# Sulfur and Selenium Compounds Related to Acetylcholine and Choline. X. Acetylthionocholine[1]

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*Received October 15, 1969*

Acetylthionocholine, the thiocarbonyl analog of acetylcholine, was synthesized. The conformation proved to be very similar to that of acetylcholine and quite different from that of acetylthiolcholine. The depolarizing activity and the enzymic hydrolysis of acetylthionocholine were investigated.

It has been proposed that acetylcholine (AcCh) plays a key role in the transmission of the nerve impulse, presumably by triggering a conformational change in a receptor–polymer thus leading to an alteration of membrane permeability to cations.[2] In view of the importance of AcCh and the simplicity of its structure, very numerous analogs of this ester have been prepared and studied.[3] However, only very recently has it been recognized that minor alterations of the structure of AcCh may alter the overall conformation of this molecule. Studies of the structure of AcCh and several related esters showed that the $-\overset{+}{N}CCO-$ grouping is in the *gauche* conformation either in the crystal[4] or in solution.[5] On the other hand, replacement of the acyloxy oxygen of acetylcholine with Se or S leads to the *trans* conformation for the $-\overset{+}{N}CCB$ (B = S, Se) grouping in acetylthiolcholine (AcSCh) and acetylselenolcholine (AcSeCh) in either environment.[6,7,8]

In a variety of pharmacological preparations the depolarizing action of AcCh was modified greatly by replacement of the chain oxygen with S or Se.[9,10,11] Since the biological actions of AcSCh and AcSeCh were quite different, while these molecules were essentially isosteric, it seemed apparent that electronic factors were at least as important as steric factors for the abilities of these compounds to exert their effects.[12]

On the basis of spectroscopic studies,[13] and on the basis of the measurement of the dipole mements of a series of isologous lactones, thiol lactones, and selenol lactones[14] it was postulated that in thiol esters and selenol esters, utilization of d orbitals by S and Se resulted in a decrease in carbonyl basicity. It was proposed that the *gauche* conformation of the $\overset{+}{N}CCO-$ group in AcCh was maintained by a "hard acid–hard base" interaction between the quaternary N and the ether O of the ester. S and Se being "soft" bases,[15] such an interaction was not likely to be favored in AcSCh and AcSeCh, which remained in the extended *trans* conformation.

In view of these considerations, the synthesis and study of the AcCh analog where the carbonyl rather than the ether O had been replaced by S seemed of interest. Acetylthionocholine was prepared by the reaction of ethyl thionoacetate with 2-dimethylaminoethanol, followed by quaternization with MeBr. The corresponding reaction of ethyl thionoacetate with 2-dimethylaminoethanethiol yielded the dithio ester analog of AcCh. The thionoselenol ester was too unstable to be isolated. The benzoylthiono analog of the product had been synthesized previously,[16] as were its thiol ester and selenol ester analogs.

X-Ray diffraction measurements were carried out on crystals of acetylthionocholine.[17] The structure of this compound turned out to be extremely similar to that of AcCh and quite different from that of its isomer, AcSCh. The *sc* conformation of the $-\overset{+}{N}CCO$ grouping in AcCh was affected very little by substitution of the carbonyl O by S.

The finding that the conformations of AcCh and acetylthionocholine are very similar but different from that of AcSCh emphasizes the importance of the interaction of the acyloxy oxygen and the quaternary N in maintaining the conformation of these compounds. It also shows that the carbonyl group does not play a major role in the maintenance of the *gauche* conformation of AcCh.

The depolarizing activity of acetylthionocholine was determined and compared with that of AcCh and AcSCh in the electroplax preparation. In this system, single cells of the electric organ of the electric eel *Electrophorus electricus* are impaled with microelectrodes, permitting the determination of the depolarizing effects of various concentrations of the compounds being tested. To prevent enzymic hydrolysis of the analogs, determinations were carried out in the presence of eserine ($5 \times 10^{-5}$ *M*). The results obtained are shown in Table I.

It can be seen that replacement of the carbonyl O with S affects neither conformation nor depolarizing activity, while replacement of the acyloxy O with S alters both.

Studies of the hydrolysis of acetylthionocholine seemed of interest since this compound forms an acetylthiono–enzyme intermediate instead of the acetyl-

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TABLE I

| Compound | Average $M$ concentration required to depolarize to 45 mV | Equipotent $M$ ratio |
|---|---|---|
| Acetylcholine | $5 \times 10^{-6}$ | 1 |
| Acetylthiolcholine | $5 \times 10^{-5}$ | 10 |
| Acetylthionocholine | $3 \times 10^{-6}$ | 1.7 |

enzyme formed during the hydrolysis of either AcCh or AcSCh.

Electric eel acetylcholinesterase was used for these studies. The $K_m$ of the thiono ester was found to be $6 \times 10^{-4}$ $M$, compared with a $K_m$ of $1 \times 10^{-4}$ for AcCh and $0.6 \times 10^{-4}$ for AcSCh. The $V_{max}$ of the hydrolysis of acetylthionocholine was significantly lower than that of AcCh or AcSCh.

## Experimental Section

**Acetylthionocholine Bromide.**—Na (200 mg) was dissolved in 5.0 g of 2-dimethylaminoethanol, followed by the addition of a solution of 8.0 g of ethyl thionoacetate[18] in 100 ml of toluene. A slow stream of $N_2$ was passed into the mixture which was heated at 97–98° for 2 hr. The reaction mixture was concentrated *in vacuo* in a 50° bath. The residue was acidified with 5 ml of concentrated HCl in 70 ml of ice-cold $H_2O$ and the mixture was filtered. The filtrate was extracted with 200 ml of $Et_2O$ to remove unreacted ethyl thionoacetate. The aqueous layer was shaken with 35 ml of ice-cold saturated $Na_2CO_3$, followed by extraction with 40-ml portions of $Et_2O$. The organic extracts were washed with $H_2O$ and dried ($MgSO_4$). Addition of 2.0 ml of MeBr to the $Et_2O$ led, after refrigeration, to the formation of 2.4 g of light yellow crystals. The product was recrystallized from 2:1 $Me_2CO$–EtOH; mp 151–152°;[19] uv $\lambda_{max}$ (EtOH) 237 mμ ($\epsilon_m \times 8440$).

*Anal.* ($C_7H_{10}BrNOS$) C, 34.71; H, 6.65; S, 13.23. Found: C, 34.81; H, 6.68; S, 12.82.

**Acetylthionothiolcholine Bromide.**—This compound was obtained in 20% yield by the reaction of 6.0 g of 2-dimethylaminoethanethiol and 8.0 g of ethyl thionoacetate in toluene followed by quaternization with MeBr. After three recrystallizations from EtOH the product melted at 169°; uv (EtOH) $\lambda_{max}$ 298 mμ ($\epsilon_{max}$ 7830).

*Anal.* ($C_7H_{16}BrNS_2$) C, H, S.

**Depolarizing Activity.**—The depolarizing activity of acetylthionocholine was measured in the isolated single cell electroplax preparation, using cells from the electric organ of *E. electricus*.[20,21] Eserine was added to prevent hydrolysis by acetylcholinesterase present in the tissue.

**Hydrolysis by Acetylcholinesterase.**—Highly purified electric eel acetylcholinesterase was used for these studies. Enzyme assays were carried out titrimetrically, using a Radiometer autotitrator. A constant pH of 7.5 ± 0.02 was maintained during enzymatic hydrolysis by the automatic addition of 0.01 $N$ NaOH to neutralize the acetic acid produced by substrate hydrolysis. Initial rates were used, the rate being constant for at least 2 min.

**Acknowledgments.**—We are indebted to Mrs. Eva Bartels of Columbia University for the electroplax assays and to Professor David Nachmansohn of Columbia University for a gift of the acetylcholinesterase used in these studies. This work was supported by grants from the National Science Foundation (GB-6835) and the National Institute for Neurological Diseases (NB-07835).

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# *In Vitro* Inhibition of Cholesterolgenesis by Various Thyroid Hormone Analogs

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Nineteen thyroid hormone analogs were tested in an *in vitro* cholesterolgenic liver homogenate system obtained from rats. $^{14}$C-Acetate was used as substrate, DMSO was used as a solvent for adding the thyroid hormone analogs to the system. Of those compounds tested, L-triiodothyronine, D-thyroxine, DL-triiodothyronine, and DL-3,5-diiodo-3′-phenylthyronine at $1.0 \times 10^{-4}$ $M$ inhibited cholesterolgenesis from $^{14}$C-acetate substrate. No effect was elicited by $T_3$ or $T_4$ when mevalonate was substrate. These studies indicated that the 3′-I or a bulky 3′-Ph associated with either D- or L-thyronine is necessary for inhibition of *in vitro* cholesterolgenesis. The D isomer is more active than the L isomer in this system. The need for higher than physiological levels of the thyroid hormones (triiodothyronine or thyroxine) in this system is in part a result of the nonspecific binding of the hormones to inert proteins in the homogenate system. When enough hormone is present to saturate the binding sites on the inert proteins the remaining hormone binds to active proteins contained in the microsomes resulting in an inhibition of cholesterolgenesis.

The alteration of blood cholesterol concentrations associated with thyroid function is well known. The hypocholesterolemia associated with thyroxine administration is attributed in part to an increased conversion of cholesterol into bile acids which overrides the increased cholesterolgenesis caused by thyroxine.[1] In thyroidectomized animals a decrease in the level of β-hydroxy-β-methylglutaryl-coenzyme A reductase (HMG-reductase) occurs whereas the administration of thyroxine increases the levels of HMG-reductase resulting in increased cholesterolgenesis.[2,3] In addition to the studies above, a new parameter was recently reported in which the addition of L-triiodothyronine ($T_3$) and L-thyroxine ($T_4$) to an *in vitro* cholesterolgenic rat liver homogenate from euthyroid rats resulted in decreased cholesterolgenesis.[4] The studies presented here are an extension of those studies and indicate that a structural specificity similar to that of $T_3$ and $T_4$ is

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