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# THE REACTION OF ACETYLCHOLINESTERASE (AChe) WITH SOME QUATERNARY HYDROXY AMINOPHENOLS*

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**Abstract**—A series of mono- and diquaternary hydroxyl derivatives of quinolinol, isoquinolinol, and stilbazole were synthesized. The dimethylcarbamyl and diethylphosphoryl analogs of these hydroxyl compounds are potent anticholinesterase agents. The anticholinesterase properties of the hydroxyl analogs were evaluated by measuring the values of their competitive and noncompetitive binding constants for the reaction with electric eel acetylcholinesterase. The p$K_a$ values were also measured.

When compared to the nonhydroxylated reference compounds, the addition of the OH group was found to increase binding of the undissociated species to free AChe (competitive component). These derivatives are generally more poorly bound to the acetyl enzyme (noncompetitive component), and the diquaternary compounds are less potent than their monoquaternary analogs.

The values of the binding constants for the reaction of the undissociated forms of the inhibitor with the enzyme were found to correlate qualitatively with the second-order rate constants for carbamylation of the enzyme by the dimethylcarbamyl analogs, but not with the constants for phosphorylation by the diethylphosphoryl compounds. The latter compounds have been shown to correlate well with the p$K_a$ value of the quaternary hydroxyl derivatives.

IT HAS RECENTLY been shown that certain diethylphosphoryl[1–2] and dimethylcarbamyl[3] derivatives of some aminophenols are potent inhibitors of purified electric eel AChe. Several of these compounds are among the most active so far reported. All of these anticholinesterase agents form relatively stable intermediate compounds with the enzyme. They are classified as "irreversible" or better, acid-transferring inhibitors.[4] The acetoxyquinolinium compounds are substrates for this enzyme.[5]

These new compounds are of interest because of the very high order of activity of some of the derivatives. Although the potency of the diethylphosphoryl analogs has been found to correlate well with the p$K_a$ values of the quaternary hydroxy derivatives, this was not true of the dimethylcarbamyl compounds. An additional factor accounting for the potency of these agents may be a high degree of binding to the enzyme. In an attempt to evaluate this component, the mono- and diquaternary hydroxy analogs of these new compounds were prepared and their reactions with acetylcholinesterase assessed. This paper will report the synthesis of these reversible inhibitors and the values of their dissociation constants with the free enzyme and acetyl enzyme. The data indicate that the values of the binding constants for the undissociated form

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of the inhibitors correlate qualitatively with the second-order rate constant values for carbamylation by the respective dimethylcarbamyl derivatives. These data did not, however, correlate with the rate constant values for the diethylphosphoryl compounds.

## METHODS

*Synthesis.* Simple quaternary quinolinols were prepared by conventional methods. For the preparation of diquaternary compounds, the quinolinols were first acetylated with acetic anhydride to protect the OH group from the very reactive alkylating agent. Bis (iodomethyl) ether was prepared by treating an acetonic solution of NaI with the chloro ether and filtering off the precipitate (NaCl). The acetoxyquinoline was added to this acetonic solution. The diquaternary compound which precipitated out was filtered and desacetylated by refluxing for 45 min in an aqueous–methanolic solution of HI (1 vol. HI conc. + 10 vol. methanol). On cooling, the phenolic diquaternary compound crystallized out. Appropriate data are recorded in Table 1.

In addition to comparing melting points for those compounds previously synthesized, the $I^-$ was titrated by the Volhardt technique. All measurements were within 1 per cent of the theoretical value.

*Enzyme.* Acetylcholinesterase (EC 3.1.1.7) was prepared from *Electrophorous electricus.*[6] In a medium of 0·1 M NaCl 0·92 M $MgCl_2$, 0·005% gelatin, $1 \times 10^{-5}$ M EDTA, 0·001 M ACh·Br, pH7, at 25°, the activity was 90 m-mole acetylcholine hydrolyzed/ml/hr. The $K_m$ had a measured value of $9{\cdot}1 \times 10^{-5}$ mole/l.

*Technique.* All kinetic measurements were made with a "pH-stat" titrator. The instrument was designed to simultaneously advance the plungers of a matched pair of 1-ml syringes delivering 0·2 $\mu$l aliquots to the reaction medium. One syringe contained NaOH standardized at 0·0466 N. The other syringe contained 0·0466 M ACh bromide adjusted to pH 7. The simultaneous addition of similar amounts of acetylcholine maintains a constant level of substrate. This device allows precise measurements to be made at constant concentrations of acetylcholine below the value of $K_m$. The usual constant temperature and $CO_2$-free techniques were used.

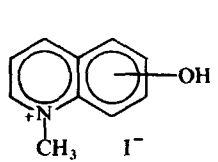
To 99 ml of a medium consisting of 0·1 M NaCl, 0·02 M $MgCl_2$, 0·005% gelatin, $1 \times 10^{-5}$ M EDTA, pH 7, at 25° was added an appropriate amount of enzyme. After recording background activity, sufficient ACh solution was added to make a final concentration of $5 \times 10^{-5}$ M. The initial rate of ACh hydrolysis at that concentration was measured; another portion of substrate was then added and the rate of hydrolysis was determined at the new concentration. In this manner, measurements were made at seven different substrate concentrations to a maximum of $1 \times 10^{-3}$ M ACh, i.e. over a 20-fold range of substrate and straddling the value of $K_m$.

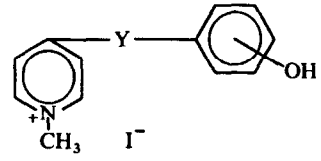
This experiment was repeated with several concentrations (varied 3- to 5-fold) of the reversible inhibitor to be tested. The data, when plotted in a double reciprocal manner (see "Kinetics" as below), will reveal the competitive, noncompetitive, or mixed kinetic behavior of the reaction. The values of the dissociation constants for these reversible inhibitors were evaluated from this type of plot. The $pK_a$ values were measured in the manner previously described.[2]

*Kinetics.* The kinetics of the reversible inhibition reaction have been described.[7, 8] The data were plotted in accordance with the equation:

$$\frac{1}{v} = \frac{I}{kE^\circ}\left[1 + \frac{(I)}{K'_I\,1 + (k_4/k_3)}\right] + \frac{K_m}{kE^\circ}\left(1 + \frac{(I)}{K_I}\right)\cdot\frac{I}{S} \qquad (1)$$

TABLE 1. MONOQUATERNARY AND DIQUATERNARY QUINOLINOLS, ISOQUINOLINOLS AND HYDROXYSTILBAZOLES




| No. | A* | B† | Y | m.p.‡ (°C) | m.p.§ (°C) | Formula | %C Calcd. | %C Found | %H Calcd. | %H Found | %N Calcd. | %N Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1‖ | ¶ | | | | Commercial cmpd. | $C_{10}H_{10}IN$ | | | | | | |
| 2 | 3 | | | 194** | 176–178†† (14) | $C_{10}H_{10}INO$ | | | | | | |
| 3 | 5 | | | 234 | 237 (5) | $C_{10}H_{10}INO$ | | | | | | |
| 4 | 6 | | | 234 | 234 (5) | $C_{10}H_{10}INO$ | | | | | | |
| 5 | 7 | | | 246 | 249 (5) | $C_{10}H_{10}INO$ | | | | | | |
| 6 | 8 | | | 173 | 180·5 (5) | $C_{10}H_{10}INO$ | | | | | | |
| 7‖ | iso¶ | | | | Commercial cmpd. | $C_{10}H_{10}IN$ | | | | | | |
| 8 | 5 iso | | | 239 | 184–87** (15) | $C_{10}H_{10}INO$ | | | | | | |
| 9 | | ¶ | —CH=CH— | 218 | 220–21 (16) | $C_{14}H_{14}IN$ | | | | | | |
| 10 | | 2 | —CH=CH— | 232 | 224–25 (17) | $C_{14}H_{14}INO$ | | | | | | |
| 11 | | 3 | —CH=CH— | 257 | 260–61 (1, 18) | $C_{14}H_{14}INO$ | | | | | | |
| 12 | | 4 | —CH=CH— | 265 | 265–66 (1, 18) | $C_{14}H_{14}INO$ | | | | | | |
| | | | Diquaternary compounds containing the bridge —$CH_2O\ CH_2$— between the two ring nitrogen atoms | | | | | | | | | |
| 13 | ¶ | | | 223 | | $C_{20}H_{18}I_2N_2O$ | 43·19 | 42·85 | 3·26 | 3·37 | 5·04 | 5·12 |
| 14 | 3 | | | 231 | | $C_{20}H_{18}I_2N_2O_3$ | 40·84 | 41·30 | 3·08 | 3·15 | 4·76 | 4·78 |
| 15 | 5 | | | 241 | | $C_{20}H_{18}I_2N_2O_3$ | 40·84 | 41·14 | 3·08 | 3·30 | 4·76 | 4·75 |
| 16 | 6 | | | 214 | | $C_{20}H_{18}I_2N_2O_3$ | 40·84 | 40·95 | 3·08 | 3·26 | 4·76 | 4·79 |
| 17 | 7 | | | 247 | | $C_{20}H_{18}I_2N_2O_3$ | 40·84 | 41·03 | 3·08 | 3·20 | 4·76 | 4·85 |
| 18 | iso¶ | | | 217 | | $C_{20}H_{18}I_2N_2O$ | 43·19 | 42·66 | 3·26 | 3·78 | 5·04 | 5·01 |
| 19 | 5 iso | | | 237 | | $C_{20}H_{18}I_2N_2O_3$ | 40·84 | 40·07 | 3·08 | 3·75 | 4·76 | 4·56 |

* Number refers to the location of the hydroxyl group on the quinoline ring.
† Number refers to the location of the hydroxyl group on the benzene ring.
‡ Melting points were taken on a Uni-Melt apparatus.
§ Reference numbers are in parentheses.
‖ The commercially available compounds are included in this list to facilitate numbering and to simplify comparison of kinetic constants appearing in Tables 2 and 3.
¶ H instead of OH.
** *p*-Toluene sulfate.
†† Methylsulfate.

The terms have the following definition:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.S \xrightarrow{k_3} E' \xrightarrow{k_4} E$$
$$\qquad\qquad\qquad\qquad\qquad\quad + p^1 \qquad + p^2$$

$$E + I \overset{K_I}{\rightleftharpoons} E \cdot I$$

$$E' + I \overset{K_I'}{\rightleftharpoons} E' \cdot I$$

$$K_m = \frac{k_2 + k_3}{k_1} \Big/ 1 + \frac{k_3}{k_4}$$

$$k = \frac{k_3 k_4}{k_3 + k_4}$$

E, S, I, E·S, E·I, E′·I have their usual meaning; and v = initial velocity; E° = total enzyme concentration; E′ = acetyl enzyme; $p^1$ and $p^2$ = products of the reaction, choline and acetic acid; $K_I$ = dissociation constant for the reaction of I with free enzyme, E; K′I = dissociation constant for the reaction of I with acetyl enzyme, E′.

All the data were plotted in accordance with equation 1. The terms $K_m$ and kE° were evaluated in the absence of the inhibitor. With inhibitor present (Fig. 1) the value of

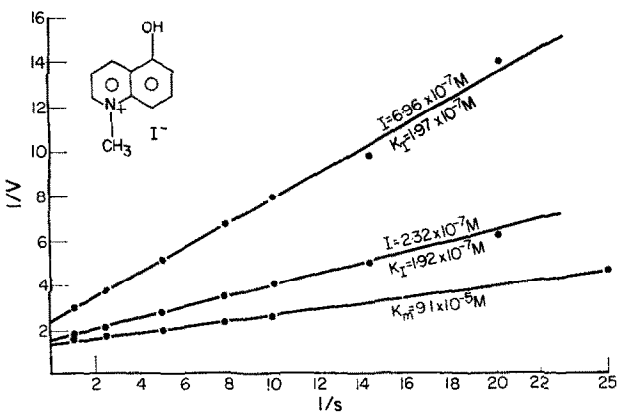



FIG. 1. This double reciprocal plot indicates the kinetics for the reaction of AChe with varying concentrations of ACh (lower plot) and in the presence of two different concentrations of compound 3 (upper plots). The values of $K_I$ calculated from the slopes represent the competitive interaction of the inhibitor with the free enzyme. The value of the noncompetitive dissociation constant can be calculated from the upward displacement of the intercept on the ordinate.

the dissociation constant $K_I$ was measured from the slope of the line. If the intercept on the ordinate is elevated above that obtained without the inhibitor, a noncompetitive component is indicated. $K'_I$ is assumed to be the dissociation constant for the reaction of the acetyl enzyme with the inhibitor.[7] Its value was calculated from the displaced intercept.

All measurements were made at pH 7. Because the p$K_a$ values of the compounds varied widely, the dissociation constant values were also corrected for the concentration of the inhibitor in the undissociated and dissociated form at pH 7. All data are tabulated in Table 2.

TABLE 2. DISSOCIATION CONSTANTS FOR THE REACTION OF SOME MONO- AND DIQUATERNARY AMINOPHENOLS WITH ACETYLCHOLINESTERASE*

| Cmpd. No. | p$K_a$ | Competitive | | | Noncompetitive | | |
|---|---|---|---|---|---|---|---|
| | | Measured $K_I$ for free enzyme | Calculated $K_I$ for undissociated form | Calculated $K_I$ for dissociated form | Measured $K_I$ for acetyl enzyme | Calculated $K_I$ for undissociated form | Calculated $K_I$ for dissociated form |
| 1 | | $6{\cdot}4 \times 10^{-6}$ | | | None | | |
| 2 | 5·25 | $1{\cdot}2 \times 10^{-5}$ | $2{\cdot}4 \times 10^{-7}$ | $1{\cdot}2 \times 10^{-5}$ | None | | |
| 3 | 6·10 | $1{\cdot}9 \times 10^{-7}$ | $2{\cdot}1 \times 10^{-8}$ | $1{\cdot}7 \times 10^{-7}$ | $1{\cdot}3 \times 10^{-6}$ | $1{\cdot}4 \times 10^{-7}$ | $1{\cdot}2 \times 10^{-6}$ |
| 4 | 7·03 | $3{\cdot}7 \times 10^{-6}$ | $1{\cdot}85 \times 10^{-6}$ | $1{\cdot}85 \times 10^{-6}$ | $4{\cdot}0 \times 10^{-5}$ | $2{\cdot}0 \times 10^{-5}$ | $2{\cdot}0 \times 10^{-5}$ |
| 5 | 5·69 | $3{\cdot}0 \times 10^{-6}$ | $1{\cdot}5 \times 10^{-7}$ | $2{\cdot}85 \times 10^{-6}$ | $2{\cdot}2 \times 10^{-5}$ | $1{\cdot}1 \times 10^{-6}$ | $2{\cdot}1 \times 10^{-5}$ |
| 6 | 6·30 | $3{\cdot}4 \times 10^{-5}$ | $5{\cdot}8 \times 10^{-6}$ | $2{\cdot}8 \times 10^{-5}$ | $1{\cdot}2 \times 10^{-4}$ | $2{\cdot}0 \times 10^{-5}$ | $1{\cdot}0 \times 10^{-4}$ |
| 7 | | $6{\cdot}8 \times 10^{-6}$ | | | None | | |
| 8 | 6·78 | $1{\cdot}1 \times 10^{-5}$ | $4{\cdot}3 \times 10^{-6}$ | $6{\cdot}7 \times 10^{-6}$ | $1{\cdot}3 \times 10^{-4}$ | $4{\cdot}95 \times 10^{-5}$ | $7{\cdot}75 \times 10^{-5}$ |
| 9 | | $8{\cdot}5 \times 10^{-5}$ | | | None | | |
| 10 | 8·28 | $5{\cdot}6 \times 10^{-6}$ | $5{\cdot}3 \times 10^{-6}$ | $3{\cdot}0 \times 10^{-6}$ | $4{\cdot}8 \times 10^{-5}$ | $4{\cdot}55 \times 10^{-5}$ | $2{\cdot}5 \times 10^{-6}$ |
| 11 | 8·81 | $3{\cdot}7 \times 10^{-6}$ | $3{\cdot}6 \times 10^{-6}$ | $1{\cdot}0 \times 10^{-7}$ | $4{\cdot}6 \times 10^{-5}$ | $4{\cdot}5 \times 10^{-5}$ | $1{\cdot}0 \times 10^{-6}$ |
| 12 | 8·27 | $2{\cdot}3 \times 10^{-6}$ | $2{\cdot}2 \times 10^{-6}$ | $1{\cdot}2 \times 10^{-7}$ | $2{\cdot}1 \times 10^{-5}$ | $2{\cdot}0 \times 10^{-5}$ | $1{\cdot}0 \times 10^{-6}$ |
| 13 | | $1{\cdot}5 \times 10^{-5}$ | | | None | | |
| 14 | 4·49 | $3{\cdot}0 \times 10^{-4}$ | $9{\cdot}0 \times 10^{-7}$ | $2{\cdot}9 \times 10^{-4}$ | None | | |
| 15 | 6·32 | $2{\cdot}0 \times 10^{-5}$ | $3{\cdot}4 \times 10^{-6}$ | $1{\cdot}7 \times 10^{-5}$ | $5{\cdot}0 \times 10^{-4}$ | $8{\cdot}5 \times 10^{-5}$ | $4{\cdot}2 \times 10^{-4}$ |
| 16 | 6·55 | $7{\cdot}8 \times 10^{-6}$ | $2{\cdot}2 \times 10^{-6}$ | $5{\cdot}5 \times 10^{-6}$ | $4{\cdot}2 \times 10^{-5}$ | $1{\cdot}2 \times 10^{-5}$ | $3{\cdot}0 \times 10^{-5}$ |
| 17 | 5·12 | $3{\cdot}7 \times 10^{-5}$ | $3{\cdot}7 \times 10^{-7}$ | $3{\cdot}6 \times 10^{-5}$ | $4{\cdot}6 \times 10^{-4}$ | $4{\cdot}6 \times 10^{-6}$ | $4{\cdot}5 \times 10^{-4}$ |
| 18 | | $8{\cdot}1 \times 10^{-6}$ | | | None | | |
| 19 | 6·34 | $5{\cdot}0 \times 10^{-6}$ | $8{\cdot}5 \times 10^{-7}$ | $4{\cdot}1 \times 10^{-6}$ | $3{\cdot}9 \times 10^{-6}$ | $6{\cdot}7 \times 10^{-6}$ | $3{\cdot}2 \times 10^{-5}$ |

* The compound numbers refer to the agents listed similarly in Table 1. The measured values of the binding constants to the free and acetyl enzyme were obtained from double reciprocal plots. These dissociation constant values were corrected for the concentration of the inhibitor in the undissociated and dissociated forms at pH 7.

## RESULTS

All compounds demonstrate reversible inhibition of acetylcholinesterase. The kinetics indicate both competitive and noncompetitive mechanisms (Fig. 1). The reference agents (compounds 1, 7, 9, 13 and 18), those without hydroxyl substitutions, were only competitive inhibitors. All hydroxyl substituted compounds except the mono- and diquarternary 3-hydroxy quinolines (compounds 2, 14) demonstrated both competitive and noncompetitive components. If the noncompetitive mechanism of inhibition is interference with the deacylation step of ACh hydrolysis,[7, 8] then the hydroxyl group appears to increase the binding of these compounds to the acetyl enzyme. The magnitude of binding to the acetyl enzyme is consistently less by factors of 4 to 25 than binding to the free enzyme.

Wilson and Quan[9] have shown that the introduction of the hydroxyl group in the 3 position of phenyltrimethyl ammonium ion increases binding to AChe by a factor of 120. This is attributed to a hydrogen bond possibly formed between the phenolic hydroxyl and some basic group at the active site. Work by Krupka substantiates this mechanism.[8] Other studies indicate that the binding of selected inhibitors to electroplax may also be similar.[10] The undissociated form of the hydroxyl substituted inhibitor is presumably the active species. The concentration of the undissociated and dissociated forms of each of the inhibitors was calculated for pH 7 from p$K_a$ values. The values of the dissociation constants were then corrected to reflect the appropriate inhibitor concentration. By comparing the unsubstituted with the hydroxyl substituted compounds, the $K_I$ values for the undissociated species indicate that the

hydroxyl group increases binding by factors ranging from 3 to 300, depending on position.

The diquaternary quinoline derivatives (compounds 13–17) in general were poorer inhibitors than the monoquaternary compounds. Presumably, by varying the length of the bridge, more potent compounds could be made.[11] The hydroxyl substituted diquaternary agents (compounds 14, 15, 16, 17) are more active than the unsubstituted derivative. They follow the monoquaternary series in this respect.

The four isoquinoline analogs (compounds 7, 8, 18, 19) show little alteration in potency except for the diquaternary hydroxyl derivative (compound 19). It is unusual because it is 10–20 times more potent than the other three.

The stilbazole series (compounds 9–12) again demonstrates the superior binding qualities of the hydroxyl derivatives. The position of the OH has only a minor effect on activity, contrary to the quinoline series. For the major competitive component the values of the binding constants for the undissociated form indicate that the stilbazoles are generally poorer inhibitors than the quinolines.

Table 3 contains data for the reversible and nonreversible inhibition of eel acetylcholinesterase under similar conditions. The $pK_a$ and $K_I$ values are taken from Table 2, columns 2 and 4 of this paper. The activity of the dimethyl carbamyl and diethylphosphoryl derivatives of these quaternary aminophenols has been previously

TABLE 3. COMPARISON OF VARIOUS CONSTANTS FOR THE REACTION OF ACETYLCHOLINESTERASE WITH SUBSTITUTED AND UNSUBSTITUTED MONO- AND DIQUATERNARY QUINOLINIUM AND ISOQUINOLINUM COMPOUNDS*

| Cmpd. No. | Position of substitution | $pK_a$ | $K_I$ (moles/l.) | $k°_2$ for carbamylation (l./mole/min) | $k°_2$ for phosphorylation (l./mole/min) |
|---|---|---|---|---|---|
| | Quinoline | | | | |
| 1 | mono | | $6·4 \times 10^{-6}$ | | |
| 13 | *bis* | | $1·5 \times 10^{-5}$ | | |
| | 3-position | | | | |
| 2 | mono | 5·3 | $2·4 \times 10^{-7}$ | $3·8 \times 10^{3}$ | $1·2 \times 10^{8}$ |
| 14 | *bis* | 4·5 | $9·0 \times 10^{-7}$ | | |
| | 5-position | | | | |
| 3 | mono | 6·1 | $2·1 \times 10^{-8}$ | $1·6 \times 10^{6}$ | $2·4 \times 10^{6}$ |
| 15 | *bis* | 6·3 | $3·4 \times 10^{-6}$ | $2·1 \times 10^{4}$ | |
| | 6-position | | | | |
| 4 | mono | 7·0 | $1·9 \times 10^{-6}$ | $3·4 \times 10^{3}$ | $9·3 \times 10^{6}$ |
| 16 | *bis* | 6·6 | $2·2 \times 10^{-6}$ | $2·3 \times 10^{4}$ | $2·7 \times 10^{8}$ |
| | 7-position | | | | |
| 5 | mono | 5·7 | $1·5 \times 10^{-7}$ | $4·25 \times 10^{5}$ | $1·2 \times 10^{8}$ |
| 17 | *bis* | 5·1 | $3·7 \times 10^{-7}$ | $5·1 \times 10^{4}$ | |
| | 8-position | | | | |
| 6 | mono | 6·3 | $5·8 \times 10^{-6}$ | $3·85 \times 10^{1}$ | $5·3 \times 10^{2}$ |
| | Isoquinoline | | | | |
| 7 | mono | | $6·8 \times 10^{-6}$ | | |
| 18 | *bis* | | $8·1 \times 10^{-6}$ | | |
| | 5-position | | | | |
| 8 | mono | 6·8 | $4·3 \times 10^{-6}$ | $3·5 \times 10^{5}$ | $6·5 \times 10^{4}$ |
| 19 | *bis* | 6·3 | $8·5 \times 10^{-7}$ | $6·85 \times 10^{5}$ | $8·1 \times 10^{5}$ |

* The compound numbers refer to the agents listed similarly in Tables 1 and 2. The $K_I$ is the value of the dissociation constant for the reaction of the undissociated form of the unsubstituted and substituted hydroxy derivatives with acetylcholinesterase; $k°_2$ for carbamylation is the second-order rate constant value for the reaction of the dimethylcarbamyl analogs with the enzyme; $k°_2$ for phosphorylation refers to the value of the second-order rate constant for the reaction of the diethylphosphoryl analogs with acetylcholinesterase.

reported.[2, 3] The second-order rate constant values ($k^\circ_2$) for carbamylation and phosphorylation of the enzyme are included here to facilitate comparison.

The $pK_a$ values for the hydroxyl substituted analogs have an inverse correlation with the $k^\circ_2$ values for phosphorylation; the lower the value of the $pK_a$ the greater the potency of the diethylphosphoryl analog.[2] No such correlation is evident between the value of the $pK_a$ and the respective activities of the hydroxyl and dimethylcarbamyl substituted analogs.[3]

As previously reported, the potency of the carbamates does not parallel the activity of the phosphates. In this study an inverse correlation between the monoquaternary hydroxyl analogs and the dimethylcarbamates is evident; the lower the values of the $K_I$ for the undissociated species the more potent the respective dimethylcarbamate derivatives. It would seem then, that the activity of these dimethyl carbamylanticholinesterase agents is dependent upon good binding characteristics, i.e. molecular complementarity. However, this does not seem critical to the potency of the diethylphosphoryl derivatives.

Although activities of the diquaternary analogs parallel those of the monoquaternary compounds, not enough were able to be synthesized to warrant significant comparisons among the principal groups of inhibitors.

These correlations between the major classes of anticholinesterase agents are expected to be qualitative, since the contributions to binding of the dimethylcarbamyl and diethylphosphoryl moieties are not considered. Their contributions may be minor.[12] However, a more meaningful quantititative relationship must await the actual measurements of the values of the binding constant of these potent anticholinesterase agents. Although kinetically this is possible,[13] sophisticated rapid reaction equipment is required for the very active compounds. We are currently studying this problem.

The data support the generalization that the activity of the quaternary diethyl phosphoryl aminophenols is principally a function of the acid character of the leaving group.[2] The latter was assessed by measuring the $pK_a$ values of the hydroxyl analogs. The activity of the quaternary dimethylcarbamyl aminophenols is principally a function of the molecular complementarity of the leaving group.[3] This quality was assessed in this study by measuring the $K_I$ values of the hydroxyl derivatives.

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## REFERENCES

1. S. Ginsburg, R. J. Kitz and I. B. Wilson, *J. mednl Chem.* **9**, 632 (1966).
2. R. J. Kitz, S. Ginsburg and I. B. Wilson, *Molec. Pharmac.* **3**, 225 (1967).
3. R. J. Kitz, S. Ginsburg and I. B. Wilson, *Biochem. Pharmac.* in press.
4. I. B. Wilson, in *Enzymes and Drug Action*, Ciba Found. Symp. Churchill, London (1962).
5. A. K. Prince, *Archs Biochem. Biophys.* **113**, 195 (1966).
6. L. T. Kremzner and I. B. Wilson, *J. biol. Chem.* **238**, 1714 (1963).
7. I. B. Wilson and J. Alexander, *J. biol. Chem.* **237**, 1323 (1962).
8. R. M. Krupka, *Biochemistry, N.Y.* **4**, 429 (1965).
9. I. B. Wilson and C. Quan, *Archs Biochem. Biophys.* **73**, 131 (1958).
10. T. R. Podleski and D. Nachmansohn, *Proc. natn. Acad. Sci. U.S.A.* **56**, 1034 (1966).
11. R. B. Barlow and G. Himms, *Br. J. Pharmac.* **10**, 173 (1955).
12. I. B. Wilson and R. Rio, *Molec. Pharmac.* **1**, 60 (1965).

13. A. R. MAIN and F. IVERSON, *Biochem. J.* **100**, 525 (1966).
14. K. J. M. ANDREWS, F. R. ATHERTON, F. BERGEL and A. L. MORRISON, *J. chem. Soc.* 1638 (1954).
15. C. F. KOELSCH and N. F. ALBERTSON, *J. Am. chem. Soc.* **75**, 2095 (1953).
16. A. J. CLAUS and J. MASSON, *J. prakt. Chem.* (2) **45**, 238 (1892).
17. L. HORWITZ, *J. org. Chem.* **21**, 1039 (1956).
18. A. P. PHILLIPS, *J. org. Chem.* **14**, 302 (1949).