Rates of Alkaline Hydrolysis and Muscarinic Activity of Some Aminoacetates and Their Quaternary Ammonium Analogs

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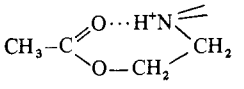
The muscarinic activity and rates of hydrolysis of a series of acetoxyethylamine derivatives were compared in an effort to explain the differences in potencies of the tertiary and quaternary amino esters. The more flexible compounds, like dimethylaminoethyl acetate (**1A**), are much weaker muscarinic agents than their corresponding quaternary analogs (**1B**), while rigid compounds, like aceclidine (**6A**), are more potent than their methochlorides. These differences may be rationalized in terms of a cyclic ammonium conformation which could readily be formed by the open chain derivatives, and which may also account for an anchimeric acceleration of the hydrolysis of the tertiary amino ester. Kinetic experiments have also provided evidence for the mechanism of this anchimerically assisted reaction.

In a study investigating the effect of pH on the muscarinic activity of some tertiary amines, it was noted that the hydronium ions of oxotremorine, arecoline, and pilocarpine were more potent muscarinic agents than their *N*-Me quaternary salts.[1] A similar relationship has been reported for the tertiary amine aceclidine (3-acetoxyquinuclidine)[2,3] and some arecoline analogs.[4] In contrast, the more classical cholinergic agents related to ACh are more active than the hydronium ions of their *N*-demethyl analogs.[5-7] In all the compounds whose tertiary derivatives are more effective than their *N*-Me quaternary analogs, the basic N is contained in a ring while in pairs of compounds in which the reverse is true the N is attached to an aliphatic chain.

These differences have been rationalized in terms of the conformations of the 2 types of compounds in aqueous solution.[1] If interaction with the receptor requires that the C=O group and positively charged N be arranged in either a transoid or open chain conformation, the ease with which the compounds could attain this conformation should be reflected in their potency. Conversely, any molecular feature that opposed this conformation would be expected to decrease potency. In a flexible molecule like dimethylaminoethyl acetate (**1A**), the attraction between the proton on the charged N and the C=O could favor a large population of conformations in which these 2 groups are close together, while the more rigid cyclic compounds could assume these conformations only with difficulty. Because of their tendency to form cyclic conformations, the flexible tertiary amino esters would be less potent than their quaternary derivatives.

This postulate was based in part on the reports of Hansen[8,9] that the cyclic ammonium structure (A) of dimethylaminoethyl acetate has an anchimeric effect on its alkaline hydrolysis. No such effect was observed with the alkaline hydrolysis of aceclidine because of the inability of this rigid molecule to attain a cyclic ammonium conformation.



This postulate was examined further in the study reported here, in which a comparison was made of the muscarinic activity and rates of hydrolysis of pairs of acetoxyethylamine derivatives with differing degrees of flexibility about the ethylamine side chain (Figure 1). Each pair consists of a tertiary amine and its corresponding *N*-Me quaternary ammonium compound. The propensity of each compound to assume a cyclic conformation in aqueous solution may be inferred from an analysis of its basic hydrolysis kinetics as a function of pH. The results provide support for the view that tertiary amines for which there is a large probability of a cyclic conformation in aqueous solution are likely to be less active as muscarinic agents than their quaternary analogs, while those in which the probability is low may be very potent in comparison to their *N*-Me derivatives.

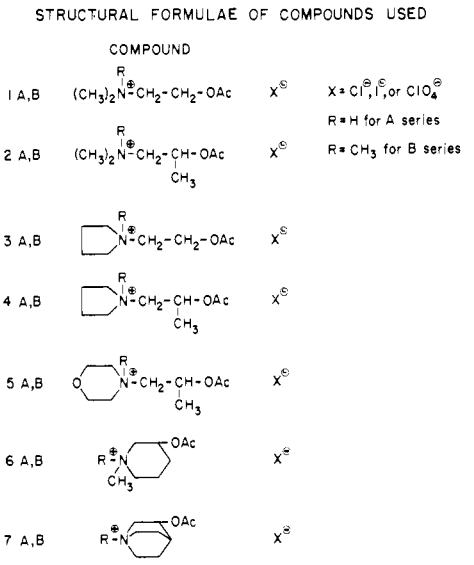


Figure 1. The A series refer to the tertiary amines and the B series refer to the quaternary ammonium derivatives.

Experimental Section

Muscarinic Activity. Strips of ileum were removed from guinea pigs weighing 350–500 g and arranged for isotonic recording in oxygenated Krebs soln contg morphine (15 μM) at pH 7.40 at 37°. After 30 min Mipafox (diisopropyl phosphorofluoridate) was added to make a concn of 50 μM. The soln was replaced after an interval of 1 hr with Krebs soln contg morphine (15 μM) which was used thereafter for all washes. Cumulative dose–response curves were obtd at intervals of 30 min followed by repeated washing until the length returned to its control value. The potency of each pair of compds relative to bethanechol as a standard was estimated by interpolation from the relative concns required to produce a 50% response. At least 2 dose–response curves were obtd with each compd. All compds except **5b** yielded the same maximum response.

Synthesis. The physical properties of the compds used in this study are presented in Table I. All of the starting amino alcohols were commercially available and were acetylated by conventional procedures. The amino alcohol hydrochlorides (prepd in Et_2O) were recrystd several times for pK_a detn.

pK_a Determinations. All pH measurements were made with a Radiometer Model 4 pH meter. The apparent acid dissociation constants were detd at 30° by measurement of the pH at 0.25, 0.50,

Table I. Physical Properties and Microanalytical Data

Compound	Mp, °C	Empirical formula[a]
1A	67.5–69.0	$C_6H_{13}NO_2 \cdot HClO_4$[b]
1B	113.5–114.5	$C_7H_{16}ClNO_6$[b]
2A	120–121	$C_7H_{15}NO_2 \cdot HCl$[c]
2B	78–79	$C_8H_{18}ClNO_6$
3A	121–123	$C_8H_{15}NO_2 \cdot HCl$
3B	65–68	$C_9H_{18}INO_2$
4A	146–148	$C_9H_{17}NO_2 \cdot HCl$
4B	99–100	$C_{10}H_{20}INO_2$
5A	188–189	$C_9H_{17}NO_3 \cdot HCl$
5B	178–180	$C_{10}H_{20}NO_3 \cdot I$
6A	143–144	$C_8H_{15}NO_2 \cdot HCl$
6B	150–152	$C_9H_{18}NO_2I$
7A	178–179	$C_9H_{15}NO_2 \cdot HCl$
7B	160–163	$C_{10}H_{18}INO_2$[d]

[a] All compds were analyzed for C, H except those noted under b, c, d. [b] Ref 10. [c] Ref 11. [d] Ref 12.

and 0.75 neutralization by addition of appropriate aliquots of 0.02 *N* KOH to a 20-ml vol of 0.02 *M* amine salt. To compensate for changes in H^+ concn due to hydrolysis during measurement, the pH was measured at 1-min intervals for 5 min after the addn of alkali, and the H^+ concn at 0 time was detd by extrapolation. The pK_a values were calcd at each neutralization point and averaged.

Hydrolysis Kinetics. Rates of hydrolysis were detd by continuous titration in a Radiometer titrator equipped with a SBR-2 Titrigraph and a SBU-1 syringe burette. The pH was monitored precisely with a Radiometer PHM4 pH meter with a common calomel electrode but with a separate glass electrode. The reaction was carried out in a water-jacketed vessel maintained at 30° with a loosely fitted cover with holes for the 3 electrodes, the burette tip, and an N_2 inlet tube. The 0.06 *N* NaOH used as a titrant was standardized daily by titration against a 1-ml aliquot of 0.02 *M* hydrogen potassium phthalate to an end point of pH 8.0.

The ester hydrolyses were started by adding 4 ml of 0.05 *M* ester salt to 14 ml of boiled glass-distd H_2O and 2 ml of 0.7 *M* KCl equilibrated to 30°. The pH was rapidly adjusted to the desired value with 1 *N* NaOH and the titration was contd automatically for 5–20 min. Several such titrations were carried out with each compd over a pH range of 1–2 units in order to obtain satisfactory confidence limits on the rate constants derived from the regression analysis (see below). The number of pH values for each tertiary amine varied from 6 to 12. In the case of quaternary ammonium compounds, the apparent rate constant k was estimated over the same pH range at 4–6 values for each compd and was found to be independent of pH in every case.

Kinetic Theory. In a constant pH titration of a reaction in which a H^+ is one of the products, the rate of addn of base is not in general the same as the reaction rate if any of the reactants or products are bases or acids. The relation between the reaction rate (V) and the apparent rate (*i.e.*, rate of addn of the titrant V_a) has been derived in previous studies[8,13] and in the present case is given by the equation $V_a/V = 1 - \{1/[1 + ([H^+]/K_a)]\} + \{1/[1 + ([H^+]/K_e)]\}$ where K_a is the dissn constant of the amino alcohol and K_e is the dissn constant of the amino ester. In the present series of compds, the pK_a of the amino alcohol in every case exceeded that of its acetate so that the actual reaction rate was always larger than the rate of addn of titrant required to maintain a constant pH. For the quaternary ammonium compds no such correction is required and the 2 rates are stoichiometrically equiv. AcOH is too strong to require a correction of this type within the pH range studied.

As expected, the hydrolysis rate decayed slowly with time and the initial rate was estimated from a tangent to the titrigraph record at 0 time. From the initical molar concn of the ester $[E]_0$ an apparent bimolecular rate constant $\bar{k} = V/([E]_0[OH^-])$ was calcd, which varied with the ester, and also with the pH at which the titration was run in the case of the tertiary amines. Two parallel reaction mechanisms must be considered: $V = k_1[OH^-][E] + k_2[OH^-][E^+]$, where [E] and $[E^+]$ refer to concns of the unprotonated and protonated amino esters respectively (see Discussion).

It follows that: $\bar{k} = k_1([E]/[E]_0) + k_2([E^+]/[E]_0) = k_1(1 - \phi) + k_2\phi$, where $\phi = ([E^+]/[E]_0)$, since $[E] + [E^+] = [E]_0$.

A linear relation therefore exists between the apparent bimolecular rate constant $\bar{k}$ and the proportion (ϕ) of amino ester which is protonated. The latter may be calcd from the pH if the pK_a of the amino ester is known. A plot of k against ϕ was found to give a straight line in every case, which was fitted by unweighted linear regression. The intercept on the ordinate provides an estimate of k_1 while the extrapolated value of $\bar{k}$ corresponding to $\phi = 1$ estimates k_2. Standard errors of the estimates of k_1 and k_2 were calcd from the equations:

$$\text{S.E.}\,(k_1) = s\left\{\frac{1}{N} + \frac{\bar{\phi}^2}{S_{\phi\phi}}\right\}^{1/2}$$

$$\text{S.E.}\,(k_2) = s\left\{\frac{1}{N} + \frac{(1-\bar{\phi})^2}{S_{\phi\phi}}\right\}^{1/2}$$

where N is the number of points from which the regression line is estimated, s^2 is the error variance estimated from derivations from linear regression, $\bar{\phi}$ is the mean value of ϕ, and $S_{\phi\phi}$ is the sum of squares of deviations of ϕ from the mean.

Table II. Relative Muscarinic Activities of Amino Esters (Guinea Pig Ileum)

Compound	Log_{10} of relative potency[a] (Bethanechol = 0) Tertiary amine	Quaternary ammonium	Quaternary / Tertiary	Corrected for ionization
1	−0.58	+1.48	2.06	2.00
2	−1.12	+1.44	2.57	2.52
3	−1.54	+1.06	2.60	2.58
4	−2.42	+0.81	3.23	3.22
5	Inactive	−1.99		
6	−1.94	−1.12	0.82	0.76
7	+0.92	−1.42	−2.34	−2.35

[a] The relative potency refers to the ratio of molar concns of bethanechol and the test compd which produce a 50% response and is expressed as the log. The quaternary/tertiary ratio is obtd from the algebraic difference of the first 2 columns. In the last column, the value for the tertiary amine has been adjusted to the concn of ionized tertiary amine for a 50% response.

Results

Muscarinic Activity. The muscarinic activities of the amino esters are shown in Table II. The values are given relative to bethanechol chloride which was used to obtain a standard response. The quaternary derivatives of **3** and **4** were comparable to the standard while aceclidine methiodide **7B** was about 0.04 of the standard in potency. The order of relative potency is partially reversed in the tertiary amines since now aceclidine (**7A**) is the most potent agent in the group. The quaternary/tertiary ratio is expressed logarithmically to indicate the change in muscarinic potency with quaternization. The arithmetic values of these ratios cover a range of 10^6. Since work with other muscarinic agents indicates that the protonated forms of the tertiary amines are responsible for the muscarinic effects rather than the free base[1,14] it seemed probable that this would be true for these compounds as well. Accordingly, the last column of Table II was calcd to show the quaternary/tertiary activity ratio when the concn of the tertiary amine was adjusted for its ionization at the pH used. These corrections were computed using the measurements of pK_a shown in Table III. Since all of the amines are relatively strong bases except for **5**, they exist at pH 7.40 mainly in the ionized form, and the corrections are small. The single exception is **5A** which is only about 3% ionized. This presumably accounts for its low muscarinic activity which was too small to be detected under the conditions used.

Kinetic Data. Figure 2 shows a representative plot of the apparent second-order rate constant against proportion ionized for the tertiary amino ester **4A**. Significant linear regression was obtained with all of the tertiary compounds,

Table III. Apparent pK_a Values of the Amino Esters and Corresponding Alcohols[a]

Compound	pK_a	
	Ester	Alcohol
1	8.25	9.42
2	8.30	9.28
3	8.82	9.63
4	8.87	10.12
5	6.03	7.30
6	8.24	8.80
7	8.93	9.88

[a]The apparent pK_a values were detd in 0.9% NaCl at 30° by titration of the hydrochloride with NaOH.

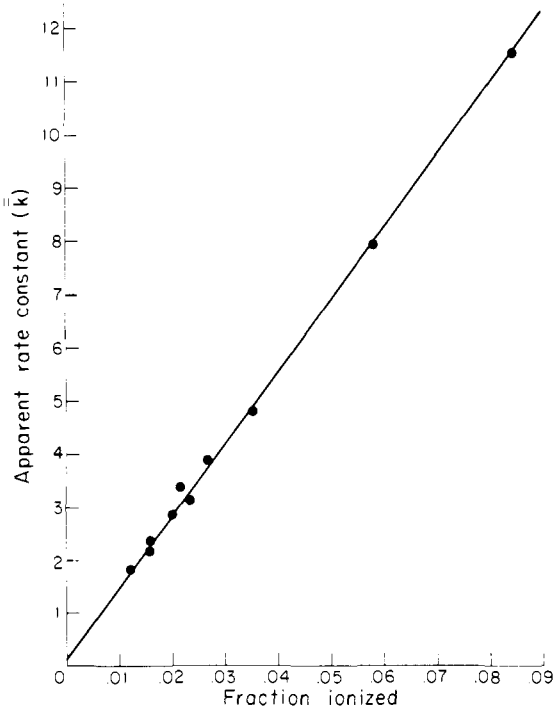


Figure 2. Effect of ionization on rate of hydrolysis of pyrrolidinoisopropyl acetate (4A). The apparent rate constant in 1 mole^{-1} sec^{-1} is plotted against the fraction ionized calcd from the pK_a value listed in Table III. The value of $\bar{k}$ at $\phi = 0$ is k_1, and that at $\phi = 1.0$ is k_2, where $\phi = E^+/E_0$. Similar data were collected for all tertiary amines in this study.

and in every case, the Y intercept differed significantly from 0. From such data the specific rate constants summarized in Table IV were calcd. The second-order rate constants for the quaternary ammonium compounds (k_4) in no case showed a significant regression on pH and the constants are also listed in Table IV.

The range of rate constants for the unionized amino esters (k_1) and the quaternary esters (k_4) vary only by a factor of about 5 while the rate constants for the protonated esters (k_2) vary by 3 orders of magnitude. The k_1 values are the lowest of the 3 rate constants k_1, k_2, and k_4 and are similar to that reported[8] for EtOAc (0.11) reflecting a minimal contribution from the neutral N to the rate of hydrolysis. When the N is permanently charged, as in the quaternary derivatives, the rate constants all increase because of the inductive effect of the positive charge. That these effects are relatively consistent from compound to compound is reflected in the small range of values for k_1 and k_4. The base strengths of all of the esters are about the same except for the morpholine derivative (5). This compound also has the largest rate constant for hydrolysis of the protonated amine.

Table IV. Kinetic Constants for Ester Hydrolysis[a]

Compound	k_1	k_2	k_4
1	0.22 ± 0.06 (6)	108.4 ± 1.4	2.42 ± 0.04 (4)
2	0.61 ± 0.15 (6)	139.4 ± 2.6	0.68 ± 0.02 (5)
3	0.50 ± 0.08 (12)	52.9 ± 1.6	2.12 ± 0.45 (5)
4	0.20 ± 0.05 (10)	133.7 ± 1.6	0.51 ± 0.02 (6)
5	0.34 ± 0.07 (6)	2127.0 ± 14.0	0.81 ± 0.05 (6)
6	0.10 ± 0.02 (10)	5.8 ± 0.21	1.31 ± 0.11 (6)
7	0.11 ± 0.01 (10)	2.1 ± 0.18	1.16 ± 0.05 (6)

[a]The constants are in units of 1 mole^{-1} sec^{-1} ± S.E. (number of measurements). k_1 and k_2 are the rate constants for the neutral and charged tertiary amino esters, respecitvely, while k_4 is the rate constant for the corresponding quaternary amino ester. The kinetics were followed by the procedure described in Methods.

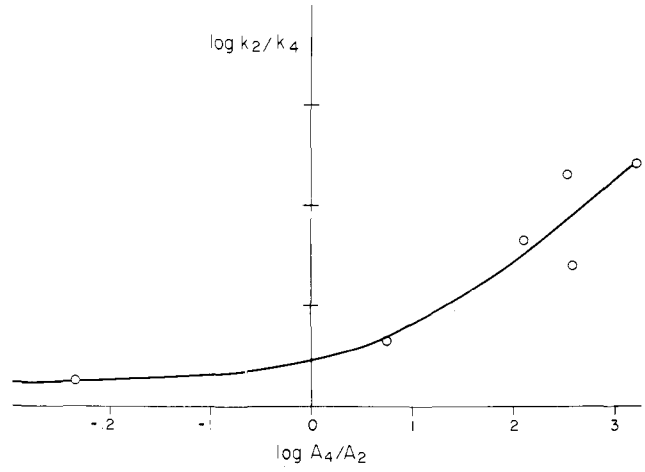


Figure 3. Relative muscarinic activities and rates of alkaline hydrolysis. k_2/k_4 is taken from Table IV and is the ratio of hydrolysis rates for the ionized tertiary amino ester and for the quaternary amino ester. A_4/A_2 is the ratio of muscarinic activities for the quaternary and tertiary amino esters, respectively.

In Figure 3 the log ratio of the relative muscarinic activities of the tertiary and quaternary ammonium compounds is plotted against log k_2/k_4. When examined by the rank correlation test[15] a significant correlation ($P < 0.025$) was found, indicating that high k_2/k_4 ratios were associated with those pairs of compounds in which the quaternary derivatives were relatively more potent.

Discussion

This study represents an attempt to explain the anomaly that while ACh and some of its analogs are much more potent muscarinic agents than the corresponding *N*-demethyl tertiary amines, the reverse is true for certain compounds which in general exhibit greater rigidity. It had been pointed out[1] that an H bond between a protonated amine group and a nucleophilic group on the receptor should have a much higher energy than the simple ionic bond which a quaternary ammonium group could make. Tertiary amines should, therefore, be capable of a greater total energy of interaction with the receptor and hence greater potency. Three possible reasons might be cited for greater muscarinic potency in *N*-Me quaternary analogs. By analogy with adrenergic systems, indirect mechanisms such as release have been proposed for some analogs of ACh and other muscarinic agents[16,17] and could be involved here. However, estimates of muscarinic activity were made in the presence of morphine, which inhibits ACh release from nerve end-

ings[18,19] and after treatment with an anticholinesterase agent in order to eliminate effects mediated by inhibition of this enzyme or differences in substrate specificity. Although these precautions do not eliminate an indirect mechanism, they make it unlikely. A second possible explanation lies in the highly directional nature of the H bond in comparison with a simple ionic bond; thus considerably greater constraints could be placed on the "fit" with the remainder of the molecule, particularly in the case of less flexible compounds. Third, intramolecular interactions in dilute solution may be sufficiently strong to preclude the adoption of a conformation appropriate for interaction with the receptor. In the present study the basis hydrolysis kinetics have been examined to provide an assessment of intramolecular interactions in a paired series of tertiary and quaternary ammonium compounds with muscarinic activity. There was a significant negative correlation (Figure 3) between an anchimeric effect on hydrolysis and muscarinic potency of the tertiary amine relative to its *N*-Me quaternary analog, and the data support the view that intramolecular interactions restricting conformational flexibility are responsible for the low muscarinic activity of tertiary amines such as dimethylaminoethyl acetate.

There are several conformational studies of ACh from which inferences have been drawn regarding the structure of the cholinergic receptor. The techniques employed have included X-ray diffraction studies in the solid state,[20] ir spectral studies in EtOH,[21] and the biological activity of derivatives of ACh with fixed stereochemistry.[22] The first 2 studies demonstrated a cyclic structure for ACh with a cisoid relationship between C-N and C-O bonds, and postulated that this conformation is important for interaction with the receptor. More recent pmr and ir studies have yielded similar data.[23] In a study with rigid cyclopropyl analogs of ACh, Chiou, *et al.*,[22] concluded that a transoid relationship between the C-N and C-O bonds is important for muscarinic activity. Those cyclopropyl derivatives cannot alter their conformation and only the trans isomer was active. If ACh must assume a transoid conformation to interact with the muscarinic receptor and produce an effect, structural and electronic features of ACh analogs which favor a cyclic or cisoid conformation should reduce the pharmacological potency. The intramolecular interactions inferred from the present study presumably lower muscarinic activity by this mechanism.

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Correlation of Antihypertensive Activity with Structure in a Series of 2*H*-1,2,4-Benzothiadiazine 1,1-Dioxides Using the Substituent Constant Approach†

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The relationship of the antihypertensive properties of an extensive series of substituted 2*H*-1,2,4-benzothiadiazine 1,1-dioxides to lipophilic, electronic, and steric effects has been examined using multiple regression analysis and the substituent constant approach of Hansch. A high degree of correlation was observed between activity and the π values of substituents at certain positions in the nucleus. Electronic effects appeared to be of minor significance. The activities of several compounds not included in the regression analyses could be calculated within satisfactory limits and structural requirements for compounds of maximum activity in the series were established. Some implications with regard to possible receptor site interactions are considered.

Following the discovery of the antihypertensive agent, diazoxide, 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (I, $R_3 = CH_3$, $R_7 = Cl$, $R_5 = R_6 = R_8 = H$),[1] many analogs were prepared in order to study structure-activity relationships.[2,3] The development and wide application of the substituent constant method to structure-activity problems by Hansch[4] prompted us to examine this approach in the antihypertensive benzothiadiazine series.

The 1,2,4-benzothiadiazine 1,1-dioxide system is capable

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