# Sulfur and Selenium Compounds Related to Acetylcholine and Choline. VII. Isologs of Benzoylthionocholine and of 2-Dimethylaminoethyl Thionobenzoate[1,2]

SHIH-HSI CHU AND HENRY G. MAUTNER

*Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut*

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In a continuing investigation of compounds isosteric, but not isoelectronic, with choline esters implicated in nerve conduction, benzoylthionocholine, benzoylthionothiolcholine, benzoylthionoselenolcholine, and their dimethylamino analogs were synthesized. The activities of these and related compounds in the giant axon of the squid and in the isolated single cell electroplax preparation were determined.

It has been suggested that acetylcholine plays its crucial role in the conduction of the nerve impulse by inducing a conformational change in its receptor polymer, thus altering membrane permeability to cations.[3,4] In an attempt to obtain information about the active sites of the depolarizing receptor, a systematic study of the depolarizing and antidepolarizing activities of sulfur and selenium isologs related to choline esters and choline has been initiated. Molecular size is altered relatively little when oxygen is replaced by sulfur and still less when sulfur is replaced by selenium. That sulfur and selenium isologs are essentially isosteric was made likely by the observation[5] that such compounds almost invariably have identical crystal structures, usually differing from those of their oxygen isologs. Sulfur and selenium isologs tend to form identical hydrates and have similar partition coefficients,[6] indicating that such compounds exhibit no major differences in solvation. X-Ray diffraction studies comparing crystalline acetylthiolcholine and acetylselenolcholine indicated the packing patterns and the conformations of these molecules to be strikingly similar.[7] While it had been shown previously by means of X-ray diffraction measurements that thio- and seleno-substituted compounds are essentially isosteric,[8,9] such similarity is even more striking in flexible molecules such as the aforementioned isologs of acetylcholine. When differences are noted in the biological activities of isosteric compounds, such differences cannot be due to differences in the abilities of such isologs to fit receptor sites, but rather due to differences in their abilities to bind to active sites or to induce configurational changes in receptor biopolymers. Since electron distribution is altered in esters as their side-chain oxygens are replaced by sulfur or selenium,[10] it may be assumed that differences in the biological activities of such isologs must be due to differences in their electron distribution.

It was noted that replacement of the side-chain oxygen of acetylcholine with sulfur and selenium[11] greatly modified the activity of the ester in the guinea pig ileum and frog rectus abdominis preparations[12] as well as in the phrenic nerve stimulated rat diaphragm.[13] These results were confirmed and extended by the use of the isolated single-cell electroplax preparation.[14,15] It was shown that introduction of sulfur and selenium into the side chain of acetylcholine progressively lowered depolarizing activity, while replacement of the oxygen of choline by sulfur or selenium resulted in a striking increase in depolarizing activity. Cholinethiol was found to be more active in the mercaptan than in the mercaptide form, while methylation of choline, cholinethiol, and cholineselenol increased the depolarizing action of these compounds.[14]

In view of the considerable changes in biological activity observed when the side-chain oxygen of cholinergic esters was replaced by sulfur and by selenium, it seemed of interest to investigate the replacement of the carbonyl oxygen by sulfur and selenium as well.

Benzoylcholine is closely related to 2-dialkylaminoethyl benzoates, the latter group of compounds being of great importance as clinically useful local anesthetics. It has been claimed that in para-substituted 2-dialkylaminoethyl benzoates the ability to block the nerve impulse could be related to the bond order of the carbonyl group.[16] Benzoylcholine itself is on the borderline of being a depolarizing agent and being an inhibitor of depolarization; in the isolated single-cell electroplax preparation it exerts a mixed effect.[17] Recently, 2-dimethylaminoethyl benzoate, the tertiary amine analog of benzoylcholine, as well as its thiolester and selenolester isologs were tested for their actions in blocking the action potential of squid giant axons[18] and for their receptor-inhibitory actions in the electroplax preparation.[19] As inhibitors of electrical activity, potency increased progressively as the side-chain oxygen was replaced by sulfur and by selenium.

For the above reasons we undertook the synthesis of the thiocarbonyl isologs of benzoylcholine, 2-dimethyl-

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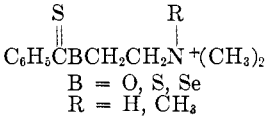
aminoethyl benzoate, and their thiolester and selenolester analogs.



B = O, S, Se
R = H, $CH_3$

2-Dimethylaminoethyl thionobenzoate was synthesized by the addition of 2-dimethylaminoethanol to thionobenzoyl chloride. A convenient method of preparing the latter compound was reported recently by Hedgley and Fletcher.[20] 2-Dimethylaminoethyl thionothiolbenzoate was prepared by the addition of 2-dimethylaminoethylthiol to thionobenzoyl chloride. Addition of 2-dimethylaminoethylselenol, freshly prepared by the sodium borohydride reduction of the corresponding diselenide, to thionobenzoyl chloride yielded the thionoselenolbenzoate. In all cases, the quaternary ammonium compounds could be prepared from the tertiary amines by the addition of methyl bromide.

## Experimental Section

**2-Dimethylaminoethyl Thionobenzoate Hydrochloride.**—A solution of 2-dimethylaminoethanol (0.455 g, 0.005 mole) in $CH_2Cl_2$ (10 ml) was added dropwise to a solution of 0.78 g (0.005 mole) of thionobenzoyl chloride[20] in 10 ml of the same solvent. The mixture was stirred at room temperature for 10 min. The yellow solution was evaporated to dryness, and the residue taken up in 10 ml of ether. Chilling the mixture, followed by introduction of dry HCl, resulted in precipitation of 1.0 g (81%) of a yellow solid. The product was recrystallized three times from EtOH, yielding yellow crystals melting at 148–149°;[21] uv spectrum $\lambda_{max}^{EtOH}$ 289, 247 mμ ($\epsilon_{max}$ 13,390, 6140).

*Anal.* Calcd for $C_{11}H_{16}ClNOS$: C, 53.75; H, 6.56; S, 13.04. Found: C, 53.75; H, 6.46; S, 13.02.

**2-Dimethylaminoethyl Thionothiolbenzoate Hydrochloride.**—A solution of 0.53 g (0.005 mole) of 2-dimethylaminoethylthiol in $CH_2Cl_2$ (10 ml) was added to a solution of 0.78 g (0.005 mole) of thionobenzoyl chloride in the same solvent. The orange mixture was worked up in the same fashion as the oxygen analog described above. A 76% yield of orange solid (1.0 g) was obtained. Three recrystallizations from EtOH yielded orange crystals melting at 162–163°, uv spectrum $\lambda_{max}^{EtOH}$ 303 mμ ($\epsilon_{max}$ 15,910).

*Anal.* Calcd for $C_{11}H_{16}ClNS_2$: C, 50.54; H, 6.16; S, 24.49. Found: C, 50.42; H, 6.16; S, 24.57.

**2-Dimethylaminoethyl Thionoselenolbenzoate Hydrochloride.**—A solution of 1.0 g of bis(2-dimethylaminoethyl) diselenide[11] was treated under $N_2$, with 0.2 g of $NaBH_4$ until the yellow color indicative of the diselenide grouping disappeared. $NaHCO_3$ (1.0 g) was added, followed by 0.78 g (0.005 mole) of thionobenzoyl chloride; the mixture was then stirred vigorously for 10 min. The product was extracted with several portions of $CH_2Cl_2$, the organic layers were combined, dried ($MgSO_4$), and evaporated to dryness. The residue was taken up in 10 ml of ether and worked up as the compounds described previously. The red product was obtained in a yield of 54%. On recrystallization from EtOH red needles were obtained; mp 166–167°; uv spectrum $\lambda_{max}^{EtOH}$ 350, 306 mμ ($\epsilon_{max}$ 4960, 13,270).

*Anal.* Calcd for $C_{11}H_{16}ClNSSe$: C, 42.79; H, 5.22; S, 10.38; Se, 25.57. Found: C, 42.80; H, 5.21; S, 10.21; Se, 25.42.

**Thionobenzoylcholine Hydrobromide.**—A solution of 1.0 g (0.0041 mole) of dimethylaminoethyl thionobenzoate hydrochloride in 10 ml of $H_2O$ was shaken with 20 ml of ice-cold saturated $Na_2SO_4$ solution. The mixture was extracted with two 20-ml portions of $CH_2Cl_2$. The organic extracts were washed (saturated $Na_2SO_4$) and dried ($MgSO_4$). Evaporation to dryness yielded a residue which was taken up in $Me_2CO$ (20 ml) mixed with MeBr (2.0 ml). A yellow precipitate weighing 0.8 g (67%) was obtained. The product was recrystallized three times from EtOH to yield yellow crystals melting at 181–182°; uv spectrum $\lambda_{max}^{EtOH}$ 289, 246 mμ ($\epsilon_{max}$ 13,623, 6698).

*Anal.* Calcd for $C_{12}H_{18}BrNOS$: C, 47.36; H, 5.96; S, 10.53; Br, 26.26. Found: C, 47.52; H, 6.16; S, 10.40; Br, 26.07.

**Thionobenzoylthiolcholine Hydrobromide.**—Quaternization with MeBr was carried out as described above. The orange product was obtained in 94% yield and recrystallized three times from EtOH. Orange crystals melting at 188–189° were obtained; uv spectrum $\lambda_{max}^{EtOH}$ 307 mμ ($\epsilon_{max}$ 15,718).

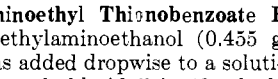
*Anal.* Calcd for $C_{12}H_{18}BrNS_2$: C, 44.98; H, 5.66; S, 20.00; Br, 24.94. Found: C, 45.06; H, 5.62; S, 19.96; Br, 24.75.

**Thionobenzoylselenolcholine Hydrobromide.**—Quaternization with MeBr, carried out as described above, yielded a purple precipitate (53%) which was recrystallized from EtOH. Purple plates melting at 193–194° were obtained; uv spectrum $\lambda_{max}^{EtOH}$ 347, 307 mμ ($\epsilon_{max}$ 5734, 15,340). Measurements were carried out using a Cary Model 15 spectrophotometer.

*Anal.* Calcd for $C_{12}H_{18}BrNSSe$: C, 39.24; H, 4.94; S, 8.73; Se, 21.52; Br, 21.76. Found: C, 39.49; H, 4.95; S, 8.96; Se, 21.40; Br, 21.72.

**Determination of Antidepolarizing Activity in the Electroplax Preparation.**—The isolated single cell electroplax preparation of the electric eel *Electrophorus electricus* was set up as described previously.[22,23] Results are summarized in Table I.

TABLE I
BLOCKING ACTIVITY IN THE ISOLATED SINGLE-CELL ELECTROPLAX PREPARATION



| R | A | B | % blockade[a] |
|---|---|---|---|
| $CH_3$ | O | O | 0 |
| $CH_3$ | O | S | 60 |
| $CH_3$ | O | Se | 100 |
| $CH_3$ | S | O | 60 |
| $CH_3$ | S | S | 90 |
| $CH_3$ | S | Se | 95[b] |
| H | O | O | 0 |
| H | O | S | 40 |
| H | O | Se | 100[b] |
| H | S | O | 55 |
| H | S | S | 60 |
| H | S | Se | 95[b] |

[a] Per cent blockade of the depolarizing action induced by $5 \times 10^{-5}$ *M* carbamylcholine. [b] Irreversible action.

## Discussion

For many years there has been considerable discussion whether acetylcholine is implicated in synaptic transmission of the nerve impulse, in axonal transmission of the nerve impulse, or in both. If, as postulated,[3,4] acetylcholine is the mediator in axonal transmission and if local anesthetics act by blocking the access of acetylcholine to axonal receptor sites, then one would expect that different analogs related to local anesthetics should exhibit similar relative blocking activities in axonal and in synaptic test preparations.

By comparing the blocking activities of the various sulfur and selenium isologs related to benzoylcholine and to 2-dimethylaminoethyl benzoate in the isolated single-cell electroplax (a synaptic preparation) with their blocking activities in the giant axon of the squid[18,24] (an axonal preparation), it can be seen that replacement of oxygen by sulfur and selenium modifies blocking action in parallel fashion in the two test systems. This observation is compatible with the postulate that similar acetylcholine-binding sites are involved in the receptor biopolymers of these preparations.

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