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Identfiication of N-Acetyl and Hydroxylated N-Acetyltranylcypromine from Tranylcypromine-Dosed Rat Urine*

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Abstract □ Mechanism of the monoamine oxidase inhibition by tranylcypromine was studied in relation to its metabolism to reactive species. A metabolic study performed to collect general biotransformation pathway in rats provided GC/MS evidence for the detection of two new metabolites, N-acetyl and hydroxylated N-acetyltranylcypromine.

Keywords □ Monoamine oxidase inhibition, 2-Phenylcyclopropylamines, Tranylcypromine, Metabolic study, N-Acetyltranylcypromine, Hydroxylated N-acetyltranylcypromine.

Tranylcypromine has been known as a competitive inhibitor of monoamine oxidase (MAO) with a long duration of action comparable to that of an irreversible inhibitor which assumed to be due to the high affinity for an enzyme.[1] Paech *et al.*[2] recently reported irreversible inhibitory nature of MAO by tranylcypromine proposing an imine or a ketone metabolic intermediate which can combine covalently with MAO protein. An analogous mechanistic explanation was also give to the inhibition by N-[2-(*o*-iodophenoxy) ethyl] cyclopropylamine.[3] When we reviewed the previous reports, those proposals were found not to be based on the confirming evidence of positive identification of a related metabolite. It was further noticed that a metabolic study of tranylcypromine had not been reported by using either *in vivo* or *in vitro* enzyme system. In addition, in view of the recent knowledge that mechanism of drug action can be rationally elucidated in connection with enzyme inhibition by its metabolite[4], a study to know general *in vivo* metabolic pathway of tranylcypromine app eared to be firstly essential in the mechanistic investigation for the MAO inhibition by 2-phenylcyclopropylamines and for pharmacology and toxicology of tranylcypromine. This communication is a first report of such an attempt and described the first GC/MS detection of N-acetyltranylcypromine and glucuronide conjugate of ring-hydroxylated N-acetyltranylcypromine in tranylcypromine-dosed rat urine.

A 40mg/kg dose of tranylcypromine sulfate (gift of SK&F, USA) was given to each of the three male Sprague Dawley rats (170-230g) through subcutaneous route. Urine (25ml from three rats) was collected over 36 hours using a metabolism cage. A urine sample (10ml, pH 7.2) was centrifuged to remove solid substances and the sample diluted with H_2O (10ml). The pH of the diluted urine was adjusted to 5.5 by adding 0.1N H_2SO_4. The solution was then extracted with $CHCl_3$ (60ml×2) and the $CHCl_3$ solution was kept for the analysis of nonconjugate metabolites extractable at pH 5.5. Any precipitate was removed from aqueous layer and the solution was made alkaline (pH 10) with dilute ammonia water. A pH 10 noncon-

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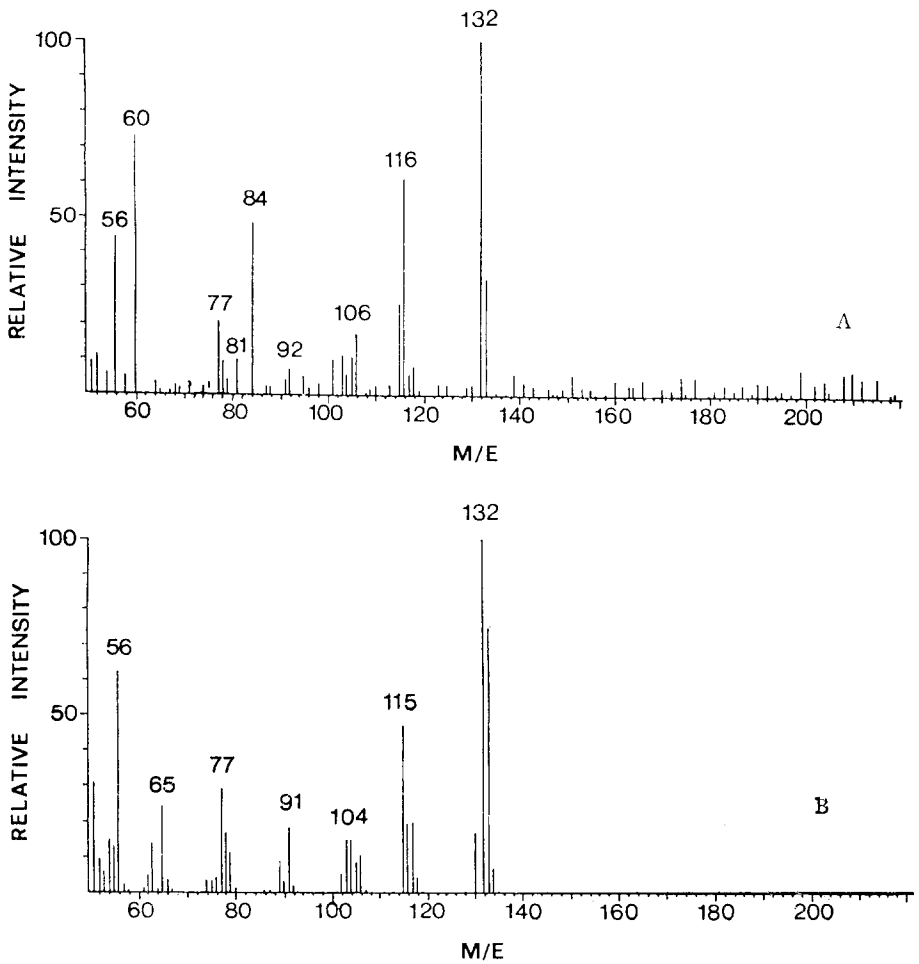
jugate fraction was obtained by extracting the sample with $CHCl_3$ (60ml×2). The aqueous layer was dried under vacuum at 40-45°C. A 10ml of 0.1M sodium acetate buffer (pH4.5) was added to the residue and the solution was incubated with 0.3ml of β-glucuronidase (Type H-3, 98,700 units/ml,Sigma). After 24 hours incubation, the solution was extracted with $CHCl_3$ (40ml×2) to obtain pH 5 conjugate fraction. The aqueous layer was further extracted with $CHCl_3$ (40ml×2) after adjusting pH to 9.5 with dilute ammonia water (pH 9.5 conjugate fraction) One of conjugate fractions obtained from duplicate experiments was treated with diazomethane (generated by adding KOH to Diazald in Carbitol). Final samples of MeOH solution (~0.2ml) were analyzed using Finnigan 4021 Gas chromatograph-Mass spectrometer with BP-5 Vitreous silica capillary column (25 m×0.33mm). The analysis condition was as follows; column temperature 100 (1 min hold)-230° C (15 min hold) (10° C/min programming), injector temperature 230° C, carrier gas (He) flow rate 5ml/min, electron energy 70 eV, scan time 0.95 sec. GC/MS data collected from the experiment was kept and will be used for



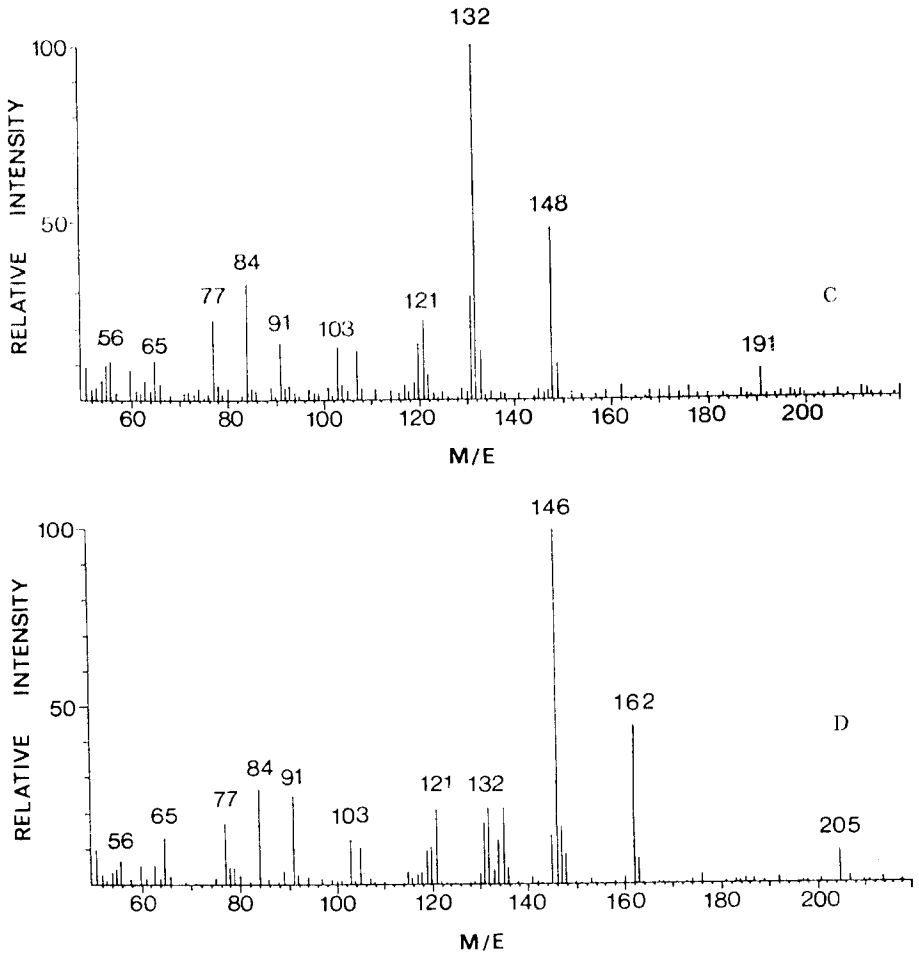


Fig. 1: Mass spectra of N-acetyltranylcypromine (A), tranylcypromine (B), hydroxylated N-acetyltranylcypromine (C), and methoxy N-acetyltranylcypromine (D).

the extensive metabolic study using deuterium labeled tranylcypromine analogs.

Analysis of GC/MS data of pH 5.5 nonconjugate fraction provided evidence for the positive detection of N-acetyltranylcypromine, which appeared at scan number 520 (Rt. 8 min 40 sec). The mass spectrum (Fig. 1A) and retention time was consistent with those from authentic N-acetyltranylcypromine. The same peak was not observed in the control urine sample. Total ion current profile (TIC) of pH 5.5 nonconjugate fraction showed tranylcypromine at scan 197 indicating that some of tranylcypromine was extractable at pH 5.5. Major peak from pH 10 nonconjugate fraction was intact tranylcypromine (Fig. 1B). Mass spectrum of the 786 scan (Fig. 1C) obtained from TIC of pH 5 conjugate fraction showed m/e 191 as a molecular ion of ring-hydrolylated N-acetyltranylcypromine. M/e 132 of M-43 (loss of $COCH_3$)

from N-acetyltranylcypromine was shifted to m/e 148 resulting from M-43 (loss of $COCH_3$). Treatment of the same sample with diazomethane gave a peak at scan 756. Mass spectrum (Fig. 1D) showed 14 mass shift to m/e 146, m/e 162, and m/e 205 which clearly indicated presence of a phenolic hydroxy group and the formation of methoxy N-acetyltranylcypromine. The metabolite was excreted as a glucuronide conjugate because it was obtained in β-glucuronidase hydrolysis fraction.

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