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Metabolic Fate of 2,5-Dimethoxy-4-methylamphetamine in the Guinea Pig and Rabbit

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Metabolic fate of 2,5-dimethoxy-4-methylamphetamine (DOM) was investigated in the guinea pig and rabbit.

DOM concentration in serum decreased rapidly after a subcutaneous injection and DOM was scarcely obtained at 3 to 4 hours later by radioimmunoassay and thin-layer chromatography.

As the metabolites of DOM, 2,5-dimethoxy-4-hydroxymethylphenyl-2-aminopropane and 2,5-dimethoxy-4-carboxyphenyl-2-aminopropane were confirmed in the urine of guinea pig and rabbit. 2,5-Dimethoxy-4-methylphenyl-2-propanol and 2,5-dimethoxy-4-methylphenyl-2-propanone, which had not been detected in rat urine but in the incubation mixture of DOM with rabbit liver microsome, were identified in guinea pig and rabbit urines. 2,5-Dimethoxy-4-methylbenzoic acid was firstly detected as a minor metabolic product of DOM in the urine of both species of animals.

Principal metabolic pathway of DOM in guinea pig was not regarded as the side-chain oxidation but as the oxidation of methyl group on benzene ring as well as in rabbit.

Keywords——metabolic fate; 2,5-dimethoxy-4-methylamphetamine; DOM; species difference; 2,5-dimethoxy-4-methylphenyl-2-propanol; 2,5-dimethoxy-4-methylphenyl-2-propanone; 2,5-dimethoxy-4-methylbenzoic acid

The present authors have reported previously the specific and sensitive methods of radioimmunoassay for 2,5-dimethoxy-4-methylamphetamine (DOM),[2,3] a powerful hallucinogen, in biological sample. From the standpoint of forensic chemistry, the detection of the metabolites of this compound in blood or urine may have a significant importance as well as the detection of DOM.

The present study was undertaken to clarify more fully the metabolic fate of ^{3}H-DOM administered subcutaneously to guinea pig and rabbit.

Experimental

Chemicals——Uniformly labelled ^{3}H-DOM was purchased from the Daiichi Radioisotope Co. and further purified by ion-exchange column chromatography on Dowex 1 in our laboratory. The specimen showed the specific radioactivity of 0.1 mCi/mg.

Authentic samples of metabolites of DOM, 2,5-dimethoxy-4-hydroxymethylphenyl-2-aminopropane, 2,5-dimethoxy-4-carboxyphenyl-2-aminopropane, 2,5-dimethoxy-4-methylphenyl-2-propanone and 2,5-dimethoxy-4-methylbenzoic acid were synthesized by the methods of Ho, *et al.*[4] and 2,5-dimethoxy-4-methylphenyl-2-propanol by Coutts' method.[5]

Other chemicals and solvents were of reagent grade available commercially.

Animals and Dosing——Male guinea pigs (520 g) and male rabbits (3.3 kg) were housed separately in the metabolic cages fitted with a urine-feces separator. Food and tap water were given *ad libitum*. The guinea pig and rabbit were injected subcutaneously 17.0 μCi/mg/kg and 8.5 μCi/0.5 mg/kg of DOM in saline to the back, respectively.

1) Location: *Kamiyoga, Setagaya-ku, Tokyo, 158, Japan.*
2) Y. Kido, K. Nagamatsu, and C. Ishizeki, *Yakugaku Zasshi*, **94**, 1290 (1974).
3) K. Nagamatsu, Y. Kido, and G. Urakubo, *Chem. Pharm. Bull.* (Tokyo), **25**, 3390 (1977).
4) B.T. Ho and L.W. Tansey, *J. Med. Chem.*, **14**, 156 (1971).
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After administration blood samples were obtained at several time intervals by heart puncture of guinea pig or from the ear vein of rabbit. Urine samples were collected every 24 hr up to 3 days after administration.

Determination of Radioactivity——Two 0.1 ml each of the blood were adsorbed to a small, round piece of filter paper (about 1.5 cm in diameter) and dried under an infrared lamp. The paper was incinerated in an automatic combustion apparatus (Aloka, ASC-111) and ^3H_2O produced was mixed automatically with 15 ml of scintillator solution (DPO 6 g, POPOP 0.4 g, naphthalene 50 g, dioxane 750 ml, toluene 150 ml and MeOH 100 ml) to prepare the counting sample.

To prepare the counting sample of serum, two 50 μl each of the serum separated from blood in usual manner were treated with 0.5 ml of Soluene-350 in counting vial at 40° for 24 hr and mixed with 15 ml of scintillator solution (DPO 5 g, POPOP 0.3 g and toluene 1 l).

After removal of the insoluble matter by centrifugation, urine was diluted with water to make a definite volume. An aliquot volume of it (0.1 to 0.5 ml) was mixed with 15 ml of scintillator solution (DPO 5 g, POPOP 0.3 g, naphthalene 50 g and dioxane 1 l) for the determination of radioactivity.

Assay of radioactivity was performed by a liquid scintillation counter (Aloka, LSC-652).

Determination of DOM in Urine——The intact DOM excreted in urine of guinea pig and rabbit was determined by isotope dilution method as follows. To a 10 ml aliquot of the 24 hr urine specimen was added 500 mg of authentic sample of DOM–HCl, and dissolved. After adjusting pH to 10 with 2 N NaOH, the solution was extracted 3 times with 30 ml each of ether and the combined extract was evaporated to dryness. The residue was dissolved in a little volume of EtOH–HCl, and the solvent was removed by distillation to afford a white crystalline mass. The mass was crystallized repeatedly from ether–EtOH up to a constant specific radioactivity, and from the radioactivity, the amount of DOM in urine was determined.

Determination of DOM in Serum——Using 10 μl of the sampled serum, radioimmunoassay of DOM was carried out by the method reported previously.[3]

Extraction of Urinary Metabolites——A definite volume of the sampled urine (20 to 30 ml) was passed through an Amberlite XAD-2 column (1 × 15 cm). After washing with 30 ml of water the column was eluted with 30 ml of MeOH, and the effluent was concentrated *in vacuo*. The residue was dissolved in 20 ml of water and processed by the procedure shown in Chart 1.

The polar fraction of urine thus obtained was adjusted to pH 5 with the acetate buffer solution and incubated overnight with β-glucuronidase (Boehringer Mannheim Co.) at 37°. The hydrolyzate was adjusted to pH 2 and extracted 3 times with 30 ml of ether. Thereafter, ether and water layers were fractionated again by differential pH solvent extraction according to Chart 1.

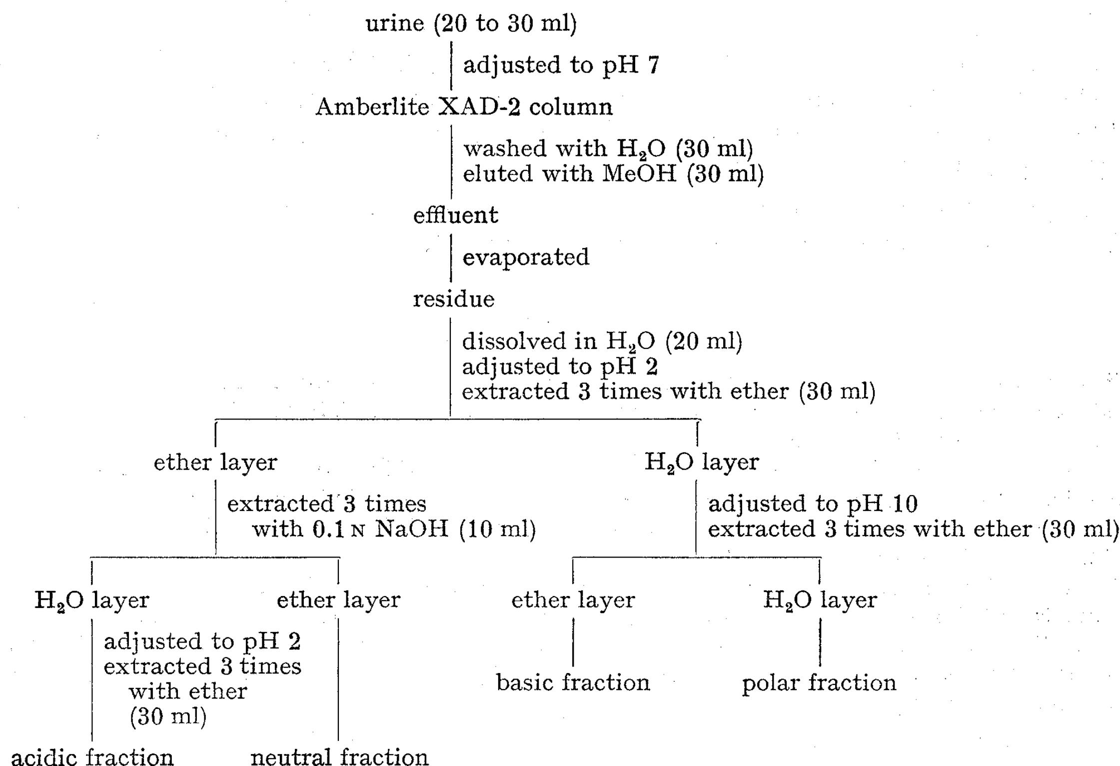

Chart 1. Fractionation of Metabolites in Urine

Acid hydrolysis was also performed by heating with 2 N HCl for 2 hr and the reaction mixture was processed in the same manner as mentioned above.

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Chromatography——For TLC, the spot film of silica gel f (Tokyo Chemical Industry Co.) was used. The developing solvents used here were (1) *n*-BuOH–AcOH–H_2O (4: 1: 1), (2) $CHCl_3$–MeOH–iso-PrOH–NH_4OH (90: 10: 95: 5), (3) *n*-hexane–AcOEt (2: 1), (4) benzene–MeOH–NH_4OH (90: 16: 8) and (5) MeOH–NH_4OH (100: 1.5).

Paper chromatography was carried out using Whatman No. 1 filter paper and developing solvent of *n*-BuOH–iso-PrOH–NH_4OH–H_2O (3: 1: 1: 1).

Results and Discussion

Radioactive Level and Concentration of DOM in Blood

Radioactive levels in blood and serum, DOM concentration in serum at several time intervals after the subcutaneous injections of ^{3}H-DOM to guinea pig and rabbit were shown in Fig. 1 and 2. The major parts of radioactivity were contained in sera of both guinea pig and rabbit, and the radioactive peaks were shown in 1.5 to 2 hr after injection.

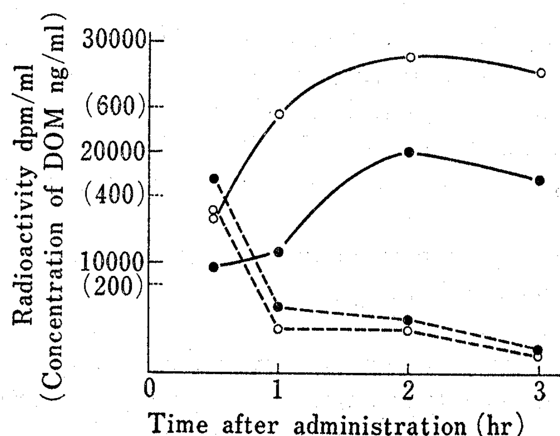

Fig. 1. Radioactive Level and DOM Concentration in Blood and Serum of Guinea Pig after Subcutaneous Injection of ^{3}H-DOM

—●—; blood-^{3}H, —○—; serum-^{3}H,
---●---; DOM (radioimmunoassay),
---○---; DOM (TLC method).

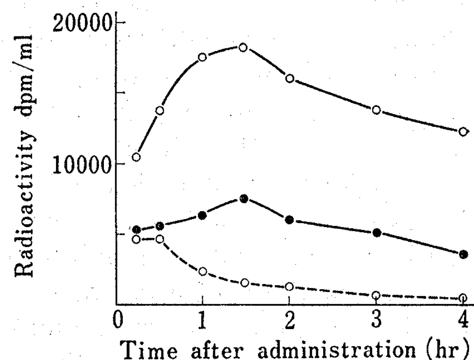

Fig. 2. Radioactive Level and DOM Concentration in Blood and Serum of Rabbit after Subcutaneous Injection of ^{3}H-DOM

—●—; blood-^{3}H, —○—; serum-^{3}H,
---○---; DOM (TLC method).

The concentration of DOM in sera of both guinea pig and rabbit, of which higher values had been noted within 30 min after injection, decreased rapidly with lapse of time, and DOM was scarcely detected at 3 to 4 hr later by radioimmunoassay[3] and TLC in the case of guinea pig and by TLC in rabbits. Sugiura, *et al.*[6] reported the rapid decrease of DOM in plasma of rat from 10 min after *i. p.* dosing by gaschlomatographic measurement. It was therefor thought that DOM was turned over from blood very rapidly in these experimental animals and the radioactivity in serum remaining at 3 to 4 hr after injection was referred to some metabolites of ^{3}H-DOM.

Urinary Excretion

Table I shows the results of the measurement of radioactivity excreted in urine in 3 days after dosing.

The radioactivities were mainly excreted in urines after the injection of ^{3}H-DOM, namely, 69% and 71% of dose to guinea pig and 64% and 71% to rabbit were recovered in urine in 24 hr and 3 days, respectively. Ho, *et al.*[7] found the excretion of 62.7% of dose in urine in

6) M. Sugiura, K. Hiraga, and T. Nakao, *Jikei Med. J.*, **20**, 23 (1973).
7) B.T. Ho, V. Estevez, L.W. Tansey, L.F. Englert, P.J. Creaven, and W.M. McIsaac, *J. Med. Chem.*, **14**, 158 (1971).

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Table I. Excretion of Radioactivity in Urine after Single *s.c.* Administration of ^{3}H-DOM to Guinea Pig and Rabbit (% of Dose)

Time (hr)	Guinea pig	Rabbit
0—24	69.31±3.17	64.32±3.53
24—48	1.82±0.40	5.35±0.71
48—72	0.95±0.24	1.92±0.24

All values represent mean ±S.E. $n=3$.

24 hr after *i. p.* injection of ^{3}H-DOM to rat, and Matin, *et al.*[8] reported the excretion of 54 to 75% of dose in urine in 24 hr after *i. p.* injection of ^{14}C-DOM to rabbit. These findings are in good agreement with the result of the present experiment.

Metabolites in Urine

Intact DOM in 24 hr urine showed 4.23% of urinary radioactivity in the case of guinea pig and 2.43% of rabbit by isotope dilution method. Ho, *et al.*[7] and Matin, *et al.*[8] also reported that the intact DOM was contained in urine, corresponding to 7.9% of rat urinary radioactivity in 24 hr after dosing and 0.5 to 2% of rabbit urinary radioactivity, respectively.

When the sampled urine was passed through the column of Amberlite XAD-2, the column was washed with water and eluted with MeOH, about 95% of radioactivity in urine was recovered in the MeOH effluent.

The major part of radioactivity in the effluent remained in the water-soluble, polar fraction as shown in Table II. Then, the fraction was hydrolyzed with β-glucuronidase and with HCl and fractionated again into the four groups.

The radioactivity of each fraction separated from the enzyme hydrolyzate was shown in Table III, indicating that the major part of radioactivity still remained in the polar fraction. The distribution ratio of radioactivity to each fraction obtained from the acid hydrolyzate was similar to that of enzyme hydrolyzate. Then, it was presumed that some polar compounds might be prominent in urine.

Table II. Distribution of Radioactivity to Basic, Neutral, Acidic and Polar Fractions of Urine (% of Urinary Radioactivity)

	Guinea pig	Rabbit
Basic fraction	7.18±0.49	5.33±0.96
Neutral fraction	2.95±0.17	3.92±0.34
Acidic fraction	1.54±0.24	2.63±0.53
Polar fraction	88.33±1.71	88.12±2.80

All values represent mean ±S.E.

Table III. Distribution of Radioactivity to Four Groups fractionated from β-Glucuronidase-treated Polar Fraction (% of Polar Fraction)

	Guinea pig	Rabbit
Basic fraction	13.54±3.60	1.99±0.28
Neutral fraction	1.77±0.36	3.49±0.34
Acidic fraction	2.37±0.49	2.19±0.42
Polar fraction	82.32±5.41	92.33±2.90

All values represent mean ±S.E.

8) S.B. Matin, P.S. Callery, J.S. Zweig, A. O'Brien, R. Rapoport, and N. Castagnoli, *J. Med. Chem.*, **17**, 877 (1974).

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The radioscanning chromatogram of the basic fraction of guinea pig urine showed two peaks with the *Rf* values of 0.51 and 0.65 by the solvent (1) and of 0.39 and 0.54 by the solvent (2) (Fig. 3) and rabbit urine also gave the same results. One with the lower *Rf* values was identified as 2,5-dimethoxy-4-hydroxymethylphenyl-2-aminopropane by comparison of the *Rf* value with that of the authentic sample. The second with the higher *Rf* value was identified as unmetabolized DOM in the same manner.

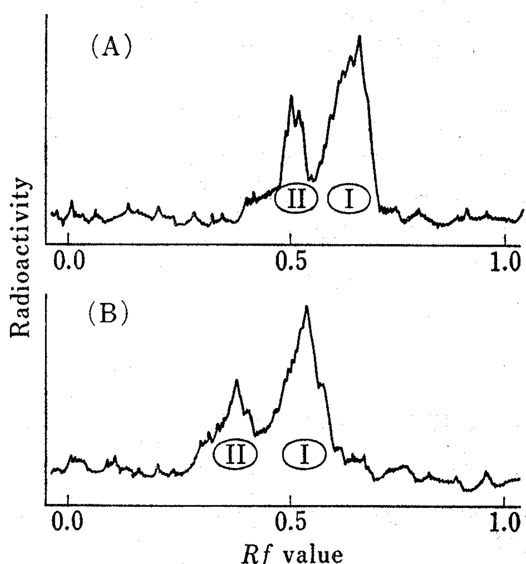

Fig. 3. Thin–Layer Chromatogram of Basic Fraction from Guinea Pig Urine

Developing solvent; (A) *n*-BuOH: AcOH: H_2O=4:1:1, (B) $CHCl_3$: MeOH: iso-PrOH: NH_4OH=90: 10: 95: 5. ⬭ spot I, II; authentic samples of DOM and 2,5-dimethoxy-4-hydroxymethylphenyl-2-aminopropane.

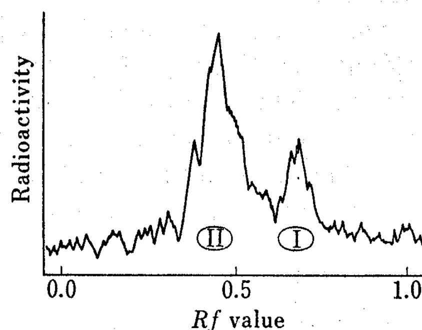

Fig. 4. Thin–Layer Chromatogram of Neutral Fraction from Guinea Pig Urine

Developing solvent; *n*-Hexane: AcOEt=2: 1. ⬭ spot I, II; authentic samples of 2,5-dimethoxy-4-methylphenyl-2-propanone and 2,5-dimethoxy-4-methylphenyl-2-propanol.

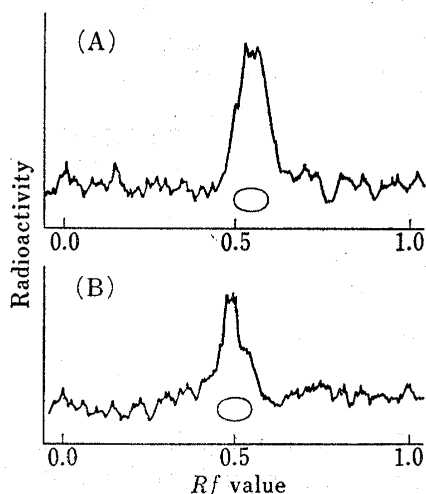

Fig. 5. Thin–Layer Chromatogram of Acidic Fraction from Guinea Pig Urine

Developing solvent;
(A) benzene: MeOH: NH_4OH=90: 16: 8,
(B) MeOH: NH_4OH=100: 1.5.
⬭ spot; authentic sample of 2,5-dimethoxy-4-methylbenzoic acid.

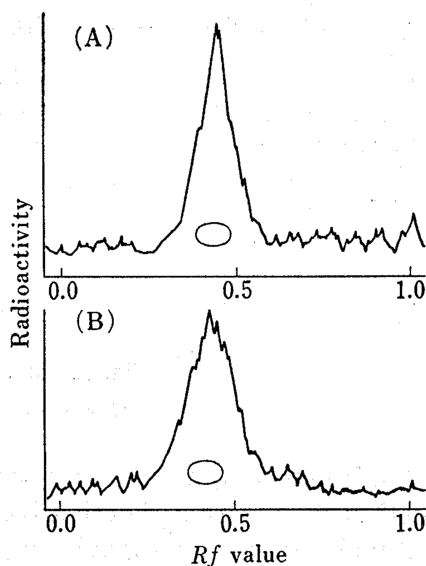

Fig. 6. Thin–Layer and Paper Chromatogram of Hydrolyzed Polar Fraction of Guinea Pig Urine

(A); TLC, developing solvent; *n*-BuOH: AcOH: H_2O=4: 1: 1.
(B); PPC, developing solvent; *n*-BuOH: iso-PrOH: NH_4OH: H_2O=3: 1: 1: 1.
⬭ spot; authentic sample of 2,5-dimethoxy-4-carboxyphenyl-2-aminopropane.

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In the neutral fractions obtained from urines of both species of animals, two radioactive spots having *Rf* values of 0.45 and 0.68 were obtained on the thin–layer chromatogram by the use of the solvent (3) (Fig. 4). These two metabolites were identified as 2,5-dimethoxy-4-methylphenyl-2-propanol for the former and 2,5-dimethoxy-4-methylphenyl-2-propanone for the latter, comparing their *Rf* values with those of authentic samples.

The metabolite in the acidic fraction of guinea pig urine was determined by TLC. One radioactive spot was detected showing the *Rf* values of 0.55 with the solbent (4) and 0.50 with the solvent (5) (Fig. 5). The *Rf* values were identical with those of the authentic sample of 2,5-dimethoxy-4-methylbenzoic acid. A small amount of the same matabolite was detected in the acidic fracion of rabbit urine.

As shown in Fig. 6, one metabolite in the hydrolyzed polar fraction of urine was detected showing *Rf* values 0.45 with solvent (1) on the thin layer chromatogram and 0.43 on the paper chromatogram. This metabolite was identified as 2,5-dimethoxy-4-carboxyphenyl-2-amino-propane by the comparison of *Rf* values with those of authentic sample.

DOM metabolites and intact DOM in urine of guinea pig and rabbit determined by TLC and possible metabolic pathway were summarized in Fig. 7.

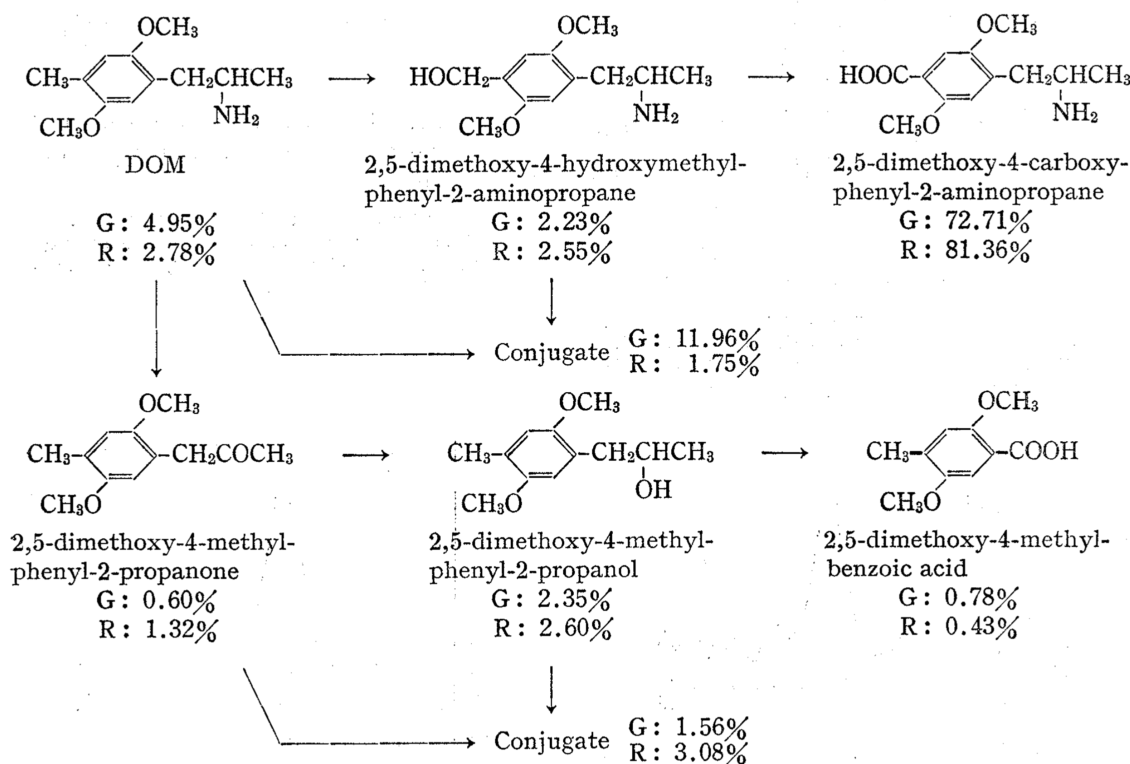

Fig. 7. Metabolites of DOM in Urine

% of 24 hour urine, G: Guinea pig, R: Rabbit.

Ho, *et al.*[7] detected, as the urinary metabolites of DOM in rat, 26.9% and 27.4% of 4-hydroxymethyl and 4-carboxyl derivatives of DOM of 24 hr urinary radioactivity, respectively. Matin, *et al.*[8] also found 4-carboxyl compound corresponding to 51% in 24 hr urine of rabbit. In the present study, it was confirmed that the principal metabolic pathway of DOM in guinea pig was also oxidation of the methyl group on the benzene skeleton as shown in Fig. 7.

Since phenyl-2-propanol and phenyl-2-propanone, deaminated metabolites of amphet-amine, were detected in rabbit urine,[9] and deamination of DOM by rabbit liver microsome, producing 2,5-dimethoxy-4-methylphenyl-2-propanol and 2,5-dimethoxy-4-methylphenyl-2-

9) L.G. Dring, R.L. Smith, and R.T. Williams, *Biochem. J.*, **116**, 425 (1970).

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propanone, has been reported,[10] the occurrence of deaminated metabolites in urine was naturally expected. Matin, *et al.*[8] failed to identify the ketone in rabbit urine, though about 10% of urinary radioactivity was found in the neutral fraction. In the present experiment, however, 2,5-dimethoxy-4-methylphenyl-2-propanol and 2,5-dimethoxy-4-methylphenyl-2-propanone were firstly detected in the urine of both guinea pig and rabbit.

2,5-Dimethoxy-4-methylbenzoic acid, which had not been confirmed in rat[7] and rabbit[8] urines, was found in the urines of both guinea pig and rabbit in this experiment.

Although the species difference has been reported on the metabolism of amphetamine,[9] there was no significant difference between guinea pig and rabbit on the metabolites of DOM.

During the course of this work, O-demethylation of DOM with rabbit liver homogenate, affording 2-O-demethyl, 5-O-demethyl and 2,5-bis(O-demethyl) metabolites, has been reported.[11,12] If O-demethylation of DOM has taken place in the present experiment, the yielded amphoteric compounds should be finally contained in the water-soluble, polar fraction in Chart I. However, since any radioactive spots other than 2,5-dimethoxy-4-carboxyphenyl-2-aminopropane could not be found on the thin-layer and paper chromatograms of hydrolyzed polar fraction (Fig. 6), O-demethylation might be minor biotransformation, even if it could be involved.

10) J. Gal, L.D. Gruenke, and N. Castagnoli, *J. Med. Chem.*, **18**, 683 (1975).
11) R.J. Weinkam, J. Gal, P. Callery, and N. Castagnoli, *Anal. Chem.*, **48**, 203 (1976).
12) J.S. Zweig and N. Castagnoli, *J. Med. Chem.*, **20**, 414 (1977).

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