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Metabolism of Benzydamine Hydrochloride: Species Differences and the Identification of Unconjugated Metabolites in Rabbit Urine

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The urinary metabolites of benzydamine hydrochloride (BZY·HCl) in rabbits were isolated by the combination of extraction with $CHCl_3$ and column or thin-layer chromatography on Amberlite XAD-2, silica gel, and aluminum oxide, and identified by thin-layer co-chromatography with authentic samples and by mass and nuclear magnetic resonance spectroscopy.

The major two metabolites are benzydamine N-oxide (BZY-NO) and 1-(*p*-hydroxybenzyl)-3-(3-dimethylaminopropoxy)-1H-indazole (HO-BZY). The minor others are demethylated BZY (nor-BZY), N-debenzylated BZY (deB-BZY), 1-benzyl-1H-indazolone (BID), and indazolone (ID). The excretion of unchanged BZY amounts about 1%. In addition, three glucuronides were found but their chemical structures are not clarified, yet.

The total amount of fluorescent metabolites excreted in 24 hr urine of rat, mouse, guinea pig, rabbit and dog received BZY·HCl was equivalent to around 30% of dose. However, 62.4 or 74.7% of fluorescent metabolites of rat or dog was extracted directly with chloroform but about 20% of mouse, guinea pig or rabbit were. After incubating chloroform extracted urine with β-glucuronidase, the fluorescent metabolites in urine was extracted with chloroform more than 70% in mouse, guinea pig or rat but less than 40% in rabbit and 10% in dog. The cat excretes fluorescent metabolites equivalent to 16.6% of dose and no glucuronide.

Benzydamine hydrochloride, 1-benzyl-3-(3-dimethylaminopropoxy)-1H-indazole hydrochloride (BZY·HCl), is a non-steroid analgestic anti-inflammatory agent[2] and has been used widely in clinical work. It has been supposed that this drug is concentrated in the inflamed tissue[3,4] but the metabolic pattern should be clarified in elucidating the mechanism of action.

BZY·HCl fluorescences strongly and it can be determined as low concentration as 0.1 μg/ml. Catanese, *et al.*[5] reported using spectrofluorometry that about 50% of the dose in men and about 30% in rats were excreted in the urine, but the conclusive information concerning the metabolites was not available.

This paper deals with the species differences in the properties of fluorescence in urine after receiving BZY·HCl and the identification of seven unconjugated metabolites. A part of this work has been preliminarily published.[6]

Material and Method

Chemicals——BZY·HCl, mp 157—159°, was the gift from Daiichi Seiyaku Co. and Yoshitomi Seiyaku Co. 1-Benzyl-3-(3-methylaminopropoxy)-1H-indazole hydrochloride (nor-BZY·HCl), 1-benzyl-3-indazolone (BID), and 3-indazolone (ID) were kindly donated by Dr. B. Silvestrini, A.C.R. Angelini, Rome, Italy.

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2) B. Silvestrini, A. Garau, C. Pozzatti, and V. Cioli, *Arzneim.-Forsch.* (*Drug Res.*), **16**, 59 (1966).
3) E. Giacalone and L. Valzelli, *Med. Pharmacol. Exp.*, **15**, 102 (1966).
4) R. Lisciani, P.S. Barcellona, and B. Silvestrini, *Eur. J. Pharmacol.*, **3**, 157 (1968).
5) B. Catanese, A. Grasso, and B. Silvestrini, *Arzneim.-Forsch.* (*Drug Res.*), **16**, 1354 (1966).
6) S. Kataoka, K. Taira, and E. Takabatake, *Chem. Pharm. Bull.* (Tokyo), **19**, 1511 (1971).

Latter two compounds were also synthesized in this laboratory according to the methods of Palazzo, *et al.*[7] and Stephenson,[8] respectively. 3-(3-Dimethylaminopropoxy)-1H-indazole (deB-BZY) was prepared from ID and dimethylaminopropyl chloride, according to the method reported by Schmutz, *et al.*[9] to give colorless viscous oil, bp_4 170—180° and colorless prisms of hydrochloride (from iso-PrOH), mp 197—199°. *Anal.* Calcd. for $C_{12}H_{18}ON_3Cl$: C, 56.35; H, 7.09; N, 16.43. Found: C, 56.28; H, 7.04; N, 16.54. 1-Benzyl-3-(3-dimethylaminopropoxy)-1H-indazole N-oxide hydrogen maleate (BZY-NO·$C_4H_4O_4$) was synthesized by means of oxidation of BZY with H_2O_2[10] to give colorless prisms (from iso-PrOH), mp 97—98°. *Anal.* Calcd. for $C_{19}H_{23}O_2N_3 \cdot C_4H_4O_4$: C, 62.57; H, 6.16; N, 9.52. Found: C, 62.46; H, 6.10; N, 9.61. All melting points are uncorrected.

β-Glucuronidase from Tokyo Zokikagaku Co. was used to hydrolyze glucuronides at 300 Fishman unit/ml of urine.

Animals——The animals used were ddN mice (30—35 g), Wistar rats (120—150 g), guinea pigs (350—450 g), white rabbits (2.5—3.0 kg), cat (1.5 kg), and dog (10 kg). All were male and maintained on commercial chow and water *ad libitum* except cat fed rice boiled with fish and soy sauce.

BZY·HCl dissolved in distilled water was administered orally by stomach tube to animals except dog and cat received tablets containing 25 mg each of drug with several pieces of sausage.

Chromatography——In ordr to separate roughly metabolites, the urine sample was applied on a column of Amberlite XAD-2 (Rohm & Haas).[11] The column was washed with H_2O and eluted with MeOH. The eluate was evaporated under reduced pressure. The residue was dissolved in H_2O and extracted with $CHCl_3$.

The $CHCl_3$ extract was concentrated under reduced pressure, mixed with a small amount of silica gel (Wakogel C-200, Wako Junyaku) or aluminum oxide (activity II—III, Merck), and dried in a rotary evaporator. The dried mixture was put on the bottom of column and suitable amount of fresh adsorbent was packed on it. The column was eluted ascendingly.

Thin-layer chromatography (TLC) was accomplished on the plates of silica gel HF_{254} (Merck) or aluminum oxide HF_{254} (basic type E, Merck) as 1.0—2.0 mm thickness after activating at 105° for 60 min for the separation of metabolites from rabbit urine. The plastic sheets of silica gel F_{254} of 0.25 mm thickness (Merck) and the aluminum plate precoated with aluminum oxide F_{254} neutral type E of 0.20 mm thickness (Merck) were used for the quantitative determinations.

The solvent systems used were A: benzene–$CHCl_3$–MeOH–EtOH–concn. NH_4OH (15:15:10:5:0.5), B: *n*-BuOH–MeOH–H_2O–AcOH (35: 20:10: 4), C: toluene–EtOH–H_2O–AcOH (20: 30:10:1 upper layer) and D: benzene–EtOH–H_2O–AcOH (20: 20:10:1, upper layer). A and B were used for silica gel and C and D were for aluminum oxide.

The location of TLC was carried on under ultraviolet (UV) light (254 nm) or by spraying with Dragendorff, Gibbs (0.1% 2,6-dichloroquinone-4-chloroimine in *n*-PrOH), Folin-Ciocalteu, or naphthoresorcinol reagents.

Mass and NMR Spectrometry——Mass spectra were obtained by direct inlet of sample at 75 eV in a JMS-01SG (Japan Electron Optics, Lab.). NMR spectra were measured using JEOL C-60 or C-100 (Japan Electron Optics, Lab.). The samples were dissolved in $CDCl_3$ or D_6-dimethyl sulfoxide and tetramethylsilane was used as an internal standard.

Determination of Fluorescence——The fluorescence was measured by means of a Farrand Spectrofluorometer SER 167. The meter reading for 1 μg/ml solution of BZY·HCl was set as 1000 (exciting at 305 nm and emission at 360 nm) and the relative fluorescence intensities of samples were measured in the same condition and calculated as BZY·HCl equivalent. The lower limit for the determination under our condition was 0.1 μg/ml of BZY·HCl in 0.1M acetate buffer pH 5.

The fluorescence intensity of synthesized metabolites was measured at the most suitable wave length for each compound and the amount of metabolites was determined comparing with respective standard.

Result

Species Differences in the Properties of Urinary Fluorescence after Receiving BZY·HCl

It was possible to determine the fluorescence of urine from animals received BZY·HCl after diluting 1000 times or more with buffer. When the fluorescence intensity of urine was

7) G. Palazzo, G. Corsi, L. Baiocchi, and B. Silvestrini, *J. Med. Chem.*, **9**, 38 (1966).
8) E.F.M. Stephenson, "Organic Syntheses," Col. Vol. III, ed. by E.C. Horning, John Wiley, and Sons, Inc., New York, 1955, p. 475.
9) J. Schmutz, F. Hunziker, and W. Michaelis, *Helv. Chim. Acta*, **47**, 1986 (1964).
10) A.C. Cope and E. Ciganek, "Organic Syntheses," Coll. Vol. IV, ed. by N. Rabjohn, John Wiley and Sons, Inc., New York, 1963, p. 612.
11) J.M. Fujimoto and V.B. Haarstad, *J. Pharm. Exptl. Therap.*, **165**, 45 (1969).

determined under the condition for BZY·HCl itself, about 30% equivalent of dose was excreted in all species except 16.6% in cat as shown in column A of Table I. When the urine was extracted with *n*-heptane at pH 8, over 96% of BZY·HCl added into urine was recovered in *n*-heptane layer. However, the intensity of fluorescenct metabolites extracted into *n*-heptane from rat urine was equivalent about 1 to 1.4% of dose and most of remaining fluorescent metabolites were extracted with *n*-BuOH (Fig. 1). This fact suggests that the renal excretion of the unchanged BZY amounts to approximately 1 to 1.4% and BZY is metabolized to more polar compounds.

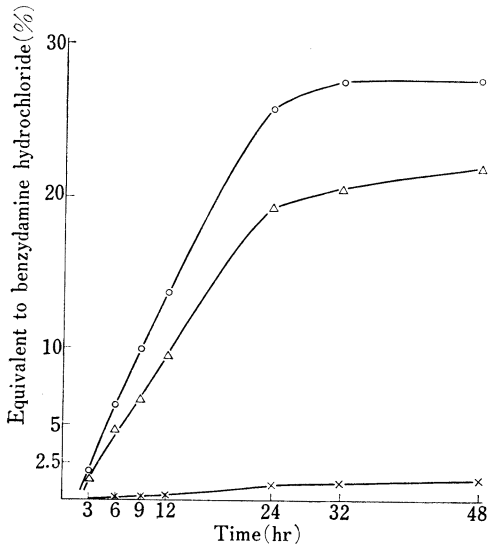

Fig. 1. Urinary Excretion of Benzydamine in Rat

Rat was dosed orally with 100 mg/kg of BZY·HCl in water.
○: total fluorescence
△: butanol extractable fluorescence
×: heptane extractable fluorescence

In order to estimate the amount of unconjugated metabolites, on the other hand, the urine was extracted with $CHCl_3$ until no more fluorescence reduced. As shown in column B of Table I, about 70% of fluorescent metabolites was extracted directly with $CHCl_3$ from the urine of rat, dog, and cat, but about 17—32% in rabbit, guinea pig, and mouse. Thereafter, the urine was incubated with β-glucuronidase at 37° for 24 hr. It is not yet elucidated whether the reduction of fluorescence intensity in a mixture during the incubation is due to the hydrolysis of glucuronide or to the decomposition of BZY structure which is essential for emitting fluorescence. Therefore, although the quantitative comparison is not adequate, there were species differences in the amount of fluorescent metabolites which was extractable with $CHCl_3$ from β-glucuronidase treated urine, as shown in column C of Table I.

These facts mean that, although the total fluorescent metabolites excreted into urine were almost same in every species except cat, the proportion of free form metabolites to conjugated metabolites varies from species to species.

Isolation of Metabolites from Rabbit Urine——The 24 hr urine (about 700 ml) from 3 rabbits received orally total dose of 1.55 g of BZY·HCl was applied on an Amberlite XAD-2

Table I. Species Differences in the Properties of Fluoresecnce in Urine of Animals received BZY·HCl

Species	Number of animals	Dose mg/kg	A Total excretion BZY·HCl equivalent % of dose	B Free form metabolite fraction[a] %	C Glucuronide fraction[b] %
Rabbit	4	200	30.8	21.9	34.8
	2	100	31.9	16.7	56.3
Guinea pig	4	100	38.9	17.7	79.2
Mouse	20	100	29.7	31.7	85.1
Rat	10	100	26.0	62.4	70.7
Dog	1	50	27.7	74.7	10.3
Cat	1	25	16.6	79.7	0

a) The percentage of reduction by extraction with $CHCl_3$ from fluorescence intensity of original urine.
b) The percentage of reduction by extraction with $CHCl_3$ from fluorescence intensity of $CHCl_3$ extracted and β-glucuronidase treated urine. Since the fluorescence intensity of urine reduced by the incubation with β-glucuronidase, the values of *a*) and *b*) in a line are not comparable. Compare the values in each column.

column (3.5×40 cm). The fluorescent metabolites in urine were almost completely adsorbed on the column. After washing with about 2 liters of water, the column was eluted with 2 liters of MeOH and the fluorescent metabolites were quantitatively eluted. After evaporating MeOH under reduced pressure, the residue was dissolved in about 100 ml of water and extracted with four 200 ml portions of $CHCl_3$. About 18% of fluorescent metabolites excreted was extracted into $CHCl_3$ layer. The TLC of this extract appeared six spots (M1—6) detected by Dragendorff reagent, whose one (M3) was detected by Gibbs reagent, too.[4] The remaining solution after extraction with $CHCl_3$ was again applied on an Amberlite XAD-2 column. The MeOH eluate from this column showed three spots positive to naphthoresorcinol reagent and the further purification is in progress. Table II shows the *Rf* values and color reaction of metabolites on TLC.

TABLE II. *Rf* Values and Reaction to Color Reagents of Reference Compounds and Urinary Metabolites in Rabbit

Metabolite	*Rf* values on TLC[a]				Color reaction					Authentic sample
	A	B	C	D	b)	c)	d)	e)	f)	
Unconjugates										
M1	0.80		0.83	0.96	+	—	—		yes	BZY (unchanged)
M2	0.40		0.26	0.60	+	—	—		yes	deB–BZY
M3	0.60		0.20	0.56	+	+ (blue)	+ (blue)		no	(HO–BZY)
M4	0.40		0.17	0.50	+	—	—		yes	nor–BZY
M5	0.40		0.10	0.30	+	—	—		yes	BZY–NO
M6	0.73		0.05	0.20	+	—	—		yes	BID
M7	0.53		0.03	0.14	—	+ (pink)	+ (blue)		yes	ID
Conjugates										
G1		0.20			+	—	—	+	no	
G2		0.35			+	—	—	+	no	
G3		0.45			+	—	—	+	no	

a) Solvent system and adsorbent, see text.
b) Dragendorff *c*) Gibbs *d*) Folin-Ciocalteu *e*) naphthoresorcinol
f) yes: indistinguishable with *Rf* value of authentic sample, no: Synthetic authentic sample is not available.

Chloroform extract was applied on a silica gel column chromatography with solvent system C and fractions of 50 ml were collected. The TLC of each fraction indicated that M6 was contained in fractions No. 1—6, M1 in No. 6—10, and M3 in No. 12—13. The fractions from No. 14 to 24 were combined, evaporated *in vacuo*, and applied on an aluminum oxide column chromatography with solvent system D. Fractions of 18 ml were collected. M2 was detected as a single spot on TLC of fractions from No. 8 to 18. M4 and M5 were separated by the preparative TLC of fractions from No. 19 to 29 on aluminum oxide with solvent system D.

The eluates from column chromatography containing a single metabolite or the MeOH extracts from adsorbents scraped from TLC corresponding to a metabolite were concentrated under reduced pressure and applied to chromatography or spectroscopy. No compound was attempted to crystallize because of small quantity available.

On the other hand, the 24 hr urine from rabbit administered 300 mg of BZY·HCl was adjusted to pH 5 and extracted with ether. The recrystallization of ether extract from MeOH gave a few mg of crystals (M7) of mp 250—252°.

The fluorometric properties of metabolites are summarized in Table III.

Identification of Metabolites——M3: M3 on TLC was visualized as deep orange by Dragendorff reagent or blue by Gibbs or Folin-Ciocalteu reagent and it was suggested that M3 was a phenolic compound. Acetic anhydride (1 ml) was added to the solution of M3 (fluoro-

Table III. Fluorometric Properties of BZY Metabolites

Compound[a]	Wave length for Excitation (nm)[b]	Wave length for Emission (nm)[b]	Relative intensity A	Relative intensity B
BZY·HCl[c]	308	350	100	100
nor-BZY·HCl	308	350	130	130
BZY-NO·$C_4H_4O_4$	308	350	96	96
deB-BZY·HCl	300	343	—	56
	308	350	44	—
BID	316	408	—	100
	308	350	11	—
ID	316	408	—	190
	308	350	22	—

a) All compounds are measured at 1.0 μg/ml of concentration in MeOH.
b) uncorrected
c) In 0.1M acetate buffer at pH 5, the fluorometric spectrum shows 305 nm for excitation and 360 nm for emission.
A) Excited at 308 nm and measured at 350 nm.
B) Measured under the condition obtaining maximum fluorescence intensity for metabolite. Values are relative intensities to BZY·HCl.

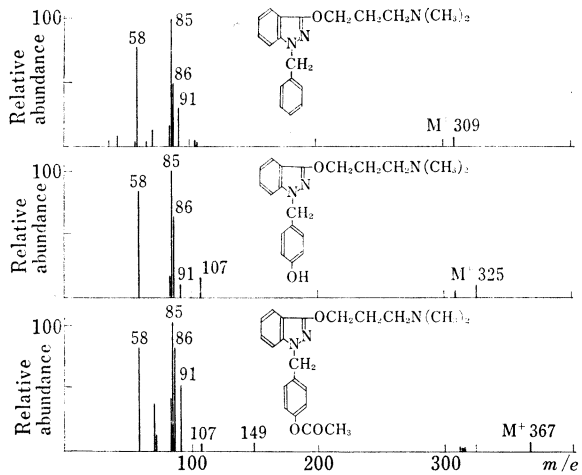

Fig. 2. Mass Spectra of BZY·HCl, HO-BZY and AcO-BZY

metrically equivalent to about 30 mg of BZY·HCl) in pyridine (1 ml). After standing for 24 hr, the reaction mixture was evaporated under reduced pressure. Both M3 and acetylated M3 were examined by mass and NMR spectroscopy as shown in Fig. 2 and 3.

The parent molecular ion in mass spectra was at *m/e* 309 for BZY, 325 for M3 and 367 for acetylated M3. These facts suggested that one oxygen atom was introduced into M3 as a hydroxy group which was able to be acetylated.

The high resolution mass spectrometry of M3 showed the parent peak *m/e* 325 (Calcd. for $C_{19}H_{23}O_2N_3$: 0.1790, Found, 0.1789 millimass unit) and a fragment peak *m/e* 107 (Calcd. for C_7H_7O: 0.0473, Found, 0.0497 millimass unit). The base peak was at 85 ($CH_2=CH-CH_2-\overset{+\cdot}{N}(CH_3)_2$) and other fragment peaks at 86 ($^+CH_2CH_2CH_2N(CH_3)_2$) and 58 ($CH_2=\overset{+\cdot}{N}(CH_3)_2$) were also shown. These data demonstrated that the dimethylaminopropoxy chain was remaining intact. The fragment ion at *m/e* 107 was presumably due to the formation of hydroxybenzyl cation, because the mass spectra of indazolone derivatives did not show this fragment.[12] According to Shannon,[13] a fragment peak at 107 appears in mass spectra of three isomeric hydroxybenzyl alcohols but is the base peak only in the para isomer. If the resulting fragment had the structure of hydroxytropylium ion, it should be identical in all three isomers. But the retention of benzyl structure would allow resonance stabilization with the participation of the electron on *para* hydroxyl group but not on *meta* or *ortho* group.

12) J. Desmarchelier and R.B. Johns, *Org. Mass Spectrosc.*, **2**, 37 (1969).
13) J.S. Schannon, *Australian J. Chem.*, **15**, 265 (1962).

NMR spectrum of M3 shown in Fig. 3 exhibited proton signals as follows: τ 2.7—3.2 (aromatic multiplet), 2.78, 3.14 (A_2B_2-type quartet, J=4.8 cps), 3.80 (singlet, OH, disappeared

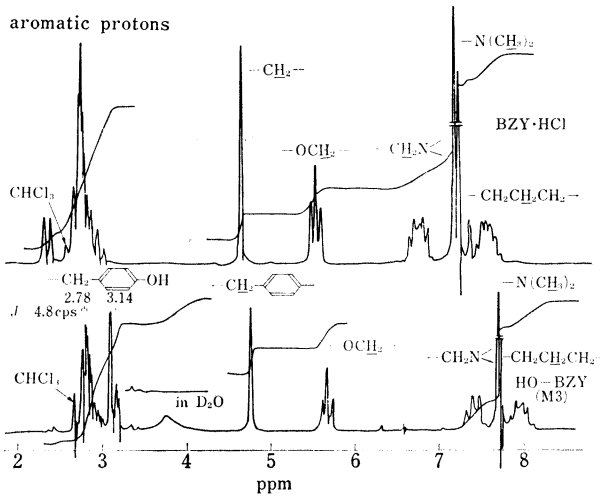

Fig. 3. NMR Spectra of BZY·HCl and HO-BZY (on τ-Value in $CDCl_3$ at 100 MHz)

TABLE IV. Nuclear Magnetic Resonance and Mass Spectra of Reference Compounds and Urinary Metabolites in Rabbit

Authentic sample	Metabolite	NMR[a] Assignment τ (ppm)					
		N-CH₂-⟨⟩	-OCH₂-	-CH₂N<	-N(CH₃)₂	-OCH₂CH₂CH₂-	-OH or >NH
BZY·HCl	M1	4.70 (2H, s)	5.54 (2H, t)	6.50 (2H, t)	7.24 (6H, d)	7.52 (2H, p)	
deB-BZY·HCl	M2	—	5.60 (2H, t)	6.72 (2H, t)	7.20 (6H, s)	7.52 (2H, p)	−2.00 (1H, s)
nor-BZY·HCl	M4	4.70 (2H, s)	5.50 (2H, t)	6.84 (2H, t)	7.40 (3H, s)	7.52 (2H, p)	
BZY-NO maleate	M5	4.66 (2H, s)	5.51 (2H, t)	6.12 (2H, t)	6.50 (6H, t)	7.52 (2H, p)	
BID	M6	4.72 (2H, s)	—	—	—	—	4.08 (—,[c] s)
ID	M7						5.90 (—,[c] s)

Authentic sample	Metabolite	Mass[b] M⁺/e	Base peak m^+/e	Other fragment peaks m^+/e			
BZY·HCl	M1	309 (4%)	85	58 (78%)	86 (50%)	91 (30%)	
deB-BZY·HCl	M2	219 (10%)	58	86 (14%)	85 (75%)		
nor-BZY·HCl	M4	295 (13%)	91	204 (12%)	72 (65%)	71 (60%)	44 (40%)
BZY-NO maleate	M5						
BID	M6	224 (14%)	91				
ID	M7	134					

a) chemical shift from tetramethylsilane, figures in parentheses indicate number of protons and multiplicity. The multiplicity: s=singlet, d=doublet, t=triplet and p=pentuplet (measured at 60 MHz).
b) measured by the direct sample introduction technic
c) be insufficient for an accurate integration

by the addition of deuterium oxide), 4.76 (singlet, $-C\underline{H}_2-C_6H_4-$), 5.67 (triplet, $-OC\underline{H}_2-$), 7.39 (triplet, $-C\underline{H}_2N$), 7.70 (singlet, $-N(C\underline{H}_3)_2$), 7.96 ppm (pentuplet, $-OCH_2C\underline{H}_2CH_2-$). NMR spectrum of acetylated M3 showed 8H aromatic protons at 2.70—3.10 (containing A_2B_2-type signals), 2H singlet signal at 4.72, 2H triplet signal at 5.66, 2H triplet signal at 7.16, 6H singlet signal at 7.54 and 3H singlet signal at 7.76 ppm which was due to acetyl group instead of hydroxyl group in M3.

In conclusion, it is reasonable to estimate M3 as 1-(*p*-hydroxybenzyl)-3-(3-dimethylaminopropoxy)-1H-indazole (HO-BZY).

Other Metabolites——The identification of other metabolites was accomplished by the co-chromatography and by the comparison of mass and NMR spectra with synthesized reference compounds. As shown in Table II, M1, 2, 4, 5, 6 and 7 were indistinguishable from BZY, nor-BZY, deB-BZY, BZY-NO, BID and ID, respectively, on TLC with three solvent systems examined. Those mass and NMR spectra were also same as the spectrum of reference compound as shown in Table IV.

The quantitative determination of urinary unconjugated metabolites in dog, rat, guinea pig and rabbit was preliminarily carried out using TLC and fluorometry. The largest metabolite was BZY–NO and 1.5, 3.0, 5.9 and 10.9% of dose in rabbit, guinea pig, rat and dog, respectively. The amount of unchanged BZY was 0.4, 0.5, 0.7 and 1.9%. Urinary excretion of other metabolites was 1% or less.

Discussion

Catanese, *et al.*[5] reported that 50 or 30% in man and rat, respectively, of administered BZY was excreted unchanged and that there was a small quantity of a metabolite which reacted with the Folin-Ciocalteu reagent in the urine. As they stated, the fluorescent metabolites equivalent to about 30% of dose were excreted in 24 hr urine in rat. However, the extraction with *n*-heptane showed that the amount of unchanged drug was small and that most of BZY was biotransformed to the more polar metabolites including glucoronides. In addition, there are considerable species differences in the proportion of free form metabolites to conjugated metabolites.

Considering the chemical structure of BZY, the following metabolic pathways are expected; N- and O-dealkylation, N-oxidation, aromatic hydroxylation and subsequent conjugation. Seven metabolites according to this expectation were identified in the unconjugate fraction of rabbit urine. The major metabolite was BZY–NO. We reported preliminarily that nor-BZY was the major metabolite from TLC data,[6] but it was ascertained that TLC on silica gel plate with solvent system A could not distinguish nor-BZY, BZY-NO, and deB-BZY. The TLC on aluminum oxide plate with solvent system C or D demonstrated that BZY-NO was separated from other two compounds and it was the major metabolite. The metabolic N-oxidation of aliphatic tertiary amines has been considered of minor importance, but recent finding showed N-oxide as major urinary metabolite for N-oxiptiline and doxepin in rat and dog,[14,15] perazine in men,[16] orphenadrine citrate in men[17] or diphenhydramine in rhesus monkey.[18]

The second largest metabolite in rabbit urine was HO-BZY, whose structure was characterized by means of mass and NMR spectra because of difficulty in synthesizing this compound and small yield from isolation work. The preliminary study shows that most of HO-BZY

14) B. Duhm, W. Maul, H. Medenwald, K. Patzschke, and L. Wegner, *Arzneim.-Forsch.*, **19**, 858 (1969).
15) D. Chobbs, *Biochem. Pharmacol.*, **18**, 1941 (1969).
16) U. Breyer, *Biochem. Pharmacol.*, **18**, 777 (1969).
17) T. Ellison, A. Snyder, J. Bolger, and R. Okun, *J. Pharm. Exptl. Therap.*, **186**, 284 (1971).
18) J.C. Drach and J.P. Howell, *Biochem. Pharmacol.*, **17**, 2125 (1968).

is excreted as glucuronide and that the amount present in free form fraction is relatively small.[6]

Few reports on the metabolic N-debenzylation were available but Imamura, *et al.*[19] demonstrated that 2-amino-3-ethoxycarbonyl-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (Y-3542) underwent N-debenzylation markedly in mice and rats *in vivo* and *in vitro* and that benzyl moiety was excreted as hippuric acid. The N-debenzylation of BZY results in deB–BZY and ID which are identified in urine.

The summary of metabolic pathway of BZY is shown in Fig. 4.

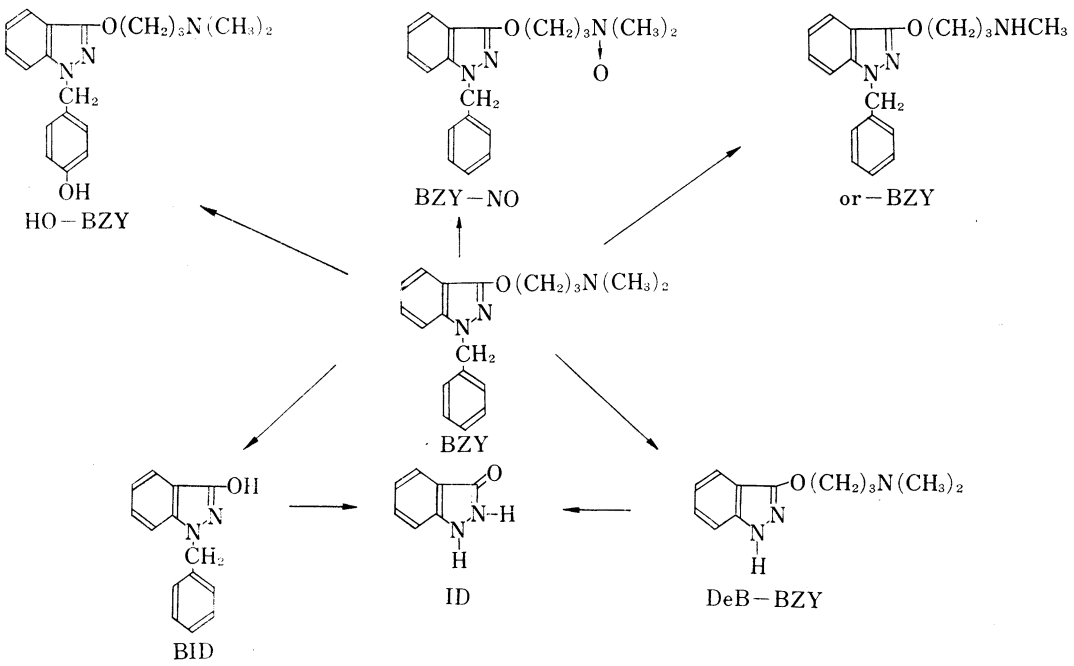

Fig. 4. Metabolic Pathways of Benzydamine

Although it is not conclusive until the studies on conjugates will be completed, the most remarkable difference in species on BZY metabolism seems to be the proportion of free form metabolites to conjugated metabolites. No glucuronide was found in cat urine as same as other cases. Dog excretes mainly as free form, especially BZY–NO. Rats excrete free form metabolites mainly, but the most conjugates are glucuronides. Guinea pigs and mice excrete a small proportion of free form, and most of conjugates are glucuronides. Rabbits excrete also a small proportion of free form, and glucuronides occupy less than half of conjugates. However, in all species except cat, the intensity of fluorescence in urine is almost same. These facts indicate the importance of species differences in the metabolism study.

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