*ACETYLCHOLINE AND ITS THIOLESTER AND SELENOLESTER ANALOGS: CONFORMATION, ELECTRON DISTRIBUTION, AND BIOLOGICAL ACTIVITY**

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Communicated by David Nachmansohn, May 16, 1969

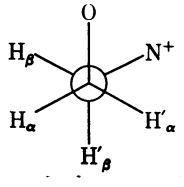
Abstract.—The crystal structures of acetylthiolcholine bromide and propionylthiolcholine iodide were elucidated. Acetylthiolcholine was found to be essentially isosteric with acetylselenolcholine, while the conformation of these compounds was quite different from that of acetylcholine.

The importance of conformation and electron distribution in the biological actions of choline esters is discussed.

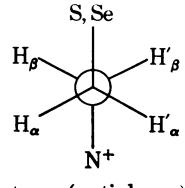
It has been proposed[1, 2] that the crucial role played by acetylcholine (AcCh) in the conduction of the nerve impulse resides in the ability of this molecule to trigger conformational changes in the receptor polymer to which it is attached, thus altering membrane permeability during electrical activity. In view of the biological importance of AcCh and the simplicity of its structure, numerous analogs of this molecule have been prepared and studied.

Even slight structural modifications may lead to changes in conformation and electron distribution throughout a molecule. This has the result that it becomes difficult to tell whether changes in biological activity induced by altering a molecule are due to changes in conformation or to an alteration of electron distribution. We have attempted to separate these factors by comparing the structures and the biological activities of isologous oxygen, sulfur, and selenium compounds. While the atomic radii of oxygen (0.66 Å) and sulfur (1.04 Å) are quite different,[3] the radii of sulfur and selenium (1.17 Å) are very similar,[3] with the result that, while structures of crystals of oxygen and sulfur analogs tend to differ, the crystal structures of sulfur and selenium isologs are so similar[4, 5] as to make such molecules isosteric.

Studies of the structure of AcCh and several related molecules in the solid state[6] showed that the N^+—C—C—O— grouping is usually in the *gauche* conformation although exceptions exist.[6a] In AcCh the *gauche* conformation also prevails in solution (D_2O).[7] In contrast, in acetylselenolcholine crystals[8] (AcSeCh), the nitrogen and selenium are *trans* to one another.[9]



gauche (synclinal)
sc



trans (antiplanar)
ap

In a variety of pharmacological preparations the depolarizing action of AcCh was modified greatly when the acyloxy oxygen of this molecule was successively replaced by sulfur and by selenium.[10-12] These compounds are hydrolyzed by acetylcholinesterase (AcCh-esterase) at similar rates.[13] Since ester, thiolester, and selenolester form the identical acyl-enzyme, this is not surprising. What is surprising is that the rate-determining step in the hydrolysis of AcSeCh is acylation,[13] while the rate-determining step in the hydrolysis of AcCh and acetylthiolcholine (AcSCh) is deacylation.[14, 15]

In view of the considerable differences in the depolarizing activities of AcCh and its S and Se analogs, it seemed of interest to establish whether these could be attributed to differences in conformation or to differences in electron distribution. For this purpose, crystallographic studies of AcSCh bromide and propionylthiolcholine iodide were carried out and the results compared with data derived from the study of AcCh and AcSeCh.

Results.—For the X-ray diffraction measurements lath-shaped crystals of both AcSCh bromide and propionylthiolcholine iodide were utilized. The following data were obtained:

Propionylthiolcholine iodide		*Acetylthiolcholine bromide*
13.886(3) Å	a	12.867(2) Å
11.911(4) Å	b	11.302(2) Å
7.791(1) Å	c	7.629(2) Å
90°	$\alpha = \beta = \gamma$	90°
1.62 gm/cm³	Density measured	1.44 gm/cm³
1.56 gm/cm³	Density calculated	1.42 gm/cm³
$Pna2_1$	Space group	$Pna2_1$

The intensities of 887 independent reflections for the propionyl derivative and 1008 reflections for AcSCh (representing *two theta* ranges of 0 to 110° and 0 to 130°, respectively) were measured by the stationary counterstationary crystal method, using Cu radiation with balanced Ni-Co filters. The high-absorption coefficient of the two compounds (μ = 69 cm^{-1} for the acetyl and μ = 211 cm^{-1} for the propionyl derivatives, coupled with the size of the crystals used (*ca.* 0.4 × 0.1 × 0.1 mm in both cases) necessitated a correction for this effect. An approximate correction was afforded by the adjustment of the measured intensities for the anisotropy of transmission of the X rays about the Phi axis of the diffractometer, as determined for a few 00l reflections at Chi = 90°. All the measurements were made on a General Electric XRD-6 diffractometer equipped with a single-crystal orientor. The intensities were converted to structure factor amplitudes ($|F_0|$) by applying absorption, Lorentz polarization corrections, and a factor to correct for the α_1-α_2 splitting.

The structures were solved by the heavy atom method. The positional and thermal parameters for the nonhydrogen atoms (no attempt was made to find hydrogens) were refined by least squares using a block diagonal approximation. The real and imaginary parts of the anomalous scattering factors for iodine, bromine, and sulfur were included in all calculations. The final R values ($\Sigma||F_0| - |F_c||/\Sigma|F_0|$) for the observed data at the conclusion of refinements were 0.102 for the propionylthiolcholine and 0.105 for the AcSCh salt. A list of the

observed and calculated structure factors and the positional parameters for the two compounds has been deposited in the Health Sciences Library of the State University of New York at Buffalo, N.Y., and will be sent on request.

The scattering factor curves utilized were taken from the International Tables for X-ray Crystallography,[16] with the exception of those for iodine and bromine.[17]

A general view of each molecule is illustrated in Figure 1, with the atomic labeling that is referred to throughout the text. A general discussion of the bond lengths and angles in these structures is felt to be inappropriate, since the errors in these parameters are estimated to be about 0.08 Å and 4° on the average. In general, the average C—N (1.49 Å), C—C (1.53 Å), and the C—O (1.19 Å) bonds of the two molecules are statistically similar to accepted values for these bonds.[18] The C5—S bond (av. 1.97 ± 0.10 Å) is longer than the S—C6 bond (av. 1.77 ± 0.02 Å) in both structures, and the average angle subtended by these bonds is 100 ± 3°.

The packing arrangement of AcSCh is very similar to that found in AcSeCh.

Discussion.—The structures of AcCh and related compounds have been under investigation for several years. It is now known[6] that the —$\overset{+}{N}$—C—C—O grouping in AcCh assumes the *gauche* (sc)[19] conformation, with the result that the ether oxygen of the ester group is rather close (3.29 Å) to the quaternary nitrogen and one of the methyl groups attached to it.

The *gauche* conformation has also been observed in choline,[20] muscarine,[21] glycerylphosphorylcholine,[22] and lactoylcholine[23] and a series of related compounds.

While the theory of "induced fit" has been applied widely to possible conformational alterations of biopolymers induced by the attachment of small molecules to receptor sites, in the case of flexible molecules (such as AcCh) the possibility that the conformation of the small molecule is altered by attachment to a receptor should also be considered. The question of rotational barriers in flexible molecules is therefore important. Calculations[24] of the total van der Waals energy of AcCh as a function of the various single-bond torsion angles indicated that the possible conformations with the acyloxy oxygen *gauche* or *trans* with re-

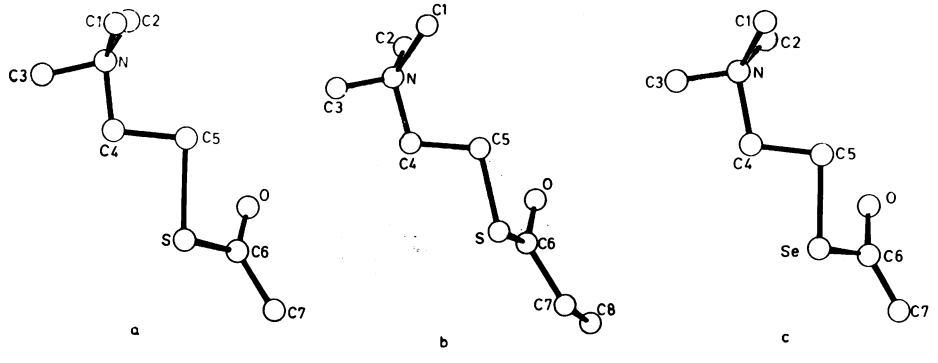


FIG. 1.—The acetylthiolcholine (*a*), propionylthiolcholine (*b*), and acetylselenolcholine (*c*) molecules as observed in the crystalline state.

spect to the quaternary nitrogen are approximately equally stable. Extended Hückel molecular orbital calculations on AcCh[25] indicated the *gauche* conformations to be more stable than the *trans* conformation, but again predicted the barriers for rotating the trimethylaminomethyl group about the methylene-methylene bond to be slight.

Thus, one is left with the question why the *gauche* conformation of the $\overset{+}{N}$—C—C—O grouping of AcCh and some related compounds is maintained both in the crystal and in solution. The conformation of part of these molecules is quite flexible; thus, in various AcCh analogs, the torsion angle of the C_6—O_1—C_5—C_4— grouping may vary from 75° to 180° depending upon the compound investigated.[21, 23]

The relative stability of the $\overset{+}{N}$—C—C—O— groupings', *gauche* conformation in AcCh has been ascribed to an electrostatic interaction between the ether oxygen and the quaternary nitrogen atom and to a hydrogen bond involving the acyloxy oxygen and a methyl proton of the cationic group.[6, 23, 26] The importance of the latter interaction may be slight, since the *gauche* conformation is also seen in ethanolamine phosphate[27] and in serine phosphate[28] even though the nitrogens in these molecules do not carry methyl groups and the H. . . . O distances exceed 2.90 Å. A dramatic alteration in the conformation of AcCh is brought about when its ether oxygen is replaced by sulfur or by selenium. AcSCh, propionylthiolcholine, and AcSeCh[8] exhibit the *trans* conformation, respectively, for the —$\overset{+}{N}$—C—C—S— and —$\overset{+}{N}$—C—C—Se— groupings.

The C_6—S—C_5—C_4 grouping in the thiolesters and the corresponding group in the selenolester exhibit torsion angles ranging from 108 to 129° in contrast to the 60° torsion angle seen in AcCh.

That the *trans* conformations of the —S—C—C—$\overset{+}{N}$— group in AcSCh and of the corresponding grouping in AcSeCh are rather stable was shown by means of nuclear magnetic resonance measurements, indicating that both these compounds retain their *trans* conformation in D_2O solution.[29]

As can be seen in Figure 1 and Table 1, the conformations of AcSCh and of AcSeCh are almost identical. In view of this, it is reasonable to assume that these compounds will have the same ability to fit receptor sites. In spite of this steric similarity, the biological effects induced by AcSCh and AcSeCh are, however, quite different, as can be seen in Table 2.

TABLE 1. *Conformation angles.*

Atomic grouping	Torsion Angles*† Propionylthiolcholine	Acetylthiolcholine	Acetylselenolcholine	Acetylcholine
N—C4—C5—O(S, Se)	176° (ap)	171° (ap)	175° (ap)	77° (sc)
C4—C5—O(S, Se)—C6	108 (ac)	129 (ac)	124 (ac)	79 (ac)
C5—O(S, Se)—C6=O	9 (sp)	16 (sp)	19 (sp)	0 (sp)
C5—O(S, Se)—C6—C7	161 (ap)	150 (ap)	155 (ap)	180 (ap)
S—C6—C7—C8	177 (ap)			

* Angles with values of 0°, 60°, 120°, and 180° are denoted as synplanar (sp), synclinal (sc), anticlinal (ac), and antiplanar (ap), respectively, in the terminology of Klyne and Prelog.[19]

† Only the magnitudes of the angles are given, as both the + and − conformations are present in the crystals.

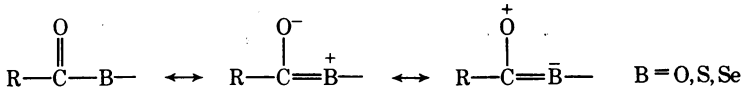
TABLE 2. *Comparison of the relative abilities of AcCh, AcSCh, and AcSeCh to induce depolarization in the isolated single cell electroplax preparation* and of their abilities to be hydrolyzed by electric eel acetylcholinesterase.†*

Compound	Electroplax‡	K_m	Acetylcholinesterase rate-determining step
AcCh	3.10^{-6}	10.10^{-5}	Deacylation
AcSCh	5.10^{-5}	6.10^{-5}	"
AcSeCh	1.10^{-2} (only to 65 mv)	3.10^{-5}	Acylation

* See refs. 11 and 30.
† See ref. 13.
‡ Av. molar conc. to depolarize to 45 mv in presence of eserine.

The differences in the abilities of AcSCh and AcSeCh to induce depolarization and the differences in the abilities of these analogues to be hydrolyzed by AcCh-esterase cannot be due to steric differences, but may be ascribed to differences in electron distribution brought about as the ether oxygen of AcCh is replaced successively by sulfur and by selenium.

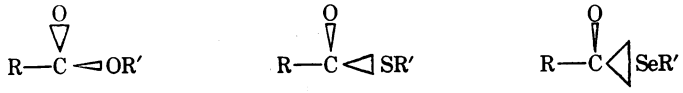
In thiolesters, as in esters, resonance interaction involving the heteroatom in the chain and the carbonyl group can take place; however, the question of which resonance form predominates has been the subject of some controversy:



Presumably, in thiolesters the sulfur atom can undergo octet expansion utilizing *d*-orbitals at the expense of the electrons of the carbonyl oxygen.[31] This proposal is based on spectroscopic and other evidence.[32, 33] Similarly, involvement of *d*-orbitals has been invoked to account for the optical rotatory behavior of selenolesters.[34] A recent comparison of the dipole moments of lactones, thiol-lactones, and selenollactones indicates that in the thio- and seleno- compounds a partial negative charge resides on the sulfur and selenium of the side chain, with a decrease in the carbonyl basicity in passing from the lactone to the thiol- to the selenollactone.[35]

The crystallographic data indicate that the length of the —S—C(=O)— bonds in AcSCh and propionylthiocholine are shorter than the lengths of the —S—CH_2— bonds in these compounds. Similar shortening was noted previously in thiol-phthalide,[36] in AcSeCh,[8] in AcCh,[6] and in other AcCh analogs,[37, 38] and presents further evidence for resonance interaction between the carbonyl group and the vicinal heteroatom.

In view of what is known about the properties of esters, thiolesters, and selenolesters, it seems reasonable to assume that the electron distribution in such compounds may be schematized as follows:



It seems that in the receptor interactions of AcCh and its S and Se analogs, electron distribution plays at least as important a role as conformation. AcSCh

and AcSeCh are isosteric both in the crystal and in solution, yet their biological effects are different in a wide range of preparations.

The fact that the —$\overset{+}{N}$—C—C—O— grouping in AcCh retains the *gauche* conformation in both the liquid and the crystal, while the corresponding —$\overset{+}{N}$—C—C—B— (B = S, Se) grouping retains the *trans* conformation in either environment suggests that the barriers for rotation around the methylene-methylene bond in these compounds may be greater than the low values predicted by calculations.[24, 25]

These results show the importance of the interaction between the ether oxygen and the quaternary group in maintaining the conformation of AcCh. The lack of participation of the carbonyl group in such interactions is emphasized by the observation that acetylthionocholine (the AcCh analog in which the carbonyl oxygen was replaced by sulfur) has a similar conformation to AcCh,[37] very different from that of its positional isomer, acetylthiolcholine.

A useful classification, introduced by Pearson,[38] is that of "hard" and "soft" acids and bases. A soft base is one in which the valence electrons are easily distorted, while in a hard base the valence electrons are held tightly. A hard acid is one of small size, high positive charge, and no readily polarized electrons, while, conversely, a soft acid is one in which the acceptor atom is large and carries electrons which are readily distorted. On the basis of these definitions, Pearson proposed that hard acids prefer to coordinate with hard bases while soft acids prefer to coordinate with soft bases. Sulfur and selenium-containing bases have low ability to accept protons or hydrogen bonds,[39] but high ability to chelate soft metals[40] or to be dissolved in soft solvents, while oxygen-containing bases, hard bases, have the opposite properties.

It seems reasonable to consider the interaction of the cationic quaternary group and the ether oxygen in AcCh and in related compounds in terms of the favored interaction of a hard acid with a hard base. Small size, concentrated electronic charge, and the lack of the possibility of octet expansion favor ionic binding; either electrostatic interactions or hydrogen bonding are thus more likely to involve "hard" oxygen atoms than they are to involve "soft" sulfur and selenium atoms. Since in AcSCh and AcSeCh the interactions favoring the proximity of the sulfur or selenium atoms to the nitrogen atom are relatively weak, while steric hindrance to such interactions appears to be even greater than it is in AcCh, these molecules assume the less-crowded *trans* conformation.

The increase in blocking activity seen when the acyloxy-oxygen of local anesthetics is replaced with sulfur or selenium agrees with the postulated importance of lipophilic interactions in the biological actions of such compounds.[41–43]

It was proposed[44] that the ability of the oxygen of muscarine and of related compounds to form hydrogen bonds may be essential for the biological actions of such compounds. Similarly, hydrogen-bond formation between the ether oxygen of AcCh and the active site of acetylcholinesterase has been postulated.[45] While such interactions are compatible with the decrease in depolarizing activity seen in passing from AcCh to AcSCh to AcSeCh, they are not compatible with the depolarizing activities of the hydrolysis products of these esters nor with the

observation that AcSCh and AcSeCh are bound more tightly to AcCh-esterase than is AcCh.

While choline is completely devoid of depolarizing activity, its analog cholinethiol, which is less likely to form hydrogen bonds than choline, is an even more powerful depolarizing agent than its parent acetyl ester. In the series methoxycholine, methylthiocholine, methylselenocholine, depolarizing activity seems to be inversely proportional to the abilities of the side-chain heteroatoms to form hydrogen bonds.

Since attachment of AcCh analogs presumably brings about conformational changes in their receptor biopolymers, the interaction is between flexible small molecules about the rotational barriers of which little is known and flexible macromolecules about the conformational barriers of which even less is known. However, some conclusions may be drawn. The observation that choline (*gauche*) is totally inactive, while AcCh (*gauche*) is an immensely powerful depolarizing agent, coupled with the observation that acetylthiolcholine (*trans*) is a much more powerful depolarizing agent than its *isosteric* (*trans*) selenolester analog, emphasizes that electron distribution and polarizability play at least as important a part as conformation in determining the "affinity" and the "efficacy" of activators of AcCh receptors.

The low biological activity of some compounds maintaining the *gauche* conformation in the $-\overset{+}{N}-C-C-B-$ grouping, coupled with the relatively high biological activity of related compounds retaining the *trans* conformation, weakens the suggestion that "the characteristic *gauche* conformation is probably associated with the biological activity of nerve amines."[46]

Finally, it should be noted that isosteric, nonisoelectronic analogs of biologically active compounds provide a powerful tool for probing the receptors to which these molecules are attached.[47] Thus, the analogs discussed above have proved to be useful for approaching the problem of the identity or nonidentity of acetylcholine receptors of depolarizing membranes and of acetylcholinesterase, as well as the problem of the identity or nonidentity of axonal and synaptic acetylcholine receptors.[48]

We wish to thank Dr. Anwar Hussain for the sample of propionylthiolcholine iodide, Mrs. Phyllis Sackman for her technical assistance, and the Computing Center of the State University of New York at Buffalo for the use of their facilities. The hospitality and encouragement of Professor David Nachmansohn are gratefully acknowledged, as is the very helpful criticism of this manuscript on the part of Professor Barbara Low and her colleagues.

* This work was supported, in part, by grants from the National Institute of Cancer (CA-10104) (E. S.) and by grants from the National Science Foundation (GB-6835) (H. G. M.) and the National Institute of Neurological Diseases (NB-07835) (H. G. M.).

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