showed that, outside of NCAIA, antibiotics were detected only in the neighborhood of Room B433A. This basement location is in the hallway between the cage-washing operation and the glassware-washing area of the Laboratory Services Branch.

The antibiotic activity found in the air on the second floor, the largest area occupied by NCAIA, frequently appeared to be due to antibiotics other than penicillin. This conclusion is based on a comparison of results from assay plates with and without penicillinase. Although two spots of penicillin were found on one plate of four exposed in Room 1016 where penicillin contamination testing was performed, the many actual and control tests performed in that room have given no indication that environmental penicillin is ever detected by these tests. Most of the air coming into this room is filtered through Cambridge absolute filters to remove particles down to 0.3 μ in diameter.

This study shows that even in rooms occupied by NCAIA, there is no widespread heavy contamination of antibiotics in the air. Significant amounts occurred only in rooms where antibiotic powders were opened for weighing or where the drugs were extracted from dosage forms by blending. However, the quantities were not great in relation to the volume of air. The highest concentration of penicillin was 0.012 unit/cu. ft. and that of CTC was 0.0185 mcg./cu. ft. These small amounts would not affect the outcome of assays being performed as part of the certification program.

CONCLUSIONS

The incidence of contamination by antibiotic dust is negligible outside of the actual laboratory rooms where testing is performed. Therefore, persons in this building not testing antibiotics are not exposed to them by way of the atmosphere. Analysts testing antibiotics are exposed to a minimal degree.

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Conformation of Some Acetylcholine Analogs as Solutes in Deuterium Oxide and Other Solvents

A. F. CASY, M. M. A. HASSAN, and E. C. WU

Abstract □ The proton magnetic resonance spectra of α- and β-methylacetylcholines in deuterium oxide are described and analyzed. Chemical shift and coupling constant data derived from these analyses and from the spectra of model compounds establish that β-methylacetylcholine shows a preference for *gauche* $^{+}$N/O conformers, while the α-isomer displays a lack of conformational preference in the solute condition. Additional IR and proton magnetic resonance studies of acetylcholine and some analogs of acetylcholine, in which one *N*-methyl is replaced by a bulkier group, uphold the preferred nature of *gauche* $^{+}$N/O conformers in the parent compound as a solute and support an intramolecular $^{+}$N. . . OCOMe interaction as a factor governing conformational preferences in these esters. The significance of the results in terms of the influence of conformational isomerism upon the duality of action of acetylcholine is discussed.

Keyphrases □ Acetylcholine analogs—conformation determination in deuterium oxide □ α-, β-Methylcholine—conformational analysis □ Conformation—acetylcholine analogs □ PMR spectroscopy—structure □ IR spectrophotometry—analysis

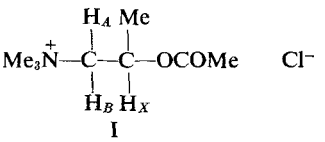
Knowledge of the conformation of acetylcholine (Ach) and related agonists has been increasingly sought during the last few years. Such information, while of potential value to the delineation of receptor characteristics and agonist–receptor interactions (1), is of additional importance in the cholinergic field because of recent postulates associating muscarinic and nicotinic effects of Ach with different conformations of the Ach molecule, recently reviewed (2). Reports upon the conformation of Ach and its congeners chiefly relate to the solid sate. Canepa *et al.* (3) showed that Ach bromide has a *gauche* $^{+}$NCCO conformation; this arrangement also occurs in many related compounds such as α-glycerophosphorylcholine and muscarine iodide (4), all in the solid state. Solute conformations of Ach in ethanol (5) and heavy water (6) have been advanced on the basis of infrared (IR) and proton magnetic resonance (PMR) spectroscopic data, respectively, the latter study in particular providing good evidence for a preferred *gauche* conformation. Molecular orbital calculations for Ach lead to the same prediction of preferred conformation (7).

This article reports some spectroscopic studies relating to the solute conformations of α- and β-methylacetylcholines analogs which retain, respectively, the nicotinic and muscarinic properties of Ach (8); the solid-state conformations of both compounds recently were described (9). Some studies of Ach analogs in which *N*-methyl is replaced by a bulkier substituent are also described.

PMR SPECTROSCOPY

PMR evidence of the conformation of substituted ethanes requires knowledge of the magnitude of spin-spin coupling between

vicinal protons (10, 11). In the case of β-methylacetylcholine (β-MeAch), this information may be obtained by analysis of the 100 MHz. resonance signal due to the methylene protons (H_A and H_B in I) by Bible's method (12); use of the methine signal (due to H_X)

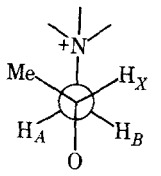


I

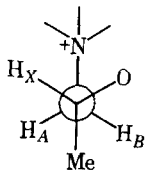
was not attempted since this formed an unresolved multiplet as a result of additional coupling with the β-methyl protons and ^{14}N (13).

Unless otherwise stated, the solvent used in the PMR work was deuterium oxide (D_2O), and the reference standard was sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The methylene signal, treated as the *AB* part of an *ABX* system, was composed of two partially overlapping quartets (Fig. 1), each being identified by the separation 14.4 Hz. (J_{AB}) which occurs four times within the 8-line signal. The distance between the centers of the two quartets is 5.6 Hz. where $|(J_{AX} + J_{BX})| = 11.2$ Hz. Construction of right-angled triangles (12), using the quantities J_{AB} (14.4 Hz.), $2D_+$ (26.8 Hz.), and $2D_-$ (20.8 Hz.) (the distances between the 1st and 3rd peaks of the two *AB* quartets), gives a $\Delta\nu_{AB}$ value of 4 or 19 Hz. and a $|(J_{AX} - J_{AB})|$ value of 37.2 or 8.2 Hz. The intensity ratio of peaks in the *X* signal was not available, so the correct alternatives could not be selected in this way; the values $\Delta\nu_{AB} = 19$ and $|(J_{AX} - J_{BX})| = 8.2$ Hz. were chosen as the more probable, however, and on the basis of comparisons with calculated spectra (14). Combination of the quantities $|(J_{AX} - J_{BX})| = 8.2$ Hz. and $|(J_{AX} + J_{BX})| = 11.2$ Hz. gives the values $J_{AX} = 9.7$ and $J_{BX} = 1.5$ Hz.

From the order of magnitude of these values (15, 16), it is clear that the preferred rotamer(s) of β-MeAch involves *trans* and *gauche* vicinal coupling constants; this conclusion supports either II or III as the preferred conformation. (In these and other Newman



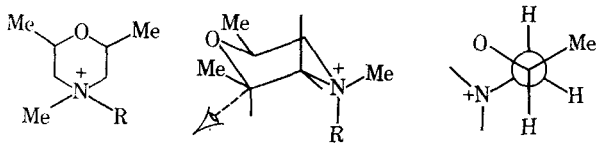
II



III

projections, the N—Me and O—COMe groups are omitted for clarity.)

The fact of a chemical shift difference between H_A and H_B of 19 Hz. (at 100 MHz.) further indicates that β-MeAch displays a marked conformational preference but does not aid in the choice of the preferred conformation because environments of the two protons differ in both conformers II and III. The morpholino derivatives, IV*a* and IV*b*, serve as rigid models for the *gauche* $^+$N/O conformer III. Data on their spectra generally support the latter as a preferred conformation, because vicinal coupling constants (for IV*a* and IV*b*) of similar orders of magnitude as those found for β-MeAch are derived from their spectra. Thus, analysis of the *X* mul-



IV

(*a*) R = H, $\bar{X} = \bar{Cl}$

(*b*) R = Me, $\bar{X} = \bar{I}$

tiplet of IV*a* gives the value $|(J_{AX} + J_{BX})| = 13.3$ Hz., where $J_{AX} = 11.2$ and $J_{BX} = 2.1$ Hz. by first-order treatment, following Booth and Gidley (17) who examined the 2,6-dimethylmorpholine base. Similar values were obtained by the same analysis of the methine signal of IV*b* (the *AB* and *X* signals of IV*a* and IV*b* virtually over-

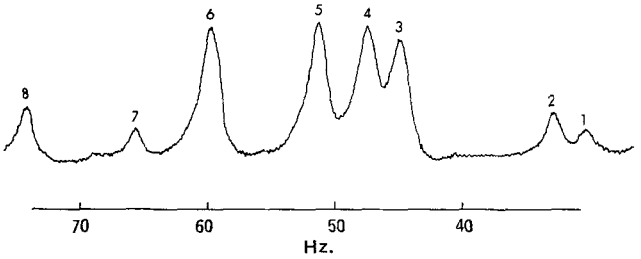


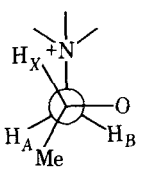
Figure 1—*100 MHz. PMR methylene signal of β-methylacetylcholine chloride in D_2O; sweep width 100 Hz. and sweep offset 370 Hz. Lines 8, 6, 4, and 2, and 7, 5, 3, and 1 form the two* AB *quarters, respectively.*

lapped); details are given in the *Experimental* section. Surprisingly, HCCN coupling was negligible in the cyclic quaternary salt IV*b*; this point is being studied further. Chemical shift data upon the secondary methyl group of β-MeAch and IV*b* also support the preferred nature of conformer III. Although the two values differ (ν_{Me}; III δ 1.33, IV*b* δ 1.23), allowance for the acylation shift (ν_{Me} in V is δ 1.24) shows that β-methyl has a similar environment in both I and IV*b*.

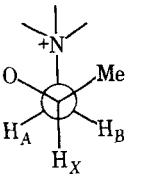
$Me_3\overset{+}{N}CH_2CHMeOH\ \bar{I}$

V

The small, but significant, differences observed between the J_{AX} and J_{BX} values of IV*b* and those of β-MeAch are best accounted for in terms of a *gauche* $^+$N/O conformer in which the NCCO dihedral angle is increased from 60° (as in III) to about 90° (as in VI); dihedral angles of this magnitude occur in solid-state conformations of Ach, α-MeAch, and β-MeAch (9) and are probably the result of a compromise between steric and electronic interactions of the nitrogen and oxygen functions. In VI, the H_XCCH_A and H_XCCH_B dihedral angles exceed and fall below, respectively, corresponding angles in the fully staggered conformation III. On the basis of the Karplus $\cos^2 \phi/J$ relationship (16), both these changes will lead to a reduction in vicinal coupling constants; hence, values lower than those in models such as IV*b*, in which dihedral angle deviations from 60° are less likely on account of constraints imposed by the cyclic structure, are to be anticipated. A significant population of the sterically unfavorable all-*gauche* conformer VII would also account for the lower J_{AX} and J_{BX} values of I compared with those of



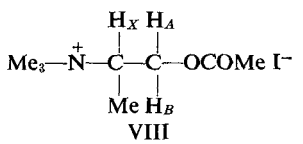
VI



VII

IV*b*, but the β-methyl chemical shift values would not be expected to correspond so closely because methyl is deshielded by charged nitrogen in VII. The population of the *trans* $^+$N/O conformer II is likely to be small since its contribution, although providing a lower J_{BX} coupling (J_{BX} is *trans* in III but *gauche* in II), would enhance the J_{AX} value.

In the case of α-MeAch, knowledge of the J_{AX} and J_{BX} coupling constant magnitudes was obtained by analysis of the H_X signal of the $ABXK_3$ system (see VIII), since this exhibited negligible coupling with ^{14}N (*cf.*, H_X signal of the β-isomer). The *AB* signal (a broad multiplet low field of the methine signal) was unsuitable for analysis because of coupling with ^{14}N and its overlap with the HDO peak; ^{14}N coupling effects were particularly clear in the α-methyl signal which formed a sharp doublet of triplets (*J* 7.0 and 2.0 Hz.)



VIII

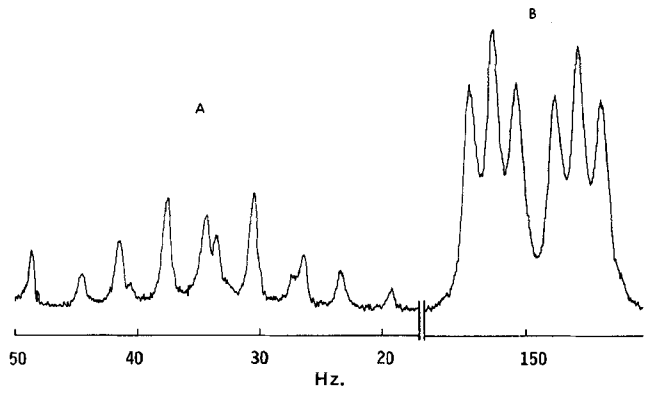
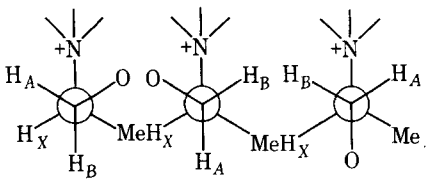


Figure 2—*100 MHz. PMR methine (A) and secondary methyl (B) signals of α-methylacetylcholine iodide in D_2O; sweep width 100 Hz. and sweep offset 420 Hz. for A and zero for B.*

(Fig. 2B). Identification of the four XK_3 quartets among the 12-line methylene signal (Fig. 2A) by the Booth and Gidley procedure (17), taking $J_{XK} = 7.0$ Hz. from the α-methyl signal, gives the quantities $|(J_{AX} + J_{BX})| = 8.8$ Hz. and the approximate values $J_{AX} = 4.2$ and $J_{BX} = 4.6$ Hz.; the latter values could not be refined by data from the *AB* portion of the spectrum because this signal was poorly resolved. The first-order values are of sufficient accuracy, however, for the present purpose. The geminal H_A and H_B protons formed a multiplet of five broad lines (partially obscured by the HDO peak) about 1.1 p.p.m. downfield of the methine signal. The overall narrowness of the signal ($W_H \sim 10$ Hz.) shows that the methylene protons have very similar chemical shifts, and the appearance of the *ABX* spectrum obtained closely approaches that calculated for the values $\Delta\nu_{AB}2$, $J_{AB}10$, and $J_{AX} = J_{BX} = 4$ Hz. (14).

The near-coincidence of the vicinal coupling constants derived by the analysis indicates that conformational preferences among α-MeAch conformers are low. *Gauche* and *trans* coupling constants for IX–XI are unknown; but values predicted on the basis of data



IX	X	XI
$J_{AX} \sim 1.5$	~ 1.5	~ 10 Hz.
$J_{BX} < 1.5$*	~ 10	~ 1.5 Hz.

* This value is judged the lowest because both coupling protons are *trans* to highly electronegative groups (18).

for β-MeAch and the models IV*a*, IV*b*, XII (the methiodide of phendimetrazine), and XIII (derived from ephedrine) should give reasonable estimates of their orders of magnitude. The probable values are listed beneath each conformer.

Similar populations of IX, X, and XI should lead to average J_{AX} and J_{BX} coupling constants of just over 4 Hz., *e.g.*, (1.5 + 1.5 + 10)/3 = 4.3 Hz., as obtained by analysis. Conformer distributions of this order would also result in similar averaged environments (and hence like chemical shifts) for the H_A and H_B protons, again in agreement with spectral observations.

The α-methyl chemical shift (δ 1.5) is lower field than that of methyl in XIV (δ 1.33) (19); this result is evidence for significant populations of IX and XI in which methyl is close to the deshielding

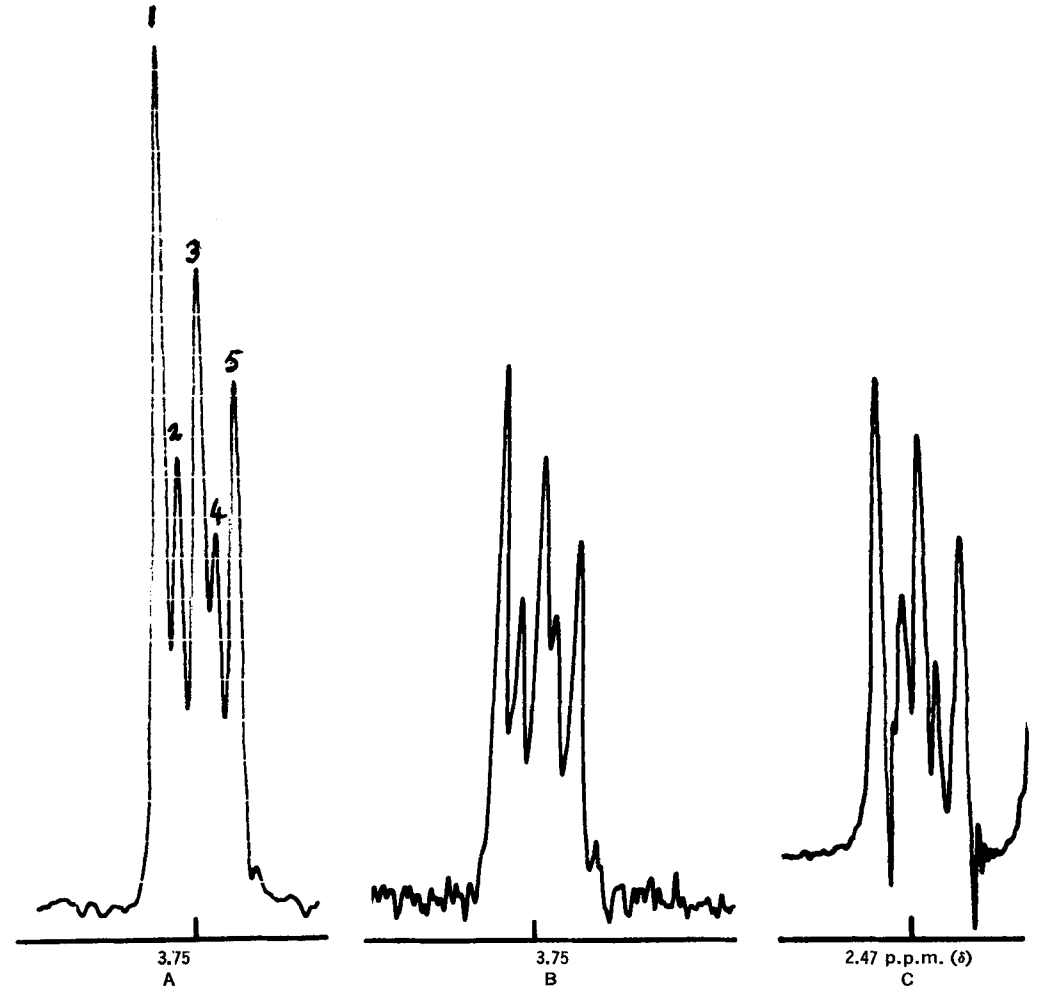
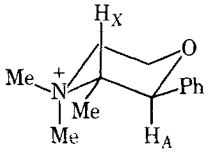


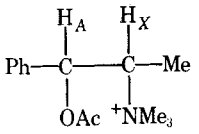
Figure 3—*60 MHz. PMR N-methylene signals of acetylcholine (A), (β-acetoxyethyl)benzyldimethylammonium bromide (B), and N-methylmorpholine (C) in D_2O.*

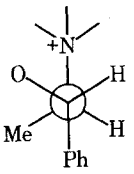
Table I—PMR Characteristics of the *N*-Methylene Protons of Some Acetylcholine Analogs in Deuterium Oxide ($RNMe_2CH_2CH_2OR'$ X^-)

Compound No.	R	R′	X	NCH_2 PMR Characteristics[a] Chemical Shift[b]	Line 1–5 Separation	Line 2–4 Separation
1	Me	COMe	Cl	3.75	9.5	5.0
				3.8	9.5	5.0[c]
				4.13	9.5	4.8[d]
2	Me	H	Cl	3.52	10.0	5.0
3	CH_2:$CHCH_2$	COMe	Br	3.68	9.8	4.8
4	CH_2:$CHCH_2$	H	Br	3.48	10.0	5.0
5	$PhCH_2$	COMe	Br	3.75	10.0	4.5
				4.2	10.0	5.8[d]
6	$PhCH_2$	H	Br	3.52	10.0	5.0
7	Me_2CH[e]	COMe	Br	3.72	10.0	4.0
8	Me_2CH	H	Br	3.48	9.5	4.0
9	*tert*-Bu[f]	COMe	I	3.67	10.0	4.0
10	*tert*-Bu	H	I	3.43	10.0	4.0
11	Morpholine based[g]			2.43	10.0	4.5
12	*N,N*-Dimethyl-morpholinium iodide			3.50	9.5	4.5

[a] Chemical shifts in p.p.m. from external tetramethylsilane; line separations in Hz. Spectra recorded at 60 MHz. at the normal operating temperature. [b] Center of multiplet. [c] In DMSO-d_6. [d] In $CDCl_3$. [e] Me signal: broad doublet (No. 7), doublet of triplet (J = 2 and 7 Hz.) (No. 8). [f] *tert*-Bu signal: broad singlet (No. 9), triplet (J = 1.5 Hz.) (No. 10). [g] Smith and Shoulders (39).

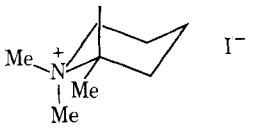






J_{AX} = 10.4 Hz. (*trans*) J_{AX} = 1.5–2.0 Hz. (*gauche*)

XII XIII XIII



XIV

oxygen function. IR evidence supports the *gauche* $^+N/O$ conformers IX and X.

To conclude the PMR studies, data are given for some analogs of Ach in which one *N*-methyl is replaced by a bulkier group. These derivatives were examined to determine whether the preference for *gauche* $^+N/O$ conformers, established for Ach itself in solvent D_2O (6), is maintained when the destabilizing steric interaction between the oxygen and nitrogen functions is increased. The original PMR analysis of Ach rests upon the extraction of the sum (N) and difference (L) of the vicinal coupling constants from the bimethylene signal treated as an $A_2'B_2'$ system. The analysis concerns, in particular, the higher field methylene multiplet (due to $^+NCH_2$), because the lower field signal (OCH_2) is complicated by coupling with ^{14}N. In the present case, no complete analysis (involving first estimation of the L and N values from line separations and then refinement by means of an iterative calculation) is attempted. Instead, the $^+NCH_2$ signal appearance and line separations are compared with those of Ach, and conformational conclusions are drawn from observed similarities or differences. Where 5-line multiplets result, separations of Lines 1 and 5 and 2 and 4 (Fig. 3) give the approximate N and L values, respectively. These data are given in Table I and the following points are made.

1. The data show that the $^+NCH_2$ methylene proton characteristics of the Ach analogs 3, 5, 7, and 9 closely mirror those of Ach itself and the model compounds morpholine and *N,N*-dimethylmorpholine (Compounds 11 and 12) which are constrained to *gauche* N/O conformations. Three examples are shown in Fig. 3. These results, following the analysis of Culvenor and Ham (6), support preferred *gauche* conformations for the analogs 3, 5, 7, and 9 in D_2O and show that replacement of an *N*-methyl group of Ach by a bulkier group has no major effect upon conformational equilibria in these quaternary salts. The same conclusions follow for the corresponding quaternary alcohols (Compounds 2, 4, 6, 8, and 10).

2. The appearance of the $^+NCH_2$ signal of Ach shows little variation when D_2O is replaced by DMSO-d_6 or $CDCl_3$ (Table I) or by D_2O–pyridine or trifluoroacetic acid (6). Hence the conformation of Ach appears to be little influenced by the solvent. This result suggests that the conformation of Ach is goverened primarily by interactions within the molecule.

3. The A_2 and B_2 methylene signals of 3,3-dimethyl-*n*-butanol and its acetoxy ester (carbocholine) in CCl_4, the tertiary base analogs of choline and Ach in D_2O, and 2-(*N*-*tert*-butyl-*N*-methylamino) ethanol (the base related to Compound 10, Table I) in $CDCl_3$ form near-symmetrical triplets of separation, 5–6 Hz., characteristic of an absence of conformational preference (11). These results indicate that *gauche* conformers are preferred in β-substituted ethanols only when the substituent is highly electronegative as in the case for a halogen (11) or a charged nitrogen atom. The spectrum of the hydrochloride of the tertiary base related to Ach in D_2O also shows a $^+NCH_2$ signal characteristic of the quaternary salts (N ~10 Hz., L ~3.8 Hz.).

4. The spectrum of acetylthiocholine (thio-Ach) was markedly different from that of Ach, the NCH_2 and SCH_2 signals being much

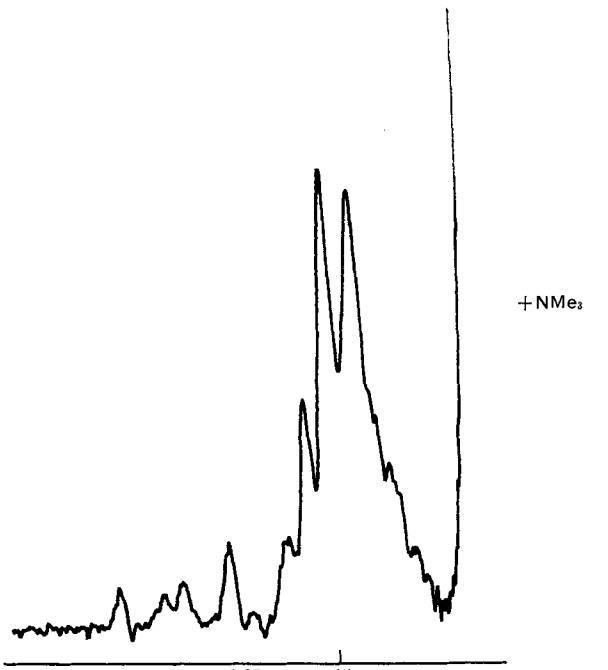


Figure 4—*60 MHz. PMR* N- *and* O-*methylene signals of acetylthiocholine chloride in* D_2O *(sweep width 250 Hz.).*

Table II—Carbonyl Stretching Frequencies of Acetylcholine and Related Compounds in Chloroform

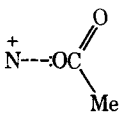
No.	Compound	$\nu_{C=O}$ (cm.$^{-1}$) 10%	$\nu_{C=O}$ (cm.$^{-1}$) 0.005 mole/l.	$\Delta\nu_{C=O}$[a]
1	$Me_3\overset{+}{N}(CH_2)_2OCOMe\ \overline{Cl}$	1750 1757[b]	1755	+20
2	$Me_2N(CH_2)_2OCOMe$	1732	1732	−3
3	EtOCOMe	1735 1745, 1725[b]	1735	—
4	$Me_2N(CH_2)_2OCOMe\cdot HCl$	1752	1757	+22
5 (β-)	$Me_3\overset{+}{N}CH_2CHMeOCOMe\ \overline{Cl}$	1745	1746	+11
	$Me_3\overset{+}{N}CH_2CHMeOCOMe\ \overline{I}$	1745	1748	+13
6	$Me_2NCH_2CHMeOCOMe$	1732	1732	−3
7 (α-)	$Me_3\overset{+}{N}CHMeCH_2OCOMe\ \overline{I}$	1757	1757	+22
8	$t\text{-}Bu\overset{+}{N}Me_2(CH_2)_2OCOMe\ \overline{I}$	1749	1753	+18
9	$CH_2{:}CHCH_2\overset{+}{N}Me_2(CH_2)_2OCOMe\ \overline{Br}$	1750	1755	+20
10	$PhCH_2\overset{+}{N}Me_2(CH_2)_2OCOMe\ \overline{Br}$	1750	1757	+22
11	$Me_2CH\overset{+}{N}Me_2(CH_2)_2OCOMe\ \overline{Br}$	1750	1750	+15
12	$Me_3C(CH_2)_2OCOMe$	1732	1732	−3
13	$Me_3\overset{+}{N}(CH_2)_2SCOMe$	1695	1695	−40

[a] $\nu_{C=O}$ (compound) − $\nu_{C=O}$ ethyl acetate. [b] In ethanol.

closer together and forming a complex multiplet approximately symmetrical about its midpoint and obscured on the high field side by the $^+NMe_3$ signal (Fig. 4). No assignment of *L* and *N* values was possible. This result is evidence that conformational preferences in the thio-derivative are different from those in Ach and its congeners; it is significant in this respect that X-ray analysis of thio-Ach bromide shows that the crystalline salt has a *trans* N/S conformation (20). Mautner (21), quoting unpublished results, recently proposed a *trans* conformation for this compound in solution.

IR SPECTROSCOPY

Several IR studies have established that the carbonyl stretching mode ($\nu_{C=O}$) of Ach is higher than that of the corresponding tertiary amine and of ethyl acetate (5, 22, 23). The shift to higher wave numbers that occurs when a β-hydrogen in ethyl acetate is replaced by trimethylammonium may be interpreted in terms of an electronic interaction between charged nitrogen[1] and a lone pair orbital of the alkyl oxygen atom of the ester function (XV); relay of the consequent drift of electrons toward nitrogen to carbonyl carbon



XV

would depress the polarization of the carbonyl group, with a consequent rise in its absorption frequency (2).[2] An interaction of the type shown in XV requires close proximity of the charged nitrogen and ester functions; demonstration of its operation thus provides support for *gauche* $^+N/O$ conformers of Ach and its congeners.

The published IR data have two limitations: (*a*) interpretation is complicated by solvent effects ($\nu_{C=O}$ values were measured in hydrogen bonding solvents such as ethanol and water), and (*b*) dilution studies, necessary to differentiate between intermolecular and intramolecular effects, were not reported. For these reasons, further IR studies of Ach and its analogs (some novel) were undertaken in this work. Spectroscopic chloroform was chosen as the reference solvent, since solvent effects should then be negligible. Esters were examined over the concentration range from 10% to 0.1 or 0.05%, the lowest concentration being 0.005 mole/l. At this dilution, intermolecular effects may be discounted (25). IR data are given in Table II, where only $\nu_{C=O}$ values at the concentration extremes are recorded. The following points are made:

1. In all cases, little change in the $\nu_{C=O}$ values of the salts occurs on dilution; this result shows that intermolecular interactions do not significantly influence the carbonyl stretching frequency of these esters.

2. The $\nu_{C=O}$ wavenumber of Ach in chloroform is similar to that of the hydrochloride of 2-dimethylaminoethyl acetate, but 23 and 20 cm.$^{-1}$ higher, respectively, than those of the corresponding tertiary base and ethyl acetate (Table I, Compounds 1–4). Carbonyl stretching frequency shifts relative to the ethyl acetate value are also seen for α- and β-MeAch (Table II, Compounds 7 and 5); the α-shift (22 cm.$^{-1}$) is similar to that of Ach, but the β-value (11 cm.$^{-1}$) is lower (a possible reason for this is given later). The corresponding β-tertiary base has a $\nu_{C=O}$ value close to that of ethyl acetate (Table II, Compounds 6 and 3). The $\nu_{C=O}$ (and $\Delta\nu_{C=O}$) values of the Ach analogs (Table II, Compounds 8 and 11), in which one of the *N*-methyl groups of the onium head is replaced by a bulkier group, are close to that of the parent compound.

3. The similar $\nu_{C=O}$ values of 3,3-dimethyl-*n*-butyl acetate (carbocholine) and ethyl acetate (Table II, Compounds 3 and 12) show that the $\nu_{C=O}$ elevation seen in Ach and related compounds is not steric in origin. In addition, results upon the free tertiary amine esters (Table II, Compounds 2 and 6) establish that the basic nitrogen function has no significant influence on the ester carbonyl stretching frequency.

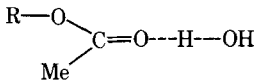
4. The very low $\nu_{C=O}$ value of thio-Ach (Table II, Compound 13) reflects the different influences of oxygen and the substantially less electronegative atom, sulfur, upon the carbonyl stretching vibration; release of a sulfur lone pair of electrons is responsible for lowering $\nu_{C=O}$ of thiol esters below the values found for ketones (26). Although the tertiary amine corresponding to thio-Ach was not available for assessing $N^+\cdots S$ interactions, the fact that the $\nu_{C=O}$ value falls within the range 1698–1690 cm.$^{-1}$ quoted for dialkyl thiolesters (26) suggests that such interactions are absent, as would be the case if a *trans* $^+N/S$ conformer was preferred (*PMR Spectroscopy* section).

5. The IR evidence for favored *gauche* $^+N/O$ conformers of Ach and its congeners relates to solute molecules in chloroform and is not necessarily relevant when D_2O or water is the solvent. The close approximation of the PMR features of Ach in $CDCl_3$ and D_2O (previously described), however, strongly suggests that Ach

[1] A reviewer points out, on the basis of molecular orbital calculations on the ammonium ion (24), that the positive charge of the onium group may be distributed over the atoms attached to the nitrogen atom. If such be the case, the interaction depicted in XV is more correctly represented as between the onium group as a whole and the ether oxygen atom; this modification does not affect the IR interpretations which follow.

[2] Fellman and Fugita (5) considered that a direct $^+N/O$ interaction was unlikely on the grounds of the smaller $\nu_{C=O}$ shifts seen in 3- and 4-carbon chain analogs of Ach; their use of planar rather than three-dimension models, however, invalidates this view. Canepa and Mooney (23) criticized their proposal of the onium influence upon the ester function being transmitted inductively through the two-carbon chain.

conformer populations are alike in polar and feebly polar solvents. The influence of the onium group upon the carbonyl stretching frequency in polar solvents may be obscured by solute–solvent interactions. Thus the lower $\nu_{C=O}$ values of Ach obtained in water (1727 cm.$^{-1}$) and 50% ethanol in water (1737 cm.$^{-1}$) by attenuated total reflectance (5, 23) show that the hydrogen bonding interaction (XVI) (which reduces $\nu_{C=O}$) is the predominant influence (2).



XVI

6. The carbonyl stretching frequency of the ester methiodide XIII, derived from ephedrine and for which there is clear PMR evidence for a preferred *gauche* $^+$N/O conformation, is 1760 cm.$^{-1}$ (0.5 and 0.1%), a value close to that of Ach and its congeners.

IR and PMR evidence is thus complementary in supporting the existence or absence of conformational preferences among quaternary esters such as Ach and the tertiary bases from which they are derived. The lack of conformational preference in the β-methyl tertiary base XVII, for example, is shown both by its having a $\nu_{C=O}$ value close to that of ethyl acetate (Table II, Compound 6) and by

$Me_2NCH_2CHMeOCOMe$
XVII

the averaged values of its CH_2CH coupling constants (J = 6 Hz. in $CDCl_3$).[3]

The small, but consistently obtained, differences between the $\nu_{C=O}$ values of Ach, α-, and β-MeAch (Table II, Compounds 1, 5, and 7) may be tentatively explained in terms of the methyl substituent aiding or opposing the close approach of the nitrogen and oxygen functions. A methyl substituent would be expected to oppose the approach of the $^+$N and OCOMe functions through the generation of unfavorable Me–H interactions (as in β-III) or assist the same approach by a buttressing effect which causes the C—$\overset{+}{N}Me_3$ bond to bend away from a geminally attached methyl group (as in α-X) and by relief of an Me–O interaction (as in α-IX). The halide ion has a negligible influence, as seen by the identical spectral properties of the chloride and iodide of β-MeAch.

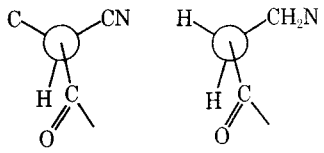
The following conclusions may be drawn from the spectroscopic evidence:

1. The additional IR and PMR studies of Ach and its *N*-substituent congeners uphold the preferred nature of the *gauche* $^+$N/O conformers of the parent compound in deuterium oxide and, by analogy, in water. The $^+$N- - -OCOMe interaction is a probable stabilization factor, since it appears capable of compensating for destabilizing steric interactions of $^+NMe_3$ and other onium groups of even greater bulk. If the positive charge on the onium function is distributed around the periphery of the group (24), the interaction might be represented more correctly by NC—$\overset{\delta+}{H}$- - -O, as proposed earlier (5), even though there is no precedent or evidence for the existence of formal hydrogen bonds of this nature (27, 28).

2. β-MeAch shows a pronounced preference for *gauche* $^+$N/O conformers and there is good evidence for a high population of the skewed form VI; the α-isomer displays a lack of conformation preference. These results correlate closely with the solid-state conformations by these analogs established by X-ray studies (9); that of the β-isomer is similar to VI, and the α-isomer exists in two forms that are related to IX and XI, respectively. There is no simple explanation for these conformational differences. It is evident from models, however, that steric interactions of the Me and $^+NMe_3$ groups in the β-conformers are of a higher order than those of Me and OCOMe in the α-forms. Hence, II and VII (β) are probably much more destabilized relative to III (or VI) than are the comparable α-conformers, IX and XI, relative to X. The preferred *gauche* conformer β-III (or VI) thus combines operation of the $^+$N- - -O interaction with minimum steric interactions of the methyl substituent.

[3] Details of the analysis of the 100 MHz. spectrum of XVII as neat liquid, which gave similar results, are given under *Experimental*. Surprisingly, the base XVII displays a pronounced conformational preference in D_2O, as is evident from the broad nature of its methine signal.

Culvenor (29) proposed use of acylation shift data (HO-CH resonance moves downfield when the OH group is acylated) as evidence of ester conformation; he suggested that normal values (1.0–1.15 p.p.m. for secondary and 0.45–0.6 p.p.m. for primary esters) are indicative of a preferred conformation in which the ester carbonyl group is eclipsed by a methine or methylene proton. Acylation shifts for α-MeAch (~0.5 p.p.m.) and β-MeAch (~1 p.p.m.) fall in the normal ranges; hence, C—C—O—C=O and H—C—O—C=O dihedral angles of about 60° are probable, as in XVIII and XIX.



XVIII XIX

While work upon rigid and semirigid analogs of Ach is subject to certain limitations, *e.g.*, fairly large deviations from the molecular dimensions of the natural agonist in the case of decalin derivatives (30) and from fully staggered *gauche* and *trans* O/N dihedral angles in the case of cyclopropane analogs (31) [a recent X-ray study established that the NCCO dihedral angle is 137° in crystalline (+)-*trans*-2-acetoxycyclopropyltrimethylammonium iodide (32)], potency variations among the various isomers provide reasonable evidence that muscarinic properties are most pronounced in isomers that approach a *trans* $^+$N/O geometry. If work on these rigid analogs is valid in terms of the acyclic agonists acetylcholine and β-methylacetylcholine, then the preferred and "active" (muscarinic) conformations of these two compounds must differ. The fact that β-methylacetylcholine is only feebly active at nicotinic sites might be taken as evidence that *gauche* conformers cannot be the active form of acetylcholine at nicotinic receptors, thereby discounting the proposal of the duality of action of Ach being related to conformational isomerism. A firm conclusion cannot be drawn, however, since the influence of the β-methyl substituent upon drug–receptor uptake at nicotinic sites is unknown.

EXPERIMENTAL

Commercial samples of acetylcholine chloride, choline chloride, β-methylacetylcholine chloride, acetylthiocholine chloride, and 2-dimethylaminoethyl acetate were used. The last ester formed a hydrochloride, m.p. 126–128°.

Anal.—Calcd. for $C_6H_{14}ClNO_2$: C, 43.0, H, 8.4. Found: C, 42.7; H, 8.4.

2-Acetoxy-1-dimethylaminopropane hydrochloride separated when acetyl chloride (15.7 g.) was added to 1-dimethylaminopropan-2-ol (10.3 g.) in toluene. The base, recovered from the hydrochloride, was distilled to give 2-acetoxy-1-dimethylaminopropane (10 g.), b.p. 47°/14 mm.; hydrobromide, m.p. 113–115°, from ethanol—ether [Tammelin (33) gave b.p. 53°/13 mm. for the base and m.p. 121° for the hydrobromide]; and methiodide, m.p. 138–139°.

Anal.—Calcd. for $C_8H_{18}INO_2$: C, 33.5; H, 6.3. Found: C, 33.5; H, 6.1.

The PMR methine signal of the base [MeCH(OCOMe)CH_2-NMe_2] formed a near sextet (J = 6.4 Hz.), symmetrical about its midpoint, near δ 4.45 (neat) and 5.1 (in $CDCl_3$); in D_2O the signal was composed of 14 lines centered near δ 5.1 and gave J values 4.6 and 7.8 Hz. by first-order analysis.

The (±)-methiodides of 2-dimethylaminopropan-1-ol, m.p. 299–300°, and 1-acetoxy-2-dimethylaminopropane (α-methylacetylcholine), m.p. 128–130° [Beckett *et al.* (34) gave 299–300° for the former and 131–132° for the latter salt] were prepared by the literature procedure.

Morpholine Derivatives—*N,N*-Dimethylmorpholinium iodide, m.p. 246–248°, from ethanol [Knorr (35) gave 251°] was obtained from *N*-methylmorpholine and methyl iodide. Methylation of a commercial mixture of *cis*- and *trans*-2,6-dimethylmorpholine with 40% formaldehyde and 88% formic acid, followed by acidification of the basic product with concentrated hydrochloric acid, gave a crude mixture of the isomeric *N*-methyl hydrochlorides. Fractional crystallization of the mixture from ethanol–ether gave pure *N*-methyl-*cis*-2,6-dimethylmorpholine hydrochloride, m.p. 222–223°.

Anal.—Calcd. for $C_7H_{16}ClNO$: C, 50.75; H, 9.7. Found: C, 50.85; H, 9.6.

The derived base with methyl iodide gave *N,N*-dimethyl-*cis*-2,6-dimethylmorpholinium iodide, m.p. 230.5–231.5°.

Anal.—Calcd. for $C_8H_{18}INO$: C, 35.4; H, 6.9. Found: C, 35.7; H, 6.8.

trans-(3-Me/4-Ph) 3,4-dimethyl-2-phenylmorpholine (phendimetrazine) (36) formed a methiodide, m.p. 175–177°, from methanol–ether.

Anal.—Calcd. for $C_{13}H_{20}INO$: C, 46.85; H, 6.05. Found: C, 46.6; H, 6.1.

Erythro-3-acetoxy-2-dimethylamino-3-phenylpropane Methiodide—The dimethylamino analog of ephedrine, obtained by treating ephedrine with excess of methyl iodide, gave 2-dimethylamino-3-hydroxy-3-phenylpropane methiodide, m.p. 198–200°, from methanol–ether.

Anal.—Calcd. for $C_{12}H_{20}INO$: C, 44.9; H, 6.3. Found: C, 44.6; H, 6.2.

This salt with acetic anhydride gave the acetate methiodide (XIII), m.p. 225–226° (from methanol–ether).

Anal.—Calcd. for $C_{14}H_{22}INO_2$: C, 46.3; H, 6.1. Found: C, 46.5; H, 5.9.

Quaternary Salts of 2-Dimethylaminoethanol—2-Dimethylaminoethanol (8.9 g.) in acetone was added dropwise to benzyl bromide (17.1 g.) in the same solvent; the solid, which separated on storage, was collected and recrystallized from ethanol–ether to give benzyldimethyl(β-hydroxyethyl)ammonium bromide (21 g.), m.p. 129–130° [Jones (37) gave 130–131°]. Allyldimethyl(β-hydroxyethyl)-ammonium bromide monohydrate, m.p. 63–65° (*Anal.*—Calcd. for $C_7H_{16}BrNO \cdot H_2O$: C, 36.85; H, 7.95. Found: C, 36.5; H, 8.35) and dimethyl(β-hydroxyethyl)*iso*-propylammonium bromide, m.p. 275–276° [Robinson (38) gave 274°] were similarly prepared. A mixture of 2-*tert*-butylaminoethanol (11.7 g.), 40% formaldehyde in water (11.2 ml.), and 88% formic acid (7.8 g.) was heated under reflux for 12 hr. and then acidified with concentrated hydrochloric acid and evaporated. The residue was made alkaline with aqueous sodium hydroxide and extracted with ether. The extract was dried (Na_2SO_4), and evaporated, and the residue was distilled to give 2-(*N*-*tert*-butyl-*N*-methylamino)ethanol (11 g.), b.p. 68–70°/17 mm. It formed a hydrochloride, m.p. 113–114°, from ethanol–ether (*Anal.*—Calcd. for $C_7H_{18}ClNO$: C, 50.1; H, 10.8. Found: C, 50.2; H, 10.8) and a methiodide, m.p. 200–202°, from methanol–ether (*Anal.*—Calcd. for $C_8H_{20}INO$: C, 35.2; H, 7.4. Found: C, 35.3; H, 7.2). The methiodide was also obtained directly from 2-*tert*-butylaminoethanol after several treatments with methyl iodide and sodium methoxide in methanol at the reflux temperature.

Quaternary Salts of 2-Dimethylaminoethyl Acetate—Allyl bromide (1.2 g.) in acetone was added to 2-dimethylaminoethyl acetate (1.3 g.) in the same solvent when (β-acetoxyethyl)allyldimethylammonium bromide (2.4 g.), m.p. 101–103°, from ethanol–ether, separated.

Anal.—Calcd. for $C_9H_{18}BrNO_2$: C, 42.9; H, 7.2. Found: C, 42.9; H, 7.2. A mixture of benzyldimethyl(β-hydroxyethyl)ammonium bromide (5.2 g.), acetic anhydride (3.1 g.), and pyridine (5 ml.) was stirred for 10 hr. at room temperature and then concentrated under reduced pressure. The residue was recrystallized from ethanol–ether to give (β-acetoxyethyl)benzyldimethylammonium bromide (5.5 g.), m.p. 125.5–126.5°.

Anal.—Calcd. for $C_{13}H_{20}BrNO_2$: C, 51.65; H, 6.7. Found: C, 51.7; H, 6.8. (β-Acetoxyethyl)dimethylisopropyl ammonium bromide, m.p. 122–124°, from ethanol–acetone–ether (*Anal.*—Calcd. for $C_9H_{20}BrNO_2$: C, 42.5; H, 7.9. Found: C, 42.6; H, 8.1) and (β-acetoxyethyl)-*tert*-butyldimethylammonium iodide, m.p. 173–174°, from methanol–ether (*Anal.*—Calcd. for $C_{10}H_{22}INO_2$: C, 38.1; H, 7.0. Found: C, 38.1; H, 7.1.), were similarly prepared.

IR spectra were recorded with a Beckman (R-10) spectrophotometer. Cells of pathlength 0.05 mm. (10–5%), 0.5 mm. (5–3%), and 2 mm. (1.0–0.1%) were used for solution studies in $CHCl_3$; spectra were calibrated with polystyrene. PMR spectra were recorded with Varian H60-D and HA-100 instruments with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the standard in D_2O and tetramethylsilane in all other solvents.

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