutyric acid was selected as a catalyst characteristic of high boiling point, which was successfully employed for the preparation of enriched imipramine.[8] It was found however that labelling on the benzene ring by the method of direct deuterium exchange was not possible in the present experimental conditions partly because of the unstable nature of tranylcypromine which did not permit the reaction temperatures exceeding 180°C.

Therefore, an alternative route to the labeled

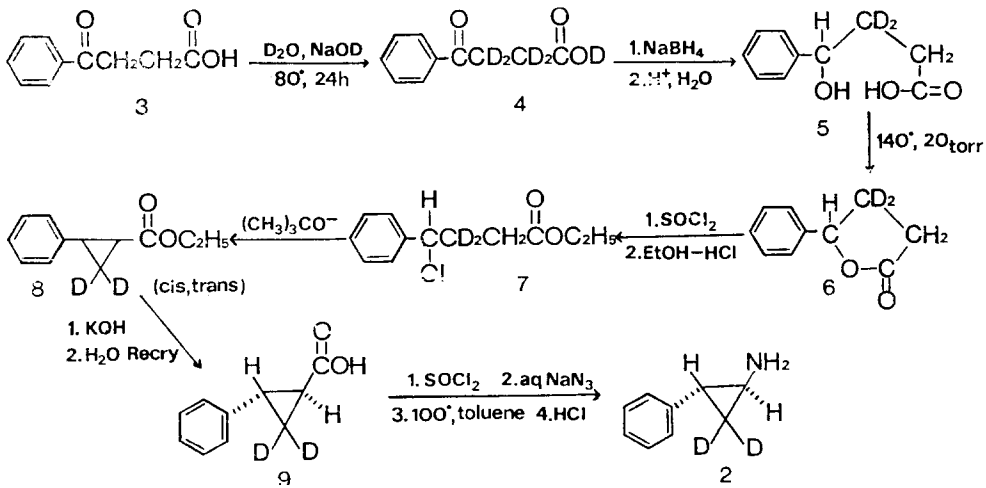


Fig. 4: A synthetic scheme for [$^2H_2$]tranylcypromine.

tranylcypromine was designed as summarized in Fig. 4. Although a similar synthetic scheme for 2-phenylcyclopropylamine derivatives was described by Kaiser et al.[9] a special consideration was given in the present work to the high enrichment in the hydrogens on cyclopropyl ring as well as the economical synthesis by the modification of reaction conditions.

Thus, the labile hydrogens of 3-benzoylpropionic acid 3 including C-3 hydrogens were exchanged with deuterium atoms by treating the compound with D_2O and NaOD. When the exchange reaction was carried out at room temperature for 24h, the degree of labelling of β,β-dideuterio-γ-phenyl-γ-butyrolactone 6 was 40%. The observed value was far below the anticipating degree of labelling; 97% which was calculated assuming that the reaction reached a complete equilibrium between exchangable protons and existing deuteriums. The labelling percent was improved eventually to the expecting value by heating the reaction mixture at 80°C for 24 h.

In order to investigate the possibility of overestimation of the labelling percent measured by NMR resulting from partial deuteration of the

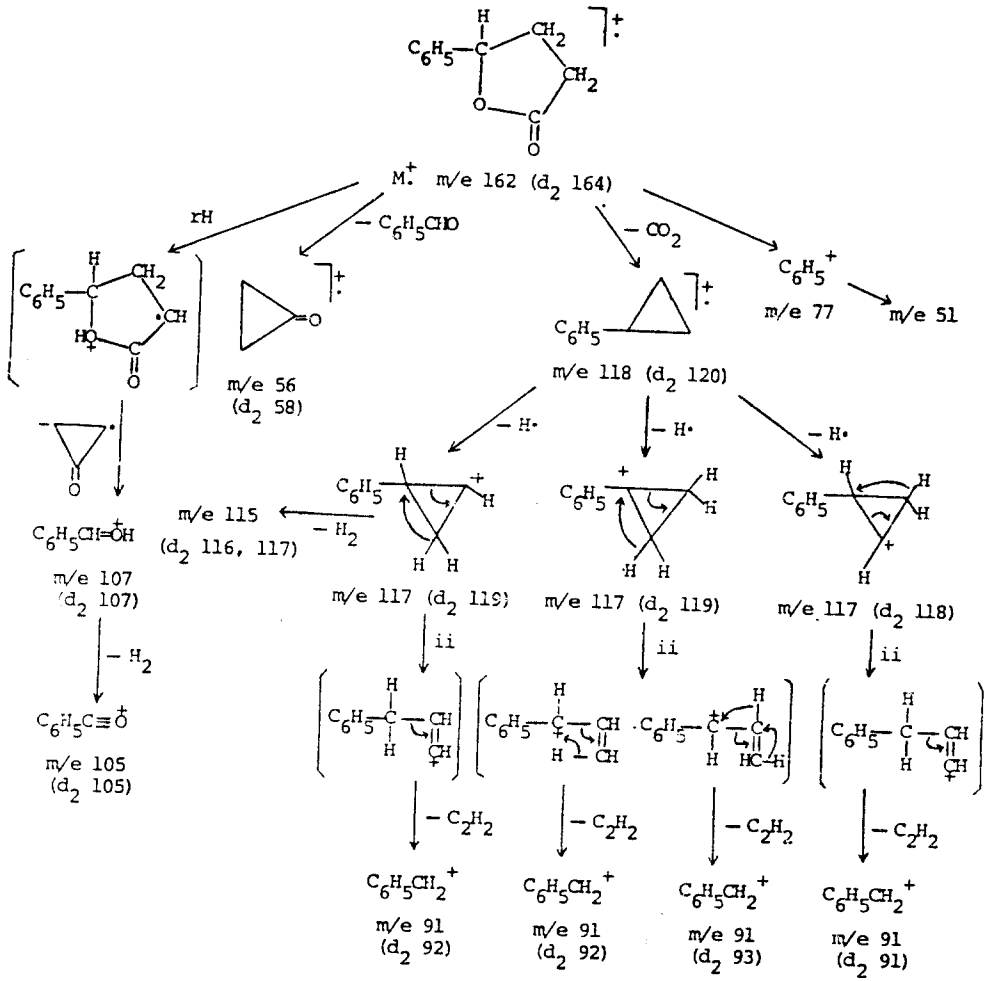


Fig. 5. Proposed mass fragmentation processes for γ-phenyl-γ-butyrolactone.

α-C hydrogens of **6**, the labeled lactone (50mg, 3 mmol) was refluxed with KOH (90mg, 16 mmol) in 50% EtOH (5 ml) for 24 h, The lactone obtained after work-up procedures was analyzed by NMR. It was found that the labelling percent was unchanged which suggests that the exchanged α-C deuteriums, if any, could be easily reverted to hydrogens during the work-up procedures.

Reduction of **4** by $NaBH_4$ and cyclization led to the isolation of **6** which contains stable deuteriums at β-C position. The $NaBH_4$ reduction products were determined to be a mixture of 3, 3-dideuterio-4-phenyl-4-hydroxybutanoic acid **5** and **6** (3:2) by NMR. Therefore, conversion of **5** in the mixture to lactone was required. The lactone was further converted via **7** to cyclopropyl compound **8** with two deuterium atoms at C-3 position. Hydrolysis of the carboxylate **8** and Curtius reaction[10] provided the synthesis of labeled tranylcypromine **2**.

Fig. 5 represents proposed mass fragmentaion pathways for γ-phenyl-γ- butyrolactone defined by comparisons between labeled and unlabeled compounds. The molecular ion at m/e 162 was shown as a base peak. The fragment ion at m/e 107 was presumably due to the α-C hydrogen rearrangement to the oxygen atom prior to the loss of cyclopropanone molecule. The ion at m/e 107 was further fragmented to m/e 105. It appeared that the lactone gave a fragment at m/e 118 by the process of CO_2 loss. Hydrogen abstraction from the fragment m/e 118 which had taken place primarily at the benzylic position resulted in the formation of m/e 117 with m/e 119 for a labeled compound. The fragment ion

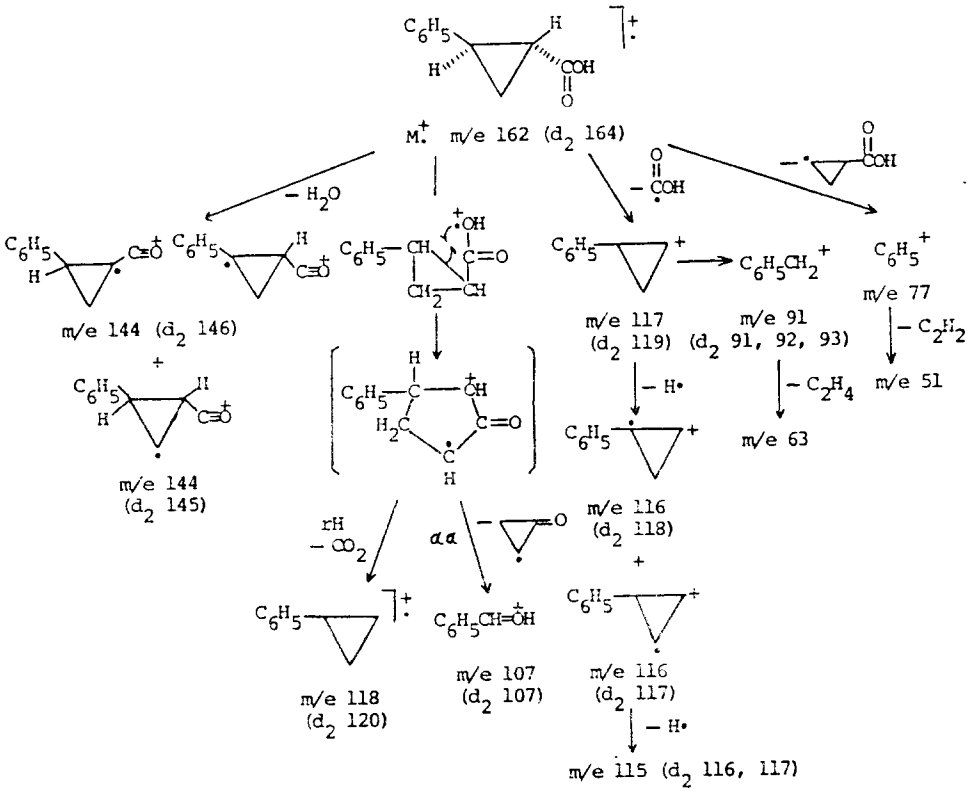


Fig. 6: Proposed mass fragmentation processes for trans-2-phenylcyclopropanecarboxylic acid.

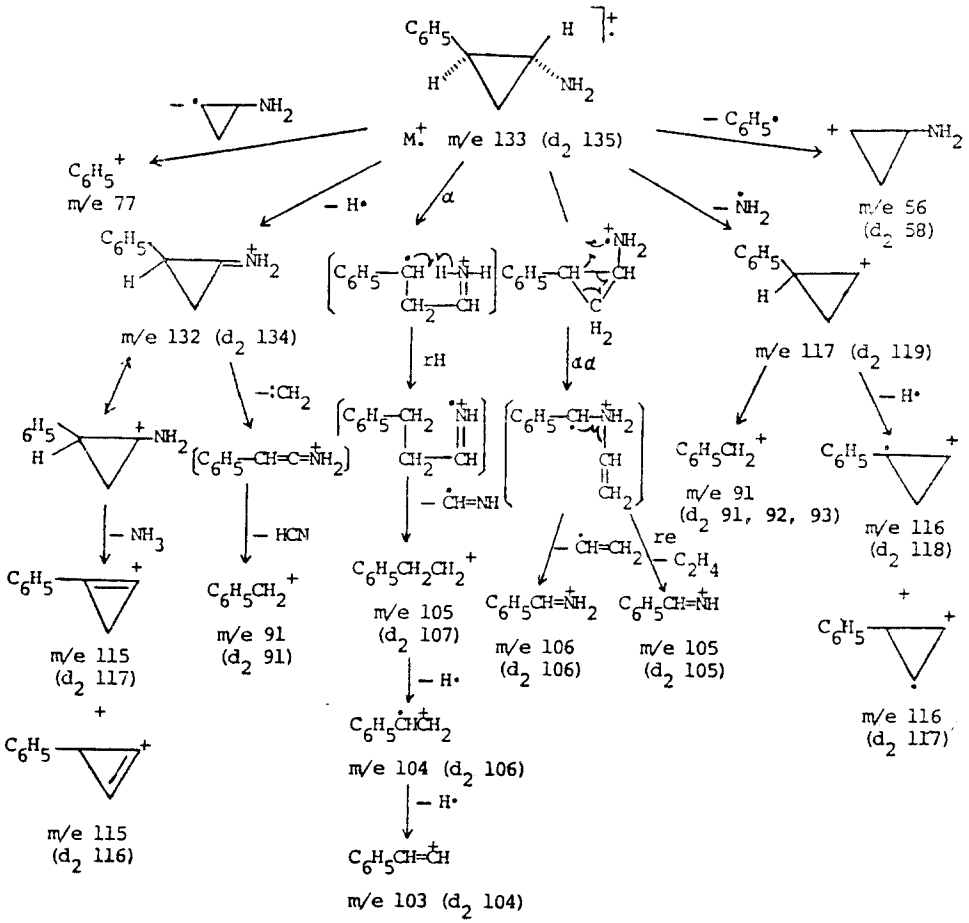


Fig. 7: Proposed mass fragmentation processes for tranylcypromine

at m/e 91 was $C_7H_5CH_2^+$ derived from m/e 117 through ii-cleavage and loss of C_2H_2 molecule. One hydrogen transfer to the benzylic carbon from β-C position appeared to be a major pathway to the formation of m/e 91 as reflected on one deuterium ion of m/e 92 for the labeled analog. According to the spectrum of the labeled compound, it was obvious that additional hydrogen scrambling processes were also involved in the formation of m/e 91. The lactone underwent $\alpha\alpha$-cleavage forming the fragment ion at m/e 56 with high abundance and retention of the β-C hydrogens.

Mass fragmentation pathways for trans-2-phenylcyclopropanecarboxylic acid were proposed in Fig. 6. A molecular ion was at m/e 162 with m/e 164 for a labeled compound. A hydrogen rearrangement from C-1 and C-2 position to oxygen atom and further loss of H_2O were a major pathway to the ion, m/e 144. The fragment ion at m/e 117 was indicative of the loss of O=Ċ-OH from the molecular ion. The presence of a fragment at m/e 107 was rationally elucidated by the fragmentation processes consisting of initial ring enlargement and cyclopropanone loss by $\alpha\alpha$-cleavage. The characteristic ion at m/e 115 was presumably derived from m/e 116 formed by a hydrogen loss from m/e 117.

Fig. 7 represents proposed mass fragmentation

processes for the tranylcypromine. A molecular ion at m/e 133 was shifted by 2 mass units for a labeled compound. An NH_3 elimination appeared to occur from the base peak ion at m/e 132 in the formation of m/e 115. The suggested pathways to the fragment, m/e 91 taking place by the loss of methylene and HCN from the base peak ion could account for the same fragment of m/e 91 observed in the labeled compound. Whereas, some of the fragments at m/e 91 might be resulted from phenylcyclopropyl ion at m/e 117 as shown by the fact that in labeled compound there were ions at m/e 92 and m/e 93. Ion clusters with the highest abundance at m/e 104 could be formed by two pathways, α- and $\alpha\alpha$-cleavages from the molecular ion. The $\alpha\alpha$-cleavage product was proposed to fragment to the ion at m/e 106 and elimination reaction product at m/e 105. Hydrogen rearrangement from the nitrogen atom to the benzylic position which had taken place at α-cleavage product could account for the complete retention of deuterium atoms at m/e 106 for a labeled compound.

ACKNOWLEDGEMENTS

The work was supported in part by a research grant from Korea Science and Engineering Foundation (1983~1985). The authors thank Mr. Byung Chul Kim of Dong-A Pharm. Co. for NMR spectrometry.

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