(14) A. O. Obaseki, M.S. thesis, University of Wisconsin, Madison, Wis., 1980.
(15) N. K. Pandit, A. O. Obaseki, and K. A. Connors, *Anal. Chem.*, **52**, 1678 (1980).
(16) A. R. Fersht and W. P. Jencks, *J. Am. Chem. Soc.*, **92**, 5432, 5442 (1970).
(17) A. Hassner, L. R. Krepski, and V. Alexanian, *Tetrahedron*, **34**, 2069 (1978).
(18) J. F. Coetzee and I. M. Kolthoff, *J. Am. Chem. Soc.*, **79**, 6110 (1957).
(19) M. K. Chantooni and I. M. Kolthoff, *ibid.*, **89**, 1582 (1967).
(20) E. Guibe-Jampel, G. Le Corre, and M. Wakselman, *Tetrahedron Lett.*, **1979**, 1157.
(21) S. A. Lapshin, V. A. Dadali, Y. S. Simanenko, and L. M. Litvinenko, *Zh. Org. Khim.*, **13**, 586 (1977).

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Metabolism of Tocainide in the Rat

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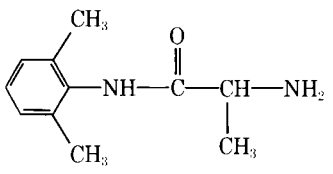
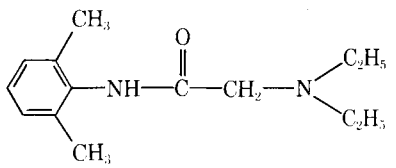
Abstract □ The metabolism of tocainide, an oral antiarrhythmic agent, was studied in male Wistar rats following oral administration of 15 mg/kg of tocainide hydrochloride. Qualitative and quantitative identification of the metabolites in urine was carried out by GC–mass spectrometry and electron capture detector gas chromatography. About 15–20% of the dose administered was excreted as intact drug in the urine. An additional 20% of the dose was present as acid hydrolysable conjugates. Enzymatic hydrolysis (β-glucuronidase) revealed half of the acid hydrolysable conjugates to be a glucuronide. The enzyme mediated hydrolysis was blocked by its specific inhibitor saccharo-1,4-lactone. *N*-acetyl tocainide, an oxidatively deaminated tocainide, an aldehyde adduct of tocainide, and a cyclic hydantoin derivative of tocainide were also identified as metabolites in the urine samples.

Keyphrases □ Tocainide—oral antiarrhythmic agent, study of metabolism, rats □ Metabolism—of tocainide, after oral administration in rats □ GC–mass spectrometry—determination of metabolism of tocainide, rats

Tocainide, 2-amino-2′,6′-propionoxylidide (I), a structural analog of lidocaine (II), is an experimental antiarrhythmic agent, presently undergoing clinical trials (1–4). In humans, tocainide is completely absorbed following oral administration (5), and most of the orally or intraperitoneally administered dose, up to 15 mg/kg, of tocainide is absorbed in rats (6). Kinetic studies carried out in rats revealed the presence of dose dependent elimination of tocainide ≥20 mg/kg (6). Identification of the pathways contributing to the nonlinearity was not possible previously due to the lack of information on the metabolism of tocainide in rats. The present report describes the metabolic fate of tocainide in male Wistar rats.



I



II

EXPERIMENTAL

Animal Experimentation—Adult male Wistar rats with an average weight of 200 g (190–210 g) were used in the present study (animals were obtained from the University of British Columbia animal care unit). The animals were maintained in 0.41- × 0.34- × 0.18-m metallic cages (6 rats/cage) in a controlled environment (24°) for at least 3 days prior to experimentation. Wooden shavings were used as bedding under elevated cages (18 cm from the bottom of the cages). The photoperiod was controlled to provide a dark cycle from 8 pm to 6 am and a light cycle from 6 am to 8 pm. The animals had access *ad libitum* to food (rat chow) and water during this period. The animals were fasted for 8–10 hours prior to and during the experiments; however, water was allowed *ad libitum*.

An aqueous solution of tocainide hydrochloride[1] or 3′,4′,5′-trideuterated tocainide hydrochloride was given orally (stomach tube) to animals under light ether anesthesia. Dosed animals were housed in separate stainless steel metabolic cages (24.5 × 17.5 × 18 cm) with facilities for collecting urine samples free of fecal contamination, into an amber-colored bottle. Twenty-four hours postdose, the sides of the cage and the collecting funnel were washed three times with distilled water to recover all excretory products. The urine samples were stored in the freezer until analyzed.

Analytical Methods—Measurement of intact tocainide in urine samples was carried out by using an electron capture detector gas chromatographic method previously reported (7).

The presence of conjugated tocainide in urine samples was determined by acidic and enzymatic hydrolysis. For the acid hydrolysis, 1 ml of urine was incubated with 1 ml of 1 *N* HCl in a sealed glass ampul for 1 hr at 100°. An aliquot of this solution was used for the analysis of tocainide.

Preliminary studies with enzymes from different sources suggested maximal hydrolysis of the conjugates to occur when bovine liver β-glucuronidase was used. The enzymatic hydrolysis involved the addition of 1 ml of 1 *M* acetate buffer (pH 5.0) and 0.2 ml of β-glucuronidase[2] to 1 ml of urine and incubation of the mixture at 37° for 24 hr. At the end of the incubation period the samples were analyzed for intact tocainide. Additional experiments were also carried out to determine the effect of saccharo-1,4-lactone[3], 0.1 m*M* final concentration, on the enzyme mediated hydrolysis of tocainide conjugates.

Multiple extraction of the urine samples (25–30 ml), without any pH adjustments or at pH 9.0 (ammonium hydroxide and ammonium carbonate) or at pH 9.0 and 12.0 using sodium hydroxide, was carried out

[1] Astra Pharmaceutical Products, Framingham, Mass.
[2] Glucurase, Sigma Chemical Co., St. Louis, Mo.
[3] Calbiochem, La Jolla, Calif.

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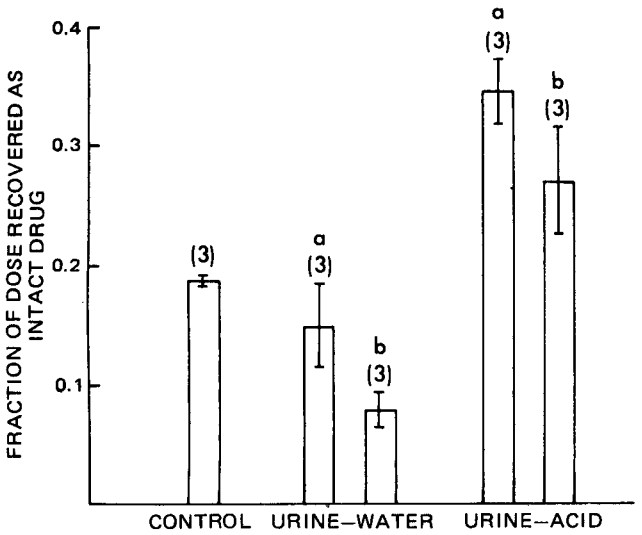


Figure 1—*Acid hydrolysis of tocainide conjugate in oven at 100°. Data presented as mean ± SD. The number in the parentheses refers to the number of experiments carried out.* a = *1-hr incubation time;* b = *2-hr incubation time.*

in a separatory funnel with an equal volume of methylene chloride[4]. In another experiment the urine samples (25 ml) were freeze-dried and the residue was dissolved in methanol (5 ml). The methanolic solution was then filtered and concentrated to ~1 ml under nitrogen at room temperature. Methylene chloride and methanolic extracts of urine were analyzed by GC–mass spectrometry[5] to determine the presence of other metabolites of tocainide. The GC component of the GC–mass spectrometric system was equipped with a 1.8-m × 2-mm i.d. coiled glass column packed with Silar 10 C[6] coated on ChromoSorb W–HP (100–120 mesh). The injection port temperature was 170° and the oven temperature was programmed from 100 to 250° at 10°/min. The temperature was held at 250° until the end of the analysis. Helium was used as a carrier gas at a flow rate of 30 ml/min. The temperature of the separator was 280°. The ionization energy was 70 eV and the accelerating voltage was 820 V. The mass spectrometer was operated in the scanning mode and the mass spectra were recorded continuously at 5-sec intervals during an entire run.

Methylation of the extracted samples was carried out by the addition of 100 μl of trimethylanilinium hydroxide[7] and subsequent incubation at 100° for ~10 min.

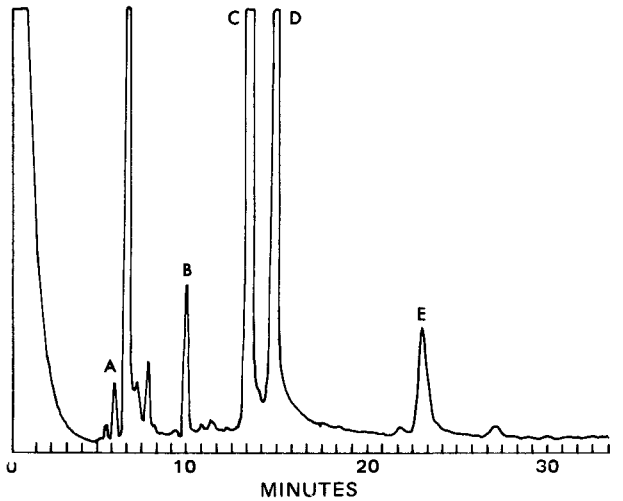


Figure 2—*GC–mass spectrometer total ion current trace of methylene chloride extract of urine sample using Silar 10-C column.*

[4] Caledon Laboratories, Ontario, Canada.
[5] Varian Mat III GC/MS with Data Base.
[6] Applied Science Laboratories, State College, Pa.
[7] Methelute, Pierce Chemical Co., Rockford, Ill.

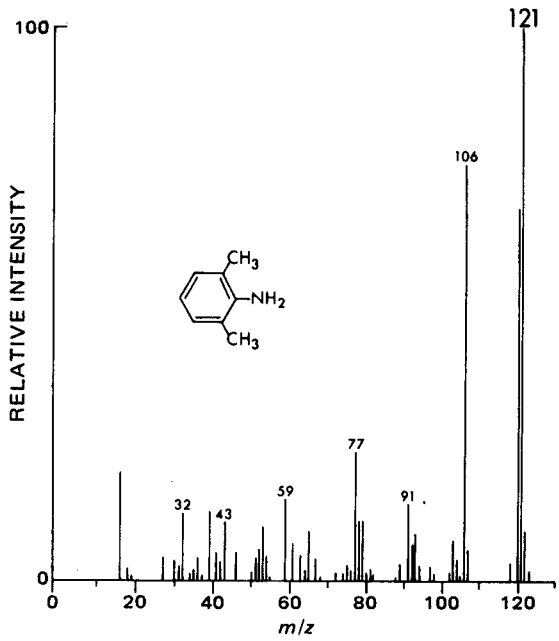


Figure 3—*Mass spectrum and the postulated structure of a metabolite of tocainide (III). This compound corresponds to peak A of Fig. 2.*

RESULTS

The results of the acid hydrolysis studies are summarized in Fig. 1. Incubation of the urine samples with hydrochloric acid liberated tocainide corresponding to >15% of the dose administered. Control experiments carried out in the absence of any acid revealed nearly a 20% loss of tocainide during the experimental process following 1 hr of hydrolysis. This would imply that the actual amount of tocainide present as conjugate would be >20% of the administered dose. Longer periods of incubation (2 hr) resulted in further reduction in the recovery of tocainide from the conjugates. This could be due to the breakdown of tocainide under the conditions of hydrolysis employed in this study.

Enzymatic hydrolysis (β-glucuronidase) of the urine, revealed the presence of nearly 7% of the dose administered as a glucuronide conjugate. The enzymatic hydrolysis mediated by β-glucuronidase was blocked by its specific inhibitor saccharo-1,4-lactone, thus confirming the conjugate to be a glucuronide. The percent of dose recovered as intact tocainide in untreated urine, β-glucuronidase treated urine, and urine treated with β-glucuronidase and saccharo-1,4-lactone were 17.5 ± 1.1 (n = 3), 24.0 ± 0.7 (n = 3), and 17.0–17.7 (n = 2).

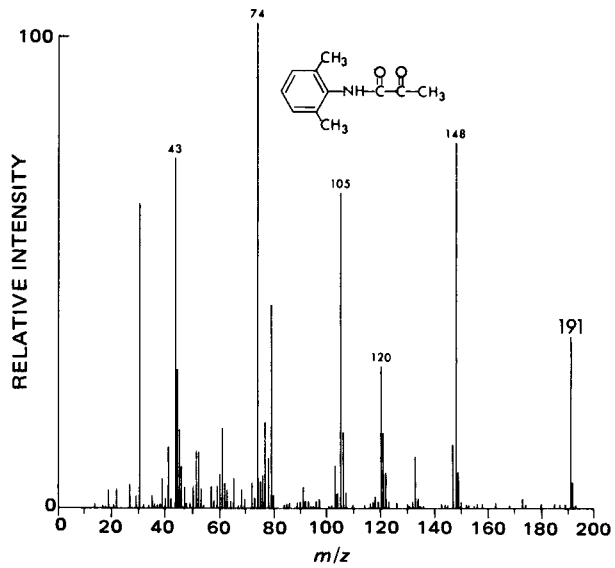


Figure 4—*Mass spectrum and the postulated structure of a metabolite of tocainide (IV). This compound corresponds to peak B of Fig. 2.*

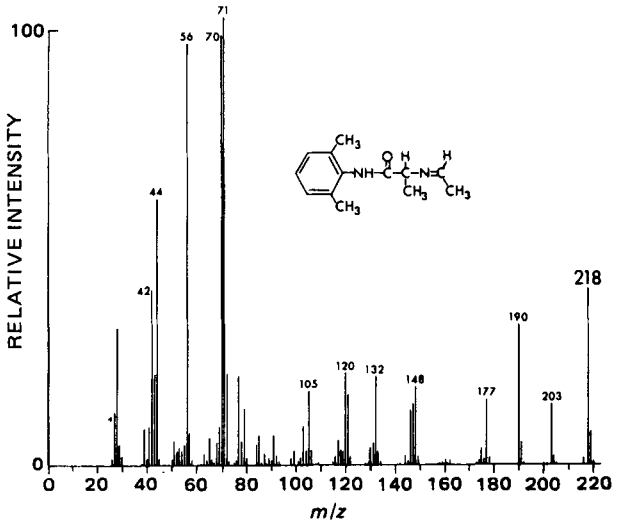


Figure 5—*Mass spectrum and the postulated structure of a metabolite of tocainide (V). This compound corresponds to peak C of Fig. 2.*

Other Metabolites of Tocainide—The chromatogram obtained following injection of the methylene chloride extract of tocainide onto a Silar 10 C column of the GC–mass spectrometer is shown in Fig. 2. Confirmation as to the origin of peaks A, B, C, D, and E were obtained from studies using trideuterated tocainide. The presence of molecular ions at *m/z* values of (M ± 3) following analysis of the urine of rats administered trideuterated tocainide pointed to tocainide as the source of these peaks. Since all three deuterium labels were present in all of these peaks, it was inferred that these peaks result from modification of the structure by metabolism at the side chain. The other peaks present in the chromatogram were from endogenous materials in the urine.

Peak A—Peak A appeared in the methylene chloride extract of urine samples at pH 9.0 as well as in the methanolic extract of the freeze-dried urine samples with a retention time of ~5 min. The mass spectrum and the postulated structure of the compound (III) are shown in Fig. 3. The molecular ion was at *m/z* 121 (124) and the base peak was at *m/z* 106 (109). The compound, however, seems to account for <5% of the dose administered.

Peak B—Peak B was present in the methylene chloride and the methanolic extract of the urine samples. The molecular ion at *m/z* 191 corresponds to a composition of $C_{11}H_{13}NO_2$ (Fig. 4). The presence of peaks at *m/z* 105, 120, and 148 suggested intactness of the carboxy-xylidide moiety of tocainide. The abundant peak at *m/z* 43 corresponded to C_2H_3O. This compound (IV) eluted at about 10 min in the chromatogram and was also present in small quantities in the urine samples.

Peak C—Peak C was present in the following extracts of rat urine: (*a*) direct extraction of urine with methylene chloride; (*b*) extracts of urine at pH 9.0 (ammonium carbonate and ammonium hydroxide) with

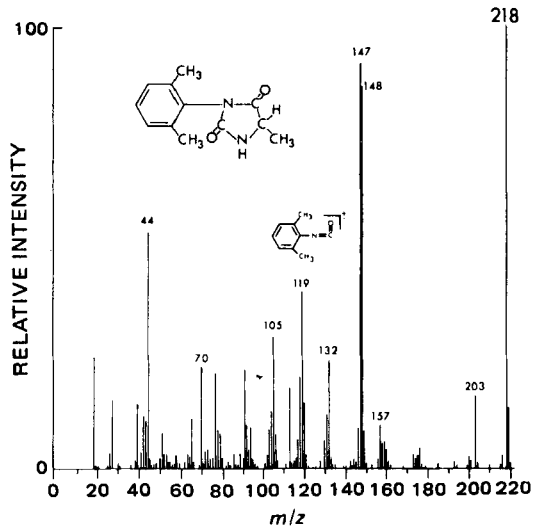
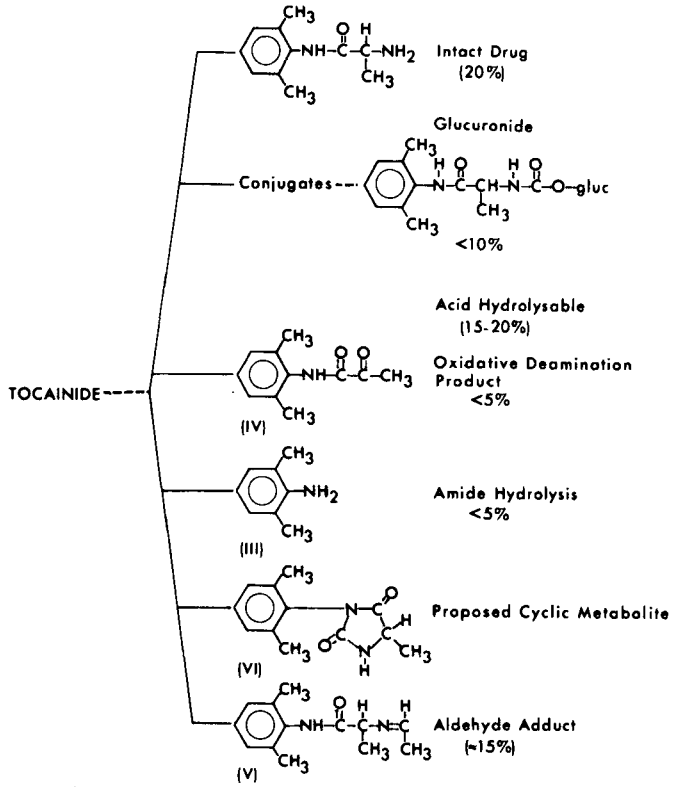


Figure 6—*Mass spectrum of the cyclic compound VI.*



Scheme I—Proposed metabolic products of tocainide. The structures and the relative amounts of the proposed metabolites of tocainide. The percentage values are the percent of dose excreted as a particular metabolite.

methylene chloride; (*c*) extracts of acid hydrolyzed urine extracted with methylene chloride at pH 9.0 (ammonium carbonate and ammonium hydroxide); (*d*) methanolic extracts of freeze-dried urine.

When subjected to mass spectral analysis, this compound revealed a molecular ion at *m/z* 218 suggesting the addition of 26 mass units to the parent compound (Fig. 5). The presence of *m/z* 221 in studies using the trideuterated tocainide suggested that all three deuterium atoms are present in this compound. The persistence of the deuterium atoms in the aromatic ring was also evident from the presence of the following peaks: *m/z* 105 (108), 120 (123), 132 (135), 148 (151), 177 (180), 190 (193), and 203 (206). The presence of ions of the following composition: C_8H_9 (105), $C_8H_{10}N$ (120), $C_9H_{10}NO$ (148), $C_{11}H_{15}NO$ (177), and $C_{11}H_{14}N_2O$ (190) indicated the intactness of the major skeleton to tocainide. The peak at *m/z* 203 (M −15) suggested the presence of a methyl group as part of the structural modification. Ions of *m/z* 28 (C_2H_4), 42 (C_2H_4N), and 70 (C_4H_8N) in samples obtained following administration of tocainide or trideuterated tocainide indicated that these fragments must arise from the side chain. The fragmentation, thus observed, corresponds to an aldehyde adduct (V) of tocainide. The adduct appears to be one of the major metabolic pathways of tocainide in rats. Preliminary estimates show nearly 15% or more of the dose of tocainide to be eliminated *via* this metabolic pathway.

Peak D—Peak D in Fig. 2 corresponded to intact tocainide. Nearly 20% of the dose administered appeared in the urine as intact drug in the 24-hr period following drug administration.

Peak E—Direct extraction of the urine samples with methylene chloride or extraction, following adjustment of the urine pH to 9.0 with ammonium carbonate and ammonium hydroxide or sodium hydroxide, resulted in peak E which eluted at ~23 min.

The parent ion at *m/z* 218 (221) suggested this compound to be a product from tocainide with the addition of 26 mass units (Fig. 6). Ions of the composition C_8H_9 (105) and C_8H_9N (122) indicate the xylidine moiety of tocainide to be intact. The presence of a peak at *m/z* 119 (122) and not at 120 (123) indicates the xylidine nitrogen to be tertiary. The abundance of *m/z* at 147 (150) indicates that the carboxylidine moiety of tocainide is also intact but for the loss of one hydrogen atom, further suggesting that the amide nitrogen is tertiary and possibly part of a cyclic system. The presence of a methyl group is indicated by *m/z* at 203 (M −15). The compound could be methylated (implying the presence of a replaceable hydrogen) but failed to form any derivative with heptafluo-

robutyric anhydride[7]. The mass fragmentation pattern of this compound was identical with the fragmentation pattern of 3-(2,6-xylyl)-5-methyl hydantoin (VI)[1].

The relative amounts of VI in the methanolic extract was quite high as compared to the methylene chloride extract. This could be due to the generation of the cyclic compound from the glucuronide conjugate that was dissolved in methanol.

DISCUSSION

The metabolic products of tocainide are summarized in Scheme I. Following oral, intravenous, or intraperitoneal administration of tocainide (15 mg/kg) to rats, ~15–20% of the dose is excreted as intact drug over a 24-hr period (6). Nearly 44% of an oral dose was excreted as free tocainide in humans while an acid or β-glucuronidase hydrolyzable metabolite accounted for nearly 23% of the tocainide dose administered (8). A novel biotransformation pathway was put forth to explain the disposition of tocainide in humans (8). The proposed structure of this metabolite was tocainide carbamoyl O-β-D-glucuronide. During preliminary work in rats, the authors identified the presence of a similar conjugate of tocainide in rat urine. Acid hydrolysis (nonspecific) as well as enzyme (β-glucuronidase) hydrolysis (specific for glucuronide conjugate) of the urine samples were carried out to determine the nature of the conjugates.

Use of β-glucuronidase corresponding to 1000 U of enzyme resulted in maximal release of tocainide from its conjugates. Addition of trace quantities (10 μl) of chloroform as suggested previously (9) did not result in any increase in the yield of tocainide from the conjugates. Under the conditions of the enzymatic hydrolysis (24 hr at 37°), no detectable degradation of tocainide was observed. Enzymatic hydrolysis resulted in the liberation of tocainide, equivalent to ~7–10% of the dose administered. Addition of saccharo-1,4-lactone into the incubation medium prevented the enzyme mediated hydrolysis of the conjugate, thus indicating the conjugate to be a glucuronide.

Acid hydrolysis of the urine sample resulted in the liberation of tocainide corresponding to ~20% of the administered dose. Simultaneous experiments showed that there is a loss of nearly 20% of tocainide in the control samples (urine sample–distilled water instead of 1 *N* HCl). This suggested that one is probably underestimating the amount of acid hydrolysable conjugates by the method used, due to the degradation of tocainide at the pH and temperature used for acid hydrolysis. Further proof for this hypothesis came about from the stability studies of tocainide carried out at different temperatures and in the presence of acid of different strength. An increase in the strength of the acid (1 *N* to 12 *N*) as well as the time of incubation resulted in a progressive reduction in the amount of tocainide remaining in an aqueous solution of the drug at 100°. Consistent with this observation, incubation of the urine samples for a longer period of time (>2 hr) or with acids of higher normality (2 *N*, 6 *N*, or 12 *N*) provided decreased estimates of the conjugates present in urine. The use of higher temperatures (autoclaving of the urine sample with water for 35 min) also degraded tocainide, but to a greater extent than that seen at 100°.

During these experiments tocainide was observed to degrade at a faster rate in the presence of urine. To determine the possible role of urea in this degradation, tocainide was incubated with 8 *M* urea for 1 hr at 100°. At these higher concentrations of urea marked degradation of tocainide was observed. It is possible that the degradation of tocainide at higher temperature is to some extent related to the urea in the urine sample. Hence, it should be noted that the estimates of the acid hydrolysable conjugates carried out in the present study are only an approximation of the actual amount present in the urine.

Experiments carried out under optimal conditions for the enzymatic hydrolysis of the glucuronide conjugates of tocainide did not account for all of the conjugates observed following acid hydrolysis. It would appear that there are other conjugates of tocainide which also yield tocainide following acid hydrolysis. Acetylation is a known metabolic pathway for the elimination of primary amines (10). GC–mass spectrometric analysis has identified the presence of small quantities of *N*-acetyl derivative of tocainide in the rat urine. It is also possible that tocainide is eliminated by an as yet unidentified conjugation pathway.

Cleavage of the amide group (amide hydrolysis) has been shown to be a metabolic pathway for the elimination of a number of lidocaine analogs (11–14). In the present study, small quantities of 2,6-dimethylaniline were observed as a metabolite of tocainide (Fig. 3).

Condensation of a metabolite of lidocaine with acetaldehyde generates N^1-ethyl-2-methyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone (14). Such condensation reactions can occur under very mild reaction conditions (14). An aldehyde adduct of tocainide was observed in relatively large quantities (nearly 15% of the dose administered in the present study). The adduct was present in the methylene chloride and the ether extract of the urine samples.

A small quantity of oxidatively deaminated tocainide was also proposed to be present as a metabolite in rats (Fig. 4).

In addition to being formed from an unstable metabolite of tocainide, the cyclic compound was also present in small quantities in the urine sample as inferred by its presence in the methylene chloride extract of the urine sample at pH < 9.0 (15). The presence of a cyclic hydantoin metabolite of etidocaine in humans was reported previously (11). This hydantoin metabolite was a close structural analog of the cyclic metabolite observed in the present study.

Thus, the metabolic pathway shown in Scheme I accounts for nearly 60–70% of the dose of tocainide. Even though not identified in the present study, hydroxylation of the aromatic ring is a possible additional pathway of elimination of tocainide.

REFERENCES

(1) D. G. McDevitt, A. S. Nies, G. R. Wilkinson, R. F. Smith, R. L. Woosley, and J. A. Oates, *Clin. Pharmacol. Ther.*, **19,** 396 (1976).

(2) R. A. Winkle, P. J. Meffin, J. W. Fitzgerald, and D. C. Harrison, *Circulation*, **54,** 884 (1976).

(3) R. L. Woosley, D. G. McDevitt, A. S. Nies, R. F. Smith, G. R. Wilkinson, and J. A. Oates, *ibid.*, **56,** 980 (1977).

(4) P. J. Meffin, R. A. Winkle, T. F. Blaschke, J. Fitzgerald, and D. C. Harrison, *Clin. Pharmacol. Ther.*, **22,** 42 (1977).

(5) D. Lalka, M. B. Meyer, B. R. Duce, and A. T. Elvin, *ibid.*, **19,** 757 (1976).

(6) R. Venkataramanan and J. E. Axelson, *J. Pharmacol. Exp. Ther.*, **215,** 231 (1980).

(7) R. Venkataramanan and J. E. Axelson, *J. Pharm. Sci.*, **67,** 201 (1978).

(8) A. T. Elvin, *et al.*, *ibid.*, **69,** 47 (1980).

(9) L. G. Richards, M. C. Castle, and G. L. Lage, *Drug Metab. Dispos.*, **5,** 469 (1977).

(10) H. G. Mandel, in "Fundamentals of Drug Metabolism and Drug Disposition," B. N. La Du, H. G. Mandel, and E. L. Way, Eds., Williams & Wilkins, Baltimore, Md. 1971, p. 149.

(11) D. J. Morgan, M. P. Smyth, T. Thomas, and J. Vine, *Xenobiotica*, **7,** 365 (1977).

(12) J. Thomas, D. Morgan, and T. Vine, *ibid.*, **6,** 39 (1976).

(13) T. J. Goehl, J. B. Davenport, and M. J. Stanley, *ibid.*, **3,** 761 (1973).

(14) S. D. Nelson, G. D. Breck, and W. F. Trager, *J. Med. Chem.*, **16,** 1106 (1973).

(15) R. Venkataramanan and J. E. Axelson, *Xenobiotica*, **11,** 259 (1981).

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