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Kinetics and Mechanism of Hydroxy Group Acetylations Catalyzed by *N*-Methylimidazole

NIVEDITA K. PANDIT * **and KENNETH A. CONNORS** ˣ

Received July 6, 1981, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.* Accepted for publication September 4, 1981. * Present address: Boehringer-Ingelheim, Ridgefield, CT 06877.

Abstract □ The kinetics of acetylation of alcohols by acetyl chloride and acetic anhydride, with *N*-methylimidazole as the catalyst, were studied in acetonitrile solution at 25°; some measurements were also made with 4-dimethylaminopyridine as the catalyst. The acetic anhydride–*N*-methylimidazole system proceeds entirely by a general base catalysis, whereas the acetyl chloride–*N*-methylimidazole system reacts entirely *via* a nucleophilic route, with the intermediate formation of the *N*-acylated catalyst. The reaction of this intermediate with the alcohol is general base catalyzed. The acetyl chloride–4-dimethylaminopyridine system also reacts *via* the nucleophilic route. In the acetic anhydride–4-dimethylaminopyridine system a small fraction of the intermediate was detected. The acetic anhydride–*N*-methylimidazole system was studied in *n*-propanol–acetonitrile solvent mixtures; no spectral evidence for intermediate formation was seen. However, the hydrolysis reaction in acetic anhydride–*N*-methylimidazole, studied over a wide range of water–acetonitrile mixtures, revealed a change in mechanism from general base in dry acetonitrile to a solely nucleophilic route at high water concentrations.

Keyphrases □ Acetylation—kinetics and mechanism, hydroxy groups catalyzed by *N*-Methylimidazole □ Kinetics—mechanism of hydroxy group acetylations, catalyzed by *N*-Methylimidazole □ *N*-Methylimidazole—kinetics and mechanism of hydroxy group acetylations, catalyst □ 4-Dimethylaminopyridine, acetylation catalyst.

Acetylations of hydroxy compounds are usually carried out with acetic anhydride or acetyl chloride as the acylating agent. Pyridine is commonly used as a catalyst. Several catalysts more powerful than pyridine are now available, most notably 4-dimethylaminopyridine, which was developed by Steglich and Höfle (1–3) as a catalyst for synthetic acetylations, and has since been applied to analytical acetylations (4–7). This study introduced *N*-methylimidazole as an analytical acylation catalyst (8–11). The relative catalytic effectiveness of pyridine–*N*-methylimidazole–4-dimethylaminopyridine is about $1:3 \times 10^2:1.7 \times 10^4$, respectively (12), in aprotic solvents.

Although the mechanisms of acyl transfer reactions have been well studied, most investigations have been of acyl transfer to water, that is, of the hydrolysis of carboxylic acid derivatives in aqueous solution. These kinetic and mechanistic results, therefore, may not be applicable to acylation reactions carried out in aprotic solvents. Because synthetic and analytical acylations usually employ nonaqueous media, it is important to understand the course of the reactions and the role of the catalyst in these systems, and several laboratories have described studies of the newer catalysts (particularly of 4-dimethylaminopyridine) in aprotic solvents. The mechanism of the catalysis is not well understood, and there is disagreement among the interpretations of reactivity data for these systems; these viewpoints are cited in the later discussion.

To achieve an understanding of the mode of catalysis by *N*-methylimidazole, the present study was designed to allow systematic kinetic measurements to be made over a wide range of conditions. The reaction systems are relatively simple but represent practical situations. Acetic anhydride and acetyl chloride are the two acylating agents studied. *N*-methylimidazole is the catalyst, though some observations were also made on 4-dimethylaminopyridine because of its importance; hence, four separate combinations of acylating agent and catalyst were studied. The acyl acceptor was an alcohol or water, and the solvent was acetonitrile, modified in some cases by the addition of alcohol or water.

EXPERIMENTAL

Materials—Acetyl chloride and acetic anhydride, analytical reagent grade[1], were used directly. *N*-Methylimidazole[2] was distilled at 10–12 mm Hg pressure; atmospheric bp 199°. 4-Dimethylaminopyridine[2] was recrystallized from *n*-hexane; mp 113°. The alcohols were of analytical reagent quality and were used directly; their purities were checked by gas chromatography.

[1] Mallinckrodt Chemical Co.
[2] Aldrich Chemical Co.

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Acetonitrile, technical grade[2], was refluxed over phosphorus pentoxide for 1 hr, then distilled from phosphorus pentoxide at 80–81°. The water content of this product was ~0.003% as determined by visual Karl Fischer titration.

Kinetic Procedure—A typical kinetic run was carried out as follows: Solutions of appropriate concentrations of the acetylating agent, the catalyst, and the hydroxy compound were prepared in acetonitrile and equilibrated at 25 ± 0.1°. Acetonitrile (4.0 ml), 3.0 ml of the catalyst solution, and 1.0 ml of the acetylating agent solution were added to a 10-ml volumetric flask. The reaction was initiated by adding 2.0 ml of the solution of hydroxy compound. The well-mixed solution was transferred to a 1-cm spectrophotometer cell, and the absorbance was monitored at a selected wavelength as a function of time[3]. The absorbance at the completion of reaction (A_∞) was measured after the lapse of ~10 half-lives.

All measurements were made at 25.0 ± 0.1°.

RESULTS AND DISCUSSION

Kinetic Scheme—The design and interpretation of the kinetic experiments were based on the model represented by Scheme I:

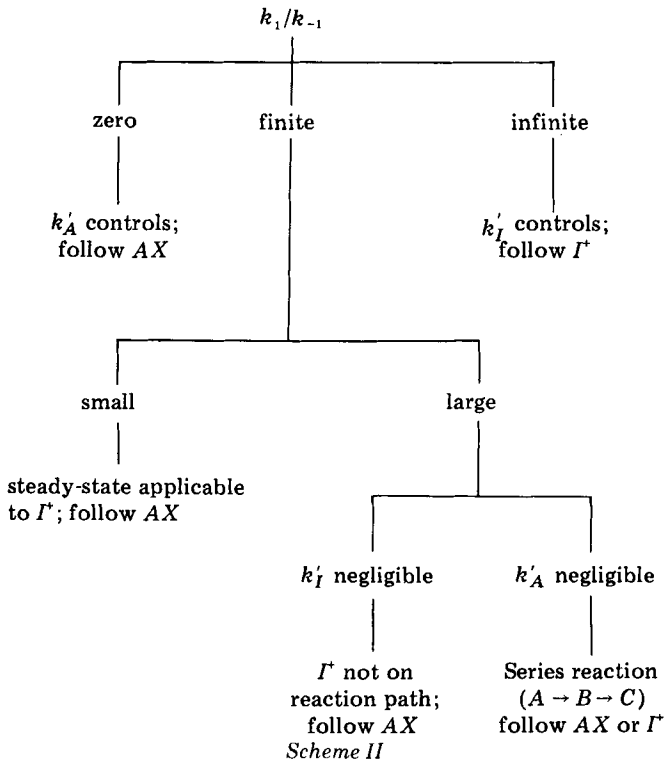
$$AX + N \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I^+ + X^-$$

$$\downarrow k'_A[\mathrm{ROH}] \qquad \downarrow k'_I[\mathrm{ROH}]$$

$$AOR + HX \qquad AOR + NH^+$$

Scheme I

where AX is the acetyl compound (acetyl chloride or acetic anhydride), N is the catalyst (N-methylimidazole or 4-dimethylaminopyridine), I^+ is an intermediate acetylammonium ion, X^- is the counterion (chloride or acetate), and ROH is the acetyl acceptor (alcohol or water). Although a single general rate equation is too complex to be useful, several important cases arise from the operation of: (*a*) the ratio k_1/k_{-1}; (*b*) the detailed nature of k'_A and k'_I; (*c*) the relative magnitudes of k'_A and k'_I; (*d*) the relative concentrations of the reactants; (*e*) the method of observation; (*f*) the nature of ROH. The experiments were designed to evaluate the validity of Scheme I and to obtain estimates of the parameters. Scheme II outlines some of the possible cases:



Scheme II

[3] Cary 14 and Cary 16 spectrophotometers, equipped with thermostatted cell compartments.

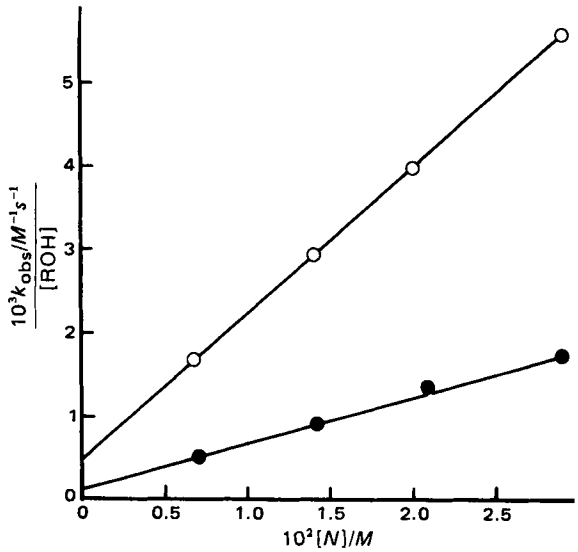


Figure 1—*Plot according to Eq. 3 for the acetylchloride–*N*-methylimidazole system. Key: (○), sec-butanol; (●), isopropanol.*

In this study the reactions were followed spectrophotometrically by monitoring the loss of AX or I^+ with time. The initial concentration of hydroxy compound usually was at least 50 times that of the acetylating agent, and in most cases good first-order behavior was observed, the apparent first-order rate constant k_{obs} being evaluated from a plot of log $(A_t - A_\infty)$ *versus* time. The precision of k_{obs} was ~2%. Deviations from first-order behavior are pointed out as appropriate.

Acetyl Chloride–*N*-Methylimidazole–Alcohols—When acetonitrile solutions of acetyl chloride and N-methylimidazole are mixed, the absorbance of the resulting solution is much greater than that calculated for the mixture of reactants. It is inferred that a very fast and quantitative formation of N-acetyl-N'-methylimidazolium ion occurs; this is the intermediate I^+ in Scheme I. At a given concentration of catalyst and varying concentrations of acetyl chloride, the same absorbance was produced as long as $[AX]_0 > [N]_0$ and *vice versa*, showing that the formation of I^+ was quantitative. The spectrum of the intermediate showed λ_{max} = 245 nm, log ϵ_{max} = 3.51. Since N-methylimidazole shows λ_{min} = 245 nm, log ϵ_{min} = 0.2, the reaction was followed at 245 nm, with initial condition $[AX]_0 < [N]_0$. In a typical reaction $[AX]_0 = 8 \times 10^{-4}$ M and $[\mathrm{ROH}]_0 = 0.5$ M.

This system therefore appears to be a case in which k_1/k_{-1} is essentially infinite (Scheme II), the observed reaction being (Scheme III):

$$I^+ + \mathrm{ROH} \xrightarrow{k'_I} \mathrm{ROAc} + NH^+$$

Scheme III

In Scheme III ROAc represents the acetylated alcohol.

It is anticipated that k'_I is given by Eq. 1, which is a testable assumption.

$$k'_I = k_I + k_{IN}[N] \qquad \text{(Eq. 1)}$$

The hypothetical rate equation is, therefore:

$$\frac{-d[I^+]}{dt} = (k_I + k_{IN}[N])[I^+][\mathrm{ROH}] \qquad \text{(Eq. 2)}$$

The experimental rate equation was found to be $-d[I^+]/dt = k_{obs}[I^+]$, the plots being linear over the course of the reactions. Therefore:

$$\frac{k_{obs}}{[\mathrm{ROH}]} = k_I + k_{IN}[N] \qquad \text{(Eq. 3)}$$

Table I—Rate Constants for the Acetyl Chloride–*N*-Methylimidazole System in Acetonitrile at 25° [a]

Alcohol	$10^3 k_I/M^{-1}s^{-1}$	$k_{IN}/M^{-2}s^{-2}$	$10^3 k_A/M^{-1}s^{-1}$
n-Propanol	0.16 (0.025)	0.50 (0.007)	4.61 (0.021)
n-Butanol	0.36 (0.026)	0.98 (0.006)	4.49 (0.032)
Isopropanol	0.15 (0.030)	0.053 (0.002)	1.49 (0.036)
sec-Butanol	0.49 (0.024)	0.17 (0.001)	3.32 (0.025)

[a] Standard deviation in parentheses, evaluated from the least-squares fit to the appropriate linear equation.

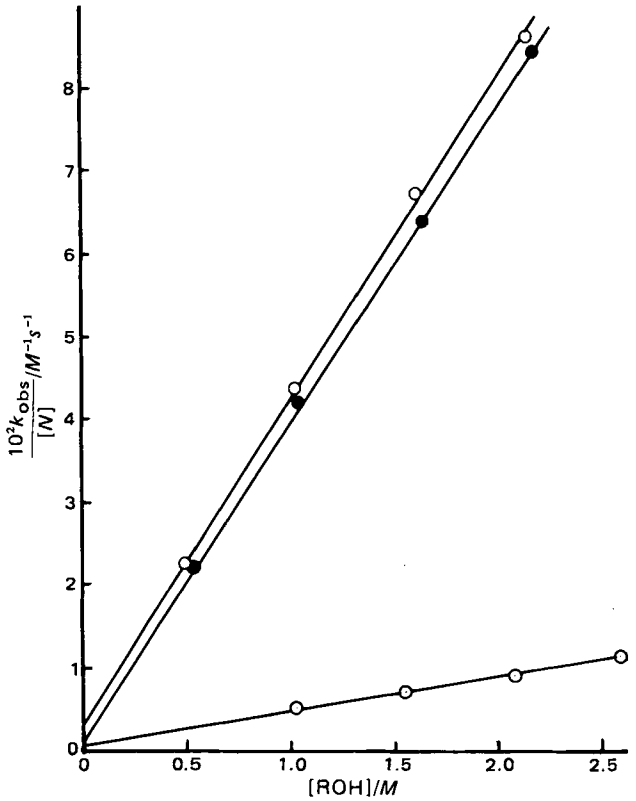


Figure 2—*Plot according to Eq. 9 for the acetic anhydride–*N*-methylimidazole system. Lines from top to bottom:* n-*propanol,* n-*butanol, isopropanol.*

Equation 3 suggests that a plot of $k_{obs}/[ROH]$ *versus* $[N]$ will be linear, where $[N]$ represents free *N*-methylimidazole. Since it was shown that conversion to the intermediate is quantitative, $[N]$ is given by:

$$[N] = [N]_0 - [AX]_0 \qquad \text{(Eq. 4)}$$

Moreover, $[N]$ should remain constant at this value throughout the course of the reaction, because each *N*-methylimidazole released from I^+ upon reaction with ROH accepts a proton, since it is the strongest base in the system.

Figure 1 shows a plot according to Eq. 3 for *sec*-butanol and isopropanol. Similar behavior was observed for *n*-propanol and *n*-butanol, and the resulting estimates of k_I and k_{IN} are listed in Table I.

To evaluate k'_A, the acetylation of these four alcohols by acetyl chloride was examined in the absence of *N*-methylimidazole. The first-order plots were linear for ~1 half-life, perhaps as a consequence of the proton release accompanying the acetylation. If k'_A is given by:

$$k'_A = k_A + k_{AN}[N] \qquad \text{(Eq. 5)}$$

evidently the rate equation in the absence of *N*-methylimidazole is $-d[AX]/dt = k_A[AX][ROH]$, or $k_{obs}/[ROH] = k_A$, where k_{obs} is evaluated from the initial linear portion of the plots. The k_A estimates obtained in this way are given in Table I.

It is evidently impossible to determine k_{AN} for this system, because acetyl chloride and *N*-methylimidazole cannot coexist in significant concentrations.

Acetic Anhydride–*N*-Methylimidazole–Alcohols—When acetonitrile solutions of acetic anhydride and *N*-methylimidazole are mixed, the resulting spectrum can be quantitatively accounted for as the sum of the spectra of these two solutes; thus, there is no spectral evidence for intermediate formation. Since the counterion should have little effect on the spectrum of the cation, and the intermediate in the acetyl chloride–*N*-methylimidazole system exhibited an intense absorption at 245 nm, it is inferred that in the present case k_1/k_{-1} is close to zero. The acetylating agent is acetic anhydride (λ_{max} = 240 nm, log ϵ_{max} = 2.0). The reaction was monitored at 245 nm, corresponding to a minimum in the spectrum of the catalyst. The apparent first-order plots were linear for at least 4 half-lives. The initial concentrations were: acetic anhydride, 0.02 *M*; alcohols, 0.5–2.6 *M*; *N*-methylimidazole, 0.011–0.11 *M*.

Table II—Rate Constants for the Acetic Anhydride–*N*-Methylimidazole System in Acetonitrile at 25° [a]

Alcohol	$10^3 k_1/M^{-1}s^{-1}$	$10^5 k_A/M^{-1}s^{-1}$	$10^2 k_{AN}/M^{-2}s^{-1}$
n-Propanol	0.70 (1.05)	2.4 (3.7)	4.03 (0.05)
n-Butanol	1.20 (1.42)	8.3 (10.2)	3.80 (0.06)
Isopropanol	0.007 (0.011)	0.15 (0.11)	0.413 (0.002)

[a] Standard deviation in parentheses.

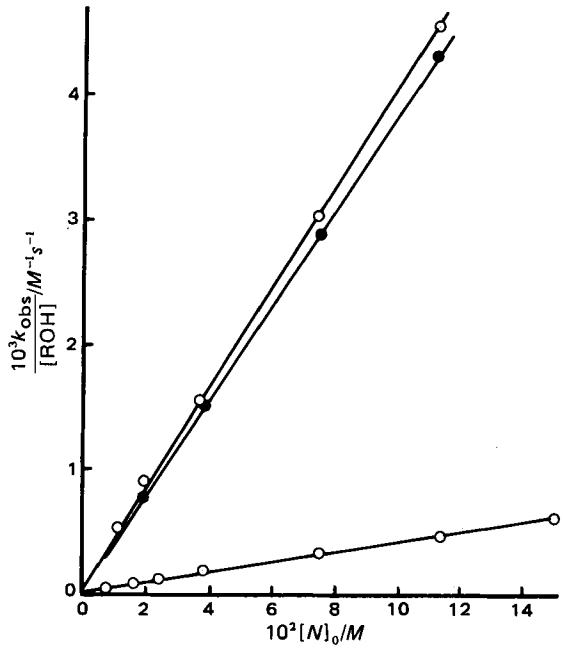


Figure 3—*Plot according to Eq. 10 for the acetic anhydride–*N*-methylimidazole system. Lines from top to bottom:* n-*propanol,* n-*butanol, isopropanol.*

Although the intermediate could not be detected spectrally, its possible presence is admitted in the rate equation for the loss of anhydride:

$$-\frac{d[AX]}{dt} = k_1[AX][N] + k'_A[AX][ROH] - k_{-1}[I^+][X^-] \qquad \text{(Eq. 6)}$$

The assumption is made that all of the H^+ produced in the acetylation is accepted by X^- (acetate); then $[X^-] = 0$, so Eq. 6 becomes:

$$-\frac{d[AX]}{dt} = k_1[AX][N] + k'_A[AX][ROH] \qquad \text{(Eq. 7)}$$

This assumption is justified in the later discussion. By hypothesis, $k'_A = k_A + k_{AN}[N]$. The experimental rate equation is $-d[AX]/dt = k_{obs}[AX]$, so:

$$k_{obs} = k_1[N] + k_A[ROH] + k_{AN}[ROH][N] \qquad \text{(Eq. 8)}$$

If in a series of experiments the catalyst concentration is held constant while the alcohol concentration is varied, the plotting form of Eq. 9 is used:

$$\frac{k_{obs}}{[N]} = k_1 + \left(\frac{k_A}{[N]} + k_{AN}\right)[ROH] \qquad \text{(Eq. 9)}$$

whereas, if [ROH] is kept constant and $[N]$ is varied, Eq. 10 can be used.

$$\frac{k_{obs}}{[ROH]} = k_A + \left(\frac{k_1}{[ROH]} + k_{AN}\right)[N] \qquad \text{(Eq. 10)}$$

Thus, from the slopes and intercepts of these two plots, the constants k_1, k_A, and k_{AN} can be evaluated.

Figure 2 is the plot according to Eq. 9, and Fig. 3 shows Eq. 10 plotted for three alcohols in the acetic anhydride–*N*-methylimidazole system. The rate constants estimated by this treatment are given in Table II; the k_1 and k_A values are not significantly different from zero.

This system was used to investigate the medium effect of the alcohol on the rate and mechanism. The reaction was studied in *n*-propanol–acetonitrile mixtures, with the results shown in Table III. Good first-order kinetics were observed, but at an alcohol concentration of 80%, sharp deviations from first-order kinetics were seen. The dependence of k_{obs}

Table III—Dependence of Rate Constant on Solvent Composition for the Acetic Anhydride–*N*-Methylimidazole System in *n*-Propanol–Acetonitrile Mixtures [a]

Concentration of *n*-Propanol		
Volume, %	Molarity	$10^3 k_{obs}/s^{-1}$
20	2.67	3.93
30	4.00	5.92
40	5.34	8.08
50	6.68	9.81
60	8.02	12.0
70	9.35	14.4

[a] $[AX]_0 = 2.95 \times 10^{-3}\ M$; $[N]_0 = 3.76 \times 10^{-2}\ M$; temp. = 25°.

upon [ROH] is linear, and no spectral evidence was seen for intermediate formation, indicating that there is no mechanism change over this range of solvent composition. The alcohol is functioning as a reactant, and has no kinetically significant medium effect over the range reported in Table III.

Acetic Anhydride–*N*-Methylimidazole–Water—In one stage of this study, the water was treated as an instance of a hydroxy compound, in relatively low concentration, undergoing acetylation in acetonitrile solution; another stage involved the hydrolysis in water–acetonitrile mixtures over a wide range of composition.

This system behaved dramatically differently from the corresponding anhydride–alcohol system, in that when water was present a strong UV absorption was observed, similar to that ascribed to intermediate formation in acetyl chloride solutions. At low water concentrations the initial absorbance was proportional to the water concentration. It is inferred that water alters the polarity of the medium, making the formation of the *N*-acetyl-*N'*-methylimidazolium intermediate possible. With this hypothesis the following kinetic treatment, based on Scheme I, describes the system. Since reaction may take place *via* both AX and I^+ (at a given water concentration both species may be present), and the change in absorbance may receive contributions from both routes, the rate $-d([AX] + [I^+])/dt = -d[AX]/dt - d[I^+]/dt$ is needed. From the kinetic scheme:

$$-\frac{d[AX]}{dt} = k_1[AX][N] + k'_A[AX][H_2O] - k_{-1}[I^+][X^-] \quad \text{(Eq. 11)}$$

$$-\frac{d[I^+]}{dt} = k_{-1}[I^+][X^-] + k'_I[I^+][H_2O] - k_1[AX][N] \quad \text{(Eq. 12)}$$

Therefore,

$$-\frac{d([AX] + [I^+])}{dt} = k'_A[AX][H_2O] + k'_I[I^+][H_2O] \quad \text{(Eq. 13)}$$

The concentrations $[AX]$ and $[I^+]$ are related by Eq. 14, where the nature of R′ is examined later:

$$[AX] = R'[I^+] \quad \text{(Eq. 14)}$$

Therefore, $-d[AX]/dt = -R'(d[I^+]/dt)$, or:

$$-\frac{d([AX] + [I^+])}{dt} = -(1 + R')\frac{d[I^+]}{dt} \quad \text{(Eq. 15)}$$

Substituting Eq. 14 into 13:

$$-\frac{d([AX] + [I^+])}{dt} = (k'_I + R'k'_A)[I^+][H_2O] \quad \text{(Eq. 16)}$$

Equations 15 and 16 are combined to give:

$$-\frac{d[I^+]}{dt} = \left(\frac{k'_I + R'k'_A}{1 + R'}\right)[I^+][H_2O] \quad \text{(Eq. 17)}$$

An alternative development in terms of AX yields:

$$-\frac{d[AX]}{dt} = \left(\frac{k'_I + R'k'_A}{1 + R'}\right)[AX][H_2O] \quad \text{(Eq. 18)}$$

It follows that:

$$-\frac{d([AX] + [I^+])}{dt} = \left(\frac{k'_I + R'k'_A}{1 + R'}\right)[H_2O]([AX] + [I^+]) \quad \text{(Eq. 19)}$$

If R′ is invariant with time, Eq. 19 has a first-order form, with the observed rate constant given by:

$$k_{obs} = \left(\frac{k'_I + R'k'_A}{1 + R'}\right)[H_2O] \quad \text{(Eq. 20)}$$

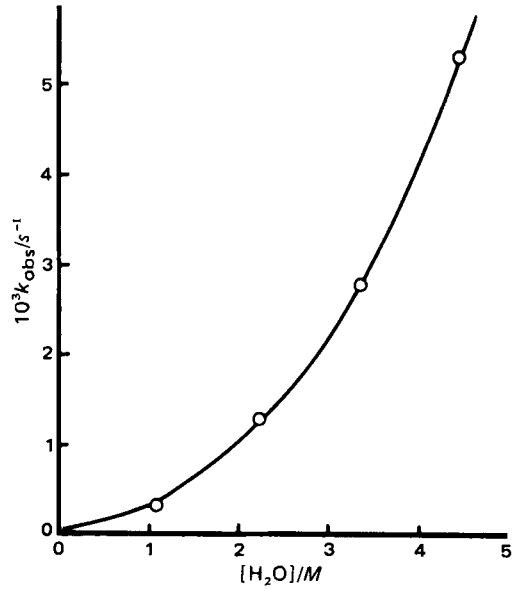


Figure 4—*Dependence of* k_{obs} *on water concentration in the acetic anhydride–*N*-methylimidazole system;* $[N]_0 = 1.5 \times 10^{-3}$ M.

It can be shown that Eq. 20 applies also when the total absorbance of the solution is followed.

The nature of R′ is now investigated. The concentrations $[AX]$ and $[I^+]$ might be controlled by the equilibrium relation:

$$K = \frac{k_1}{k_{-1}} = \frac{[I^+][X^-]}{[AX][N]} \quad \text{(Eq. 21)}$$

or $[AX] = R[I^+]$, where $R = [X^-]/K[N]$. Another possibility is that the steady-state approximation may be applied to $[I^+]$, giving:

$$[AX] = \left(\frac{k_{-1}[X^-] + k'_I[H_2O]}{k_1[N]}\right)[I^+] \quad \text{(Eq. 22)}$$

Comparison with Eq. 14 gives:

$$R' = \frac{[X^-]}{K[N]} + \frac{k'_I[H_2O]}{k_1[N]} \quad \text{(Eq. 23)}$$

The quantity R′ is therefore a function of water concentration through $[H_2O]$, k_1, and K, all of which increase as the water content of the medium increases. As $[H_2O]$ increases, R′ will approach zero and k_{obs} will approach $k'_I[H_2O]$. At very low $[H_2O]$, R′ will become large and k_{obs} will approach $k'_A[H_2O]$. Equations 22 and 23 include equilibrium control as a special case.

In general, R′ is also a function of time (at given water concentration), so first-order kinetics may not be observed when R′ is finite. This means that deviations from first-order kinetics are most likely to be observed at water concentrations where R′ is making a significant contribution to k_{obs}, that is, where both k'_A and k'_I are contributing.

Deviations from first-order kinetics were seen at water concentrations <20% v/v (4.45 *M*); above this concentration good first-order plots were obtained. Figures 4 and 5 show the dependence of k_{obs} on water concentration in the low and high water concentration regions. From the slope at high water concentration, k'_I is found to be $1.05 \times 10^{-3}\ M^{-1}\ s^{-1}$ (at $[N]_0 = 3.76 \times 10^{-4}\ M$). An estimate of the slope at $[H_2O] = 0$ gives $k'_A \approx 2 \times 10^{-4}\ M^{-1}\ s^{-1}$ ($[N]_0 = 1.50 \times 10^{-3}\ M$).

The dependence of k_{obs} on $[N]$ can be investigated by combining Eq. 20 with the detailed expressions for k'_A, k'_I, and R′, but the results are complex and difficult to interpret (13). The experimental observation is that k_{obs} shows positive curvature as a function of $[N]_0$ at low water concentration and negative curvature at high water concentration.

The linear portion of Fig. 5 suggests that R′ becomes negligible above ~5–10 *M* water; presumably above this value the medium is polar enough to support the extensive formation of the intermediate. Below 5–10 *M* water the reaction proceeds *via* both the anhydride and the intermediate.

The acetyl chloride–*N*-methylimidazole system was also studied in the presence of water, but very complicated kinetics were observed. This is thought to be a consequence of the unanticipated production of acetic anhydride in the system, which can occur in this way. Acetyl chloride hydrolyzes (*via* the intermediate) to give acetic acid, which transfers a proton to *N*-methylimidazole yielding acetate ion. At low water con-

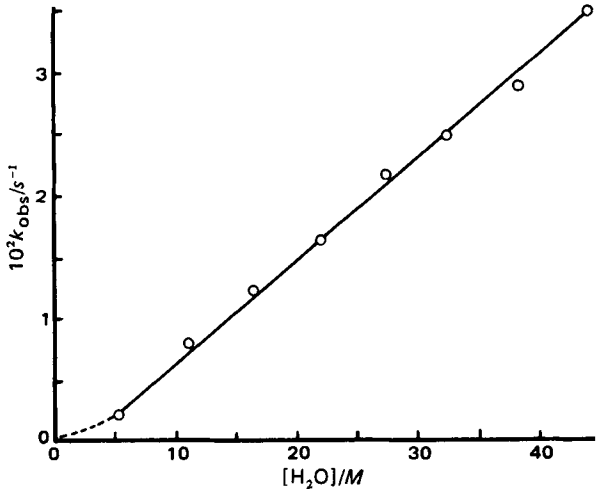


Figure 5—*Dependence of* k_{obs} *on water concentration for the acetic anhydride–N-methylimidazole systems;* $[N]_0 = 3.76 \times 10^{-4}$ M. *The dashed line represents the behavior seen in Fig. 4, but the two figures were obtained at different catalyst concentrations.*

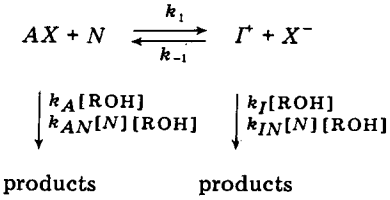
centrations, acetate will react with the intermediate I^+ to generate acetic anhydride, according to Scheme I. Further study of this complex system did not seem warranted.

Catalysis by 4-Dimethylaminopyridine—A mixture of acetyl chloride and 4-dimethylaminopyridine in acetonitrile shows a rapid and quantitative conversion to the intermediate, with λ_{max} = 315 nm, log ϵ_{max} = 4.5. The acetylation of isopropanol was followed at 315 nm. The reactions did not exhibit good first-order kinetic behavior; the reason is unknown. By estimating rate constants from the initial stage of the reaction, data treatment according to the method described for the acetyl chloride–*N*-methylimidazole system yielded the approximate estimates $k_I = 6.4 \times 10^{-4}\ M^{-1}\ s^{-1}$ and $k_{IN} = 1.3\ M^{-2}\ s^{-1}$.

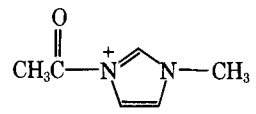
When acetonitrile solutions of acetic anhydride and 4-dimethylaminopyridine are mixed, a rapid but small absorbance increase is obtained at λ_{max} = 315 nm. This is attributed to intermediate formation. Using the molar absorptivity found in the acetyl chloride–dimethylaminopyridine system, it is estimated that 5–10% conversion to the intermediate occurs. The acetylation of isopropanol was studied, the loss of anhydride being followed. Deviations from first-order kinetics were observed, though the deviations were less pronounced than in the acetyl chloride system. Estimates of k_{obs} from the initial portions of the plots led to the estimates $k_A \simeq 0$ and $k_{AN} \simeq 0.5\ M^{-2}\ s^{-1}$. The estimate of k_{AN} may include a contribution from the k_{IN} route.

Mechanism of *N*-Methylimidazole Catalysis—This study has shown that catalysis of acetylations by *N*-methylimidazole can be accounted for in terms of Scheme IV. The intermediate I^+ is the *N*-acetyl-*N'*-methylimidazolium ion (I). The rate constants can be described as follows: k_A = the uncatalyzed reaction; k_{AN} = general base catalysis; k_I = the nucleophilic route; and k_{IN} = general base catalysis of the nucleophilic route.

When *AX* is acetyl chloride, k_1/k_{-1} is very large, the reaction occurs essentially only *via* the I^+ route. When *AX* is acetic anhydride, k_1/k_{-1}

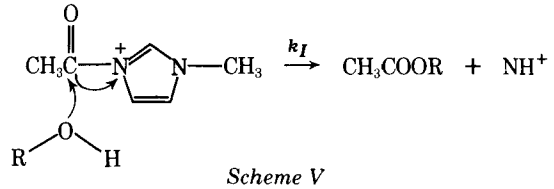


Scheme IV

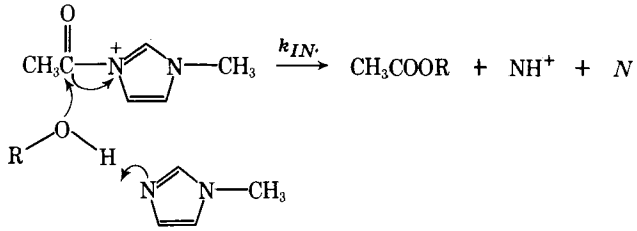


I

is zero, and the reaction occurs entirely *via* the *AX* route. The k_I and k_{IN} reactions are conjectured to occur as shown in Schemes V and VI, where *N* represents *N*-methylimidazole:



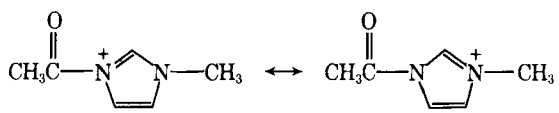
Scheme V



Scheme VI

The mechanisms for acetyl chloride and acetic anhydride are different because chloride is a better leaving group than acetate. When *N*-methylimidazole is replaced by the more powerful catalyst 4-dimethylaminopyridine, some intermediate formation is detected even in the acetic anhydride system. When water is added to the acetonitrile medium, intermediate formation occurs in the acetic anhydride–*N*-methylimidazole system, which constitutes a case in which the mechanism changes from the pure *AX* route (in dry acetonitrile) to the pure I^+ route (in acetonitrile containing $\geq$10 *M* water). This effect can be ascribed to the increase in the polarity in the medium, which will promote formation of the polar intermediate. The dielectric constant of acetonitrile is 36 and that of water is 78. In contrast, incorporation of *n*-propanol into acetonitrile led to no detectable intermediate formation. This is consistent with the view that alcohol–acetonitrile mixtures are not more polar than pure acetonitrile; the dielectric constant of *n*-propanol is 20.5. Besides the spectral and kinetic evidence described here, these mechanistic conclusions are supported by other results. *N*-acetyl-*N'*-methylimidazolium acetate could not be synthesized in acetonitrile, but in a previous report it was found that the chloride salt could be prepared (14). The rate of acetylation of isopropanol by acetic anhydride–*N*-methylimidazole has been investigated in a series of aprotic solvents, with a very small solvent effect being observed (15). This is consistent with the general base (k_{AN}) route but not with the formation of the polar intermediate. It may be noted that the third-order rate constants determined titrimetrically for acetic anhydride–*N*-methylimidazole acetylations in earlier work (8, 15) may now be identified as k_{AN} in Scheme IV.

Comparison of k_A and k_I for the acetyl chloride–*N*-methylimidazole system (Table I) shows that $k_A > k_I$ for each alcohol studied. This means that diversion through the unassisted nucleophilic route (k_I) actually inhibits the reaction relative to the uncatalyzed (k_A) process, possibly because of stabilization of the intermediate by electron delocalization as indicated in II. (Similar delocalization can occur in the 4-dimethylaminopyridine intermediate.)



II

Table IV—Rate Constants for Acetylation of Isopropyl Alcohol in Acetonitrile at 25° [a]

	Acetyl Chloride				Acetic Anhydride			
	k_A	k_{AN}	k_I	k_{IN}	k_A	k_{AN}	k_I	k_{IN}
N-Methylimidazole	0.0015	—	0.00015	0.053	0	0.0041	—	—
4-Dimethylaminopyridine	—	—	0.00064	1.3	0	0.5	—	—

[a] Units of rate constants as in Tables I and II.

Although it has not been possible to measure both k_{AN} and k_{IN} for the same system, Table IV gathers rate constants that have been obtained for both acetylating agents and both catalysts with isopropanol. Analogy with the k_A/k_I ratio suggests that k_{AN} may be $>k_{IN}$, whereas Table IV indicates that $k_{IN} > k_{AN}$ (although these data are not for precisely the same system).

These observations may account for the puzzling report (3) that 1-ethynylcyclohexanol is acetylated faster by acetic anhydride than by acetyl chloride in the presence of 4-dimethylaminopyridine (in $CDCl_3$ solution). If the mechanisms are as described here, the intermediate is quantitatively formed from acetyl chloride, with the concomitant consumption of an equimolar amount of 4-dimethylaminopyridine; thus, the concentration of free catalyst is depleted and is very low under the conditions described. The reaction occurs by the k_I and k_{IN} routes. In the acetic anhydride system, on the other hand, no intermediate is formed, with the result that the catalyst concentration remains high, and the reaction proceeds *via* the k_{AN} route. Under the reported conditions (3), using the rate constants in Table IV, it is calculated that the rate in the anhydride system is greater than the rate in the acetyl chloride system.

Effect of the Solvent—The rates and mechanisms of acylation reactions can be affected by the reaction medium through the operation of several effects.

The Ratio k_1/k_{-1}—This ratio describes the formation of the polar final state ($I^+ + X^-$) from the less polar initial state ($AX + N$). An increase in solvent polarity should increase k_1/k_{-1}; this effect was seen in the system acetic anhydride–*N*-methylimidazole–water, in which k_1/k_{-1} changes from practically zero at low water concentration to very large in substantial water concentrations. The mechanism is changed as a consequence. In the intermediate range where k_1/k_{-1} is finite, both reaction routes are accessible and the kinetics are complex.

Since much prior kinetic study of acylation mechanisms has emphasized fully aqueous systems, the nucleophilic route has often been implicated for those reactions, as in the pyridine-catalyzed hydrolysis of acetic anhydride (16). As the present study shows, however, the extent of *N*-acylammonium intermediate formation can be very sensitive to the solvent.

The Rate Constants k_A, k_{AN}, k_I, *and* k_{IN}—These will be expected to respond to solvent polarity in accord with the postulate that increased solvent polarity will increase the rate if the transition state is more polar than the initial state and *vice versa*. For example, the k_{AN} route will involve some charge separation in the transition state, so k_{AN} may increase slightly as the solvent is made more polar.

The initial state for the k_I and k_{IN} routes is highly polar, being I^+ or the ion-pair I^+X^-. The effect of the solvent will depend upon the relative polarity of the transition state. Greater acylation rates in nonpolar solvents, in terms of a favored breakdown of the charged intermediate to neutral products, has been accounted for (17). This requires the following reaction (in the symbols of Scheme IV): $I^+X^- + ROH \rightarrow AOR + N + HX$. The proton transfer must also be part of (or take place prior to) the rate determining step. If the reaction is $I^+ + ROH \rightarrow AOR + NH^+$, the solvent effect should be modest. In interpreting a solvent effect in these terms, the concurrent behavior of k_1/k_{-1}, which may alter the route of the reaction, must be taken into account.

The Basicity of the Catalyst (N) *and the Counterion* (X^-)—Upon this property depend the proton-accepting abilities of these bases, the effectiveness of X^- as a leaving group, and the nucleophilicity of *N*. As the solvent is changed, the relative basicity of a pair of bases may change.

Acetonitrile is a weaker base than water, so acids are weaker in acetonitrile than in water (as measured by their acid dissociation constants). For carboxylic acids, the relationship between pKa in acetonitrile and water is (18):

$$\text{pKa (acetonitrile)} = \text{pKa (water)} + 4.4$$

whereas for the conjugate acids of amines it is:

$$\text{pKa (acetonitrile)} = 0.47\ \text{pKa (water)} + 2.2$$

The different behavior for acids RCOOH and RNH_3^+ is a consequence of the lower dielectric constant of acetonitrile and its poor anion-solvating capability. The base strengths of acetate and *N*-methylimidazole in acetonitrile, as calculated with these relationships, are:

Acetic acid: pKa (acetonitrile) = 9.15, pKa (water) = 4.75

N-Methylimidazole: pKa (acetonitrile) = 5.5, pKa (water) = 7.0

In water, *N*-methylimidazole is a stronger base than acetate, but in acetonitrile, acetate is the stronger base. This conclusion was used in the derivation of Eq. 7.

The possibility of nucleophilic catalysis is largely determined by the relative basicities of the leaving group (X^-) and the attacking nucleophile (*N*). If pKa (NH^+) > pKa (HX), then the nucleophile is a stronger base than the leaving group, and the nucleophilic route (*via* formation of the *N*-acylated nucleophile) is favored, whereas if pKa (HX) > pKa (NH^+) the nucleophilic route is not favored, and the direct general base route is more probable. As described previously, acetate is a much stronger base than is *N*-methylimidazole in acetonitrile, hence, formation of *N*-acetyl-*N'*-methylimidazolium acetate is not probable from acetic anhydride. The observation of reaction solely *via* the k_{AN} route is consistent with this description. In the acetyl chloride case, however, *N*-methylimidazole is a stronger base than chloride in acetonitrile [chloride is solvated in acetonitrile, whereas larger anions are not (19)], so the nucleophilic route predominates.

Since 4-dimethylaminopyridine is a stronger base than is *N*-methylimidazole, it would be expected to form the acyl intermediate to a greater extent (with a given AX). This behavior was observed: with acetic anhydride, *N*-methylimidazole gave no detectable intermediate, whereas 4-dimethylaminopyridine gave 5–10% conversion to the intermediate.

Molecular Aggregation—In solvents of low dielectric constant, ion-pairs and higher ionic aggregates can be detected. The reactivity of an ion-pair will in general differ from that of a dissociated ion, so ion-pair formation may have kinetic consequences. Acetonitrile is a solvent of moderate polarity, and ion-pairing will not be as extensive as in solvents of much lower dielectric constant. It has not been found necessary to invoke the ion-pair I^+X^- in the present kinetic treatment. Greater reactivity of acetic anhydride than of acetyl chloride (with 4-dimethylaminopyridine catalyst) in nonpolar solvents by postulating the I^+X^- ion-pair has been reported (3), the explanation being that the I^+Cl^- ion-pair is tightly bound and therefore less reactive than the more loosely bound I^+OAc^- ion-pair. An alternative explanation for these results was given previously.

A recent study (20) questioned whether the intermediate I^+ is on the reaction path in 4-dimethylaminopyridine-catalyzed acetylations, and proposed a mechanism in which the catalyst attacks a molecular complex of anhydride and alcohol formed in a pre-equilibrium, giving a solvated ion-pair. No kinetic evidence for this mechanism was presented. The presence of molecular complexes is very likely in these systems, hydrogen-bonded complexes of alcohols with H-bond acceptors being probable in nonpolar solvents. In highly aqueous systems, complex formation of the hydrophobic type can occur. The *N*-methylimidazole systems studied here did not require the mechanistic presence of complexes. It is possible that such species may be responsible for some of the anomalous kinetic behavior seen with 4-dimethylaminopyridine, but this was not investigated[4].

Unusual rate equations for the reaction of anilines with acetyl halides in the presence of *N*-methylimidazole, in nonpolar solvents has been reported (21). Rate terms included the quantities $[I^+X^-]^2$ and $[I^+X^-][AX]$. Such terms may arise because of the presence of ion-pairs and molecular complexes.

REFERENCES

(1) W. Steglich and G. Höfle, *Angew. Chem. Int. Ed.*, **8**, 981 (1969).

(2) G. Höfle and W. Steglich, *Synthesis*, **1972**, 620.

(3) G. Höfle, W. Steglich, and H. Vorbrüggen, *Angew. Chem. Int. Ed.*, **17**, 569 (1978).

(4) K. A. Connors and K. S. Albert, *J. Pharm. Sci.*, **62**, 845 (1973).

(5) E. L. Rowe and S. M. Machkovech, *ibid.*, **66**, 273 (1977).

(6) V. Fell and C. R. Lee, *J. Chromatogr.*, **121**, 41 (1976).

(7) F. de Fabrizio, *J. Pharm. Sci.*, **69**, 854 (1980).

(8) K. A. Connors and N. K. Pandit, *Anal. Chem.*, **50**, 1542 (1978).

(9) R. Wachowiak and K. A. Connors, *ibid.*, **51**, 27 (1979).

(10) S. L. Wellons, M. A. Carey, and D. K. Eider, *ibid.*, **52**, 1374 (1980).

(11) A. S. Bittner, L. E. Harris, and W. F. Campbell, *J. Agr. Food Chem.*, **28**, 1242 (1980).

(12) S.-F. Lin and K. A. Connors, *J. Pharm. Sci.*, **70**, 235 (1981).

(13) N. K. Pandit, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1980.

[4] Side-reactions in this system may be responsible for the kinetic behavior. Several products of such reactions have been identified (3).

(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

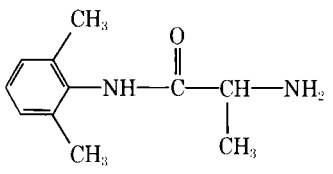
RAMAN VENKATARAMANAN *, FRANK S. ABBOTT, and JAMES E. AXELSON ˣ

Received June 24, 1981, from the *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., Canada V6T IW5.* Accepted for publication August 28, 1981. * Present address: University of Pittsburgh, 3501 Terrace Street, Pittsburgh, PA 15261.

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.



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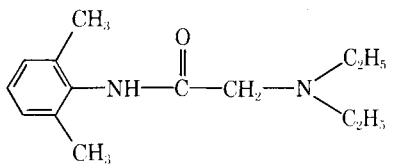

II

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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