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Conformational Relationships Between Analogs of Acetylcholine and Those of Local Anesthetics in Solution

(NMR conformational analysis)

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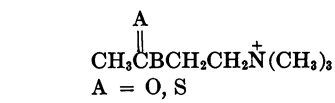
ABSTRACT **Conformations of analogs of acetylcholine and of related local anesthetics, in which either of the oxygen atoms had been replaced by other heteroatoms, were studied in solution by nuclear magnetic resonance. Several correlations between structure and conformation could be made.**

Acetylcholine (AcCh) plays an essential role in transmission of nerve impulses, presumably by inducing a conformational change in the receptor biopolymer to which it is attached, thereby leading to an alteration of cation permeability. On the other hand, it has been postulated that local anesthetics block the conduction of nerve impulses by attachment to axonal AcCh receptors (2, 3).

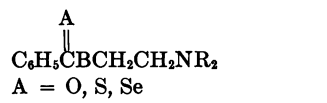
In view of these considerations, it seemed of interest to investigate the following: (*a*) Effects of modifying the ester grouping on the conformations of analogs of AcCh and of local anesthetics in crystals and in solution, (*b*) factors involved in maintaining the conformations of such molecules, and (*c*) relationships between the conformations and biological actions of molecules, either triggering or blocking conduction of nerve impulses.

One of the problems encountered when the structures of biologically active molecules are modified, is that even replacement of one atom by another may alter overall conformation and electron distribution throughout the molecule. Therefore, it is often difficult to decide whether a change in biological activity induced by modification of the structure of an active compound is due to an alteration of steric or of electronic factors.

In an attempt to dissect steric from electronic factors, we synthesized a series of analogs of AcCh and local anesthetics in which either or both of the oxygens of the ester grouping were replaced by sulfur or by selenium (4–7).



A = O, S
B = O, S, Se



A = O, S, Se
B = O, S, Se, NH
R = —CH_3, —CH_2CH_3

It was found that replacement of the acyloxy oxygen by sulfur or selenium in crystals resulted in a drastic alteration of the conformation of either AcCh or of 2-dialkylaminoethyl benzoates. In either case, the *gauche* (sc) conformation of the —O—C–C—N-grouping (8, 9) was altered to the fully extended *trans*(*ap*) conformation (10, 11). On the other hand, replacement of the carbonyl oxygen of AcCh with sulfur affected the *gauche* conformation of the —O—C–C—N-grouping to a very minor extent (12).

It has been noted that in the case of AcCh (13) and its thiolester (14, 15) and selenolester (14) analogs, the conformation observed in the crystal also predominates in solution. The present study is concerned with the factors responsible for the conformations observed as well as with the relationships between analogs of AcCh and analogs of local anesthetics.

Abbreviation: AcCh, acetylcholine.

RESULTS

Compounds were synthesized according to methods given in references in Tables 1–3.

^1H Resonance spectra were recorded at 60 MHz or 100 MHz in Hitachi R-20-B or Varian HA-100 spectrometers. Compounds were examined in about 1 M solutions at 34°. Chemical shifts are recorded in ppm and measured from internal tetramethyl silane or sodium 3-trimethyl-silylpropionate-2,2,3,3-d_4 references.

XCH_2CH_2Y groups of the compounds studied gave spectra of the AA'BB' type, except for unprotonated 2-dimethylaminoethyl benzoate. Analyses were performed initially by assuming that the spectra were of the AA'XX' type. Parameters obtained were refined by a LAOCOON III computer program (16).

In the case of the diethylamino compounds, double resonance spin decoupling of the methyl protons in the *N*-ethyl groups was used to uncover any CH_2N portion of the spectrum overlapping with the *N*-ethyl methylene protons.

Relative rotamer populations were obtained from the empirical relationships between the vicinal H–H coupling constants and electronegativities of the substituents as described by Abraham and Gatti (17). Substituent electronegativities used were those given by Huggins (18). Thus, J_{AB} and J_{AB}' were obtained as follows:

$$J_{AB'} = n_t J_t' + n_g J_g'$$
$$J_{AB} = n_t J_t' + n_g (J_g' + J_g'')/2$$

Data are summarized in Tables 1–3. If the rotamers were energetically equivalent, a *gauche*:*trans* distribution of 67%: 33% would be expected. Distribution values are only approximate with an error of about 10%, although small deviations from statistical distribution of rotamers can be detected with ease. For solubility reasons most spectra were obtained in CD_3OD; they were substantially the same when determined in D_2O.

TABLE 1. *Conformation of acetylcholine and of related esters*

Compound	H_A	H_B	H_C	J_{AB}	$J_{AB'}$	% Conformation Gauche	% Conformation Trans
$(CH_3)_3\overset{+}{N}CH_2CH_2O\overset{O}{\overset{\|}{C}}CH_3$	*4.55	3.74		7.21	2.51	100	0
	†4.45	3.80		6.80	2.75	100	0
	‡4.59	4.16		7.00	3.00	97	3
$(CH_3)_2\overset{+}{S}CH_2CH_2O\overset{O}{\overset{\|}{C}}CH_3$ (19)	§4.54	3.82		7.15	3.35	99	1
	H_A	H_B	H_C	J_{AB}	$J_{AB'}$		
$(CH_3)_3\overset{+}{N}(CH_2)_3O\overset{O}{\overset{\|}{C}}CH_3$	§4.16	2.13	3.55	6.0	6.0	67	33
				J_{BC}	$J_{BC'}$		
				4.65	11.85	10	90

* In D_2O (14).
† In $(CH_3)_2SO$-d_6.
‡ In $CDCl_3$.
§ In CD_3OD.

In the case of acetylhomocholine

$$CH_3\overset{O}{\overset{\|}{C}}O—CH_2—CH_2—CH_2—\overset{+}{N}(CH_3)_3$$

it is necessary to distinguish between rotamer distribution in the —O—C_A—C_B—C— and rotamer distribution in the —C—C_B—C_C—$\overset{+}{N}$-groupings.

DISCUSSION

The results are summarized in Tables 1–3. Several generalizations can be made:

(*1*) The conformation of AcCh and of related esters is not induced by structured solvent, since the quasi-cyclic structures of such molecules predominate not only in D_2O but also in solvents as nonpolar as chloroform. The lack of a compact structure in the three-carbon analog, acetylhomocholine, also speaks against solvent-induced hydrophobic interactions.

(*2*) It appears that the *gauche* conformation of the –OCCN- grouping is favored by interactions between the acyloxy oxygen of the ester group and the positively charged group in the β-position. This interaction is seen whether the latter group is a trimethylammonium, a dimethylammonium, or a dimethylsulfonium group. In amides related to local

TABLE 2. *Conformation of 2-dimethylaminoethyl benzoate and related esters*

Compounds*	H_A	H_B	J_{AB}	$J_{AB'}$	% Conformation Gauche	% Conformation Trans
$(CH_3)_2\overset{+}{N}HCH_2CH_2O\overset{O}{\overset{\|}{C}}C_6H_5$	4.66	3.64	7.08	3.10	100	0
$(CH_3)_2NCH_2CH_2O\overset{O}{\overset{\|}{C}}C_6H_5$	4.40	2.73	6.20	6.20	67	33
$(CH_3)_2\overset{+}{N}HCH_2CH_2O\overset{S}{\overset{\|}{C}}C_6H_5$	5.04	3.79	7.00	2.90	96	4
$(CH_3)_2\overset{+}{N}HCH_2CH_2S\overset{S}{\overset{\|}{C}}C_6H_5$	3.83	3.43	5.63	9.97	30	70
$(CH_3)_2NCH_2CH_2S\overset{S}{\overset{\|}{C}}C_6H_5$	3.28	2.99	6.25	9.25	41	59
$(CH_3)_2\overset{+}{N}HCH_2CH_2S\overset{Se}{\overset{\|}{C}}C_6H_5$	3.70	3.47	5.30	11.30	19	81

For the following compounds spectral analysis proved impossible due to insufficient difference in chemical shift between H_A and H_B:

$(CH_3)_2\overset{+}{N}HCH_2CH_2S\overset{O}{\overset{\|}{C}}C_6H_5$, $(CH_3)_2\overset{+}{N}HCH_2CH_2Se\overset{O}{\overset{\|}{C}}C_6H_5$, and $(CH_3)_2\overset{+}{N}HCH_2CH_2Se\overset{S}{\overset{\|}{C}}C_6H_5$

* See ref. 6 and footnote § in Table 1.

TABLE 3. *Conformation of 2-diethylaminoethyl benzamide and related compounds*

Compounds*	H_A	H_B	J_{AB}	$J_{AB'}$	% Conformation Gauche	% Conformation Trans
$(C_2H_5)_2NCH_2CH_2NHC(=O)C_6H_5$	3.52	2.74	6.00	8.40	45	55
$(C_2H_5)_2\overset{+}{N}HCH_2CH_2NHC(=O)C_6H_5$	3.75	3.40	6.60	5.70	74	26
$(C_2H_5)_2NCH_2CH_2NHC(=S)C_6H_5$	3.94	2.88	5.95	8.45	45	55
$(C_2H_5)_2\overset{+}{N}HCH_2CH_2NHC(=S)C_6H_5$	4.19	3.51	5.85	7.25	58	42
$(C_2H_5)_2NCH_2CH_2NHC(=Se)C_6H_5$	4.11	3.07	6.05	8.15	48	52
$(C_2H_5)_2\overset{+}{N}HCH_2CH_2NHC(=Se)C_6H_5$	4.27	3.57	6.35	7.85	51	49
$(CH_3)_2\overset{+}{N}HCH_2CH_2NHC(=S)C_6H_5$	4.21	3.53	6.50	6.00	71	29

* See ref. 7 and footnote § in Table 1.

anesthetics, the *gauche* conformation is favored to a lesser degree than in analogous esters.

(3) In the case of 2-dimethylaminoethyl benzoate, studied at pH values at which its amino group is not protonated, no specific rotamer is favored. The same is true in 2-dimethylaminoethyl acetates (20).

(4) If the acyloxy oxygen of esters is replaced by sulfur or by selenium, the *trans* conformation is favored in 2-trimethylammoniumethyl, in 2-dimethylammoniumethyl, and in 2-dimethylaminoethyl compounds.

(5) The carbonyl oxygens of AcCh and of related esters play a relatively minor role in stabilization of the *gauche* conformation of the –OCCN-grouping. Acetylthionocholine (12) or thiocarbonyl analogs of local anesthetics retained their quasi-cyclic conformation, quite different from the conformation of their thiolester isomers.

(6) In amide analogs of local anesthetics, however, replacement of the carbonyl oxygen by sulfur and by selenium does affect conformation, with more *trans* conformer being present on passing from amide to thiocarboxamide and selenocarboxamide. It seems likely that this observation can be attributed to the increasing importance of the resonance form $-\overset{+}{N}H=\overset{B-}{\overset{|}{C}}-$ (B=O, S, Se) on descending the periodic table (21). Such resonance would counteract any stabilization of the *gauche* conformation for the –NCCN-grouping.

(7) 2-Dimethylaminoethyl benzoates and their thiolester and selenolester analogs show the same conformer predominating in solution as their acetate analogs. In other words, AcCh analogs and protonated local anesthetics are conformationally similar.

(8) Replacement of methyl with ethyl groups on the cationic end affects conformation (Table 3).

(9) In all cases studied here, the conformation predominating in solution is that favored in the crystal.

Several attempts have been made to relate the conformations of molecules triggering or blocking conduction of the nerve impulse with the conformations of the postulated active sites of the biopolymers to which they are attached in exerting their functions. Our data and the very recent data of Partington *et al.* (22) do not show simple relationships between the conformation of molecules affecting electrically excitable membranes (11) and their potencies, although conformations as determined either in the crystal or in polar or nonpolar solvents may differ from conformations induced by attachment to biopolymers. However, even in rigid molecules in which conformational flexibility is minimized, relationships between conformation and ability to affect excitable membranes have proved to be very elusive (23). In view of the observation that the triggering and blocking activities of thiolesters and selenolesters differ widely (11), even though these molecules are essentially isosteric both in the crystal and in solution, one must assume that factors such as electron distribution, polarizability, or ability to interact with lipophilic residues are at least as important as conformation.

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