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# INHIBITION OF THE PSEUDOCHOLINESTERASE IN HORSE SERUM BY SOME CHOLINE ANALOGUES

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**Abstract**—The inhibitory properties of the reversed ethyl esters of choline and α- and β-methylcholine towards the hydrolysis of butyrylcholine by the pseudocholinesterase (PCE) in horse serum were determined.

The spontaneous decomposition of ethyl 2-dimethylamino propionate methiodide in bicarbonate buffer at pH 7·4 was investigated.

LEVINE[1] showed that increase in the length of the alcohol chain of a choline ester results in a decrease in the rate of hydrolysis by pseudocholinesterase (PCE) (acylcholine acylhydrolase-3.1.1.8). It was considered probable that reversal of the ester group of propionylcholine (PrCh) would produce a compound having an affinity for the active site comparable to that of PrCh, but a reduced rate of hydrolysis so that it would inhibit the hydrolysis of BuCh by PCE. The inhibition of the hydrolysis of BuCh by the PCE in horse serum was therefore determined for ethyl 2-dimethylamino propionate methiodide (EMP) and also for the α- and β-methyl derivatives.

During the enzymological investigations, considerable spontaneous decomposition of EMP occurred on addition of bicarbonate buffer, pH 7·4; the nature of this decomposition was investigated.

## METHODS AND RESULTS

(A) *Substrates*

(a) Butyrylcholine iodide, m.p. 92°[2] BuCh.

(b) Ethyl 2-dimethylamino propionate methiodide, m.p. 178·5°.[3]

(c) Ethyl 2-dimethylamino-2-methyl propionate methiodide m.p. 131·5–132°.[4]

(d) Ethyl 2-dimethylamino-1-methyl propionate methiodide, m.p. 115–115·5° (Found: C, 36·0; H, 6·6%; Equiv.300. $C_8H_{18}INO_2$ requires C, 36·0; H, 6·6%; Equiv. 301).

(B) *Enzyme studies*

The hydrolysis and inhibitor studies were conducted using the standard Warburg manometric technique[5, 6] under conditions described elsewhere.[7, 8] The enzyme preparation was diluted horse serum obtained from the Wellcome Research

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Laboratories and stored at 0°. Serum dilutions (3:100) were used to give an enzyme solution such that 1·5 ml hydrolysed approximately 2 mg of BuCh ([S] = $10^{-2}$M) in 30 min.

The inhibition of the hydrolysis of BuCh by the PCE present in horse serum was investigated for ethyl 2-dimethylamino propionate methiodide (α-MeEMP) and ethyl 2-dimethylamino-1-methyl propionate methiodide (β-MeEMP).

The results obtained are recorded in Table 1. Graphs of 1/V against [I] were plotted (Fig. 1 shows a typical example) and the $K_i$ and $I_{50}$ values obtained from these graphs

TABLE 1. THE HYDROLYSIS OF BuCh BY DILUTED HORSE SERUM ALONE, AND IN PRESENCE OF EMP, α-MeEMP OR β-MeEMP

| | EMP | | α-MeEMP | | β-MeEMP | |
|---|---|---|---|---|---|---|
| [BuCh] | $b^{1.5}_{30}$ | [I] | $b^{1.5}_{30}$ | [I] | $b^{1.5}_{30}$ | [I] |
| 3 | 50·3 | 2·82 | 37·7 | 3·01 | 34·2 | 3·00 |
| 3 | 56·5 | 1·88 | 47·0 | 2·00 | 43·1 | 2·00 |
| 3 | 66·0 | 0·93 | 71·1 | 0·50 | 56·9 | 1·00 |
| 3 | 78·3 | 0 | 78·3 | 0 | 78·3 | 0 |
| 5 | 60·7 | 2·81 | 52·3 | 3·00 | 46·5 | 3·00 |
| 5 | 68·0 | 1·88 | 57·4 | 2·00 | 56·9 | 2·00 |
| 5 | 79·3 | 0·93 | 69·0 | 1·00 | 66·7 | 1·00 |
| 5 | 90·0 | 0 | 90·0 | 0 | 90·0 | 0 |
| 10 | 75·0 | 2·81 | 67·1 | 3·00 | 56·7 | 3·00 |
| 10 | 81·7 | 1·88 | 75·5 | 2·01 | 67·8 | 2·00 |
| 10 | 89·2 | 0·93 | 84·4 | 1·00 | 80·5 | 1·00 |
| 10 | 95·0 | 0 | 95·0 | 0 | 95·0 | 0 |

Values for the data include: [BuCh] = M × $10^2$; $b^{1.5}_{30}$ = μl; [I] = M × $10^3$.

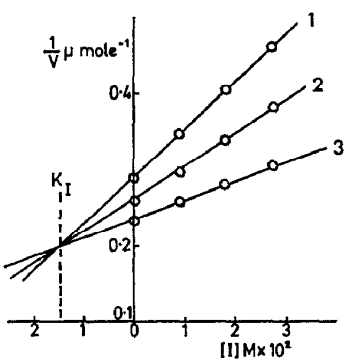


FIG. 1. Inhibition of the enzymic hydrolysis of Bu Ch. ([S] = 3 × $10^{-3}$ (1); 5 × $10^{-3}$ (2); $10^{-3}$ (3) M) by diluted horse serum in the presence of EMP. (see Table 1).

are presented in Table 2. The hydrolysis of these compounds was studied using serum dilutions of the same activity as that used in the inhibitor studies and was negligible.

(C) *Investigation of the spontaneous decomposition of EMP*

(i) *Autohydrolysis.* Autohydrolysis determinations were carried out by the standard technique, using a buffer blank instead of the enzyme solution. The results

obtained for the autohydrolysis of EMP in the presence of BuCh ([S] = $3 \times 10^{-2}$M), at three concentrations—$3 \times 10^{-2}$, $2 \times 10^{-2}$ and $10^{-2}$M—are given in Fig. 2. The data obtained showed high rates of autohydrolysis. The results obtained in the inhibitory studies using EMP, were corrected accordingly. Substitution of a methyl group in the $\alpha$- or $\beta$- positions reduced the rates of authohydrolysis to negligible proportions.

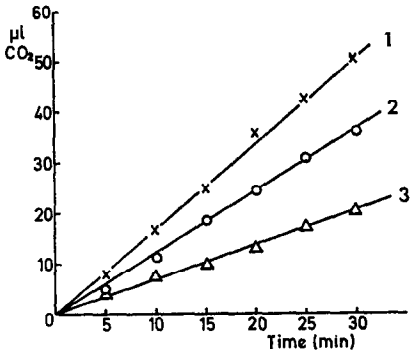


FIG. 2. Autohydrolysis of EMP ($3 \times 10^{-2}$ (1); $2 \times 10^{-2}$ (2); $10^{-2}$ (3) M) in the presence of BuCh ($3 \times 10^{-3}$ M).

TABLE 2. $K_i$ AND $I_{50}$ VALUES FOR THE INHIBITION OF THE HYDROLYSIS OF BuCh BY PCE USING EMP, $\alpha$-MeEMP AND $\beta$-MeEMP

| Inhibitor | $K_i$ | $I_{50}$ values (M) at 3 BuCh concn: | | |
|---|---|---|---|---|
| | M $\times 10^{-2}$ | $3 \times 10^{-3}$M (1) | $5 \times 10^{-3}$M (2) | $10^{-2}$M (3) |
| EMP | 1·5 | $4{\cdot}75 \times 10^{-2}$ | $6{\cdot}1 \times 10^{-2}$ | $9{\cdot}9 \times 10^{-2}$ |
| $\alpha$-MeEMP | 0·5 | $2{\cdot}83 \times 10^{-2}$ | $3{\cdot}8 \times 10^{-2}$ | $7{\cdot}45 \times 10^{-2}$ |
| $\beta$-MeEMP | 0·725 | $2{\cdot}38 \times 10^{-2}$ | $3{\cdot}25 \times 10^{-2}$ | $4{\cdot}5 \times 10^{-2}$ |

(ii) *Elimination.* The nature of the elimination reaction occurring in solutions of EMP in bicarbonate buffer, pH 7·4, was confirmed by gas chromatography. In order to eliminate the majority of the components of the system the vapour above the sample rather than the sample itself, was used for chromatography. Two columns were used:

Column A: Support, Chromosorb G;
Liquid phase, Carbowax 20 M 2%, KOH 5%;
Length, 1 m.

Column B: Support, Glass beads 60–85 mesh;
Liquid phase, SE 301 0·1%;
Length, 2 m.

Determinations were carried out using a Perkin–Elmer F11 cromatograph with a flame ionisation detector. The EMP used was $3{\cdot}5 \times 10^{-2}$M and the buffer pH was 7·4.

The results obtained are as follows:

Column A: $T_r$—sample, 0·98 min; ethylacrylate, 0·95 min; alcohol, 0·69 min.

Column B; $T_r$—sample, 8·04 min; ethylacrylate, 8·01 min; alcohol, 1·76 min.

It was impossible, from these results, to derive correction factors that could be used in analysis of the inhibitory studies as the data were not quantitative.

The vapour above solutions containing the α- and β-methyl analogues of EMP in bicarbonate buffer (pH 7·4) and EMP in distilled water (pH 6·5) were analysed using column B. The results obtained were as follows:

(i) using the α-methyl analogue—

$T_r$ sample, 11·2 min; ethyl crotonate, 11·6 min,

(ii) using the β-methyl analogue—

no detectable peak of ethyl methacrylate.

No peak corresponding to ethyl acrylate was detected when a solution of EMP in distilled water was used (pH 6·5).

## DISCUSSION

The high rate of autohydrolysis observed for EMP (Fig. 2) is probably due to the influence of the quaternary nitrogen group supplementing the positive charge on the ester carbon atom, thus increasing its susceptibility to nucleophilic attack. Methyl substitution in the α- or β-positions reduces the rate of autohydrolysis observed, due partly to the electron donating effect of the methyl group, and partly to steric hindrance impeding the approach of the hydroxyl nucleophil.

The gas chromatographic studies described in Section C (ii) indicate that solutions of EMP in bicarbonate buffer pH 7·4, decompose to give ethyl acrylate,

$$C_2H_5OCCH_2CH_2\overset{+}{N}(CH_3)_3 \xrightarrow[37^\circ]{\text{pH } 7{\cdot}4} C_2H_5OC(=O)CH=CH_2 + H_2O + N(CH_3)_3$$

It is suggested that the mechanism involved is an $E_2$ *trans* elimination and that the unusual facility of the reaction is due to the strongly electron withdrawing ester carbonyl group on the β carbon atom, and to an electrostatic attraction between the quaternary nitrogen and the carbonyl oxygen, which will tend to keep the molecule in the required configuration. β-MeEMP gave no detectable volatile ester due to the steric and inductive effects of β-methyl substitution. α-Methyl substitution gave a reduced degree of elimination as shown by the increased analytical sensitivity required to detect it.

The inhibitory studies (see Table 2) imply that the affinity of EMP for the enzyme is lower than the affinities of either of the two methyl substituted derivatives, although in view of the probable steric effect of methyl substitution, the opposite conclusion was expected. The degree of spontaneous elimination occurring was not estimated quantitatively but was greater for EMP than for its α- and β-methyl derivatives, and it is suggested that application of accurate correction factors to the inhibitory data would show that the affinity of EMP for PCE is, in fact, greater than the affinities of either methyl derivative.

The results emphasise the importance of spatial features in the interaction of substrates with enzymes. Reversal of the ester link gives compounds which show affinity for the enzyme. The compounds act as inhibitors but do not constitute substrates for the enzymic reaction.

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