THE CONFORMATION OF ACETYLCHOLINE

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SUMMARY

The infrared absorption of the carbonyl peak of acetylcholine exhibits a shift toward the higher energy. 2-Dimethylamino ethyl acetate, 3-dimethylaminopropyl acetate, 4-dimethylaminobutyl acetate show the expected absorption spectra for the carbonyl peak in that they are precisely like ethyl acetate. 3-Dimethylaminopropyl acetate methiodide and 4-dimethylaminobutyl acetate methiodide do not exhibit absorption for the carbonyl peak like acetylcholine, but rather behave like the unquaternarized esters. *dl, cis* 2-Dimethylamino-cyclohexanol acetate methiodide shows a similar shift in the infra-red absorption for the carbonyl peak of acetylcholine, the *trans* isomer does not.

The above evidence was advanced in support of a cyclic conformation of acetylcholine which can explain the observed kinetic data for alkaline hydrolysis and acid catalysis of this substance, as well as explain the rates of acylation of hydroxylamine observed.

INTRODUCTION

The knowledge that acetylcholine is unstable in alkaline solution and relatively stable in acid solution has been held for sometime by neurophysiologists working with this ester, but it was not until recently that the hydrolysis velocity constants were determined which quantitated this fact. In their discussion of their data, BUTTERWORTH, ELEY AND STONE[1] offered an explanation for the discrepancy between the velocity constants of acetylcholine and simple esters such as ethyl acetate under conditions of acid catalysis or basic hydrolysis. At the core of their argument is the proposition that the charge on the nitrogen atom in the choline electrostatically influences the attacking base or proton and thus alters the rates of hydrolysis.

In this report, we propose a cyclic conformation for acetylcholine in solution, of ion-dipole character, brought about by an electrostatic attraction between the quaternary nitrogen and a polarized carbonyl oxygen which can account for the discrepancy in the hydrolysis rates observed and we present evidence for its existence.

MATERIALS AND METHODS

Acetylcholine iodide was purchased from the California Corporation for Biochemical Research. 2-Dimethylaminoethyl acetate was prepared according to the method of

JONES AND MAJOR[2], 3-Dimethylaminopropyl acetate, 3-dimethyl-aminopropyl acetate methiodide, 4-dimethylaminobutyl acetate and 4-dimethylaminobutyl acetate methiodide were also prepared with this procedure.

d,l trans 2-dimethylaminocyclohexyl acetate methiodide was prepared by the method of BALDRIDGE, MCCARVILLE AND FRIESS[3]. The *d,l cis* 2-di-methylaminocyclohexyl acetate methiodide was the generous gift of Dr. S. L. FRIESS.

The infrared spectra were determined with a Perkin-Elmer 221G instrument programmed for maximum resolution. A fixed path sodium chloride cell and a variable sodium chloride reference cell were used throughout the measurements. The esters were dissolved in absolute ethanol immediately before measurements were carried out.

The acylation velocity rates of the homologues of acetylcholine were carried out at 25° in water. At zero time, 10 ml of 2 *M* hydroxylamine hydrochloride was dissolved in 0.2 *M* borate buffer which had been adjusted to pH 10.2 and added to approx. 10 mg of the ester in 10 ml of solvent. 1-ml samples were removed at intervals and immediately pipetted into 0.2 ml 4 *N* HCl to stop the reaction. After all of the samples were taken, 0.5 ml of 10% $FeCl_3$ in 0.1 *N* hydrochloric acid followed by 2 ml of ethanol, were added to the samples to develop the hydroxamate color. The absorbancy is read in a spectrophotometer at 520 mμ.

The evaluation of the biological activity of acetylcholine and its homologues, on the frog rectus preparation, was carried out according to standard assay procedure[4]. The derivatives were bracketed between the known standards of acetylcholine which elicited identical contraction of the rectus muscle. Muscle contraction was measured with a strain gauge transducer and polygraph.

RESULTS

The spectra of the various esters observed in the region of 2000 cm^{-1} to 1600 cm^{-1} are shown in Fig. 1. This region was devoid of all but the weakest solvent absorption peaks which could easily be balanced out. One observes that 2-dimethylaminoethyl acetate, 3-dimethylaminopropyl acetate and 4-dimethylaminobutyl acetate like ethyl acetate exhibit a double peaked carbonyl absorption complex at 1728 cm^{-1} and 1748 cm^{-1} while acetylcholine absorbs sharply at 1760 cm^{-1}. Of striking interest is that the 3-dimethylaminopropyl acetate methiodide and the 4-dimethylaminobutyl acetate methiodide show the same carbonyl complex as their unquaternarized ester relatives and as seen in ethyl acetate. It is noteworthy that the 3-dimethylpropyl acetate methiodide exhibits a slight spectral shift toward the higher energy of the spectrum.

The *d,l cis* 2-dimethylaminocyclohexyl acetate methiodide like acetylcholine absorbs sharply but at 1753 cm^{-1} while *d,l trans* 2-dimethylaminocyclohexyl acetate methiodide exhibits a broader peak at the lower energy 1740 cm^{-1}.

The first-order rate constants for the acylation of hydroxylamine by homologues of acetylcholine were obtained by plotting the absorbancy of the ferric hydroxamate against time as shown in Fig. 2 a and b. The reaction velocity depends only on the concentration of the acetylcholine and is thus first order when the hydroxylamine concentration is maintained at a maximum; the value of "*k*" may be determined, since the absorbancy is a linear function of the hydroxamate formed, by the expression $k = 2.303/t \log a/(a-x)$.

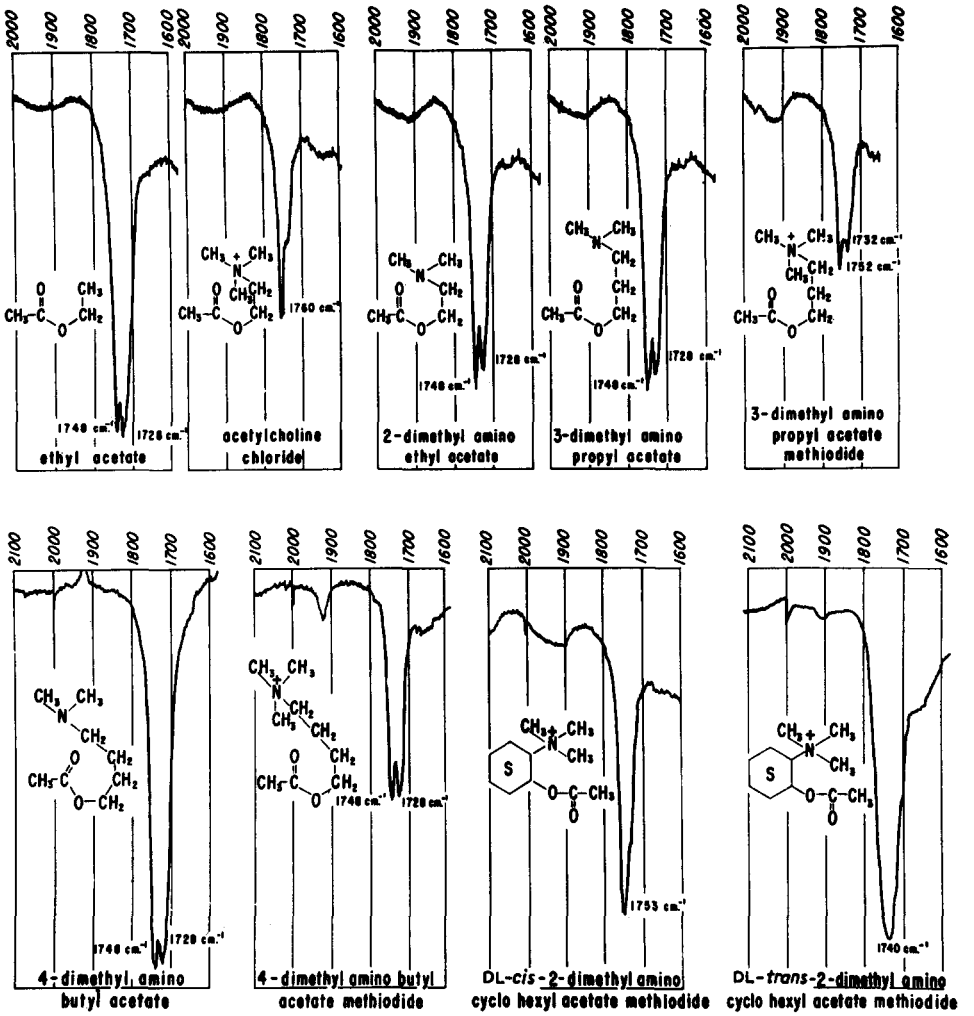


Fig. 1. Infrared-absorption spectra of various acetic acid esters.

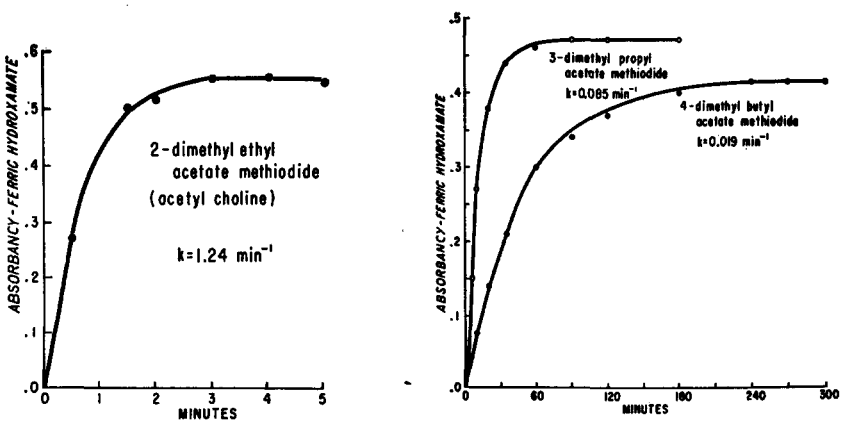


Fig. 2. Absorbancy *versus* time plots of ferric hydroxamate of some acetic acid esters.

The effect of the homologues of acetylcholine on the isolated frog rectus muscle were compared. 3-Dimethylaminopropyl acetate methiodide was 60 times less active than acetylcholine, while 4-dimethylaminobutyl acetate methiodide was 91 times less active. No activity could be elicited with the *d,l cis* 2-dimethylaminocyclohexyl acetate methiodide at concentration of 1000 times that of acetylcholine.

DISCUSSION

There is little doubt, from the data presented, that the infrared carbonyl absorption peak for acetylcholine is different from other simple esters and the shift to the higher energy observed suggests that the positively charged quaternary nitrogen alters polarization of this group. That this is an intramolecular effect is deduced from the observation that the butyl homologue exhibits the common and expected ester carbonyl absorption spectrum while only a very small shift is seen with the propyl homologue. If there were an intermolecular effect from the quaternary group of other esters in solution, one would expect the butyl homologue to exhibit the same distortion of the carbonyl peak as acetylcholine. Thus it would seem that the structure in Fig. 3 best accounts for these observations. The cyclic character of this configuration is brought about by an electrostatic attraction between the quaternary nitrogen and a polarized carbonyl group. This orientation would be favored in a 6 membered ring such as this and furthermore, would not be expected to be as stable in the 7 or 8 membered ring configuration. An interesting verification of this hypothesis was obtained from the investigations with the dimethylaminocyclohexyl acetate methiodides. The *cis* configuration would be expected to exhibit this phenomena while the *trans* configuration would be less favored.

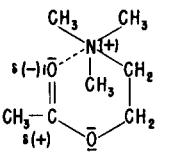
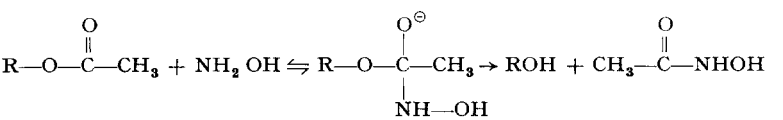


Fig. 3. Proposed cyclic structure of acetylcholine.

The marked influence of the quaternary nitrogen on the hydrolysis velocities can easily be appreciated from the cyclic structure proposed. This orientation would be expected to increase the rate of hydrolysis in alkaline solution since the polarized carbonyl group would facilitate the attack of the hydroxyl ion. In acid catalysed hydrolysis, the influence of the proton would not be expected to be markedly different from its effect on other esters. This hypothesis adequately explains the results of BUTTERWORTH *et al.*[1].

A reasonable mechanism for the hydroxamate formation would be the rate-determining addition of hydroxylamine to the ester carbonyl and fast elimination of $RO^{\ominus}$ to form product.



Since a full negative charge is being developed in the transition state leading to the intermediate, it would be expected that any group tending to stabilize that charge will lower the energy requirements of the transition state. The shift of the carbonyl band in the infrared spectrum of acetylcholine toward higher energy may be a field effect and if indeed it is, due to a cyclic conformation where the carbonyl oxygen and quaternary nitrogen are interacting, it seems reasonable to assume that this effect would be much more pronounced in the transition state than in the ground state. The net result of this interaction would be the enhancement of reaction rate. This would explain the velocity constants for acetylcholine $\gg$ trimethylaminopropyl acetate iodide $>$ trimethylaminobutyl acetate iodide.

In this regard, our results, from experiments on the acylation of hydroxylamine, indicate that the facilitation of reaction due to the cyclic configuration holds as a general rule. If the increase of the rate of acylation were due only to the presence of the quaternary nitrogen, then we would expect all three homologues to behave similarly if not identically. The fact that acetylcholine exhibits a pronounced facility above the other homologues, supports the explanation advanced above.

The observed rates of enzymic acylation of acetylcholine esterase detailed by WILSON AND CABIB[5] may in part be an expression of this configuration. Of course, the order of magnitude of the acylation rates obtained with acetylcholine and 2-dimethylamino acetate, in their enzyme studies, demands that some special enzymic affinity exists beyond the inherent physical differences of these substances as discussed above, nevertheless, the *difference* between the two enzymic rates can be explained from the foregoing discussion.

ACKNOWLEDGEMENTS

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REFERENCES

[1] J. BUTTERWORTH, D. D. ELEY AND G. S. STONE, *Biochem. J.*, 53 (1953) 30.
[2] L. W. JONES AND R. T. MAJOR, *J. Am. Chem. Soc.*, 52 (1940) 307.
[3] H. D. BALDRIDGE Jr., W. J. MCCARVILLE and S. L. FRIESS, *J. Am. Chem. Soc.*, 77 (1955) 739.
[4] J. H. GADDUM, *Methods in Medical Research*, Vol. 3, The Year Book Publish. Inc., Chicago, 1950, p. 87.
[5] I. B. WILSON AND E. CABIB, *J. Am. Chem. Soc.*, 78 (1956) 222.