Notes

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Metabolism of Drugs. LXXV.[1] The Enzymatic Degradation of Isoprophenamine

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In the previous paper[3] we have reported the *in vivo* metabolism of isoprophenamine (1-*o*- chlorophenyl-2-isopropylaminoethanol) in mice, rabbits and humans after oral administration. In mice the drug was converted to *o*-chloromandelic acid which was excreted into urine as one of the main metabolites. In rabbits and humans, on the other hand, the drug was converted to *o*-chlorobenzoic acid and *o*-chlorohippuric acid as acidic metabolites. On the basis of these results, a scheme for the metabolism of isoprophenamine was postulated that this drug may be metabolized to 1-*o*-chlorophenyl-2-aminoethanol which in turn is metabolized to *o*-chloromandelic acid and further to *o*-chlorobenzoic acid.

The present *in vitro* investigation was undertaken in order to confirm the metabolic pathway of isoprophenamine more exactly using 9000 *g* supernatant fractions of the rabbit liver.

Experimental

Preparation of 9000 *g* Supernatant Fractions——Adult male rabbits (average body weight 3.0 kg) were killed, exanguinated, and the livers were homogenized in two volumes of cold 0.2 M potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 9000 *g* for 30 min and the supernatant containing the microsomal and soluble fractions was used for all experiments.

Enzyme Assay——Incubations were carried out in air for 1 hr at 37° using metabolic incubator. The incubation mixture consisted of 30 μmoles of isoprophenamine (as base), 12 ml of 9000 *g* supernatant, 600 μ moles of nicotinamide, 450 μmoles of $MgCl_2$, 1.2μ moles of NADP and 0.1 M potassium phosphate buffer of pH 7.4 to make a final volume of 30 ml. In the control experiment was used the boiled 9000 *g* supernatant instead of enzyme source.

Extraction of Metabolites——The reaction mixture was heated on a boiling water bath and centrifuged. The supernatant was made alkaline by adding of 1 ml of 1 N NaOH and was extracted three times with 10 ml of AcOEt (basic AcOEt extract). The aqueous phase was then acidified by adding 3 ml of 1 N HCl and was extracted with AcOEt as same as above (acidic AcOEt extract). Both basic and acidic AcOEt extracts were evaporated to dryness *in vacuo* and the residues were dried on P_2O_5. To the residues were added 0.35 ml of pyridine, 0.1 ml hexamethyldisilazane and 0.05 ml of trimethylchlorosilane. The mixtures were heated for 10 min at 80° and submitted to gas chromatography.

Gas Chromatography——A Shimadzu Model GC-1C Gas Chromatograph (hydrogen flameionization detector) was employed in this study. A glass column of 2.625 m×4 mm was packed with 1.5% OV-1 on Shimalite W (80—100 meshes). The operation conditions were as follows: sensitivity 100, range 1.6, column temp. 155°, detector temp. 230°, flow rate of carrier gas (N_2) 45 ml/min (2.6 kg/cm^2), flow rate of H_2 45 ml/min.

Result and Discussion

As shown in Fig. 1, both gas chromatograms of the basic AcOEt extract and its control exhibited a peak of isoprophenamine at retention time of 2.1 min. The peak in the former was evidently smaller than that in the latter, and this represents that a part of isoprophenamine was enzymatically degradated by 9000 *g* supernatant during incubation.

1) Part LXXIV: K. Tatsumi, N. Arima and H. Yoshimura, *Yakugaku Zasshi*, **91**, 680 (1971).
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3) K. Tatsumi, N. Arima, C. Yamato, H. Yoshimura and H. Tsukamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 1254 (1970).

Furthermore, the gas chromatogram of the basic AcOEt extract revealed a new peak with retention time of 3.6 min, which was not observable in the control. This peak corresponded to that of the primary amine, 1-*o*-chlorophenyl-2-aminoethanol.

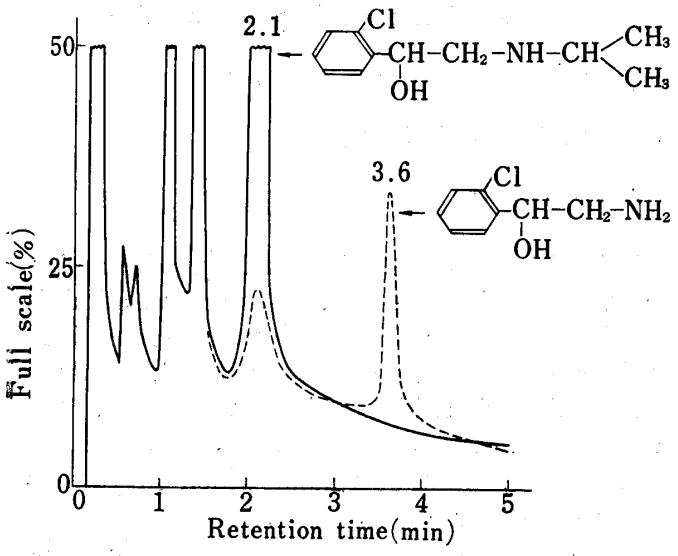

Fig. 1. The Gas Chromatogram of the Basic AcOEt Extract and the Control

-------: basic AcOEt extract, ———: control

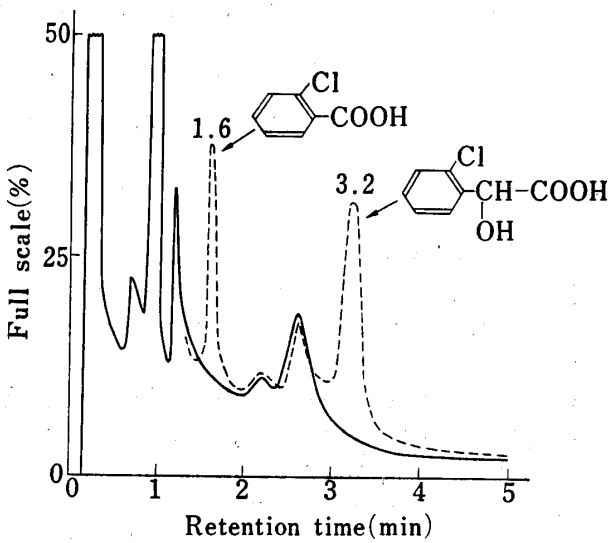

Fig. 2. The Gas Chromatogram of the Acidic AcOEt Extract and the Control

-------: acidic AcOEt extract, ———: control

The formation of this primary amine was expected previously as the first metabolite of isoprophenamine in side chain oxidation pathway, but could not be detected *in vivo*.[3] This has now been demonstrated in the *in vitro* system as described above. With this respect, Bond and Howe[4] also reported that the primary amine, a metabolite of pronethalol (2-isopropylamino-1-(2-naphthyl)ethanol), produced only in the *in vitro* system, and Conway, *et al.*[5] demonstrated that considerably small amount of ^{3}H-metanephrine, as compared with the amount of neutral and acidic metabolites, was excreted in urine of dog given ^{3}H-isoproterenol. From these results, it seems reasonable to consider that 1-aryl-2-aminoethanol wich is formed by N-dealkylation, is easily attacked by monoamine oxidase prior to its excretion into urine.

Fig. 2 shows the gas chromatogram of the acidic AcOEt extract and its control.

The gas chromatogram of the acidic AcOEt extract revealed two new peaks with retention times of 1.6 min and 3.2 min, which were not observable in the control. These peaks corresponded to those of authentic *o*-chlorobenzoic acid and *o*-chloromandelic acid, respectively.

In a previous study[3] on the *in vivo* metabolism of isoprophenamine in rabbits, *o*-chlorobenzoic acid and its conjugate, *o*-chlorohippuric acid, were found as the urinary acidic metabolites, but not *o*-chloromandelic acid. The present *in vitro* work demonstrated the formation of *o*-chloromandelic acid, in addition to *o*-chlorobenzoic acid. From these results, it seems reasonable to consider that 1-*o*-chlorophenyl-2-aminoethanol is presumably acted upon by monoamine oxidase followed by an aldehyde dehydrogenase to give *o*-chloromandelic acid, which by dehydrogenation and oxidative decarboxylation would give *o*-chlorobenzoic acid.

Summarizing these results, it is concluded that isoprophenamine is metabolized first to 1-*o*-chlorophenyl-2-aminoethanol and then to *o*-chloromandelic acid which, in turn, was converted to *o*-chlorobenzoic acid in rabbits.

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4) P.B. Bond and R. Howe, *Biochem. Pharmacol.*, **16**, 1261 (1967).
5) W.D. Conway, H. Minatoya, A.M Lands and J.M. Shekosky, *J. Pharm. Sci.*, **57**, 1135 (1968).