

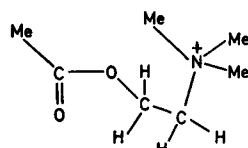
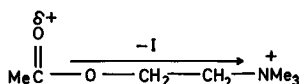
The possible role of conformational isomerism in the biological actions of acetylcholine: spectral studies

M. MARTIN-SMITH, G. A. SMAIL AND J. B. STENLAKE

Infrared studies of the NC-H stretching frequencies of selected trimethylammonium salts, including acetylcholine, were made in an attempt to obtain evidence that intramolecular NC-H - - - O hydrogen bonding is responsible for the existence in solution of a *quasi*-ring conformation for acetylcholine. Acetylcholine and related compounds were also examined by nuclear magnetic resonance spectroscopy. The carbonyl stretching frequencies of an homologous series of acetoxy alkyl sulphones were measured in dioxan solution to gain information on the effect of electron withdrawing substituents in the absence of intermolecular hydrogen bonding. The observed high ester carbonyl absorption frequency in acetylcholine may be interpreted in terms of the inductive effect from the onium nitrogen atom but the results are not incompatible with the existence of a *quasi*-ring conformation involving C-H - - - O hydrogen bonding between one of the *N*-methyl groups and the acetoxy oxygen atom.

RECENT publications of infrared (Fellman & Fujita, 1966) and nuclear magnetic resonance (Culvenor & Ham, 1966) spectroscopic evidence relating to the conformation of acetylcholine in various solvents prompt this report of our independent spectral studies on the neurohormone and related compounds.

In contrast to other comparative infrared studies (Fellman & Fujita, 1962, 1963, 1965, 1966) which were primarily concerned with establishing the influence of the inductive effect from the quaternary ammonium group (1), our studies were undertaken in an attempt to establish whether or not the cyclic conformation (2) of acetylcholine owed its stability to intramolecular $\overset{+}{\text{N}}\text{C-H} - - - \text{O}$ hydrogen bonding as suggested by Sutor (1962, 1963) and Whittaker (1963). The evidence leading to the conclusion that the cyclic conformation 2, or the similar conformation 3,



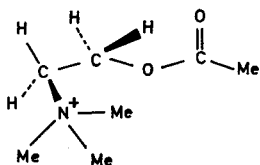
for acetylcholine may exist in solution as well as in the solid state (Canepa, Pauling & Sörum, 1966; Culvenor & Ham, 1966) has been summarized in our earlier paper (Martin-Smith, Smail & Stenlake, 1967) together with speculations about the possible role of these conformations as the "muscarinic" conformer of acetylcholine.

Although the inductive effect of the quaternary ammonium group as shown in (1) would explain (compare Jones & Sandorfy, 1956) the observed high carbonyl absorption frequency in the infrared spectrum of acetylcholine (Fellman & Fujita, 1962, 1963, 1965, 1966) as well as the

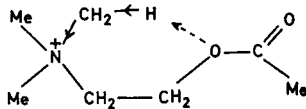
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high electrophilicity of the ester carbonyl carbon atom as evidenced by kinetic studies (Butterworth, Eley & Stone, 1953; Fellman & Fujita, 1962) [despite challenge of this interpretation by Canepa & Mooney (1965)], it is also possible that the kinetic results and the infrared carbonyl frequency are the result of electron withdrawal from the carbonyl group *via* the acyloxy oxygen atom due to hydrogen bonding (4). This would serve to depress the permanent polarization of the carbonyl group with consequent rise in its absorption frequency (Henbest & Lovell, 1957; West, Korst & Johnson, 1960; Bruce & Fife, 1962; Biggins, Cairns & others, 1963).

Hydrogen bonding involving C-H groups as proton donors is not without parallel and it has been concluded that intramolecular C-H --- O bonds exist in the crystal structures of a number of compounds (Sutor, 1962, 1963; Canepa & others, 1966). Hydrogen bonding involving the C-H group has also been suspected in other instances (Arshid, Giles & Jain, 1956; Cummings, Garven & others, 1959; Pinchas, 1963) and the various arguments have been well reviewed (Pimentel & Maclellan, 1960). Allerhand & Schleyer (1963) have shown that the ability of C-H groups to act as proton donors in intermolecular hydrogen



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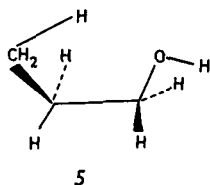


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bonds depends upon the hybridization of the carbon atom ($sp > sp^2 > sp^3$) and increases with the number of electron withdrawing groups. They further concluded that the C-H group of an sp^3 -hybridized carbon atom will only display proton donor propensities in those instances where that carbon atom is attached to at least two strongly activating groups. At variance with these generalizations and more pertinent to the present situation is the recent infrared study of Krueger & Mettee (1964). This has been interpreted as showing the conformational heterogeneity of propan-1-ol (5) in dilute carbon tetrachloride solution and evidence is presented for the existence of a weak intramolecular C-H --- O hydrogen bond by interaction of the C-H of the methyl group with one of the oxygen lone-pair orbitals. The favourable geometry of the n-propanol molecule is considered to be responsible for the existence of the C-H --- O hydrogen bond. In the *quasi*-ring conformation of acetylcholine (2) analogous features pertain in the favourable disposition for C-H --- O hydrogen bonding of one of the *N*-methyl groups and the acyloxy oxygen atom (Canepa & others, 1966; Culvenor & Ham, 1966). This, together with the reinforcing effect of an electron-withdrawing substituent, *viz.* the quaternary nitrogen atom, would rationalize the stabilization of 2 by the proposed intramolecular mechanism as in 4.

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The further application of infrared spectroscopy appeared to offer a promising method of establishing whether or not conformation 2 of acetylcholine existed in solution. In addition to the effect of C-H - - - O hydrogen bonding on the carbonyl stretching frequency, the effect of such bonding should also be reflected in the nature of the C-H stretching and deformation frequencies of the *N*-methyl groups of acetylcholine. Accordingly an infrared study of these absorptions in acetylcholine and several other selected trimethylammonium salts was undertaken. Acetylcholine and related compounds were also examined by nuclear magnetic



resonance spectroscopy. The carbonyl stretching frequencies of the sulphones $\text{MeCH}_2\text{SO}_2[\text{CH}_2]_n\text{OCOMe}$ (where $n = 1-3$) were measured in dioxan solution to gain information on the effect of electron-withdrawing substituents on these absorptions in a situation uncomplicated by intramolecular hydrogen bond formation. Fellman & Fujita (1966) made their measurements of the carbonyl stretching frequencies of the series $\text{Me}_3\text{N}^+[\text{CH}_2]_n\text{OCOMe}$ (where $n = 1-4$) in ethanolic solution and in dimethyl sulphoxide solution. In the former solvent, intermolecular hydrogen bonding between solvent and solute must be presumed to occur and indeed the split carbonyl absorptions observed by these workers (Fellman & Fujita, 1962) could well have their origin in such hydrogen bonding. Although similar intermolecular effects are not possible with dimethyl sulphoxide, the marked hygroscopic properties of this solvent make it far from ideal. Since all of our quaternary ammonium salts were insoluble in solvents incapable of acting as hydrogen donors, the series of sulphones (which can be regarded as analogues of the ω -acetoxyalkyltrimethylammonium series in which the sulphone grouping replaces the onium function as an electron withdrawing substituent) were employed as suitable model compounds.

Experimental

INSTRUMENTS

Infrared spectra were measured in rigorously dried dioxan solution, liquid film and solid state (KCl disc) employing a Unicam SP 100 double beam spectrophotometer equipped with an SP 130 sodium chloride prism-grating double monochromator operated under vacuum conditions. Nuclear magnetic resonance spectra were recorded in deuterium oxide solution employing a Perkin-Elmer R 10 spectrometer operating at 40 Mc/sec and using 3-trimethylsilylpropionylsulphonic acid as internal standard.

TERTIARY BASES (Table 2)

Dimethyloctylamine and dimethylaminoacetone were prepared by the addition of octyl bromide and monochloroacetone respectively to an ethereal solution of anhydrous dimethylamine. 2-Dimethylaminoethanol was redistilled from the commercial product through a 9 inch Vigreux column, b.p. 134.5° at 760 mm.

QUATERNARY SALTS (Table 1)

Acetylcholine chloride, (\pm)-acetyl- β -methylcholine chloride and succinylcholine chloride were obtained by repeated recrystallization of commercial samples from ethanol-acetone-ether followed by drying *in vacuo* over phosphorus pentoxide. Tetramethylammonium iodide, octyltrimethylammonium iodide, choline iodide and acetyltrimethylammonium iodide were all prepared by the addition of iodomethane to an ethanolic solution of the appropriate amine followed by repeated recrystallizations from aqueous ethanol, ethanol, ethanol-acetone or ethanol-acetone-ether.

SULPHONES (Table 4)

Acetoxymethyl ethyl sulphone. Acetoxymethyl ethyl sulphide (Böhme, Fischer & Frank, 1949), prepared from chloromethyl ethyl sulphide (Böhme, 1936) and anhydrous sodium acetate, was oxidized with an ethereal solution of monoperoxyphthalic acid. The solution was evaporated to dryness under reduced pressure at room temperature and the residue extracted with dry chloroform. Distillation yielded the product, b.p. 98–100° at 0.5 mm. [Böhme & others (1949) record b.p. 107° at 0.7 mm] (Calc. for $C_5H_{10}O_4S$: C, 36.1; H, 6.1; S, 19.3%. Found: C, 36.4; H, 6.0; S, 19.1%.)

2-Acetoxyethyl ethyl sulphone. Ethyl 2-hydroxyethyl sulphone (Exner, 1954) (6.9 g), acetic anhydride (12.5 ml) and concentrated sulphuric acid (3 ml) were heated for 1 hr at 100°. The reaction mixture was cooled, crushed ice (6 g) added and the whole extracted with chloroform (2 \times 100 ml, 2 \times 50 ml). The combined extracts were dried (Na_2SO_4), the chloroform removed and the residue distilled to give 2-acetoxyethyl ethyl sulphone (1.5 g) as an almost colourless oil, b.p. 126–127° at 0.2 mm. (Found: C, 40.2; H, 6.8; S, 17.5%. $C_6H_{12}O_4S$ requires C, 40.0; H, 6.7; S, 17.8%.)

3-Acetoxypropyl ethyl sulphone. Ethyl 3-hydroxypropyl sulphide (Rothstein, 1937) (10 g) in glacial acetic acid (25 ml) was cautiously treated with aqueous hydrogen peroxide (30% w/v, 18 ml) at such a rate that the temperature did not exceed 40°, and the mixture was then left overnight. The solvent was removed *in vacuo* and the residue distilled to give crude ethyl 3-hydroxypropyl sulphone (7 g), b.p. 161–163° at 0.7 mm, m.p. 36–37° (from chloroform-carbon tetrachloride and cooling to –10°). A mixture of the sulphone (7 g), acetic anhydride (14 ml) and concentrated sulphuric acid (3 ml) treated as described in the preparation of 2-acetoxyethyl ethyl sulphone afforded 3-acetoxypropyl ethyl sulphone (2 g), b.p. 147–149° at 0.2 mm. (Found: C, 43.5; H, 7.3%. $C_7H_{14}O_4S$ requires C, 43.3; H, 7.3%.)

Results

The absorption frequencies in the 3,100–2,700 cm^{-1} region (ν_{CH}) of representative quaternary ammonium salts in the solid state (KCl disc) and of some of the corresponding tertiary bases as thin liquid films are shown in Tables 1 and 2, respectively. The absorption frequencies in the region 1,500–1,390 cm^{-1} (δ_{CH}) measured from the solid state spectra of the quaternary salts are given in Table 3. Table 4 shows the infrared data ($\nu_{\text{C=O}}$) obtained from solution spectra (dioxan) of the sulphones formally related to acetylcholine and its higher homologues.

Discussion

EXAMINATION OF CH ABSORPTIONS

For the unambiguous interpretation of the ν_{CH} and δ_{CH} absorptions in solution the selection of solvent is necessarily restricted to one incapable of accepting a hydrogen bridge proton in order to eliminate the possibility of hydrogen bonding between solute and solvent molecules. Since no such solvent also capable of dissolving the quaternary salts could be found, a comparison was made between the infrared spectrum of a given trimethylammonium salt in the solid state and its corresponding tertiary base in the liquid state. Any conclusions would be complicated by possible intermolecular hydrogen bonding and differences in solid and liquid state spectra but such a comparison might indicate any influence of the positively charged nitrogen atom on the *N*-methyl CH stretching and deformation frequencies. Also, a comparison of the spectra of quaternary salts theoretically capable of forming C–H --- O hydrogen bonded quasi-rings with those unable to do so might indicate whether hydrogen bonding was indeed occurring.

CH stretching frequencies. No effect unequivocally attributable to the influence of C–H --- O bonding on ν_{CH} is apparent from Tables 1 and 2 but nevertheless the data are not incompatible with such bonding. The spectra of both the quaternary salts and the tertiary bases exhibit a multiplicity of peaks in the 3,100–2,700 cm^{-1} region making detailed interpretation difficult. Simplification of the spectra by deletion of overtones of lower frequency absorption modes did not prove feasible. Ebsworth & Sheppard (1959) have made the assignments ν_{CH_3} asymm. 3,005 cm^{-1} and ν_{CH_3} sym. 2,925 cm^{-1} for the tetramethylammonium ion and indeed in all the quaternary salts (Table 1) strong absorption is observed at 3,010–3,000 cm^{-1} (3,019 cm^{-1} for succinylcholine chloride) corresponding to ν_{CH_3} asymm. No similar consistency is observed in the 2,925 cm^{-1} region but there does appear to be a regularity in the absorptions observed at 2,952–2,940 cm^{-1} (2,957 cm^{-1} in succinylcholine chloride). Fellman & Fujita (1966) recorded absorption at 3,010–3,008 cm^{-1} for the CH stretching mode in acetylcholine which would thus appear to be a normal ν_{CH} of trimethylammonium compounds. The C–H stretching absorption in *N*-methyl compounds occurs at lower frequency than in *C*-methyl compounds (Cross, 1961) due to the greater

TABLE 1. SOLID STATE SPECTRA (KCl DISC)

| Compound | Absorption frequencies in 3100–2700 cm ⁻¹ region cm ⁻¹ | | | | | | | | | | Carbonyl stretching frequency cm ⁻¹ | |
|------------------------------------|--|--------|--------|------|------|------|--------------|--------------|------|------|--|--------------|
| | 3100 3054 | 3010 | 3005sh | | | 2949 | 2928 2905 | 2880 | 2830 | | | 2780 2730 |
| 1. Tetramethylammonium iodide | | | | | | | | | | | | — |
| 2. Trimethyloctylammonium iodide | 3024 | 3010 | | | | 2940 | 2917 | 2867 2851 | | | 2781 | — |
| 3. Choline iodide | 3026 | | 3005 | 2985 | | 2950 | 2930 | 2881 | 2820 | | 2770 | — |
| 4. Acetylcholine chloride | 3023 | 3010 | 3000sh | 2977 | 2960 | 2946 | 2920 | 2852 | 2825 | 2807 | 2790 | 1737 |
| 5. Acetyl-β-methylcholine chloride | 3043 | 3010sh | 3000 | 2982 | 2965 | 2938 | 2920 | | | | 2796 | 1738 |
| 6. Succinylcholine chloride | 3048 3024sh 3019 | | | 2973 | 2957 | 2933 | | | | | | 1740 |
| Acetoniltrimethylammonium iodide | 3055 3046 | 3009 | | 2952 | | | 2915 | | | 2806 | 2776 2740 | 1734 |

sh denotes shoulder.

TABLE 2. THIN LIQUID FILM SPECTRA

| Compound | Absorption frequencies in 3100–2700 cm ⁻¹ region cm ⁻¹ | | | | | | | | Carbonyl stretching frequency cm ⁻¹ |
|------------------------------|--|------|------|------|------|------|------|--|--|
| | 2952 | 2925 | 2868 | 2853 | 2810 | 2778 | 2760 | | |
| 8. Dimethyloctylamine .. | 2952 | 2925 | 2868 | 2853 | 2810 | 2778 | 2760 | | — |
| 9. 2-Dimethylaminoethanol .. | 2967sh | 2944 | | 2855 | 2820 | 2777 | | | — |
| 10. Dimethylaminoacetone .. | 2970 | 2944 | 2873 | | 2823 | 2776 | | | 1725sh 1715 |

sh denotes shoulder

electronegativity of the nitrogen atom, whereas in quaternary ammonium compounds the C–H stretching absorption occurs at higher frequency than in tertiary amines (Braunholtz, Ebsworth & others, 1958) despite the still greater inductive effect of the positively charged nitrogen atom. The weak bands in the region 2,800–2,400 cm⁻¹ of the spectrum of the tetramethylammonium ion have been interpreted as combination frequencies involving probably both infrared- and Raman-active fundamentals (Ebsworth & Sheppard, 1959). Compounds 3–7 (Table 1), in which C–H---O hydrogen bonding is possible, are characterized by absorptions between 3,000 and 2,950 cm⁻¹ (cf. compounds 1 and 2). These peaks in compounds 3–7 could represent absorption frequencies corresponding to those just above 3,000 cm⁻¹ (ν_{OH_3} asym.) in compounds 1 and 2 lowered due to hydrogen bonding, although absorption at *ca* 2,985 cm⁻¹, common to the solid state spectra of all choline esters examined, has been tentatively ascribed to superposition of C–H stretching from the acyl group on weak C–H stretching originating in the choline

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moiety (Whittaker, 1963). Whereas choline, acetylcholine, (\pm)-acetyl- β -methylcholine and succinylcholine show well-defined bands at 2,985, 2,977, 2,982 and 2,973 cm^{-1} respectively, the methiodide of dimethylaminoacetone shows absorption at 2,952 cm^{-1} which would not be inconsistent with NC-H --- O hydrogen bonding because the greater inherent proton-accepting propensity of a carbonyl oxygen, as compared with an hydroxylic or acyloxy oxygen (Jaffé, 1953; Stewart & Yates, 1960), would be expected to give a greater decrease in the bond order of the C-H bond concerned with consequent lowering of the absorption frequency.

The fundamental C-H stretching absorptions of the dimethylamino-compounds (Table 2) are observed at 2,823–2,810 cm^{-1} and 2,778–2,766 cm^{-1} . These values are thus in close agreement with the literature assignments of 2,825–2,810 cm^{-1} and 2,775–2,765 cm^{-1} for the $-\text{NMe}_2$ group in amines (Braunholtz & others, 1958; Hill & Meakins, 1958). Once again the complexity of the spectra prevents simple correlation in the terms sought.

CH deformation frequencies. If C-H --- O hydrogen bonding is involved in the stabilization of the proposed quasi-ring conformation (4) then the effect of such bonding might also be expected to affect the CH deformation frequencies. The frequency shift should be towards higher wave numbers due to an increase in the restoring force tending to keep the C-H bond directed towards the oxygen atom (Hallam, 1963). The relative upward shift is, however, smaller than the relative downward shift in the corresponding stretching modes (Hallam, 1963) although in

TABLE 3. SOLID STATE SPECTRA (KCl DISC)

| Compound | Absorption frequencies in 1500–1390 cm^{-1} region cm^{-1} | | | | | | | | | | |
|--|--|------------------|--------|------|------|------|----------------|------|------|------|--------|
| 1. Tetramethylammonium iodide | 1484 | | | | | | | | | 1403 | 1396 |
| 2. Octyltrimethylammonium iodide | 1484 | 1478sh 1470sh | 1462sh | | | 1433 | | | | 1408 | 1395 |
| 3. Choline iodide | 1485 | | 1466sh | 1455 | | | 1423 | 1410 | | | |
| 4. Acetylcholine chloride | 1492sh | 1487 | 1475 | 1455 | 1445 | 1435 | 1425 | 1412 | | | |
| 5. Acetyl- β -methylcholine chloride | 1495sh | 1483 | 1465 | | 1442 | | | | | 1409 | 1390sh |
| 6. Succinylcholine chloride | 1497 | 1483 | | 1455 | 1448 | | 1429 1421sh | 1415 | | | |
| 7. Acetyltrimethylammonium iodide | 1485 | 1470 | | | 1449 | 1435 | | 1416 | 1401 | | |

sh denotes shoulder.

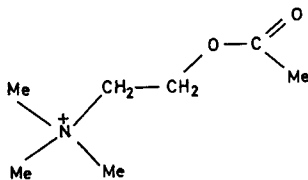
only a few instances have the effects of H-bonding on deformation modes been well substantiated. Ebsworth & Sheppard (1959) made the assignments δ_{CH_3} asym. 1,483 cm^{-1} and δ_{CH_3} sym. 1,403 + 1,397 cm^{-1} for the tetramethylammonium ion.

The solid state spectral data of acetylcholine, (\pm)-acetyl- β -methylcholine and succinylcholine in the 1,500–1,390 cm^{-1} region are compared

with a number of quaternary ammonium salts in Table 3. Thus tetramethylammonium iodide shows three well defined peaks at 1,484, 1,403 and 1,396 cm^{-1} in complete agreement with the assignments of Ebsworth & Sheppard (1959). Octyltrimethylammonium iodide shows well-defined absorptions at comparable frequencies, although in this instance considerable broadening coupled with a series of shoulders on the low frequency side of the 1,484 cm^{-1} peak (a manifestation of the eight carbon atom chain) can be seen. In the remaining compounds of Table 3 the δ_{CH_3} sym. modes cannot be identified with certainty but there is a well-defined band at $1,485 \pm 2 \text{ cm}^{-1}$ corresponding to δ_{CH_3} asym. There is broadening in succinylcholine, which shows a second well-resolved peak at 1,497 cm^{-1} , also in acetylcholine and (\pm)-acetyl- β -methylcholine which show shoulders at 1,492 and 1,495 cm^{-1} respectively. As evidence for C-H - - - O hydrogen bonding this is equivocal in view of the poor documentation of this region. Moreover deformation modes frequently couple with other vibrational modes in the lower "finger print" region of the spectrum (Hallam, 1963). Choline iodide (Table 3, No. 3) and acetyltrimethylammonium iodide (No. 7), although theoretically capable of C-H - - - O hydrogen bonding, do not show similar characteristics.

EXAMINATION OF CARBONYL ABSORPTIONS OF ACETYLCHOLINE AND (\pm)-ACETYL- β -METHYLCHOLINE

Since liquids containing hydrogen atoms capable of hydrogen bond formation could not be employed as solvents and insolubility of the compounds limited the selection of solvent, spectra were determined in dry dioxan, in which acetylcholine and (\pm)-acetyl- β -methylcholine proved sparingly soluble. Both showed split carbonyl absorptions at



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1,753 cm^{-1} and 1,732 cm^{-1} possibly due to the co-existence of conformations akin to 4 and 6. However, low intensity absorption at *ca* 1,720 cm^{-1} . (indicative of traces of free acetic acid despite rigorous precautions to exclude moisture) prevented firm assignment of these dual ester carbonyl absorptions to conformation effects. Ethyl acetate and diethyl succinate used as standards of reference showed single $\nu_{\text{C}=\text{O}}$ in dioxan solution at 1,743 cm^{-1} and 1,737 cm^{-1} respectively.

EXAMINATION OF CARBONYL ABSORPTIONS OF THE SULPHONES

Table 4 shows the carbonyl stretching frequencies of the series of sulphones $\text{EtSO}_2[\text{CH}_2]_n\text{OCOMe}$ ($n = 1-3$) in dry dioxan solution. These compounds may be regarded as analogues of the ω -acetoxyalkyl-

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trimethylammonium compounds in which the sulphone group replaces the onium function as an electron withdrawing substituent. But in contrast, being soluble in solvents incapable of acting as hydrogen donors, they were expected to yield information on the effect of -I substituents on the carbonyl stretching frequency in a situation uncomplicated by intermolecular hydrogen bonding between solvent and solute. All show a higher ester carbonyl stretching frequency than ethyl acetate or diethylsuccinate (Table 4), and only acetoxymethyl ethyl sulphone ($n = 1$)

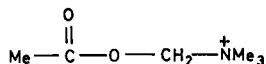
TABLE 4. CARBONYL STRETCHING FREQUENCIES OF SULPHONES IN DRY DIOXAN SOLUTION

| Sulphone $\text{EtSO}_2(\text{CH}_2)_n\text{OCOMe}$ | Carbonyl stretching frequency cm^{-1} | |
|---|--|------|
| $n = 1$ | 1775sh | 1764 |
| $n = 2$ | | 1751 |
| $n = 3$ | | 1745 |
| Ethyl acetate | | 1743 |
| Diethyl succinate | | 1737 |

sh denotes shoulder.

shows any indication of a split carbonyl absorption—there being a shoulder on the high frequency side at $1,775 \text{ cm}^{-1}$. The progressive decrease in the ester carbonyl stretching frequency as n increases from 1 to 3 agrees with the expected decrease in the influence of the inductive effect of the sulphone group and supports the conclusions of Fellman & Fujita (1963, 1966) in the series $\text{Me}_3\text{N}^+[\text{CH}_2]_n\text{OCOMe}$, where $n = 1-4$. It also provides an analogy for the observations of Nakamoto, Morimoto & Martell (1962) who showed that protonation of the nitrogen atom of the iminodiacetic acid di-anion produced a shift to higher frequency of the carboxylate carbonyl absorption.

The arguments of Fellman & Fujita (1963) to account for the higher frequency of the carbonyl group in acetylcholine in terms of the inductive effect of the quaternary nitrogen atom have been stated by Canepa & Mooney (1965) to be unsatisfactory. From earlier infrared studies (Gerrard, Mooney & Willis, 1961) of substitution patterns in mono-substituted benzene derivatives, Canepa & Mooney (1965) argue that the contribution from (*I*) is not expected to be important and they show by attenuated total reflection and transmission measurements that the



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carbonyl frequency in acetylcholine is of the order expected for aliphatic esters. The high frequency carbonyl absorptions observed by Fellman & Fujita (1963) [and omitted by Canepa & Mooney (1965) in their criticism] at *ca* $1,780 \text{ cm}^{-1}$ for trimethylammonium-methyl acetate (7) and observed at $1,775 \text{ cm}^{-1}$ and $1,764 \text{ cm}^{-1}$ for acetoxymethyl ethyl sulphone (Table 4) are difficult to rationalize if one accepts the conclusions of Canepa & Mooney (1965) that the mesomeric and/or inductomeric interaction between alkoxy and acyl groups are negligible.

NUCLEAR MAGNETIC RESONANCE STUDIES

A comparison of the nmr spectra of tetramethylammonium iodide, octyltrimethylammonium iodide, acetylcholine chloride and (\pm)-acetyl- β -methylcholine chloride in deuterium oxide solution failed to reveal any evidence of C-H --- O hydrogen bonding (compare Fellman & Fujita, 1966). The tetramethylammonium ion showed a 1:1:1 triplet structure similar to that observed in the tetraethylammonium ion (Bullock, Tuck & Woodhouse, 1963) due to coupling of the protons to the ^{14}N nucleus ($I = 1$). The other three compounds all showed unresolved *N*-methyl proton absorptions at 6.88τ , 6.76τ and 6.81τ respectively. Culvenor & Ham (1966) deduced that in deuterium oxide solution acetylcholine appears to exist largely in one conformation (3). These spectra were recorded at 60 Mc/sec whereas those in the present study being recorded at 40 Mc/sec were insufficiently resolved to be of the same diagnostic value. Even at 60 Mc/sec the *N*-methyl proton absorption of acetylcholine at 6.76τ (Culvenor & Ham, 1966) remains unresolved. Thus if C-H --- O hydrogen bonding is present in acetylcholine in deuterium oxide solution then interchange between hydrogen bonded and non-hydrogen bonded species is probably too fast at room temperature to be detectable by nmr spectroscopy (Pople, Schneider & Bertstein, 1962).

CONCLUSIONS

From the above discussion it is apparent that the results are not incompatible with the existence of intramolecularly hydrogen bonded forms such as the quasi-ring structures (2 and 3) which exist in the crystalline state (Canepa & others, 1966) and in aqueous solution (Canepa, 1965; Culvenor & Ham, 1966), respectively. The high ester carbonyl stretching frequency of acetylcholine although perhaps best interpreted in terms of an inductive effect from the onium nitrogen atom can also be interpreted in terms of C-H --- O hydrogen bonding between one of the *N*-methyl groups and the acetoxy oxygen atom. Thus, while the studies involving the sulphones indicate that inductive effects exert a big influence on the ester carbonyl absorptions, the absorptions occurring between $3,000$ and $2,950\text{ cm}^{-1}$ in the selected choline esters may represent ν_{OH} absorptions lowered in frequency as a result of C-H --- O hydrogen bonding. The δ_{OH} absorptions in the choline esters also provide circumstantial evidence for C-H --- O hydrogen bonding but unequivocal interpretation of these absorptions must await better documentation of this spectral region.

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