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Studies on Absorption, Biotransformation and Excretion of Drug. II. Metabolism of 2-Indanamine

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The metabolites of IA (2-indanamine hydrochloride) have been investigated in rats and rabbits. When IA (100 mg/kg) was administered to rats, following metabolites were found together with unchanged IA: *cis*-, *trans*-1-hydroxy-2-indanamine, *cis*-, *trans*-1,2-indandiol, 5-hydroxy-2-indanamine. In the urine of rabbits dosed 100 mg/kg of IA, the presence of 2-indanol precursors of 2-indanone and was recognized together with the above metabolites. From the investigation about the sequence of metabolites of IA, it became evident that the oxidative deamination of IA was only occurred in rabbits, but not in rats.

2-Indanamine (IA) and its derivatives were first synthesized by Huebner and co-workers in 1961,[2] and their pharmacological properties were investigated by Witkin, *et al.*[3] It was found that IA had a strong analgetic activity comparable to morphine in case of oral administration and did not show addiction.[3,4] Afterwards, the facts that IA caused some stimulation similar to amphetamine (AP) at high doses and moderate hypertension were reported.

In addition to these interesting pharmacological properties, the structure of IA is quite similar to that of AP which has been studied from the various view points due to its remarkable properties. Thus, IA is essentially β-phenethylamine derivative with the end carbon of the three carbon aliphatic side chain attached back on to the aromatic ring.

In spite of their structural similarity, all the pharmacological properties do not correspond to each other. Therefore, we thought that it would be of interest to investigate whether the same thing can be observed in metabolism of IA and AP, and present paper will deal with the metabolic fate of IA in rats and rabbits comparing with that of AP.

Material and Method

2-Indanamine hydrochloride was synthesized according to the method of Huebner, *et al.*[2]

Synthesis of the Proposed Metabolites——1) 2-Indanone: This compound was synthesized from indene according to the method described by Rosen,[5] mp 57—58°. 2-Indanone 2,4-dinitrophenylhydrazone was synthesized according to the method described by Brooks, *et al.*[6] mp 205—206°.

2) 2-Indanol: To an ethereal solution (20 ml) of 2-indanone (300 mg) was added dropwise an alcoholic solution (20 ml) of sodium borohydride with stirring under ice cooling. After the addition was completed, the solution was allowed to stand for 1 hr at room temperature to complete reaction. The solution was treated by the usual way to give white needles, recrystallized from ethanol, mp 69°.

3) *cis*-1,2-Indandiol (*cis*-diol): This compound was synthesized according to the method described by Rosen, *et al.*[5] mp 99—101°.

1) Location: *2-12-3 Sakurashinmachi, Setagaya-ku, Tokyo.*

2) C.F. Huebner, D. Donoghue, P.W. Strachan, P. Beak and E. Wenkert, *J. Org. Chem.*, **27**, 4465 (1962).
3) L.B. Witkin, C.F. Huebner, F. Galdi, E. O'Keefe, P. Spitaletta and A.J. Plummer, *J. Pharmacol. Exptl. Therap.*, **133**, 400 (1961).
4) G. deStevens (ed.), "Medical Chemistry," A Series of Monographs. Analgetics," Vol. 5, Academic press, New York and London, 1965.
5) W.E. Rosen, L. Dorfman and M.P. Linfield, *J. Am. Chem.*, **29**, 1723 (1964).
6) C.J.W. Brooks and L. Young, *Biochem. J.*, **63**, 264 (1956).

4) *trans*-1,2-Indandiol (*trans*-diol): This compound was synthesized according to the method described by Porter and Suter,[7] mp 158°.

5) *cis*-1-Hydroxy-2-indanamine hydrochloride (*cis*-OH–IA): This compound was synthesized according to the method described by Thrift,[8] mp 206°.

6) *trans*-1-Hydroxy-2-indanamine hydrochloride (*trans*-OH–IA): This compound was synthesized according to the method described by Thrift,[8] mp 216—217°.

7) Homophthalic Acid: This compound was synthesized according to the method described in Organic Syntheses,[9] mp 180—181°.

8) 2-Indanylacetamide (IA-Ac): This compound was synthesized according to the method described by Huebner, *et al.*[2] mp 125—126°.

9) 5-Hydroxy-2-indanamine (5-OH-IA): This compound was synthesized according to the following reaction steps; *p*-anisaldehyde + malonic acid $\longrightarrow$ *p*-methoxycinnamic acid,[10] mp 172° $\xrightarrow{PtO_2}$ *β*-*p*-methoxyphenylpropionic acid,[10] mp 100—104° $\xrightarrow{PCl_5\text{–}AlCl_3}$ 6-methoxy-1-indanone,[11] mp 108° $\xrightarrow[2)\ \text{Reduction(Pd-C)}]{1)\ \text{iso-amylnitrite/HCl}}$ 5-methoxy-2-indanamine hydrochloride,[12] mp 232° $\xrightarrow{\text{pyridine hydrochloride}}$ 5-hydroxy-2-indanamine. 5-methoxy-2-indanamine hydrochloride (450 mg) was added to completely dried pyridine hydrochloride at bath temperature 220° and gently stirred for 15 min. After cooling, an aqueous solution of sodium bicarbonate was added to neutralize hydrochloric acid, followed by evaporation to dryness under reduced pressure. The residue was washed with ethanol, which was evaporated to give white crystals, mp 220°. UV λ_{max}^{EtOH} mμ: 268 sh., 282. IR ν_{max}^{KBr} cm^{-1}: 3300 (-OH,-NH_2), 1600 (phenyl group), 1459 (phenyl group). NMR (D_2O) ppm: 7.26 (1H, doublet, J=8 Hz, aromatic proton), 6.94 (1H, singlet, aromatic proton), 6.86 (1H, doublet, J=8 Hz, aromatic proton), 4.14 (1H, multiplet, >CH–NH_2), 3.06 (4H, multiplet, benzylic proton). Mass Spectrum: Calcd. for $C_9H_{11}ON$ (molecular peak): 149.0826. Found: 149.0841.

10) 2-Amino-1-indanone Hydrochloride: This compound was synthesized according to the method described by Thrift,[8] mp 200° (decomp.).

Animal——Male rats (SD strain) weighing 300 g and male albino rabbits weighing 3 kg were used.

Administration of Compounds——Aqueous solutions of IA hydrochloride, *cis*-OH-IA, *trans*-OH-IA and 2-amino-1-indanone hydrochloride and 30% alcoholic solutions of 2-indanone and 2-indanol were administered orally at a dose level of 100 mg/kg to the animals. After administration, animals were housed in metabolic cages and 48 hr urines were pooled for extraction of metabolites.

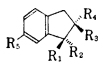
Extraction of the Metabolites——The pooled urine sample was extracted with an equal volume of chloroform two times at pH 7.2 (fraction A). One portion of the extracted urine was hydrolyzed with *β*-glucuronidase (Bovine liver, 3000 units) at pH 4.5 for 24 hr. After neutralization, the urine was extracted with chloroform (fraction B). Another portion was adjusted to pH 1.0 with hydrochloric acid and hydrolyzed by heating on boiling water bath for 30 min. The urine was neutralized with sodium hydroxide, and followed by extraction with chloroform (fraction C). In the case of IA and 2-indanone, in addition to above treatment, the extracted urine was adjusted to pH 10 with 2N sodium hydroxide, heated at 45—50° for 15 min, and followed by extraction with chloroform (fraction D). From above fractions, the solvent was evaporated to dryness under reduced pressure after drying over anhyd. Na_2SO_4, and the resulting residues were used for detection of the metabolites. The urine collected before administration was used as a negative control.

Thin-Layer Chromatography (TLC)——TLC was carried out on thin-layer plates, 0.25 mm thick (Silica gel B-5F, Wako) which were activated at 120° for 30 min. The solvent systems used were 1) $CHCl_3$, 2) benzene–hexane (80:20), 3) AcOEt–$CHCl_3$ (80:20), 4) benzene–AcOEt–MeOH–conc. NH_3 (80:20:20:2), 5) AcOEt–MeOH–conc. NH_3 (80:20:3), 6) $CHCl_3$–acetone–MeOH–conc. NH_3 (80:10:10:2), 7) AcOEt–acetone–MeOH–conc. NH_3 (80:10:10:2), 8) $CHCl_3$–MeOH–AcOH (90:10:2) and 9) AcOEt–cyclohexane–AcOH (80:10:2).

The chromatograms were visualized under ultraviolet (UV) light or by following methods; The detection of 2-indanol was carried out by dropping conc. H_2SO_4 on thin-layer plate to give immediately yellow colour. The detection limit was about 1 μg. 2-Indanone was visuallized by the same method using conc. H_2SO_4 after it was reduced to 2-indanol by spraying ethanolic solution of sodium borohydride (5% w/v). The detection of primary amines was carried out by spraying ninhydrin reagent. The detection of phenolic compounds was carried out by spraying $FeCl_3$ reagent. The detection of diols was carried out according to the method described by Brooks and Young.[6]

7) H.D. Porter and C.M. Suter, *J. Am. Chem. Soc.*, **57**, 2022 (1935).
8) R.I. Thrift, *J. Chem. Soc.* (*C*), **1967**, 288.
9) O. Grumidt, R. Egan and A. Buck, "Organic Syntheses," Vol. 29, ed. by C.S. Hamilton, John Wiley and Sons, Inc., New York, N.Y., 1949, p. 76.
10) W.S. Johnson and Shelberg, *J. Am. Chem. Soc.*, **67**, 1853 (1945).
11) W.S. Johnson and H.J. Glenn, *J. Am. Chem. Soc.*, **71**, 1092 (1949).
12) H. Richter and M. Schenck, *C.A.*, **53**, P2190e (1959).

TABLE I. The $Rf \times 100$ Values and Colouration of IA and Its Proposed Metabolites

	IA	5-OH-IA	*cis* OH-IA	*trans* OH-IA	*cis* Diol	*trans* Diol	2-Indanol	2-Indanone	Homophthalic acid	IA-Ac
R_1	H	H	OH	H	OH	H	H	H	—	H
R_2	H	H	H	OH	H	OH	H	H	—	H
R_3	NH_2	NH_2	NH_2	NH_2	OH	OH	OH	O	—	NH-Ac
R_4	H	H	H	H	H	H	H	O	—	H
R_5	H	OH	H	H	H	H	H	H	—	H
Solv. System 1	—	—	—	—	—	—	24	84	—	—
Solv. System 2	—	—	—	—	—	—	—	28	—	—
Solv. System 3	—	—	—	—	—	—	70	—	—	—
Solv. System 4	54	43	96	35	46	31	—	—	—	64
Solv. System 5	58	52	—	—	55	37	—	—	—	85
Solv. System 6	86	46	92	66	—	—	—	—	—	93
Solv. System 7	—	—	98	40	—	—	—	—	—	—
Solv. System 8	—	—	—	—	—	—	—	—	24	—
Solv. System 9	—	—	—	—	—	—	—	—	33	—
conc. H_2SO_4	—	—	—	—	—	—	yellow	blue	—	—
Ninhydrin	purple	purple	yellow	yellow	—	—	—	—	—	—
$NaIO_4$-Shiff base	—	—	—	—	white[a]	white[a]	—	—	—	—
$FeCl_3$	—	blue	—	—	—	—	—	—	—	—
B. C. G.[b]	—	—	—	—	—	—	—	—	yellow[c]	—

a) yellowish white on purple ground *b*) B.C.G.=bromocresol green *c*) yellow on blue ground
See text for solvent system.

Result

(1) Identification of Urinary Metabolites of IA

TLC of the fraction A revealed the presence of five and six metabolites in rats and rabbits, respectively. All the metabolites found in rats were also recognized in rabbits and showed *Rf* values at 0.96 (metabolite 1), 0.54 (metabolite 2), 0.46 (metabolite 3), 0.35 (metabolite 4) and 0.31 (metabolite 5) with solvent system 4. Metabolites 1, 2 and 4 were positive to ninhydrin reagent to give yellow, purple and yellow colour, respectively. Metabolite 3 and 5 were positive to diol-detecting reagent to give yellowish white colour on purple ground. Another metabolite observed only in rabbits urine had *Rf* value at 0.24 with solvent system 1 and gave yellow colour with conc. H_2SO_4 (metabolite 6).

In fraction B obtained from rabbits, phenolic (metabolite 7) and neutral metabolites (metabolite 8) were recognized together with above metabolites 1, 3, 4, 5 and 6. Metabolite 7 had *Rf* value at 0.43 with solvent system 4 and positive to ninhydrin reagent. Metabolite 8 had *Rf* value at 0.84 with solvent system 1 and gave blue colour with conc. H_2SO_4. In the case of rats, the presence of all the above metabolites except 6 and 8 was observed.

In fraction C, the presence of metabolites 6, 7 and 8 was observed in rabbits. On the other hand, in the case of rats, metabolite 6 was not recognized.

Investigation of fraction D with TLC using various solvent systems showed the presence of metabolite 8 in the case of rabbits. This could not be detected in rats urine.

Metabolite 1——The crystallization of this metabolite was unsuccessful because of its limited amount, but its structure was determined to be *cis*-OH-IA by comparison of its UV spectra (λ_{max}^{EtOH} mμ: 258, 266, 273), mass spectra (molecular peak composition: $C_9H_{11}ON$) and chromatographic behaviours (Table I) with those of authentic sample.

Metabolite 2——Metabolite 2 was isolated by TLC using solvent system 4 and 6. It was crystallized as a hydrochloride to give white needles, which had mp 241°, undepressed on

admixture with IA hydrochloride. Its spectral (infrared (IR), UV and nuclear magnetic resonance (NMR)) and chemical properties (Table I) were all coincident with those of authentic IA hydrochloride.

Metabolite 3——This metabolite was isolated and purified by TLC using solvent system 4 and 5, and crystallized from cyclohexane to afford white needles, mp 98°. The mass spectral data showed a molecular composition ($C_9H_{10}O_2$) which differed from that of IA by $-NH+O_2$. The UV absorption spectra (λ_{max}^{EtOH} mμ: 258, 266, 273) was almost identical with that of IA. From these result, it was assumed that the oxidative deamination and hydroxylation occurred in IA to give metabolite 3. The determination of configuration of hydroxy groups was carried out by comparison of its spectral (UV, IR and mass) data and chromatographic properties (Table I) with those of authentic *cis*-diol. The mp was not depressed on admixture with authentic *cis*-diol.

Metabolite 4——The isolation of metabolite 4 was carried out by TLC using solvent system 4 and 6 in turn. This was crystallized as a hydrochloride to give white leaflets, mp 159—160°. The mass spectra showed a molecular peak at *m/e* $C_9H_{11}ON$ which differed from that of IA by the addition of one oxygen atom. The UV absorption spectra (λ_{max}^{EtOH} mμ: 258, 266, 273) was almost identical with that of IA. From these results, it seemed likely that benzylic position of IA was attached by hydroxy group to yield this metabolite. The *trans*-configuration of hydroxy group to amino group was determined by comparison of these spectral (UV, IR and mass) data and chromatographic properties (Table I) of metabolite 5 with those of *trans*-OH–IA. The depression of mp was not observed on admixture with authentic *trans*-OH–IA hydrochloride.

Metabolite 5——Metabolite 5 was isolated by the same method described in metabolite 3. Recrystallization from water afforded white leaflets, mp 158—159°. The mass spectra showed a molecular peak at *m/e* $C_9H_{10}O_2$ which differed from that of IA by $-NH+O_2$. The UV absorption spectra (λ_{max}^{EtOH} mμ: 258, 266, 273) was almost identical with that of IA. From these results, it was evident that the occurrence of oxidative deamination and hydroxylation of five membered-ring of IA yielded metabolite 5. The location and *trans*-configuration of hydroxy groups were determined by comparison of the spectral (UV, IR and mass) data and chemical properties with those of authentic *trans*-diol. The depression of mp was not observed on admixture with authentic sample.

Metabolite 6——Metabolite 6 was isolated as crystals by sublimation from fraction A, B and C. This metabolite was present only in the extracts of the rabbit urine. It was recrystallized from ethanol to give white needles, mp 69°. The spectral (UV, IR and NMR) data and chemical properties (Table I) were all identical with those of authentic 2-indanol. The depression of mp was not observed on admixture with authentic sample.

Metabolite 7——This metabolite was isolated and purified by TLC using solvent system 4, 5 and 6, and crystallized as a hydrochloride, mp 220°. The UV absorption spectra (λ_{max}^{EtOH} mμ: 268 sh., 282. $\lambda_{max}^{2N\ NaOH}$mμ: 302) indicated the presence of phenolic hydroxy group. The mass spectral analysis showed a molecular peak at *m/e* $C_9H_{11}ON$ which differed from that of IA by the addition of one oxygen atom. The NMR and IR spectra were identical with those of 5-OH–IA. The depression of mp was not observed on admixture with authentic 5-OH–IA.

Metabolite 8——This metabolite migrated as same as 2-indanone on TLC with various solvent systems. In order to isolate a sufficient quantity of this metabolite for characterization, the large amount of the extracted urine was steam-distilled under acidic condition, and followed by extraction with ether. After evaporation of the ether under reduced pressure, this metabolite was crystallized as 2,4-dinitrophenylhydrazone.[6] The spectral (UV and IR) data and chemical properties were all identical with those of authentic 2-indanone 2,4-dinitrophenylhydrazone. The depression of mp (205—206°) was not observed on admixture with authentic sample. From fraction B and D, 2-indanone was extracted with ether. The identi-

fication was carried out using TLC with various solvent systems (Table I).

The presence of homophthalic acid and IA-Ac could not be detected in the both species.

(2) Identification of Urinary Metabolites of 2-Indanol

Fraction A and B contained three metabolites which were identified 2-indanol, *cis*- and *trans*-diol by the same procedure described above. From fraction C, 2-indanone and 2-indanol was obtained. These metabolites were found in both species.

(3) Identification of Urinary Metabolites of 2-Indanone

After administration of 2-indanone, the presence of 2-indanone (fraction A, B and C), *cis*- and *trans*-diol (fraction A and B) was observed in the both species as reported by Lewis,[13] and the identification of these metabolites was carried out by the same procedure described above. In the fraction D obtained from rabbits, the presence of 2-indanone was detected on TLC with various solvent systems, but the isolation was unsuccessuful due to its limited amount. In addition to above metabolites, a new metabolite was recognized in fraction A, B and C, and it was identified 2-indanol by the same procedure described above.

(4) Identification of Urinary Metabolites of *cis*- and *trans*-OH-IA

When *cis*-OH–IA was administered, *cis*-, *trans*-diol and *trans*-OH–IA were found in fraction A and B together with unchanged *cis*-OH–IA. In the case of *trans*-OH–IA, the presence of above metabolites was observed. These metabolites were excreted in the both species. In fraction C, 2-indanone was present. The isolation of *cis*-OH–IA was carried out by TLC using solvent system 6 and 7. This was crystallized as a hydrochloride to give white leaflets, mp 205° (decomp.). The mass spectra (molecular peak at *m/e* $C_9H_{11}ON$), UV absorption spectra (λ_{max}^{EtOH} mμ: 258, 266, 273) and chemical properties were identical with authentic *cis*-OH–IA. The mp was not depressed on admixture with authentic sample.

(5) Identification of Urinary Metabolites of 2-Amino-1-indanone

The administration of 2-amino-1-indanone yielded four metabolites which were identified to be *cis*-, *trans*-diol, *cis*- and *trans*-OH–IA by the same procedure described above.

Discussion

2-Indanone found in fraction C seems an artefact arising from conjugates of *cis*- and *trans*-diol, since the acid treatment of these conjugates readily gives free forms which were, in turn, converted to the ketone.[6] However, another possibility to be considered is that a part of 2-indanone in fraction C may arise from the other acid-labile precursor or precursors of the ketone such as enol-gluculonide or enol-sulfate. Because such examples were observed in the enol-sulfate of 1-phenyl-2-propanone and enol-glucuronide of 4–hydroxycoumarin.[14] In order to study this point, moderate hy-drolysis with β-glucuronidase or sodium hydroxide was carried out (fraction B and D, re-spectively). Under these conditions, it was confirmed that the conversion of the diols and the aminoalcohols into 2-indanone could not occur. From these experiments it becomes evident that IA and 2-indanone were partly excreted in ra bbits as precursors of the ketone whereas in the case of rats the ketone was only observed in the fraction B after administration of 2-indanone. These results give no definite evidence for the structure of the precursors of 2-indanone but suggest that a part of the ketone was excreted in rabbits as indene-2-yl glucuro-nide and sulphate. In rats, however, excretion of the sulphate could be ruled out since the ketone could be detected only in the fraction B. Williams, *et al.* reported that 22% of dosed AP was excreted as 1-phenylprop-1-ene-2-yl hydrogen sulphate

13) D.A. Lewis, *Nature*, **210**, 1046 (1966).
14) *a*) L.G. Dring, R.L. Smith and R.T. Williams, *Biochem. J.*, **116**, 425 (1970); *b*) J.A.R.M ead, J.N. Smith and R.T. Williams, *Biochem. J.*, **68**, 61 (1958).

in rabbits.[14a] But in the case of IA and 2-indanone, the excretion of these metabolites was minor, if any. This fact could be interpreted by the different amount of enol forms in 2-indanone and 1-phenylpropane-2-one.[15]

Since IA is transformed in the both species into a number of metabolites, the problem is to decide their sequence. It is necessary to study the metabolism of each possible intermediate and all of the substances isolated in order to determine how they are related to each other. Lewis reported that the interconversion of *cis*- and *trans*-diol occurred *in vivo* and 2-hydroxy-1-indanone was the intermediate necessary for this transformation.[13,16] The interconversion of *cis*- and *trans*-OH–IA and the transformation of 2-amino-1-indanone into these amino-alcohols were also observed in the present investigation. The aminoketone could not be detected due to its instability, but it seems reasonable to assume that this compound was the intermediate in the aminoalcohol interconversion. As described by Lewis,[13] 2-indanone was biotransformed to the diols in the both species, but it was observed in the present study that 2-indanol (one of the metabolites of 2-indanone) was also converted into the diols. From these findings, it is uncertain whether 2-indanone or 2-indanol is their precursor. However, it appears that 2-indanone was first reduced *in vivo* to the corresponding alcohol which was followed by hydroxylation to the diols. Because such biotransformation was shown in the metabolism of methylcyclohexanone, cyclohexanone, α- and β-tetralone, and the model system developed by Elliott, *et al.* to account for the reductions of the alicyclic ketones they have studied might well serve to account for the reduction observed in the present work.[17]

In the urine of rabbits dosed IA, the excretion of 2-indanol and the precursors of 2-indanone was observed, whereas, in the case of rats dosed IA, these metabolites could not be detected. In addition, the administration of 2-indanone gave 2-indanol and precursors of the ketone in the both species. This fact seems to indicate that the oxidative deamination of IA does not occur in rats. Although the diols were obtained from the urine of rats as well as from that of rabbits after administration of IA, this result is not compatible with above thought when it is taken into consideration that the diols are the metabolites of the amino-alcohols as well as 2-indanol. Based on these considerations, possible metabolic pathway of IA might be presented in Chart 1.

The known metabolites of AP in rabbits are *p*-hydroxyamphetamine (6% of dose), 1-

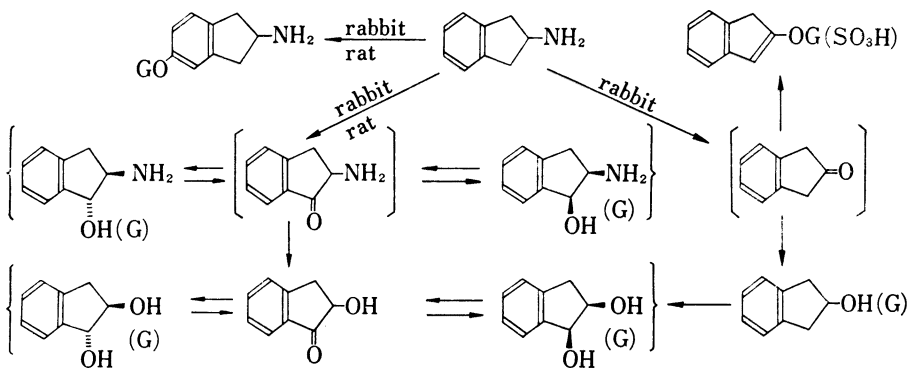


Chart 1. Proposed Metabolic Pathway of IA in Rabbits and Rats

[ ]: Shows the compounds which could not be isolated.

15) A. Gero, *J. Org. Chem.*, **19**, 1960 (1954).
16) D.A. Lewis, *Biochem. J.*, **99**, 694 (1966).
17) *a*) C.C. Tao and T.H. Elliott, *Biochem. J.*, **84**, 389 (1962); *b*) T.H. Elliott and J. Hanam, *ibid.*, **108**, 551 (1968); *c*) T.H. Elliott, R.C.C. Tao and R.T. Williams, *ibid.*, **95**, 70(1965); *d*) K.L. Cheo, T.H. Elliott and R.C.C. Tao, *ibid.*, **104**, 198 (1967); *e*) T.H. Elliott, D.V. Parke and R.T. Williams, *ibid.*, **72**, 193 (1959).

phenyl-propane-2-one (22%), benzoic acid (25%) and 1-phenyl-propane-2-ol (7%).[14] On the other hand, AP is metabolized in rats to *p*-hydroxyamphetamine (60% of dose), N-acetyl-amphetamine (2%), norephedrine (0.3%) and *p*-hydroxynorephedrine (0.3%).[14] In the metabolism of IA, the oxidative deamination was only occurred in rabbits but not in rats as was observed in AP. Although large species difference was recognized in the excretion of *p*-hydroxyamphetamine after administration of AP, the hydroxylation on aromatic ring of IA was observed in rabbits as well as in rats in almost equal amount (*ca.* 10%). The urinary excretion of *cis*- and *trans*-OH–IA, which are corresponding to norephedrine, was observed in the both species, whereas the biotransformation of AP into norephedrine only occurred in rats. These results show that there are wide species variations in the metabolism of IA but these variations do not correlated to those of AP. Therefore, it can be said that the side chain of AP does not form five-membered ring as IA in the interaction of drug metabolizing enzyme.

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