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Chem. Pharm. Bull. 24(7)1637—1640(1976) UDC 615.216.2.015.4 : 615.076.9

A New Metabolite of Tetracaine[1]

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(Received July 24, 1975)

The metabolism of tetracaine in rats, mice, rabbits and horses has been investigated. It was found that a considerable amount of a new metabolite was excreted in the urine of all the animals examined. The metabolite was isolated as crystals from the urine of the tetracaine-administered rabbits and horses, and was characterized as tetracaine N-oxide on the basis of analytical and spectral data.

Tetracaine has been used as a local anesthetic for a long time and its metabolic fate in blood has been investigated by several workers.[3-5] It will be shown in this paper that a considerable amount of ingested tetracaine is oxidized to a new metabolite, tetracaine N-oxide, and excreted in the urine of horses as well as other experimental animals (rats, mice and rabbits).

Materials and Method

Chemicals——Tetracaine hydrochloride was obtained commercially and all solvents used in this study were of reagent grade.

Animals and Dosing——The animals used were ddN mice (30—50 g), Wistar rats (220—240 g), rabbits (2.5—3.0 kg) and horses (450—500 kg); only male animals were used.

Tetracaine hydrochloride was dissolved in 10% glucose solution and administered (5 mg/kg body weight) to the experimental animals subcutaneously (*s.c.*). A dose of 200 mg/head was administered *s.c.* to the horses.

Urine samples from the experimental animals other than horses were collected for 24 hr after the dosing. Since it was not practical to collect 24 hr urine samples from horses, spot samples were taken at 3, 6, 12, and 24 hr after the dosing.

Extraction of the Metabolites——After the administration of tetracaine hydrochloride, urine samples from each species were extracted with $CHCl_3$ at pH 9. The $CHCl_3$ phase was dried over anhydrous Na_2SO_4 and evaporated to dryness under a reduced pressure. Urine collected from each species before the medication was used as a negative control.

Thin-Layer Chromatography (TLC)——TLC was carried out on silica gel plates, 0.25 mm thick (Wakogel B-5FM), which were activated at 110° for 30 min. The solvent systems used were 95% ethanol (solvent A) and chloroform–MeOH–conc.NH_4OH (95: 5: 0.5; solvent B). The resulting chromatograms were examined by UV light and Dragendorff reagent. Tetracaine and its metabolite gave brown color with the reagent.

Preparation of Tetracaine N-Oxide——Five hundred milligrams of tetracaine was added to 30 ml of 10% H_2O_2. The mixture was stirred for 6 hr and allowed to stand overnight to separate white powders which were collected by filtration. The powders were purified on dissolving in ethyl acetate to give 200 mg of crystals, mp 115—116°. *Anal.* Calcd. for $C_{15}H_{24}O_3N_2 \cdot 3H_2O$: C, 53.87; H, 9.04; N, 8.38. Found: C, 53.86; H, 9.06; N, 8.50.

Results and Discussion

Detection of Urinary Metabolites

Metabolites in the urine of the tetracaine-administered animals were studied by TLC. It appeared from the chromatograms that a metabolite alone was excreted in the urine of the

1) Presented in part at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.
2) Location: *4-37, Kamiyoga, Setagaya-ku, Tokyo, 159, Japan.*
3) W. Kalow, *J. Pharmacol.*, 104, 112 (1952).
4) I. Porush, A. Shimamura, and L.T. Takahashi, *J. Pharm. Sci.* **54**, 1809 (1965).
5) F.F. Foldes, G.M. Davidson, D. Duncalf, and K. Shigeo, *Clin. Pharmacol. Therp.*, **6**, 328 (1965).

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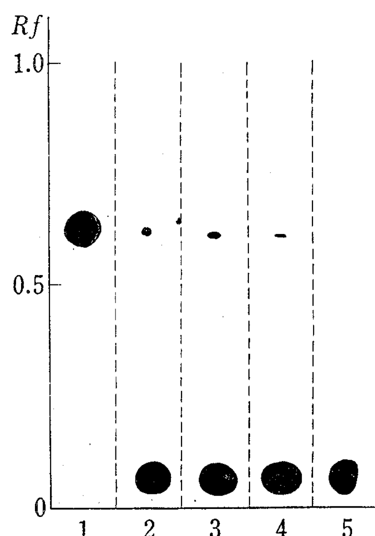

Fig. 1. Thin-Layer Chromatogram of the Urine Extracts of Various Animals after the Administration of Tetracaine (Solvent B)

1: tetracaine (control)
2: rats 4: rabbit
3: mice 5: horse

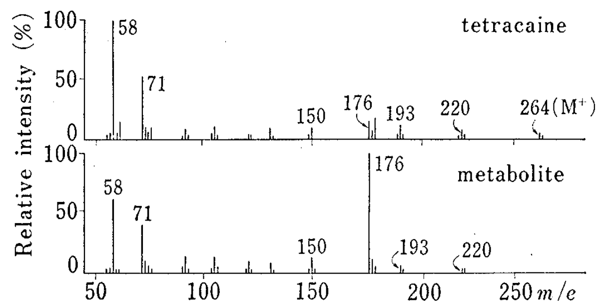

Fig. 2. Mass Spectra of Tetracaine and Its Metabolite

The spectra were measured by the direct sample introduction technique on a Hitachi RMU-7 mass spectrometer. The ionizing voltage was maintained 70 eV and the sample heating temperature were 150° (tetracaine) and 250° (metabolite).

horses, while the unchanged tetracaine and a metabolite were in the urine of the mice, the rats and the rabbits (Fig. 1).

Isolation of the Metabolite from the Urine of the Tetracaine-administered Rabbits

The 24 hr urine (about 1500 ml) from 5 rabbits which were administered *s.c.* dose of 5 mg/kg of tetracaine hydrochloride was passed through an Amberlite XAD-2 resin (20—50 mesh, Rohm & Haas Co.) column (3.5×40 cm). Most metabolites in the urine were adsorbed to the column. The column was washed with about 5 liters of water and then it was eluted with 2 liters each of 10, 20, 30, 50, 70 and 90% MeOH, successively. TLC of each fraction showed that a metabolite and unchanged tetracaine were found in 50 and 70% MeOH fractions. These fractions were combined, evaporated under a reduced pressure and submitted to TLC (20×20 cm) with solvent A. The band of the metabolite was detected under UV light, scraped off the plate and eluted with MeOH. The eluent was concentrated *in vacuo* and the residue was submitted to rechromatography using solvent B. The above described isolation procedures were repeated and the residue was crystallized from ethylacetate. Recrystallization from ethylacetate gave a few mg of the crystals of the metabolite, mp 115—116°.

Identification of the Metabolite

The ultraviolet absorption spectrum of the metabolite shows absorption maxima at 228 and 312 nm in 0.1N HCl and at 310 nm in 0.1N NaOH. This spectrum was similar to that of tetracine (maxima at 225, 312 nm in acid and at 308 nm in alkali) and suggested that the disubstituted phenyl skeleton should remain intact in this metabolite.

Mass spectrum of the crystals of metabolite is shown in Fig. 2. As the temperature of the sample heater was required to be high (250°), no molecular ion was observed and the fragment ions appeared similar to those of tetracaine. From this observation it may be considered that thermal degradation of the parent amine occurred during the measurement. UV and mass spectra of the metabolite indicate that the *p*-N-butylaminobenzoyl and dimethylaminoethyl groups remain intact.

In the nuclear magnetic resonance spectrum of the metabolite, the signals due to the dimethylaminoethyl group of tetracaine at 2.30 (6H, t, N–Me_2), 2.64 (2H, t, CH_2–N) and 4.35

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ppm (2H, t, CH_2) were shifted to at 3.28, 3.60 and 4.85 ppm, respectively, and a new signal corresponding to two protons appeared at 2.25 ppm. This signal disappeared when D_2O was added. All other signals of the metabolite, corresponding to aromatic protons of *p*-substituted phenyl group (6.4—7.9 ppm), a butyl group (0.93, 1.50 and 3.12 ppm) and a secondary amino group (4.08 ppm) remained at the same ppm as those of tetracaine.

These results strongly suggested that the metabolite would be tetracaine N-oxide hydrate. Then, the structure was definitely established by direct comparison with the authentic sample (see experimental section).

Determination of Tetracaine N-Oxide in the Urine

Concentration of the excreted tetracaine and its N-oxide in the urine of the rabbits and the horses were determined according to the method of Fukuda and Momose,[6] as shown in Table I and II.

Table I. Concentrations of Tetracaine and Its N-Oxide in Rabbit Urine after Dosing[a]

Rabbit No.	Urine Vol 0—24 hr, ml	Tetracaine		Tetracaine N-oxide	
		μg/ml	%[b]	μg/ml	%[b]
1	320	—[c]	—	5.8	14.1
2	305	—	—	4.2	9.7
3	370	0.5	1.4	3.6	10.1
4	300	0.4	0.9	4.5	10.2

a) Fifteen milligrams/kg were administered.
b) percentage of dose
c) not detected

Table II. Concentration (μg/ml) of Tetracaine N-Oxide Horse Urine after Dosing[a]

Hours after dosing	Horse					
	1	2	3	4	5	6
3.0	6.1	3.4	9.7	3.2	6.5	7.0
6.0	1.6	2.5	3.0	1.8	2.0	1.7
12.0	0.2	0.4	0·5	0.3	—[b]	—
24.0	—	—	—	—	—	—

a) Two hundred milligrams/head were administered.
b) not detected

The urinary excretion of the unchanged local anesthetics of ester type is negligible because of their rapid breakdown in the body. Brodie, *et al.*[7] studied the recovery of procaine from the urine of man administered with intravenous procaine. Only approximately 2% of the administered procaine were excreted unchanged in the urine within 24 hr.

It has been believed to date that tetracaine is largely metabolized to *p*-butylaminobenzoic acid and diethylaminoethanol by the esterase in serum.[8] From the present study it became evident that tetracaine was oxidized to tetracaine N-oxide in various animals. The amount of the N-oxide in the urine of the rabbits was about 11% of the total dose administered. Moreover, our results obtained by spot sampling indicated that tetracaine N-oxide persists in the urine of the horses for the first 12 hr if measured by the described method. On the other

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7) B.B. Brodie, P.A. Lief, and R. Post, *J. Pharmacol.*, **94**, 359 (1948).
8) J.R. Dipalma, "Drill's Pharmacology in Medicine," 4th ed., Mc Graw-Hill Book Co., New York, 1971, p. 196.

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hand, oxidation of tetracaine to tetracaine N-oxide by the urine or the extraction solvents were not occurred in our experimentals. This result shows that the tetracaine N-oxide obtained from the urine samples is not an artificial product.

Kalow[3] reported that tetracaine was also hydrolyzed by the procaine esterase in serum, and that it was hydrolyzed four to five times more slowly than procaine. On the other hand, it was known that the procaine esterase activity in the blood of various animals including horses were lower than man.[9,10] Therefore, presence of the tetracaine N-oxide in the urine of various animals, it may be considered that tetracaine was slowly hydrolyzed in body.

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Chem. Pharm. Bull. 24(7)1640—1645(1976)

UDC 547.445.04 : 547.785.5.04

Studies on Ketene and Its Derivatives. LXXIX.[1] Reaction of Diketene with Benzimidazole and 2-Methylbenzimidazole Derivatives

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(Received August 13, 1975)

Reaction of benzimidazole and N-acetylbenzimidazole with diketene in acetic acid or acetic anhydride at room temperature resulted in the formation of 2-acetonyl-1,3-diacetyl-2,3-dihydrobenzimidazole (II), and 5-acetyl-4a,5-dihydropyrido[1,2-*a*]benzimidazol-1,3(2H,4H)-dione. On the other hand, reactions of 2-ethoxycarbonylmethylbenzimidazole (XIIIa), 2-carbamoylmethylbenzimidazole (XIIIb), 2-cyanomethylbenzimidazole (XIIIc) with diketene in acetic acid at room temperature resulted in the formation of 4-substituted-3-methylpyrido[1,2-*a*]benzimidazol-1(5H)-one (XIIIa—c) in good yields, respectively.

We have previously reported that[3,4] reaction of diketene with pyridine afforded the Wollenberg compounds. This reaction involves the addition of diketene to the C=N double bond of pyridine followed by acetoacetylation and cyclization to give the pyronoquinolizine derivative. Similarly, aromatic N-heterocycles such as quinoline, isoquinoline, and phenanthridine reacted with diketene to give Wollenberg type compounds.[5] Recently, Yamanaka, *et al.*[6] reported the reaction of isoquinoline with diketene in acetic acid to afford 1-acetonyl-2-acetyl-1,2-dihydroisoquinoline.

However, reactions with five membered aromatic N-heterocycles, in view of the above reactions, have not been studied yet. In the present paper we wish to report the reaction of diketene with benzimidazole and its derivatives.

Reaction of Diketene with Benzimidazole in Acetic Acid

When benzimidazole was allowed to react with diketene in acetic acid or acetic anhydride, colorless prisms of mp 140—141°, $C_{14}H_{16}O_3N_2$ (II), were obtained together with pale yellow

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2) Location: *Aobayama, Sendai, 980, Japan.*
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4) O. Wollenberg, *Chem. Ber.*, **67**, 1675 (1934).
5) T. Kato and Y. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **14**, 752 (1966).
6) H. Yamanaka, T. Shiraishi, and T. Sakamoto, *Heterocycles*, **3**, 1069 (1975).

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