BIOORGANIC CHEMISTRY 2, 202–220 (1973)

The Reaction of Tertiary Amino Alcohols with Active Esters

I. Acylation and Deacylation Steps

MOSHE M. WERBER[1] AND YECHIEL SHALITIN[2]

Department of Biology, Technion–Israel Institute of Technology, Haifa, Israel

Received August 17, 1972

The reactions of triethanolamine and four other tertiary amino alcohols with six active ester substrates were studied in the pH range 6–10 at 30°C. The reaction products were in all cases the respective *O*-acyl-amino alcohols. Analysis of the effects of substituents in the leaving group as well as in the acyl moiety of the substrates showed that the ester product was formed by direct attack of the nucleophilic hydroxyl group. Comparison with reactions of tertiary amines with the same substrates supports this conclusion. The reactions of tertiary amino alcohols were also compared with those of zwitterionic quaternary amino alcohols and 3-quinuclidinol, a "rigid" tertiary amino alcohol. On the basis of these comparisons, it is proposed that one of the pathways for the predominant effect of the neutral species of tertiary amino alcohols involves intramolecular general base assistance by the tertiary amino group to the nucleophilic attack of the hydroxylic oxygen on the substrate. The contribution of this pathway to the rate of reaction is evaluated.

In several systems the first product of the reaction, an *O*-acyl-amino alcohol, undergoes relatively rapid deacylation, the overall reaction being thus hydrolysis of active esters, catalyzed by the amino alcohol via an acylation–deacylation mechanism.

INTRODUCTION

Bioorganic models of the mechanism of action of "serine enzymes" (*1*, *2*) should undergo an acylation–deacylation reaction according to the following scheme:

$$\text{E-OH} + \text{RCO—X} \rightarrow \text{E-OCOR} + \text{HX} \qquad (1)$$

$$\text{E-OCOR} \xrightarrow{\text{H}_2\text{O}} \text{E-OH} + \text{RCOO}^- + \text{H}^+ \qquad (2)$$

where E-OH stands for the enzyme model. Models for this reaction, or for parts of it, have been the object of extensive and fruitful studies (*3–6*). The models planned and studied fall into two classes. One class contains nucleophiles which efficiently attack active ester substrates to form stable acylated compounds. These reactions can serve as models for the enzyme-acylation step. In the other class a catalytic group is built into the substrate molecule in a favorable steric position and it intramolecularly catalyzes the hydrolysis of the susceptible group, thus imitating the deacylation step of the enzymatic mechanism.

[1] From the D.Sc. Thesis of M. M. W., Technion, 1969. Present address: Department of Polymer Research, The Weizmann Institute of Science, Rehovot, Israel.

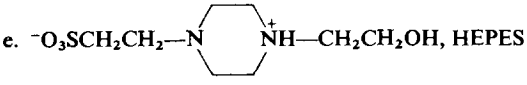
[2] To whom correspondence should be addressed.

Tertiary amino alcohols are simple and straightforward bifunctional models, because in the course of their reaction with substrates only one acylated product, an ester, can be formed.

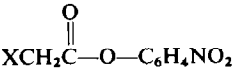
The reactions of tertiary amino alcohols (I), and triethanolamine in particular, with several phenyl (II) and *p*-nitrophenyl esters (III) have been investigated in detail, and the reaction mechanism has been elucidated by comparing it to analogous reactions with nucleophiles such as tertiary amines and quaternary ammonium alcohols.

I

a. $R_1 = R_2 = -CH_2CH_2OH$, TEA
b. $R_1 = R_2 = -CH_2CH_3$, DEAE
c. $R_1 = CH_2CH_2OH$, $R_2 = -CH_2COOH$, DHEG
d. $R_1 = R_2 = -CH_2COOH$, HEIDA

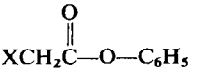


e. HEPES



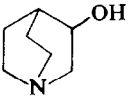
III

a. X = H—, pNPA
b. X = CH_3OCONH—, NPCMG
c. X = CH_3OCOO—, NPCMGO



II

a. X = H—, PA
b. X = CH_3O—, PMA
c. X = Br—, PBA



IV

3-quinuclidinol (quol)

EXPERIMENTAL

Materials. Reagent grade inorganic compounds were used. Triethanolamine (TEA), dihydroxyethylglycine (DHEG), nitrilotriacetic acid (NTA), and triethylamine were from Fluka (puriss). Diethyl aminoethanol (DEAE) (Eastman white label) was redistilled (bp 54–55°C/16 mm Hg). 3-Quinuclidinol (Quol) was used as the hydrochloride (Aldrich). 2-Hydroxyethyl-piperazino-ethane-sulfonic acid (HEPES) was from Sigma (high purity). 2-Hydroxyethyl iminodiacetic acid (HEIDA) was from Pfaltz and Bauer (high purity). 3,3-Diethylglycinamide (DEGA) was synthesized from diethylamine and 2-chloroacetamide in dimethylformamide (DMF) or dichloroethane: 23.4 g (0.25 mole) of chloroacetamide were dissolved in 100 ml DMF; 130 ml (1.25 mole) of diethylamine were added, and the reaction mixture was stirred (initially in the cold) for several hours. The diethylamine hydrochloride precipitate was filtered off and the diethylamine and DMF were evaporated *in vacuo*. The product was purified by sublimation and had mp 76.5–77°C (lit. (7) 76–77°C). Calculated molecular weight for $C_6H_{14}N_2O$: 130; observed molecular weight by titration with HCl: 129. To prepare *N*-methyl triethanolammonium iodide, (MTEAI), 7.5 g (0.05 mole) of triethanolamine and 7.8 g (0.055 mole) of methyl iodide were dissolved in 30 ml ether and refluxed for 2 hr. The solvent was

removed *in vacuo* and the resulting material washed several times in ether and dried. A viscous oil was obtained which did not contain any basic or acidic groups.

Anal. Calcd for $C_7H_{18}NO_3I$: N, 4.87%; I, 43.6%. Found: N, 4.78%; I, 42.3%.

p-Nitrophenyl esters were synthesized using the following method: 0.01 mole of a carboxylic acid and 0.01 mole of recrystallized *p*-nitrophenol were dissolved in 100 ml of ethyl acetate (previously dried over Na_2SO_4) and 0.01 mole of dicyclohexyl carbodiimide (DCC) (Fluka) was added and the reaction mixture stirred for several hours (4–16) at room temperature under protection ($CaCl_2$) against air moisture. A few drops of acetic acid were then added to destroy unreacted DCC, the precipitated urea was filtered off, and the solvent evaporated *in vacuo*. The product was taken up in ethanol and precipitated with water. The esters were recrystallized from aqueous ethanol or from benzene–petroleum ether (bp 30–60°C). The ester content was checked by measuring the absorbance of 400 nm of the *p*-nitrophenolate anion released upon treatment of a weighed amount of the ester with alkali (this content was in all cases 97–100%). This method was used in the synthesis of *p*-nitrophenyl *O*-carbomethoxyglycolate (NPCMGO, mp 119°C) and *p*-nitrophenyl carbomethoxyglycinate (NPCMG, mp 80–83°C).

p-Nitrophenyl acetate (pNPA, mp 76–77°C) was obtained from Yeda, and phenyl bromoacetate (PBA) was from Eastman (white label). The following acids (needed for ester synthesis) were synthesized: *O*-carbomethoxy glycolic acid from glycolic acid and carbomethoxy chloride (*8*), and carbomethoxy glycine from glycine and carbomethoxy chloride, using a general method for the synthesis of *N*-acyl-amines (*9*). *trans*-Cinnamoyl-imidazole (CI) was synthesized from cinnamoyl chloride and imidazole in benzene (*10*). Phenylacetate (PA) was synthesized from phenol and acetic anhydride in alkaline aqueous solution (*11*). Phenyl methoxyacetate (PMA) was synthesized from phenol and methoxyacetic acid with DCC as coupling reagent, using the above-mentioned method for *p*-nitrophenyl esters.

Deionized fresh glass-distilled water was used to prepare the solutions. D_2O (99.8%) was from Biorad.

Apparatus and methods. pH measurements were taken with a Radiometer pH meter (Model 28 or 26) equipped with a small-diameter glass electrode (Radiometer G-222B) and a Radiometer calomel electrode. The pH of the solutions was measured before and after the kinetic experiments, and runs showing drifts exceeding 0.03 pH unit were discarded. In the D_2O solutions a correction of +0.36 was added to the pH observed at 30°C (*12*). Potentiometric titrations were performed on a Radiometer Titrator Type TTT1c equipped with a Titrigraph Type SBR2c and a syringe–buret-type SBU1a, (using Radiometer glass G-202B and calomel electrodes). The titrations were carried out at 30°C in a volume of 10 ml and at the same ionic strength as in the kinetic experiments ($I = 0.5$). Kinetics was followed spectrophotometrically on a Beckman DB spectrophotometer thermostated at 30°C ± 0.1. The ionic strength was kept at $I = 0.5$ by the addition of calculated amounts of $NaNO_3$. Rates were measured by following the disappearance of substrate or appearance of product. In the case of CI the decrease in absorbance due to the conversion of the imidazole derivative ($\lambda_{max} = 307$ nm) to an alkyl ester ($\lambda_{max} = 281$ nm) or to a cinnamate ion ($\lambda_{max} = 269.5$ nm) (*10, 13*) was recorded. With phenyl esters the increase in absorbance at 268 nm was recorded. With *p*-nitrophenyl esters the appearance of the *p*-nitrophenolate anion ($\lambda_{max} = 400$ nm) was usually

followed, but at pH values below the pK of *p*-nitrophenol (pK = 7.15) (*14*) the isosbestic wavelength for the *p*-nitrophenol–*p*-nitrophenolate pair (350 nm) (*15*) was chosen to follow the reaction.

The amino alcohols usually served both as nucleophiles and as buffers. In experiments with tertiary amines and choline, where a buffer had to be used, 5×10^{-3} M or 1×10^{-2} M of HEPES buffer was added for pH 8 and carbonate for pH 9. These small concentrations of buffers were not kinetically important when compared with the nucleophile contribution to the rate. Stock solutions of amino alcohols (1 M) were found to be stable for months at 4°C. Substrates ($2–6 \times 10^{-3}$ M) were routinely dissolved in highly pure acetonitrile and kept at −18°C. The reactions were initiated by the addition of 0.05 ml of substrate solution to 2.45 ml of the nucleophile solution, at the desired concentration, pH, and ionic strength, in a cuvette (optical path 1.0 cm). (The acetonitrile concentration was thus 2% in all experiments.) The cuvette was quickly stirred and the recording started. Nucleophile concentrations (0.05–0.5 M) were in large excess over substrate concentrations (5×10^{-5}–5×10^{-4} M). Thus, pseudo-first-order kinetics prevailed. Final absorbance values, A_∞, were determined in all cases (after at least seven half-times).

The hydroxamic acid method for esters (*16*) was generally used for determining the product. The optical density of the hydroxamic acid–$FeCl_3$ complex was measured at 540 nm on a Bausch and Lomb spectronic model 20. The same method was used to follow deacylation reactions, i.e., the conversion of the initial ester product to hydrolytic products. This was done in the following manner: To 10 ml of nucleophile solution at 30°C 0.02 ml of substrate in acetonitrile was added. One-milliliter aliquots were withdrawn at intervals and analyzed for ester content. Since the substrate concentration was much higher ($2–4 \times 10^{-3}$ M) than in normal kinetic experiments, the rate of *p*-nitrophenolate production was generally verified with high concentration of substrates, by measuring the absorbance of aliquots after dilution.

In the case of cinnamoyl imidazole, the nature of the product could be determined with the aid of the characteristic spectral properties of the different cinnamoyl derivative: CI, $\lambda_{max} = 307$ nm, $\varepsilon_M = 2.52 \times 10^4$ M^{-1} cm^{-1}; cinnamate anion, $\lambda_{max} = 269.5$ nm, $\varepsilon_M = 2.03 \times 10^4$ M^{-1} cm^{-1}; cinnamate ester, $\lambda_{max} = 281$ nm, $\varepsilon_M = 2.21 \times 10^4$ M^{-1} cm^{-1} (*10*, *13*). A convenient way to evaluate the amount of ester formed as product of the reaction was to run a spectrum of the reaction solution after the absorbance at 320 nm (due to the substrate) had ceased to change, and to determine λ_{max}. The percentage of ester moiety in the product was obtained (within 5% accuracy) using the following expression:

$$\text{Ester percent} = \frac{100(\lambda_{max} - 269.5)}{(\lambda_{max} - 269.5) + 1.09(281 - \lambda_{max})} \tag{3}$$

Calculations. The first-order constant, k_{obs}, was calculated from the absorbances. Values of $\log(A–A_\infty)$ (in the case of higher absorption by the substrate) or of $\log(A_\infty–A)$ (in the case of higher absorption by the product) were plotted versus time. From the slopes of the curves k_{obs} values were calculated. Some of the k_{obs} values were obtained using an Elliott 503 computer (using a linear least-square program, written in Algol 60). The values calculated by those two methods were in excellent agreement.

At each pH, k_{obs} values were measured at several concentrations of nucleophile. By

plotting k_{obs} vs [N], the concentration of the nucleophile, at any pH, a straight line was obtained whose slope yielded the apparent second-order rate constant, k'_N, at the specific pH, and whose intercept gave $k_0 (= k_{OH}[OH^-])$. In those cases in which the pH dependence of k'_N was found to follow a normal titration curve, true k_N values were calculated by dividing the k'_N values by α, the fraction of the unprotonated active species of N present in solution at this pH.

In determining the rate constants for alkaline hydrolysis (in which the active species is the hydroxide ion) pK_w (30°C) was taken as 13.833 (*17*) and therefore $a_{OH} = K_w/a_H = 1.48 \times 10^{-14}/a_H$. Activity was converted to concentration by calculating the activity coefficient from the following expression (*18*):

$$\log \gamma^{\pm} = -0.507 [Z_+ Z_- I^{1/2}/(1 + I^{1/2})] + 0.1 I \quad (4)$$

For univalent ions at $I = 0.5$, $\gamma = 0.69$.

In experiments with D_2O, $a_{OD} = K_{D_2O}/a_D$ and pK_{D_2O} at 30°C was taken as 14.65 (*19*).

pK values were determined by plotting the values of $\Delta T_{OH}/\Delta pH$, where T_{OH} is the amount of hydroxide added to the solution, after correction for titration of a blank solution. The maximum of such a plot is the pK of the titrated compound (*20*). The pK values of the nucleophiles used in this study are listed in Table 1.

TABLE 1

pK VALUES OF NUCLEOPHILES USED IN THE REACTIONS WITH ACTIVE ESTERS (30°, $I = 0.5$)

Nucleophile	pK	Nucleophile	pK
TEA (Ia)	7.80	Diethyl glycine ethyl ester	7.98
DEAE (Ib)	9.87[a]	Diethyl glycinamide	8.37
DHEG (Ic)	8.11	Nitrilotriacetate	9.70[b]
HEIDA (Id)	8.72[c]	Triethylamine	10.77[d]
HEPES (Ie)	7.32	Quinuclidinol (Quol)	9.77[e]

[a] B. A. Timini and D. H. Everett, *J. Chem. Soc.* (*B*), 1380 (1968).

[b] Calculated from the value for $I = 3.0$ [T. Moeller and R. Ferrus, *Inorg. Chem.* **1**, 49 (1962)].

[c] In this case $I = 1.2$; pK value calculated for conditions of the kinetic work from K. S. Rajan and A. E. Martell, *J. Inorg. Nucl. Chem.* **26**, 789 (1964).

[d] M. C. Cox, D. H. Everett, D. A. Landsman, and R. J. Munn, *J. Chem. Soc.* (*B*), 1373 (1968).

[e] Computed from the value at 25°, $I = 1.0$; [W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.* **90**, 2622 (1968)].

RESULTS

Acylation of Tertiary Amino Alcohols

1. *Triethanolamine (TEA).* The rate of acylation of TEA by five substrates was measured in the pH range 6–9 (see Fig. 1 with NPCMG and CI as substrates). The observed pseudo-first-order constants—k_{obs}—were dependent on TEA concentration and pH.

$$k_{obs} = k_0 + k'_N[N], \quad (5)$$

where

$$k_0 = k_{OH}[OH^-]. \quad (6)$$

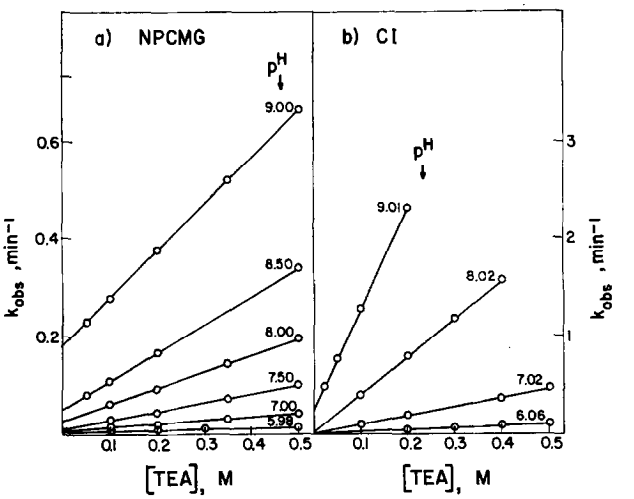


FIG. 1. pH dependence of the rate constants for the acylation of TEA by NPCMG and by CI at 30°, $I = 0.5$.

The pH dependence of the reaction of Tris with esters was analyzed by Bruice and York (*21*). They found that the second-order constant at each pH, k'_N, consisted of two terms, a neutral term k_n and a basic term k'_b.

$$k'_N = (k_n K + k'_b K a_{OH})/(K + a_H). \tag{7}$$

K being the (acidic) dissociation constant of the base. By plotting $k'_N(K + a_H)$ as a function of a_{OH}, one can obtain k'_b from the slope and k_n from the intercept. In the present study, when the data for the reactions of TEA with those five substrates were plotted according to Eq. (7) (see Fig. 2 in the case of NPCMG), an upward curvature was observed at pH values below the pK (7.80). This behavior is due to the occurrence of an acidic term, k_a, in the expression for k'_N:

$$k'_N = (k_n K + k'_b K a_{OH} + k_a a_H)/(K + a_H). \tag{8}$$

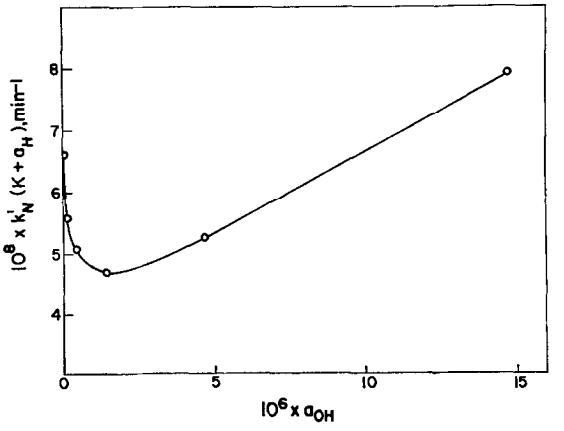


FIG. 2. Kinetic analysis of the acylation reaction of TEA by NPCMG according to the equation of Bruice (Eq. 8). Reactions were run at 30°, $I = 0.5$.

Since the contribution of the acidic term was negligible at values of pH > pK, one could evaluate the values of k_n and k_b' from the linear portion of Fig. 2 (the value of k_n was obtained by extrapolation to $a_{OH} = 0$).

After subtraction of the neutral and basic terms from the values of $k_N'(K + a_H)$, the resulting values were plotted against a_H. The slopes of the straight lines thus obtained yielded the values of k_a. The values of k_n, k_b', and k_a for the five substrates studied are summarized in Table 2. (Included for comparison are the values of k_{OH}, calculated from Eq. (6)).

TABLE 2

RATE CONSTANTS FOR THE ACYLATION OF TEA by active substrates (30°, $I = 0.5$)[a]

Substrate	$10^{-3} \times k_b'$ ($min^{-1} M^{-2}$)	k_n ($min^{-1} M^{-1}$)	$10^3 \times k_a$ ($min^{-1} M^{-1}$)	$10^{-3} \times k_{OH}$ ($min^{-1} M^{-1}$)
pNPA	2.55	0.081	1.85[b]	0.71[c]
NPCMG	25.0	0.50	2.40	8.43
NPCMGO	144	2.96	15.2	34.6
PBA	118	5.15	—	37.4
CI	294	5.57	88.5[b]	6.08[d]

[a] Obtained from experiments in the pH range 6–9.

[b] Approximate value, calculated from 2 points only.

[c] To be compared with $k_{OH} = 570$ $min^{-1} M^{-1}$ at 25°, $I = 1.0$. [J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.* **86**, 837 (1964)].

[d] To be compared with $k_{OH} = 5600$ $min^{-1} M^{-1}$ at 25°, $I = 0.1$, Ref. (*13*).

2. *Other tertiary amino alcohols.* Four other tertiary amino alcohols were used in this study: DEAE, DHEG, HEIDA, and HEPES.

The pH dependence of the reactions of the amino alcohols with their substrates was analyzed in the same manner as for TEA (Table 3). Although with two of these amino alcohols (DEAE and DHEG) an acidic term was observed, the values of k_a could not be calculated because the data were insufficient. Values of k_b' and k_n were obtained according to Eq. (7). In the case of HEIDA it was found that the contribution of the basic term was negligible at pH 9.0.

3. *Kinetic isotopic effect.* As a test for a possible intramolecular general base catalysis of the tertiary nitrogen of the amino alcohols on the hydroxylic proton (scheme V), the kinetic isotopic effect (*3*, *4*) was studied. The rates of the acylation of TEA by NPCMG and NPCMGO were measured at three pD values: 8.46, 8.96, and 9.46. k_N' values were obtained at these pD values and the pD dependence of the reaction was analyzed according to Eq. (7),[3] using the value of p$K(D_2O) = 8.28$ that was found for TEA in D_2O. The values of k_n^D and $k_b'^D$ were calculated from this plot and the kinetic isotope effects k^H/k^D obtained were summarized in Table 4.

[3] There was no need to use Eq. (8), since this was performed at pD > p$K(D_2O)$.

TABLE 3

RATE CONSTANTS FOR THE ACYLATION OF TERTIARY AMINO ALCOHOLS AND CHOLINE BY ACTIVE SUBSTRATES AT (30°, $I = 0.5$)

	DEAE[a]		DHEG[b]		HEIDA[c]	HEPES[d]		Choline[e]
Substrate	k_n ($min^{-1} M^{-1}$)	$10^{-3} \times k_b$ ($min^{-1} M^{-2}$)	k_n ($min^{-1} M^{-1}$)	$10^{-3} \times k_b'$ ($min^{-1} M^{-2}$)	k_n ($min^{-1} M^{-1}$)	k_n ($min^{-1} M^{-1}$)	$10^{-3} \times k_b'$ ($min^{-1} M^{-2}$)	$10^{-3} \times k_b'$ ($min^{-1} M^{-2}$)
pNPA	1.65	29.6	—	—	0.22	—	—	—
NPCMG	14.8	—	0.50	3.72	0.94	0.050	5.46	60.5
NPCMGO	—	—	2.64	21.5	4.20	0.50	8.50	450
PA	0.23	<1	—	—	—	—	—	—
PMA	5.54	64.5	—	—	—	—	—	—
CI	141	—	—	—	2.16	—	—	670

[a] pH range 8–10.5.
[b] pH range 7–9.
[c] At pH 9 only.
[d] pH range 8–9.
[e] pH range 7–9.

TABLE 4

KINETIC ISOTOPE EFFECT FOR THE ACYLATION OF TEA BY NPCMG AND NPCMGO IN H_2O AND D_2O (30°, $I = 0.5$)[a]

Substrate	k_n^H/k_n^D	$k_b'^H/k_b'^D$
NPCMG	1.075	0.735
NPCMGO	1.095	0.733

[a] k_n^H and $k_b'^H$ values from Table 2.

Product of the Acylation of Tertiary Amino Alcohols and Rate of Deacylation

The primary product of the acylation reaction of TEA and of the other tertiary amino alcohols was identified as an ester by the hydroxamic acid method, and in the case of the substrate CI also by its spectral characteristics (see Methods). In some cases (especially with esters containing an active acyl moiety) a second step of the reaction was observed, leading to hydrolysis of the *O*-acyl-amino alcohols formed in the first step.

$$(HOCH_2CH_2)_3N + R\overset{O}{\overset{\|}{C}}OR' \longrightarrow R'OH + (HOCH_2CH_2)_2NCH_2CH_2O\overset{O}{\overset{\|}{C}}R \xrightarrow{OH^-} (HOCH_2CH_2)_3N + R\overset{O}{\overset{\|}{C}}O^- + H^+ \quad (9)$$

In these cases the rate of deacylation was also measured and the maximal amount of ester formed calculated according to an integrated kinetic expression for consecutive and competitive reactions (*22*). In all the cases checked there was an excellent agreement between the calculated values (assuming that the primary product of the amino alcohol-catalyzed reaction is an ester) and the observed values of ester content. The product of the acylation reaction was checked in the following systems: TEA with CI (pH 6–9), pNPA (pH 8–9), PBA (pH 7–8), NPCMG (pH 8); DEAE with NPCMG (pH 9), PMA (pH 9), PA (pH 9.5); DHEG with NPCMG and CI (pH 8); HEIDA with NPCMG and CI (pH 9); HEPES with CI (pH 9).

The rate of deacylation was measured in several systems. Under all the conditions used, deacylation was the rate-determining step, so that the catalysis acceleration factor (F) could be obtained by dividing the rate of deacylation k_d', by the rate of alkaline hydrolysis at the pH of the experiment (Table 5). As can be seen in some of the systems real catalysis ($F > 1$) of the hydrolysis of a substrate can be achieved via this acylation–deacylation mechanism. In the system containing DEAE and PA the rate of deacylation was measured at pH 9.5 at two additional values of ionic strength: at $I = 0.35$, $k_d' = 1.775 \times 10^{-2}$ min^{-1} (in 10% acetonitrile); at $I = 0.07$, $k_d' = 2.36 \times 10^{-2}$ min^{-1} (to be compared with a value calculated from Hansen's work (*6*) for *O*-acetyl-diethylamino-ethanol: $k_d' = 2.38 \times 10^{-2}$ min^{-1} at 25°C and $I = 0.07$).

TABLE 5

EFFICIENCY OF CATALYZED HYDROLYSIS OF VARIOUS SUBSTRATES BY TERTIARY AMINO ALCOHOLS (30°, $I = 0.5$)

Amino alcohol	Substrate	pH	$10^2 \times k'^{a}_{ac}$ (min^{-1})	$10^2 \times k'^{b}_{d}$ (min^{-1})	$10^2 \times k_0$ (min^{-1})	F^c
TEA	PBA	7.0	36.2	5.5	0.8	6.9
	PBA	8.0	163	10.6	8.0	1.3
DEAE	PMA	9.0	37.4	20.9	4.3	4.9
	PA	9.5	4.24	1.63	0.56	3.9

[a] First-order rate constant for the acylation step at amino alcohol concentration of 0.5 M corrected for alkaline hydrolysis at the same pH.

[b] Rate constant for deacylation.

[c] F = acceleration factor, the ratio between the deacylation rate k'_d and that of alkaline hydrolysis – k_0.

Comparison of tertiary amino alcohols with similar systems. The rates of reaction of tertiary amino alcohols with *p*-nitrophenyl and phenyl esters were compared with those of molecules possessing one of the two functional groups present in the model system: 1. tertiary amines; 2. a "rigid" tertiary amino alcohol (3-quinuclidinol); 3. quaternary amino alcohols.

1. *Tertiary amines.* The amines used in this study and their pK values are listed in Table 1. k_N values, obtained according to Eq. (10), are summarized in Table 6. As can be seen, the k_N values for the different substrates are lower than k_n values in the amino alcohol systems of comparable pK (Tables 2 and 3).

$$k'_N = k_N \cdot \alpha = k_N \cdot K/(K + a_H) \tag{10}$$

TABLE 6

k_N VALUES FOR THE REACTION OF TERTIARY AMINES WITH ACTIVE SUBSTRATES (30°, $I = 0.5$)[a]

Amine	k_N ($min^{-1}\ M^{-1}$)				
	pNPA	NPCMG	NPCMGO	PBA	CI
Diethylglycine ethyl ester[b]	—	0.028	0.14	0.075	0.028
Diethylglycinamide[c]	—	0.031	0.17	0.081	0.036
Nitrilotriacetate[d, e]	—	0.27	1.8	—	0.22
Triethylamine[f]	6.1	37	330	—	45.8

[a] pH range of measurements 8–9, amine concentrations 0.1–0.5 M.

[b] At pH 8.5, concentrations above 0.3 M could not be used because of the development of turbidity.

[c] At pH 9.0 the values of k_{obs} at an amine concentration of 0.5 M showed negative deviations, probably due to association of amine molecules.

[d] In this case $I = 3.0$.

[e] At pH 8 no effect was actually observed for NPCMG and CI and at pH 9.0 negative deviations occurred with 0.5 M amine (cf. *c*).

[f] For pNPA, rates were measured only at pH 10 and 11. For the other substrates the rates were measured at 8 and 9.

2. *3-Quinuclidinol.* Quol is a "rigid" tertiary amino alcohol, in which the hydroxyl and amino groups are unable to "co-operate," because they are rigidly held at a distance exceeding that of a possible hydrogen bond. This tertiary amino alcohol is generally considered to function as an amine (*23*). The rate of reaction of Quol with several substrates was studied at pH 9.0, 9.5, and 10, under the same conditions as those of the amino alcohols. k'_N values obtained at these three pH values were divided [according to Eq. (10)] by the fraction of free base calculated at each pH [using a pK value of 9.77 (Table 1)], and the resultant values were found to be pH independent. It was concluded that in the case of Quol neither the basic nor the acidic term of Eq. (8) contributes significantly to the rate of reaction at those pH values. The values thus calculated can, therefore, be taken as second-order rate constants, k_N, of the neutral species of Quol with the substrates (Table 7). However, variable amounts of ester product could be detected

TABLE 7

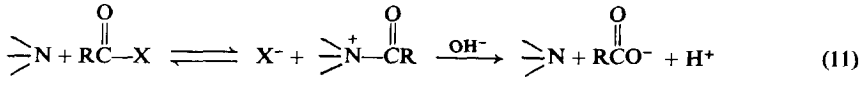
k_N VALUES AND PERCENTAGE OF ESTER PRODUCT FOR THE REACTIONS OF QUOL (30°, $I = 0.5$)

Substrate	k_N ($min^{-1}\ M^{-1}$)	% Ester product
NPCMG	9.1[a]	35
PA	0.056[b]	—
CI	26.0	70

[a] Experiments performed at a single pH value.

[b] To be compared with $k_2 = 0.076$ $min^{-1}\ M^{-1}$ at 25° and $I = 1.0$ (*23*).

in the reactions of Quol with all the substrates, due to *O*-attack of the zwitterionic ammonium–alkoxide species of Quol on these substrates. On the other hand, the behavior of Quol as an amine expresses itself in the dependence of the reaction rate on substrate concentration. Thus, in the reaction of 0.5 M Quol pH 9.0 with 6×10^{-3} M pNPA, the corrected first-order constant, i.e., $k_{obs} - k_0$, was reduced to about 50% of its value with 6×10^{-5} M substrate and similar effects were observed with all the substrates. This behavior can be interpreted, as was done by Jencks and Gilchrist (*23*) in a similar case, according to Eq. (11)



Quol (11)

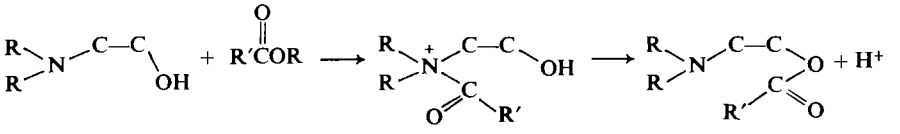
that is by reversal of the formation of the quaternary acyl-ammonium.

3. *Quaternary ammonium alcohols.* The neutral constant k_n for tertiary amino alcohols can be divided into two terms, k^0 contributed by the uncharged amino alcohol and $k^{\pm}$ contributed by the zwitterionic form. In order to evaluate the contribution of the zwitterionic pathway to the overall rate in the case of tertiary amino alcohols, the same

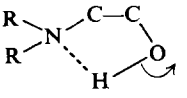
substrates were studied with the quaternary ammonium alcohols choline and *N*-methyl triethanolammonium iodide in the pH range of 7–9. Plots of k'_N as a function of a_{OH} gave straight lines with zero intercepts indicating that the only important species for the reaction is the zwitterionic alkoxide ion. The k'_b values obtained for choline are listed in Table 3. The value of k'_b for the reaction of methyl-TEA with PBA was found to be 1.25×10^6 min^{-1} M^{-2}.

DISCUSSION

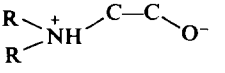
The principal pathway of the acylation reaction is via the neutral term, at least in the pH range studied. For example, using the rate constants of Table 1, it can be shown that between pH 8 and 10 more than 90% of the reaction rate of DEAE with CI can be accounted for by the neutral pathway. The important question concerning the mechanism of this reaction is whether one has N-attack, followed by a rapid N → O shift, Eq. (12), or O-attack of the bifunctional nucleophile on the substrate. In the latter case one has to consider two alternative mechanisms (V and VI):



(12)



V



VI

By comparing the characteristics of the reactions of tertiary amino alcohols with those of tertiary amines on one hand and those of oxyanions on the other, it was possible to distinguish between N-attack and O-attack. The following arguments can be put forward in favour of O-attack and, therefore, exclude N-attack, Eq. (12):

(*a*) The reactivity of amino alcohols is proportional to their number of hydroxyl groups. Linear Brønsted plots (Eq. 13) were obtained

$$\log k'_n = \beta pK + C \tag{13}$$

only after dividing k_n values by the number of hydroxyl groups in the various amino alcohols (Fig. 3). The values of β and C obtained from these plots are summarized in Table 8.

TABLE 8

PARAMETERS OF BRØNSTED'S EQUATION FOR TERTIARY AMINO ALCOHOLS

Substrate	pNPA	NPCMG	NPCMGO	PMA	CI
β	0.89	0.90	0.67	0.93	0.90
C	−8.49	−7.73	−6.91	−8.04	−6.65

(*b*) The observed order of substrate reactivity as reflected by the *C* values

$$\text{CI} > \text{NPCMGO} > \text{NPCMG} > \text{PMA} > \text{pNPA}$$

is the same as in the case of oxyanions, such as choline (cf Table 3) and is different from that observed with tertiary amines (Table 6). The same order of reactivity has also been found for nontertiary amino alcohols, but not for nontertiary amines (*24*).

The outstanding reactivity of CI toward basic oxyanions is probably due to the high sensitivity of the reactions of a substrate with a poor leaving group, the imidazole anion, (pK_a 14.2) (*25*) to the basicity of the nucleophile (*26*). On the other hand, in the

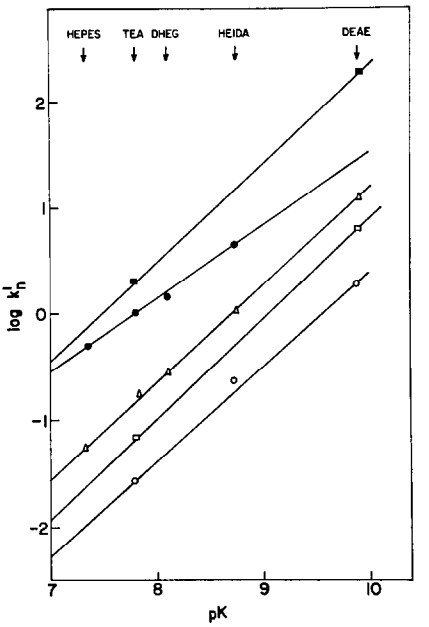


FIG. 3. Brønsted plots for the reaction of tertiary amino alcohols with esters at 30°, $I = 0.5$. ○–○ pNPA; □–□ PMA; △–△ NPCMG; ●–● NPCMGO; ■–■ CI. k_n' is k_n divided by the number of hydroxyls in the amino alcohol.

reactions of oxyanions with substrates possessing a good leaving group, such as *p*-nitrophenolate, there is a leveling off of the dependence of the reaction rate on basicity (*23*, *27*), while this behavior is not observed, or is at least much less pronounced, for neutral amines. The combination of these two effects causes acyl-imidazoles to be more sensitive than *p*-nitrophenyl esters toward oxyanions. Being thus much more reactive toward negatively charged oxyanions than toward neutral amines, the position of CI in the order of reactivity of substrates may be used, in ambiguous cases, as a criterion with regard to the nature of the nucleophile. In the case of amino alcohols its position among the substrates with respect to reactivity supports the contention that these reactions involve nucleophilic attack by the hydroxyl group and not by the amine group.

(*c*) Applying Hammett's Eq. (*28*)

$$\log k/k_0 = \rho\sigma \qquad (14)$$

a value of $\rho = 0.85$ was obtained for the neutral term of the reaction of DEAE with phenyl acetates, using a value of $\sigma = 1.0$ for the p-NO_2 substituent on the phenyl ring (*29*). This value falls within the range observed for typical oxyanion reactions (0.6–1.0), while the ρ values of amine reactions are higher (2.1–2.9) (*3*). A similar value ($\rho = 0.71$) had been found (*21*) for the neutral term of the reaction of Tris with phenyl acetates, thus lending support to the contention that even in the case of nontertiary amino alcohols O-attack is the predominant pathway of the reaction. Experiments with diethanolamine (secondary amine) and Tris indicate that their reactivity is higher than that of comparable amines and that ester products are formed through a neutral form of the amino alcohol (*24*).

(*d*) The sensitivity of the reaction to electronic effects of the substituents in the acyl moiety of the substrate can be expressed by the inductive ρ_I values.[4]

The following values of σ_I were used for the acyl substituents of the phenyl esters, PA, 0, PMA, 0.25, and PBA, 0.45, and for those of the p-nitrophenyl esters: pNPA-0: NPCMG-0.276 (*30*), and NPCMGO-0.43. Plots of $\log k_n$ as a function of σ_I yielded straight lines, whose slopes are the ρ_I values (Table 9). The almost identical sensitivity

TABLE 9

ρ_I VALUES FOR THE REACTIONS OF NUCLEOPHILES WITH PHENYL AND p-NITROPHENYL ESTERS

Nucleophile	OH^- (k_{OH})	DEAE (k_n)	TEA (k_n)	TEA (k_b')
ρ_I (phenyl esters)	5.56	5.50	—	—
ρ_I (p-nitrophenyl esters)	3.94	2.96	3.48	3.84

of the reactions of the hydroxide ion and of the basic and neutral species of amino alcohols to electronic effects in the acyl moiety of the substrates indicates that they are all mainly oxyanion reactions. It was shown previously (*31–33*) that the ρ_I values for the amine reactions differ from those for the oxyanion reactions (including hydroxide ion) for several series of substrates. For o-nitrophenyl acetates, for example, the values of ρ_I obtained for amines were 3.2 ± 0.4, while those for oxyanions were 5.2 ± 0.4. Our values for the reaction of oxyanions with phenyl esters are in agreement with the above-mentioned, whereas with p-nitrophenyl esters the values were found to be lower ($\sigma_I = 3.5 \pm 0.5$), probably indicating that these reactions, which involve more bond breaking of the leaving group in the transition state, may be less sensitive to the bond-making process (*34*). In agreement with this assumption, the ρ_I values obtained for the reactions of amines with those p-nitrophenyl esters [$\rho_I = 1.1$ with diethylamine (*24*)] are similarly lower (by about two units) than the comparable values for phenyl esters (*33*, *34*).

(*e*) The second-order rate constants of reaction of tertiary amino alcohols were not found to be dependent on substrate concentration, as was the case with some tertiary amines (such as Quol).

[4] Similarly to Hammett's equation, one can write for the inductive ρ_I value: $\log k/k_0 = \rho_I \sigma_I$.

(*f*) The values of k_n for amino alcohols are 10- to 40-fold higher than those of k_N for comparable amines with polar substituents, such as diethylglycinamide and nitrilotriacetate (Fig. 3 for the substrate NPCMG). Brønsted law is obeyed with $\beta = 0.8$ in this series, but the reactivity of these amines is about 10-fold lower than that of triethylamine and Quol (*23*). However, diethylglycinamide and nitrilotriacetate are more adequate systems for evaluating the contribution of N-attack in tertiary amino alcohols to the total rate of reaction. This can be deduced from the fact that negligible amounts of hydrolysis products are found with the amino alcohols, indicating that N-attack hardly

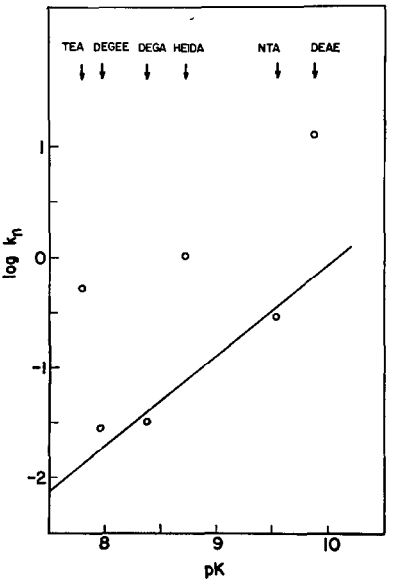


FIG. 4. Brønsted plots for the reactions of tertiary amines with NPCMG, at 30°, $I = 0.5$. (DEGEE, diethylglycine ethyl ester; DEGA, diethylglycinamide; NTA, nitrilotriacetate.)

contributes to the reaction rate. On the other hand, hydrolysis products are expected if N-attack is an important pathway, since the quaternary *N*-acyl-amino alcohol would partition between the hydroxyl groups and hydroxide ions.

With respect to the question of the mechanism of O-attack, i.e., the differentiation between mechanisms V and VI, the following points should be considered: Introduction of a negative charge into an amino alcohol molecule (carboxylate derivatives) was found to reduce the rate constant for the basic term of the reaction, k_b', by a factor of 6 (compare k_b' values for TEA and DHEG, Tables 2 and 3). If the neutral species of the amino alcohol behaved like a zwitterion containing an alkoxide anion, the rate of reaction should be similarly influenced by negative charges. That this is not so can be seen by comparing the k_n values for DHEG and HEIDA (Table 3), and from Fig. 3, where no deviations were observed in the Brønsted plots, which included both neutral and negatively charged amino alcohols. On the other hand, relatively small kinetic isotope effect (1.08—see Table 4) is not in agreement with mechanism V. Larger isotope effects are expected for reactions involving proton transfer in the rate-determining step. However, several

general base-catalyzed reactions involving good leaving groups are known, in which the kinetic isotope effect is either small or nonexistent (*29*, *35*).

In order to estimate the contribution of the uncharged species to the neutral mechanism, the rate constant of solvolysis catalyzed by the neutral amino alcohol was compared to that of quaternary ammonium analogs. In Quol, the amino group cannot "cooperate" with the hydroxyl group; the only pathway yielding ester products is that of the tertiary ammonium alcohol zwitterion. Thus, the rate constant of ester formation, i.e., $k_N \times$ ester fraction (Table 7), with Quol can also be used as a criterion for the zwitterionic mechanism (VI). Assuming that the reaction rate of the quaternary zwitterion $R_3\overset{+}{N}CH_2CH_2O^-$ and of Quol zwitterion are the same as that of the zwitterionic tertiary ammonium alcohol $R_2\overset{+}{N}HCH_2CH_2O^-$, it was possible to estimate for tertiary amino alcohols both k^0, the rate constant of the uncharged species $R_2NCH_2CH_2OH$, and $k^{\pm}$, which is the contribution of its zwitterionic isomer $R_2\overset{+}{N}HCH_2CH_2O^-$. [See Appendix Eqs. (4A) and (5A)]. The results of such an analysis are summarized in Table 10. It can, therefore, be concluded that the hydrogen-bonded amino alcohol certainly provides one of the pathways for the reaction, in addition to that contributed by the zwitterionic mechanism.

TABLE 10

EVALUATION OF THE CONTRIBUTION OF THE UNCHARGED SPECIES OF TERTIARY AMINO ALCOHOLS TO THE NEUTRAL MECHANISM[a]

System	k^0/k_n[b]	$k^0/k_\pm$[b]	k^0/k_n[c]	$k^0/k_\pm$[c]
DEAE + pNPA	0.57	1.3		
DEAE + NPCMG	0.55	1.2	0.78	3.6
DEAE + CI			0.86	6
DEAE + PA			0.75	3
TEA + PBA	0.77	3.3		

[a] k_n values for TEA from Table 2, k_n values for DEAE, and k_b values for choline—Table 3, k_b values for *N*-methyl-TEA see text.

[b] Comparison of DEAE with choline and TEA with *N*-methyl-TEA iodide.

[c] Comparison of DEAE with Quol.

The basic term of the reaction. The mechanism effective in this case is specific base catalysis, where the hydroxide ion converts the amino alcohol into an oxyanion in an equilibrium process preceding the rate-determining step:

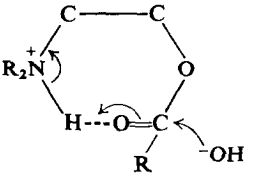
$$R_2NCH_2CH_2OH + OH^- \overset{K_{OH}}{\rightleftharpoons} R_2NCH_2CH_2O^- + H_2O \qquad (15)$$

Consistent with this mechanism are the dependence of the reaction rate on the negative charge of the amino alcohol and the kinetic isotope effect value of 0.73 (Table 4). This value includes the ratio of equilibrium constants for alcohol ionization—K_{OH} in H_2O and D_2O, where $K_{OH} = K/K_W$.

$$\frac{k_b'^{H}}{k_b'^{D}} = \frac{k_b{}^{H} K_{OH}^{H}}{k_b{}^{D} K_{OH}^{D}} \qquad (16)$$

While the ratio K_w^H/K_w^D at 30°C is 6.6 (*19*) the ratio of the alcohol dissociation constants K^H/K^D can be evaluated from Rule and La Mer's plot[5] as 4.35, thus leading to a value of K_{OH}^H/K_{OH}^D of 0.66. The small value for the ratio of the rates of attack of the alkoxide of TEA on substrates, $k_b^H/k_b^D = 1.11$, is due to different general solvent effects in H_2O and D_2O (*36*). Our value of $k_b'^H/k_b'^D$ is thus in the range of values observed for basic terms of alcohol reactions using an identical rate law (*3*, *36*).

Deacylation mechanism. The most plausible mechanism for deacylation of the acylated amino alcohols is the one suggested by Hansen (*6*) (VII) in which the protonated ammonium group causes polarization of the ester carbonyl, thus facilitating its attack by the hydroxide ion (*6*).



(VII)

A similar mechanism could be suggested for the acidic term of the acylation step, where general base and/or general acid catalysis are likely to assist the attack of the undissociated hydroxyl group.[6]

In addition to Hansen's arguments (*6*, *37*) in favor of the deacylation mechanism VII, the effect of ionic strength upon the rate of reaction as found in this work (see above) is also consistent with the suggested mechanism. The rate of deacylation was observed to decrease with increasing ionic strength, as should be expected for the reaction of two ions with opposite charges (*38*).

CONCLUSION

The data presented here establish that tertiary amino alcohols are true catalysts of the hydrolysis of active esters (Table 5). In addition, they function through a two-step mechanism of acylation and deacylation of an hydroxyl group and in the neutral pH range. Although the rate acceleration is modest, it is of the same order of magnitude as those of similar models (*39*, *40*). The failure to observe higher catalytic acceleration factors (Table 5) is due to the fact that the deacylation step is rate limiting and its rates are lower than or similar to those found for spontaneous hydrolysis of activated esters. It is likely that by using worse leaving groups, acylation might become rate limiting, thereby enabling one to attain higher catalytic factors at high concentration of amino alcohols.

[5] By extrapolation from Fig. 2 of C. K. Rule and V. K. La Mer, *J. Amer. Chem. Soc.* **60**, 1981 (1938), using pK 14 for the hydroxyl groups of TEA as for those of pentaerythritol (*21*).

[6] Hansen has found that *O*-acetyl dimethylaminoethanol in its protonated form undergoes base-catalyzed hydrolysis about 400 times faster than the unprotonated form, and about 30 times faster than *O*-acetyl choline.

APPENDIX

The contribution of the "uncharged" mechanism to the neutral term of the reaction. The protonated form of a tertiary amino alcohol, denoted $\overset{+}{H}A\overset{+}{H}$, can dissociate to the uncharged species AH^0 and to zwitterionic species $HA^{\pm}$.

$$HA^{\pm} + H^+ \overset{K_Z}{\rightleftharpoons} \overset{+}{H}A\overset{+}{H} \overset{K_0}{\rightleftharpoons} H^+ + AH^0$$

$$[H^+][HA^{\pm}]/[\overset{+}{H}A\overset{+}{H}] = K_Z \qquad [H^+][AH^0]/[\overset{+}{H}A\overset{+}{H}] = K_0$$

hence,

$$[HA^{\pm}]/[AH^0] = K_Z/K_0 \simeq \alpha_Z \tag{1A}$$

As generally $K_Z \ll K_0$ (for DEAE $K_0 = 10^{-9.87}$), and assuming that K_Z is the same as for choline ($K_Z = 10^{-13.9}$), Eq. (1A) gives α_Z, the fraction of the zwitterionic species in the neutral form. ($\alpha_Z \simeq 0.93 \times 10^{-4}$ for DEAE.)

The rate of the reaction between substrate and basic alcohol is given by:

$$v = k_b'[S][ROH][OH^-] = k_b[S][RO^-]$$

hence,

$$k_b = \frac{k_b'[ROH][OH^-][H^+]}{[RO^-][H^+]} = k_b'\frac{K_W}{K_Z} \tag{2A}$$

Assuming that the neutral term k_n is composed to two specific rate constants, k^0, contributed by the uncharged species, and $k^{\pm}$, contributed by the zwitterionic species, we can write:

$$k_n([AH^0] + [HA^{\pm}]) = k^0[AH^0] + k^{\pm}[HA^{\pm}]$$

$$= k^0([AH^0] + [HA^{\pm}])\frac{K_0}{K_Z + K_0} + k^{\pm}([AH^0] + [HA^{\pm}])\frac{K_Z}{K_Z + K_0}$$

hence,

$$k_n = k^0\frac{K_0}{K_Z + K_0} + k^{\pm}\frac{K_Z}{K_Z + K_0} \tag{3A}$$

As $K_0 \gg K_Z$

$$k_n = k^0 + k^{\pm}K_Z/K_0 \tag{4A}$$

For the zwitterionic species $k^{\pm} = k_b$. Substituting Eq. (2A) in (4A), we obtain

$$k_n = k^0 + k_b K_Z/K_0 = k^0 + k_b' K_W/K_0 \tag{5A}$$

If $k^{\pm}$, the second-order rate constant for the zwitterion, and K_Z can be determined, k^0 is obtainable from Eq. (4A). Generally, however, it is easier to find k_b', the third-order rate constant, and K_0, the dissociation constant of the amino alcohol, and k^0 can then be calculated from Eq. (5A).

ACKNOWLEDGMENT

We express our thanks to Drs. W. P. Jencks, S. A. Bernhard, and P. Greenzaid for helpful discussions.

REFERENCES

1. L. CUNNINGHAM, "Comprehensive Biochemistry," (M. Florkin and E. H. Stotz, Eds.), Vol. **16**, p. 85, Elsevier, Amsterdam, 1965.
2. H. NEURATH, *Sci. Amer.* **211**, 68 (1964).
3. T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. **1**, Chap. 1, Benjamin, New York, 1966.
4. W. P. JENCKS, "Catalysis in Chemistry and Enzymology," Part 1, McGraw-Hill, New York, 1969.
5. T. C. BRUICE AND S. J. BENKOVIC, *J. Amer. Chem. Soc.* **85**, 1 (1963).
6. B. HANSEN, *Acta Chem. Scand.* **16**, 1927 (1962).
7. G. B. BACHMAN AND D. E. WELTON, *J. Org. Chem.* **12**, 221 (1947).
8. E. FISHER AND H. O. L. FISHER, *Ber.* **47**, 771 (1914).
9. "Organic Syntheses," (E. C. Horning, Ed.), Coll. Vol. **3**, p. 168, Wiley, New York, 1955.
10. R. SCHONBAUM, B. ZERNER, AND M. L. BENDER, *J. Amer. Chem. Soc.* **84**, 2540 (1962).
11. A. I. VOGEL, "A Textbook of Practical Organic Chemistry," 3rd Ed., p. 669, Longmans, London, 1964.
12. T. H. FIFE AND T. C. BRUICE, *J. Phys. Chem.* **65**, 1079 (1961).
13. S. A. BERNHARD, Y. SHALITIN, AND Z. H. TASHJIAN, *J. Amer. Chem. Soc.* **86**, 4406 (1964).
14. R. A. ROBINSON AND A. I. BIGGS, *Trans. Faraday Soc.* **51**, 901 (1955).
15. J. McD. ARMSTRONG, D. V. MYERS, J. A. VEERPORTE, AND J. T. EDSALL, *J. Biol. Chem.* **241**, 5137 (1966).
16. S. HESTRIN, *J. Biol. Chem.* **180**, 249 (1949).
17. R. A. ROBINSON AND H. H. STOKES, "Electrolyte Solutions," 2nd Ed., p. 544, Butterworths, London, 1959.
18. E. A. GUGGENHEIM, *Phil. Mag.* **22**, 322 (1926).
19. T. C. BRUICE AND J. J. BRUNO, *J. Amer. Chem. Soc.* **83**, 3494 (1961).
20. J. T. EDSALL AND J. WYMAN, "Biophysical Chemistry," Vol. **1**, p. 419, Academic Press, New York, 1958.
21. T. C. BRUICE AND J. L. YORK, *J. Amer. Chem. Soc.* **83**, 1382 (1961).
22. A. A. FROST AND R. G. PEARSON, "Kinetics and Mechanism," 2nd Ed., Wiley, New York, 1961.
23. W. P. JENCKS AND M. GILCHRIST, *J. Amer. Chem. Soc.* **90**, 2622 (1968).
24. M. M. WERBER AND Y. SHALITIN, unpublished results.
25. G. YAGIL, *Tetrahedron* **23**, 2855 (1967).
26. D. C. OAKENFULL AND W. P. JENCKS, *J. Amer. Chem. Soc.* **93**, 178, 188 (1971).
27. W. P. JENCKS AND M. GILCHRIST, *J. Amer. Chem. Soc.* **84**, 2910 (1962).
28. L. P. HAMMETT, "Physical Organic Chemistry," Chaps. 3, 4, 7, McGraw-Hill, New York, 1940.
29. T. C. BRUICE AND S. J. BENKOVIC, *J. Amer. Chem. Soc.* **86**, 418 (1964).
30. M. CHARTON, *J. Org. Chem.* **29**, 1222 (1964).
31. B. HOMQUIST AND T. C. BRUICE, *J. Amer. Chem. Soc.* **91**, 2985 (1969).
32. K. KOEHLER, R. SKORA, AND E. H. CORDES, *J. Amer. Chem. Soc.* **88**, 3577 (1966).
33. J. F. KIRSCH AND A. KLINE, *J. Amer. Chem. Soc.* **91**, 1841 (1969).
34. T. C. BRUICE, A. F. HEGARTY, S. M. FELTON, A. DONZEL, AND N. G. KUNDU, *J. Amer. Chem. Soc.* **92**, 1370 (1970).
35. M. L. BENDER, E. J. POLLOCK, AND M. C. NEVEU, *J. Amer. Chem. Soc.* **84**, 595 (1962).
36. P. BALLINGER AND F. A. LONG, *J. Amer. Chem. Soc.* **81**, 2347 (1959).
37. B. HANSEN AND A. FLORMARK, *Acta Chem. Scand.* **17**, 1481 (1963).
38. E. S. AMIS, "Solvent Effects on Reaction Rates and Mechanisms," Academic Press, New York, 1966.
39. T. C. BRUICE AND G. L. SCHMIR, *J. Amer. Chem. Soc.* **79**, 1663 (1957).
40. W. B. GRUHN AND M. L. BENDER, *J. Amer. Chem. Soc.* **91**, 5883 (1969).