Metabolites of Bromazepam, a Benzodiazepine, in the Human, Dog, Rat, and Mouse

MORTON A. SCHWARTZ*▲, EDWARD POSTMA*, STANLEY J. KOLIS*, and ARTHUR S. LEON†

Abstract □ The biotransformation of bromazepam, 7-bromo-1,3-dihydro-5-(2-pyridyl)-2*H*-1,4-benzodiazepin-2-one, was studied in four species. Four metabolites were identified and their excretion was quantitatively determined. The major urinary metabolites excreted by three women given single 12-mg. oral doses of bromazepam-5-^{14}C were the conjugated forms of 3-hydroxybromazepam (I), accounting for 13–30% of the dose, and 2-(2-amino-5-bromo-3-hydroxybenzoyl)pyridine (IV), accounting for 4–25% of the dose. Conjugated I was also the major metabolite in three dogs and four groups of mice administered ^{14}C-bromazepam, but its excretion by four rats was very limited. Together with I, Metabolite IV and 2-(2-amino-5-bromobenzoyl)pyridine were detected in the excreta of the human, dog, rat, and mouse. Although the pyridyl *N*-oxide derivative of bromazepam was not detected as a metabolite in any species, the N_4-oxide was a minor metabolite in dog urine. This is the first reported instance of metabolic N_4-oxidation of a 1,4-benzodiazepin-2-one.

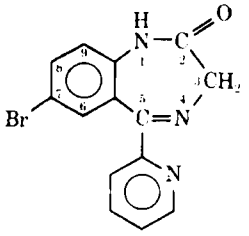
Keyphrases □ Bromazepam (a benzodiazepine) metabolism in human, dog, rat, and mouse, identification of metabolites, mechanisms discussed □ Benzodiazepine derivatives—metabolism of bromazepam in human, dog, rat, and mouse, identification of metabolites, mechanisms discussed □ Biotransformation—identification of bromazepam metabolites in human, dog, rat, and mouse □ Metabolites, bromazepam—identified in urine and feces of humans, rats, dogs, and mice

Bromazepam [7-bromo-1,3-dihydro-5-(2-pyridyl)-2*H*-1,4-benzodiazepin-2-one and formerly designated Ro 5-3350] was reported (1) to possess central nervous system activity in animals and antianxiety activity in humans. This drug is a member of the benzodiazepine class of compounds whose metabolism was recently reviewed (2).

In this study, the metabolites of bromazepam were identified and quantitated in various species to: (*a*) detect species differences in metabolic pathways, and (*b*) compare the biotransformation of bromazepam with that of other benzodiazepines. Bromazepam labeled with ^{14}C in either the C_2- or C_5-position was used. Bromazepam-5-^{14}C was preferred (but was not available for the initial studies in dog and mouse) because if metabolic cleavage of the diazepine ring occurred, resulting in the loss of atoms 2, 3, and 4, the label would remain with the benzoylpyridine moiety. A chemical cleavage of this ring by acid hydrolysis was used in the development of a GLC assay of bromazepam (3).

EXPERIMENTAL

Labeled Drug and Determination of ^{14}C—Prior to preparing each drug formulation, TLC with System 1 (described below) and the determination of the ^{14}C migrating with authentic bromazepam confirmed that the radiochemical purity of both C_5- and C_2-labeled bromazepam remained at 97 and 98%, respectively, throughout the study. Furthermore, at the end of the study, the compounds identified as metabolites were cochromatographed with each labeled bromazepam. Only one compound, 3-hydroxybromazepam (I), was found to be a possible contaminant of the labeled drugs,



bromazepam

but only to the extent of <0.7% of the ^{14}C of bromazepam-5-^{14}C and <0.3% of the ^{14}C of bromazepam-2-^{14}C.

The determination of radioactivity in urine, in homogenates of feces in 50% ethanol, in ethyl acetate extracts of both, and on particles of silica gel was accomplished by liquid scintillation spectrometry as previously described (4).

Design of Human and Animal Studies—Three Negro females[1] (Subjects 1, 2, and 3) were not given any medication chronically for at least 3 weeks prior to labeled bromazepam administration. After obtaining informed consent, each subject received two tablets, each containing 6 mg. of bromazepam-5-^{14}C with a specific activity of 4.3 μc./mg. The excipients (starch, lactose, microcrystalline cellulose[2], magnesium stearate, and FD&C certified dyes) and the type of tablet compression [single compression using a 0.86-cm. (0.34-in.) flat-faced punch] were chosen to duplicate the formulation and size of the tablets currently used in the clinical trials. Subject 1, 51 years of age and weighing 88 kg., developed influenza 4 days after the labeled drug was given and recovered without treatment with drugs. Subject 2, 36 years of age and weighing 57 kg., also developed influenza after a week on this study and was treated with phenazopyridine hydrochloride. Subject 3, 48 years of age and weighing 85 kg., received aspirin and hydrochlorothiazide (50 mg.) on several occasions but no chronic drug treatment during the study. Daily collections of urine and feces were made until the excretion of ^{14}C decreased to barely measurable levels.

Four nonfasted 11–12-kg. male dogs (beagles) were given single oral doses of ^{14}C-bromazepam in gelatin capsules. Dog 1 was given 5 mg./kg. of bromazepam-2-^{14}C (specific activity 2.3 μc./mg.), whereas the remaining dogs received the same dose of bromazepam-5-^{14}C (specific activity 1.0 μc./mg.). Urine and feces were collected daily for as long as readily measurable amounts of ^{14}C were excreted.

Bromazepam-5-^{14}C (1.0 μc./mg.) was dissolved in a vehicle consisting mainly of 50% propylene glycol, 10% ethanol, 1.5% benzyl alcohol, and an aqueous acetate buffer[3] and was administered to four nonfasted male Charles River rats at a dose of 2 mg./kg. Two 230-g. rats received an intravenous dose and two 285-g. rats were given the drug orally by intubation. Solutions of bromazepam-2-^{14}C (27 μc./mg.) and bromazepam-5-^{14}C (35 μc./mg.) in the same parenteral vehicle, after dilution with water to avoid toxicity due to the ingredients of the vehicle, were injected intravenously into nonfasted Carworth Farms 1S 20-g. male mice at a dose of 1.0 and 0.3 mg./kg., respectively. Two groups of three mice each (Groups I and II) received the C_2-labeled drug, and two groups (Groups III and IV) were given bromazepam-5-^{14}C. Each group was placed in a separate cage. Urine and feces were collected from the four rats and four groups of mice until excretion of ^{14}C was barely measurable.

[1] Hospitalized in the Special Treatment Unit, Newark Beth Israel Hospital, Newark, N. J.

[2] Avicel.

[3] This Hoffmann-La Roche Inc. formulation (F-31) was developed by Mr. H. Newmark.

Table I—Excretion of Radioactivity Derived from ^{14}C-Bromazepam by Human, Dog, Rat, and Mouse

Subject or Animal	Dose Administered and Route	Duration, Days	Excretion of ^{14}C in Percent of Dose Administered Urinary	Fecal	Total
Human					
1	12 mg. bromazepam-5-^{14}C p.o.	14	59.1	2.1	61.2
2	12 mg. bromazepam-5-^{14}C p.o.	11	74.0	2.0	76.0
3	12 mg. bromazepam-5-^{14}C p.o.	17	78.6	5.9	84.5
Dog					
1	5 mg./kg. bromazepam-2-^{14}C p.o.	24	46.7	18.5	65.2
2	5 mg./kg. bromazepam-5-^{14}C p.o.	10	52.0	25.8	77.8
3	5 mg./kg. bromazepam-5-^{14}C p.o.	4	15.5	58.7	74.2
4	5 mg./kg. bromazepam-5-^{14}C p.o.	10	37.0	27.0	64.0
Rat					
1	2 mg./kg. bromazepam-5-^{14}C i.v.	4	28.0	59.1	87.1
2	2 mg./kg. bromazepam-5-^{14}C i.v.	4	32.0	58.2	90.2
3	2 mg./kg. bromazepam-5-^{14}C p.o.	6	17.1	58.3	75.4
4	2 mg./kg. bromazepam-5-^{14}C p.o.	6	19.9	65.0	84.9
Mouse					
Group I	1 mg./kg. bromazepam-2-^{14}C i.v.	4	49.9	24.2	74.1
Group II	1 mg./kg. bromazepam-2-^{14}C i.v.	4	62.3	17.3	79.6
Group III	0.3 mg./kg. bromazepam-5-^{14}C i.v.	6	58.3	20.5	78.8
Group IV	0.3 mg./kg. bromazepam-5-^{14}C i.v.	6	60.9	19.7	80.6

Extraction of Labeled Metabolites—Aliquots of urine from each species and of fecal homogenates (further diluted 1:5 with water) from the dog, rat, and mouse, each containing at least 10^5 d.p.m. of ^{14}C, were extracted with ethyl acetate. Following the initial extraction at pH 9.0, the aqueous phase was incubated with a commercial preparation of β-glucuronidase and arylsulfatase[4], which was added to a final concentration of 1% (v/v). This incubation at pH 5.5 and 37° for 2–3 hr., which previously yielded maximum hydrolysis of conjugated benzodiazepine metabolites (5), was followed by serial extraction at pH 9.0, 7.0, and 2.0. All pH adjustments were made by the addition of dilute acid or alkali; at each designated pH, two consecutive volumes of ethyl acetate, each equaling the aqueous volume, were shaken with the aqueous phases for approximately 10 min. each. The combined extract at each pH was analyzed for ^{14}C and the extracts obtained at pH 9.0 were concentrated and examined by TLC.

TLC for Metabolite Separation, Identification, and Quantitation—Silica gel containing a fluorescent indicator[5] was used for TLC with the following solvent systems: 1, heptane–ethyl acetate–ethanol–concentrated ammonia (50:50:10:3); 2, heptane–chloroform–ethanol–concentrated ammonia (50:50:20:1); 2*a* and 2*b*, same solvents in the proportions of 25:75:10:0.5 and 50:50:50:1, respectively; 3, isopropanol–concentrated ammonia (100:1); 4, heptane–chloroform–ethanol (5:5:1); 5, ethyl acetate–methyl ethyl ketone–ethanol–concentrated ammonia (90:10:10:0.5); 6, benzene–ethyl acetate–ethanol–concentrated ammonia (80:20:20:1); 7, heptane–methylene chloride–ethanol–concentrated ammonia (50:50:25:1); 7*a*, same solvents in proportions of 50:50:50:1; 8, ethyl acetate–ethanol–concentrated ammonia (95:5:1); 9, chloroform–ethyl acetate–ethanol–concentrated ammonia (50:50:12:3); 10, heptane–benzene–ethanol–concentrated ammonia (50:50:20:0.5); 11, heptane–chloroform–ethanol–acetic acid (10:10:1:2); and 12, chloroform–ethanol–concentrated ammonia (95:5:0.5).

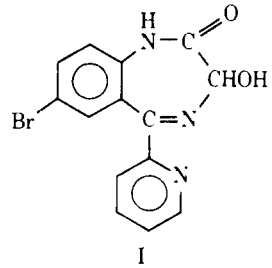
The use of one-dimensional TLC for metabolite separation followed by identification of the isolated metabolites by cochromatography with reference compounds or by spectroscopic techniques has been described (2). Individual urinary and fecal metabolites were determined by comparative two-dimensional TLC. In this analysis, both identification and quantitation result from the demonstration that, on two-dimensional TLC with at least two different solvent system pairs, a consistent amount of ^{14}C extracted from urine or feces migrates as a reference compound added as an internal standard. The solvent system pairs were designated by the number of each system in the order used, and the reference compounds were located by viewing the developed chromatoplate under shortwave UV light. Silica gel containing each reference compound was suspended in the fluor for the determination of ^{14}C.

In additional experiments, each identified metabolite added to water was shown by spectrophotometric assay to be quantitatively extracted at the pH used in its extraction from the biological sample. This demonstrated that no correction factor for incomplete extraction was required in calculating the excretion of each metabolite.

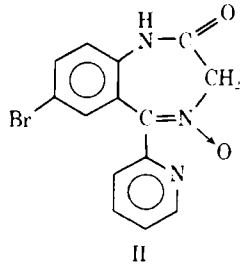
RESULTS

Excretion of Radioactivity by Each Species—The excretion of ^{14}C is summarized in Table I. The total excretion of ^{14}C by the human subjects ranged from 61 to 85% of the dose, with almost all of this radioactivity excreted in the urine. While the total excretion of ^{14}C by dogs was in this same range, a greater amount of the dose was excreted in the feces. The markedly high fecal excretion of orally administered ^{14}C by Dog 3 (59% of the dose) suggested that absorption of labeled bromazepam was impaired in this animal. In rats, the high fecal excretion of radioactivity was seen after both oral and intravenous administration of C_5-labeled drug, suggesting that biliary excretion was important in this species. In mice given either bromazepam-2-^{14}C or bromazepam-5-^{14}C intravenously, the excretion of radioactivity was similar to that obtained with man and dog; urinary excretion of ^{14}C predominated and the recovery of the dose was not complete.

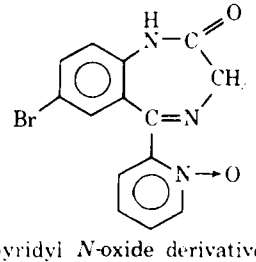
The reason for the incomplete excretion of all of the administered radioactivity, most evident in the human and dog, is not clear at this time. Since this loss is not limited to the administration of the C_2-labeled drug, it is not due to extensive metabolic cleavage of the diazepine ring and elimination of the C_2-carbon as carbon dioxide.



I



II



pyridyl *N*-oxide derivative

[4] Glusulase, Endo Labs., Garden City, N. Y.

[5] Precoated chromatoplates, Camag DF-B (Camag Inc., New Berlin, Wis.) and Q4F (Quantum Industries, Fairfield, N. J.), were used for two-dimensional TLC with Solvent Systems 5–6 and 10–6, respectively; chromatoplates prepared with SilicAR 7-GF-5 (Mallinckrodt Chemical Works, St. Louis, Mo.) were used with all other systems.

Table II—Metabolite Identification by Comparative Two-Dimensional TLC

Reference Compound	Labeled Metabolite Source[a]	TLC Systems	Percent of ^{14}C Migrating as Reference Compound
I	Dog 1 urine	1–2	95
		3–1	90
	Dog 2 urine	5–6	73
		8–9	80
		2*b*–7*a*	74
IV	Subject 3 urine	10–6	44
		2*a*–3	45
		2–11	45
III	Dog 2 feces	5–6	12
		2–7	14
		8–9	12

[a] Ethyl acetate extracts obtained by extraction at pH 9.0 of the glusulase-treated urine or feces were cochromatographed with reference compounds.

Apparently, a portion of the drug-derived ^{14}C is excreted very slowly.

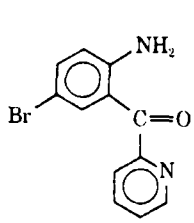
Identification of Urinary Metabolites—Reference compounds of interest were 3-hydroxybromazepam [I, 7-bromo-1,3-dihydro-3-hydroxy-5-(2-pyridyl)-2*H*-1,4-benzodiazepin-2-one], 4-oxide derivative [II, 7-bromo-1,3-dihydro-5-(2-pyridyl)-2*H*-1,4-benzodiazepin-2-one 4-oxide], pyridyl *N*-oxide derivative [7-bromo-1,3-dihydro-5-(2-pyridyl-2-oxide)-2*H*-1,4-benzodiazepin-2-one], 2-(2-amino-5-bromobenzoyl)pyridine (III), and 2-(2-amino-5-bromo-3-hydroxybenzoyl)-pyridine (IV). Evidence for I being a metabolite had been obtained in an earlier investigation (unpublished) of bromazepam metabolism in the dog. Evidence that IV is a urinary metabolite in the dog and rabbit was reported (6) during the present studies. Compound II and the pyridyl *N*-oxide derivative allowed differentiation between metabolic oxidation at the 4-nitrogen and pyridyl nitrogen, respectively.

Preliminary one-dimensional TLC of extracts of dog urine suggested that I, III, and IV were metabolites. Representative results from two-dimensional TLC, which confirmed their status as metabolites and allowed for their quantitation, are shown in Table II. The consistent migration of ^{14}C with the designated reference compound in extracts chosen because of their relatively high content of the metabolite in question is evident.

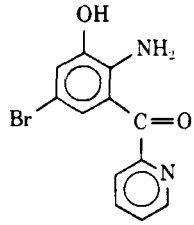
Also detected in the initial examination of dog urine by one-dimensional TLC was a directly extractable metabolite, which was purified first by TLC with System 1 and then by TLC of the eluted metabolite with System 2. The spectra resulting from low-resolution mass spectrometry[6] of a 4-mcg. sample of this metabolite showed that oxygen had been added to the bromazepam molecule and indicated, from the strong loss of *m/e* 16, that this metabolite was either the 4-oxide (II) or a pyridyl *N*-oxide derivative of bromazepam. Two-dimensional TLC, in which the isolated metabolite was cochromatographed with each isomer, clearly supported the identification of this metabolite as II.

This result was unexpected because metabolic *N*-oxidation of a benzodiazepine at the 4-position had not previously been reported. Further evidence for this structure was obtained by characterizing the benzoylpyridine formed on acid hydrolysis of the metabolite. A 50-mcg. sample of isolated metabolite was hydrolyzed in 6 *N* sulfuric acid, and the benzoylpyridine was extracted as previously described (3). When tested by TLC (System 4) and GLC[7] [using a 1.82-m. (6 ft.) 3% OV-17 column with inlet, column, and flame-ionization detector temperatures of 275, 240, and 290°, respectively], the metabolite hydrolysis product chromatographed as authentic III, supporting the identification of the intact metabolite as II.

The pyridyl *N*-oxide derivative was sought as a urinary or fecal metabolite in all four species investigated. However, no measurable or consistent amount of the ^{14}C of any extract examined migrated with this reference compound, thus indicating that it was not a metabolite of bromazepam.



III



IV

Quantitative Profile of Bromazepam and Metabolite Excretion—The extractability of the urinary metabolites of each species is presented in Table III. The urinary metabolites of man and dog are excreted mainly as conjugates hydrolizable by glusulase to labeled compounds extractable at pH 9.0. This is not true for the rat and mouse. In the rat, roughly one-quarter of the urinary ^{14}C is extractable at pH 9.0, both before and after glusulase treatment; in the mouse, the directly extractable ^{14}C represents the major metabolite fraction. An appreciable amount of urinary ^{14}C is extracted at pH 2.0 in both the rat and mouse. Fecal samples from dogs, rats, and mice were also extracted and, in general, less ^{14}C was extractable at each pH than was found for the respective urine samples.

The metabolites were quantitated in the pH 9.0 extracts obtained before and after glusulase treatment. As seen in Table IV, the excretion of unchanged bromazepam by each species accounted for only 1–2% of the dose. The major urinary metabolites excreted by the human subjects were the conjugated forms of I and IV. Subject 2 differed from the other two in excreting a larger amount of I (31% of the dose) and a smaller amount of IV (only 4% of the dose).

In Dog 3, in which most of the ^{14}C was excreted in the feces, 73% of the fecal ^{14}C was found to be unchanged bromazepam. Since the absorption of bromazepam in this animal was severely limited, only data from the other three dogs were used to compile the average excretion values shown in Table IV. The major urinary metabolite was conjugated I (20% of the dose). A small amount of urinary II was also excreted by dogs, and the dog was the only species in which measurable amounts of II were found in the urine.

Compound I was not excreted as a major metabolite by the rat, in contrast to the human and dog. Since only small amounts of III and IV were also found in the urine, most of the ^{14}C excreted by rats remained unidentified. The dominant metabolite excreted by mice was I, accounting for 36% of the dose. However, in contrast to its excretion mainly as a urinary conjugate by the human and dog, urinary I was excreted by the mouse primarily unconjugated. Very little III and IV and no detectable amounts of II were excreted by the mice; almost all radioactivity not excreted as I remained unidentified.

DISCUSSION

The excretion of conjugated 3-hydroxylated derivatives is prominent in humans and dogs, but not in rats, administered various 1,4-benzodiazepin-2-ones (2). The present results show that bromazepam also fits this pattern. In addition, the finding that Metabolite I, although mostly unconjugated, is a major urinary metabolite of bromazepam in the mouse suggests that this species metabolizes bromazepam as do the human and dog. The difference in the pattern of metabolites excreted by the mouse and rat may hold true for benzodiazepin-2-ones in general. Recently, a considerable fraction of an intravenous dose of either diazepam[8] or desmethyldiazepam was found (7) to be secreted in the bile of the mouse, but not in the bile of the rat, as the conjugated 3-hydroxy metabolite, oxazepam[9].

In the rat the preferred metabolic reaction apparently is hydroxylation of benzodiazepines at the *para*-position of the 5-phenyl ring (2). This is also true for oxazepam metabolism in the rat (8). However, since the major metabolites of bromazepam in the rat were not

[6] A Consolidated Electronics Corp. 21-110 mass spectrometer produced the spectra, which were interpreted by Ms. J. Pao and Dr. W. Benz.

[7] Micro-Tek gas chromatograph, model MT-220, Tracor, Inc., Austin, Tex.

[8] Diazepam is the active ingredient in Valium, Hoffmann-La Roche Inc., Nutley, N. J.

[9] Oxazepam is the active ingredient in Serax, Wyeth Labs., Inc., Radnor, Pa.

Table III—Extractability of the Urinary Radioactivity Excreted by Human, Dog, Rat, and Mouse[a]

Serial Extraction with Ethyl Acetate	Percent of Urinary Radioactivity in Each Extract[b]			
	Human (3)[c]	Dog (4)[c]	Rat (4)[c]	Mouse (4)[c]
Preglusulase at pH 9.0	6.4 ± 0.75	10 ± 1.9	24 ± 2.8	48 ± 1.9
Postglusulase at pH 9.0	63 ± 7.4	63 ± 9.7	21 ± 6.2	22 ± 2.4
at pH 7.0	6.5 ± 2.0	5.6 ± 1.0	8.0 ± 3.6	3.1 ± 0.2
at pH 2.0	9.2 ± 1.8	4.0 ± 1.1	18 ± 2.5	17 ± 2.2
Total	85	83	71	90

[a] In each instance, the sample extracted was pooled urine representing over 90% of the final amount of ^{14}C eliminated in the urine. [b] Since the extractability within each species appeared to be unaffected by the position of ^{14}C labeling or the route of ^{14}C-bromazepam administration, the mean ± standard deviation is presented. [c] Number of individual subjects or animals whose urine was extracted.

Table IV—Excretion of Bromazepam and Metabolites by Human, Dog, Rat, and Mouse

Excreted Component	Percent of Dose Excreted[a]								
	Human Urine			Dog (3)		Rat (4)		Mouse (4)	
	Subject 1	Subject 2	Subject 3	Urine	Feces	Urine	Feces	Urine	Feces
Bromazepam	1.1	1.2	1.8	Negl.[b]	1.6 ± 0.2	0.7 ± 0.4	1.0 ± 0.3	0.6 ± 0.1	0.5 ± 0.1
I (Nonconjugated)	0.5	1.6	0.7	Negl.	1.7 ± 0.7	1.6 ± 0.3	Negl.	22.7 ± 2.6	4.1 ± 0.9
I (Conjugated)	12.8	29.8	12.5	19.9 ± 8.9	1.3 ± 0.5	Negl.	Negl.	8.2 ± 1.3	1.1 ± 0.4
II (Nonconjugated)	Negl.	Negl.	Negl.	2.5 ± 1.4	Negl.[c]	Negl.	Negl.	Negl.	Negl.
III[d]	1.7	Negl.	3.5	Negl.[c]	0.9 ± 0.4[c]	0.6 ± 0.1	Negl.	1.6 ± 0.3[c]	Negl.
IV[e]	10.2	3.9	24.6	2.5 ± 1.8[c]	1.7 ± 1.6[c]	1.4 ± 0.6	Negl.	0.9 ± 0.1[c]	Negl.
Unidentified ^{14}C	31.4	36.8	33.4	19.6 ± 1.8	16.7 ± 3.0	18.2 ± 5.6	55.8 ± 5.7	22.0 ± 2.0	13.2 + 1.9
Total ^{14}C	57.7	73.3	76.5	44.5 ± 7.7	23.9 ± 4.6	22.5 ± 6.6	56.8 ± 5.6	56.0 ± 5.1	18.9 ± 2.9

[a] The values presented, individual values for the three subjects and mean ± standard deviation for the number of animals shown in parentheses for each species, were obtained from the analysis of pools of urine or feces which accounted for at least 90% of the ^{14}C excreted by each route. In addition to the solvent systems shown in Table II, System 11–12 was used (particularly in the quantitation of Metabolite IV). [b] Negl. indicates that less than 0.5% of the dose was excreted. [c] Average of results from two animals. [d] Compound III was found mainly in the postglusulase extract, indicating its apparent excretion as a conjugate. [e] Compound IV was found mainly in the postglusulase extract of urine and in the preglusulase extract of feces.

identified, there is no evidence for hydroxylation of the 5-pyridyl ring in this species.

Metabolic cleavage of the diazepine ring of medazepam[10] (9) and nitrazepam[11] (10) to form both a benzophenone and a phenolic benzophenone hydroxylated *ortho* to the amino function has been found in the human. Therefore, the excretion of III and IV as metabolites of bromazepam in the human is not surprising. From the magnitude of conjugated IV excretion by the three women (4, 10, and 25% of the dose), it is evident that both 3-hydroxylation and benzoylpyridine formation must be considered important pathways in the human.

One novel pathway, the formation and urinary excretion of bromazepam N_4-oxide (II), occurred in the dog but not in the human, rat, or mouse. Although the reduction of the N_4-oxide function of a benzodiazepin-2-one 4-oxide (demoxepam) was shown to occur in the dog (11), oxidation of this diazepine ring nitrogen has not been reported until now. Interestingly, the pyridyl nitrogen of bromazepam was apparently not oxidized since the pyridyl *N*-oxide derivative was not detected as a metabolite in any of the four species. Whether or not N_4 oxidation is a metabolic reaction unique to bromazepam cannot be known until a thorough search for the corresponding N_4-oxide metabolites of other benzodiazepines is made.

The present quantitative findings are similar to recent data[12] obtained from two human subjects given 6 mg. of bromazepam-5-^{14}C intravenously; the subjects excreted 10 and 15% of the dose in the urine as I (mostly conjugated) and 16% as IV (again mostly conjugated) in 24 hr. In addition, the rat excreted I, III, and IV as urinary metabolites, with IV, at 4–5% of the dose, being excreted to a greater extent than the other two.

On the basis of the results obtained to date, it is apparent that the metabolites excreted by humans were also excreted to some extent by the dog, rat, and mouse, whereas only the dog excreted II.

[10] Medazepam is the active ingredient in Nobrium, F. Hoffmann–La Roche and Co., A. G., Basle, Switzerland.

[11] Nitrazepam is the active ingredient in Mogadon, F. Hoffmann–La Roche and Co., A. G., Basle, Switzerland.

[12] J. Raaflaub and J. Speiser, F. Hoffmann–La Roche and Co., Basle, Switzerland, unpublished data.

REFERENCES

(1) G. Zbinden and L. O. Randall, *Advan. Pharmacol.*, **5**, 254(1967).

(2) M. A. Schwartz, in "Benzodiazepines," S. Garattini, E. Mussini, and L. O. Randall, Eds., Raven Press, New York, N. Y., 1973, pp. 53–74.

(3) J. A. F. de Silva and J. Kaplan, *J. Pharm. Sci.*, **55**, 1278 (1966).

(4) M. A. Schwartz and E. Postma, *ibid.*, **61**, 123(1972).

(5) M. A. Schwartz, B. A. Koechlin, E. Postma, S. Palmer, and G. Krol, *J. Pharmacol. Exp. Ther.*, **149**, 423(1965).

(6) H. Sawada, *Experientia*, **28**, 393(1972).

(7) P. Bertagni, F. Marcucci, E. Mussini, and S. Garattini, *J. Pharm. Sci.*, **61**, 965(1972).

(8) S. F. Sisenwine, C. O. Tio, S. R. Shrader, and H. W. Ruelius, *Arzneim.-Forsch.*, **22**, 682(1972).

(9) M. A. Schwartz and J. J. Carbone, *Biochem. Pharmacol.*, **19**, 343(1970).

(10) J. Rieder and G. Wendt, in "Benzodiazepines," S. Garattini, E. Mussini, and L. O. Randall, Eds., Raven Press, New York, N. Y., 1973, pp. 99–127.

(11) M. A. Schwartz, E. Postma, and S. J. Kolis, *J. Pharm. Sci.*, **60**, 438(1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 16, 1973, from the *Department of Biochemistry and Drug Metabolism and the †Department of Medical Pharmacology, Hoffmann-La Roche Inc., Nutley, NJ 07110

Accepted for publication July 18, 1973.

The authors thank Dr. H. H. Kaegi and Mr. W. Burger for the synthesis of bromazepam-2-^{14}C and F. Hoffmann-La Roche and Co., Basle, Switzerland, for supplying bromazepam-5-^{14}C. They are also indebted to Mr. J. Vance for preparing the tablets containing bromazepam-5-^{14}C for use in the human study and to the Chemical Research Department for the synthesis of the authentic reference compounds.

▲ To whom inquiries should be directed.