SYNTHESIS OF MEDICINAL SUBSTANCES WITH CONTROLLABLE LENGTH OF ACTION (IN THE CASE OF CURARE-LIKE COMPOUNDS)

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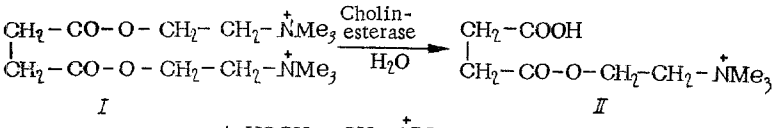
Control of the length of action of medicinal substances is an important pharmacological problem. From the chemical standpoint, its successful solution would be possible with the aid of inactivators which act selectively on the medicinal substances and convert them into inactive compounds. Such substances, introduced at the right moment, would stop the action of the medicinal agent.

If one searches for analogies with nature, it is possible to point out the inactivation of ditilin by cholinesterase [1]



I

II

+ $HOCH_2-CH_2-\overset{+}{N}Me_3$

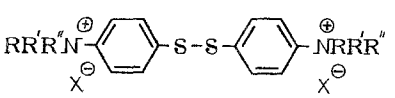
IIa

A characteristic feature of ditilin is the presence of two weak bonds (the ester bonds). Cholinesterase acts on them selectively. The decomposition products II and IIa are practically free of the curare-like activity of I. In this case, the inactivator is not introduced from without, but is present in the neuromuscular synapse. The result is shortness of action of ditilin.

We have synthesized a medicinal substance which contains a specific link in its molecule that may be broken up under the action of inactivators introduced from without. To do this, we took compounds from the myorelaxant class. As a specific link we selected the disulfide bond, which possesses suitable lability.

The structure of the myorelaxant was chosen on the basis of the following considerations: it should contain two quaternary ammonium groups; the spacing between the quaternary nitrogen atoms should be 14-15 Å; and there should be a S—S bond between the ammonium groups.

These conditions were satisfied by a diphenyl disulfide structure with two quaternary ammonium groups in the 4,4'-positions (compound III), which was taken as a model:



III

The disulfide bond in this compound possesses enhanced lability, since it connects two aromatic nuclei which contain electro-acceptor ammonium groups in the para position. It might be hoped that a suitable chemical reagent could break the S—S bond, which would lead to removal of the neuro-muscular blockade.

Starting from dimethylaniline, we prepared 4,4'-bis-(dimethylaminophenyl) disulfide and a number of its mono- and diquaternary derivatives, by the following scheme:

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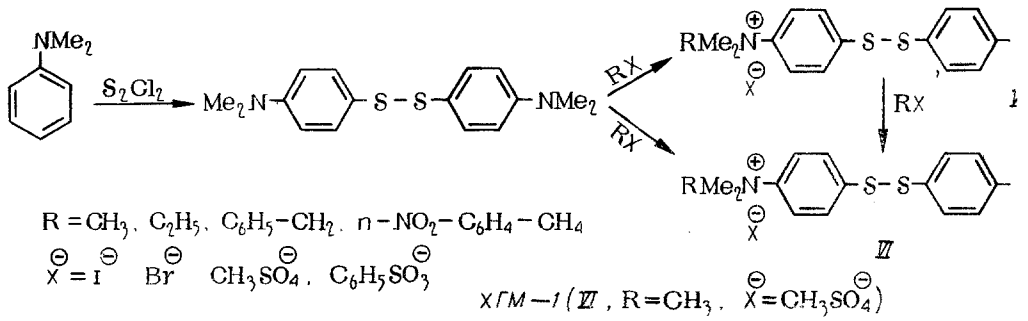


The ditertiary diamine (IV) was prepared by the method of Merz [2]. Alkylation of product IV leads to formation of both the mono- and also the diquaternary product (compounds V and VI), wherein the ratio of these depends on the character of the solvent, the reaction temperature, and the form of alkylating agent. Thus, with dimethyl sulfate or methyl iodide in benzene at 15-20°, IV forms practically pure V; from these same reagents, but in acetone at 15-20°, VI is formed; and in acetone at 55-60°, a mixture of V and VI in approximately equal amounts by weight is formed.

By selecting reaction conditions we also prepared mono- and diquaternary compounds of IV with benzyl or p-nitrobenzyl radicals. In connection with the low reactivity of ethyl iodide, the corresponding ethiodide from product IV was prepared by the action of ethyl benzenesulfonate on IV, with subsequent replacement of the benzenesulfonate anion by the iodide anion.

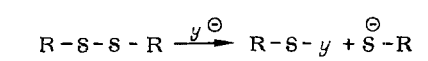
The results obtained are presented in Table 1.

The diquaternary derivatives can be prepared not only from the ditertiary amine but also from the corresponding monoquaternary compounds. For example, upon action of dimethyl sulfate on the monoquaternary product (V), $R = CH_3$, $X^- = CH_3SO_4^-$, the diquaternary derivative (VI) is formed ($R = CH_3$, $X^- = CH_3SO_4^-$). Thus, it is possible to prepare unsymmetrical diquaternary derivatives.

Pharmacological investigation of the preparations made showed that the original model was correctly chosen [3]. For example, the diquaternary product, VI, $R = CH_3$, $X^- = CH_3SO_4^-$ (KhGM-1) proved to be a typical curare-like compound of mixed type of action. It possesses characteristic cholinomimetic activity, which was studied in experiments on isolated straight frog stomach muscle. This compound causes a contraction amounting to 50% of the maximum possible, in a concentration of $2.9 \cdot 10^{-5}$ M. This same preparation causes blockade of neuro-muscular transfer in experiments on isolated rat phrenic diaphragm preparation in a concentration of $3 \cdot 10^{-5}$ M.

Further work consisted in the choice of such a chemical agent that, possessing sufficient selectivity, it could get, unchanged, to the myorelaxant molecule present in the neuro-muscular synapse, and, along with this, would possess enough activity to cleave the miorelaxant at the S—S bond.

As is known, the mechanism of heterolytic cleavage of the S—S bond is similar to the mechanism of nucleophilic substitution at a carbon atom, with this sole difference, that the so-called S-nucleophilic agent is not attacking a carbon atom, but a sulfur atom, adding to it and dislodging the appropriate mercaptide;



One of the active S-nucleophilic agents is the sulfite anion, SO_3^{-2}. Experimentation showed that the reaction of sodium sulfite with KhGM-1 actually leads to breakdown of the KhGM-1 at the point of the disulfide bond, with formation of the zwitterion (VII) and the mercaptan (VIII); the reaction takes place in water at room temperature:

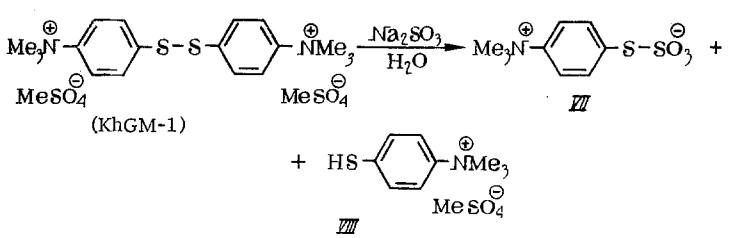


TABLE 1. Mono- and Diquaternary Derivatives (V and VI, Respectively) of 4,4'-Bis-(dimethylaminophenyl) Disulfide (IV), Prepared by Reaction of IV with Alkylating Agents RX: $[(CH_3)_2N-C_6H_4-S-S-C_6H_4-N(CH_3)_2]\ (RX)_n$; n = 1 (V) or 2 (VI)

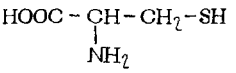
RX	Solvent	Product obtained	Found, in %					Empirical formula	Calcd, in %				
			C	H	N	I(Br)	S		C	H	N	I(Br)	S
CH_3I	Benzene	V	45,43 45,29	5,64 5,79	6,47 6,54	28,89 29,00	14,21 14,12	$C_{17}H_{23}IN_2S_2$	45,70	5,16	6,27	28,50	14,33
	Acetone	VI	36,62 36,68	4,80 4,91	4,75 4,84		10,68 10,61	$C_{18}H_{26}I_2N_2S_2$	36,85	4,42	5,76		10,87
$(CH_3)_2SO_4$	Benzene	V	50,04 50,48	6,12 6,38	6,43 6,62		22,24	$C_{18}H_{26}N_2O_4S_3$	50,20	6,05	6,51		22,30
	Acetone	VI	43,46 43,00	5,58 5,99	5,06 5,15		22,96 22,80	$C_{20}H_{32}N_2O_8S_4$	43,15	5,75	5,03		23,00
$C_6H_5CH_2I$	Benzene	V	53,08 53,33	5,42 5,42	5,28 5,30	24,72		$C_{23}H_{27}IN_2S_2$	52,90	5,17	5,36	24,35	
$C_6H_5CH_2Br$	Acetone	VI	48,36 48,42	4,68 4,89	3,66 3,57			$C_{30}H_{34}I_2N_2S_2$	48,60	4,60	3,78		
	Acetone	VI	55,79 55,81	5,62 5,99	4,07 4,00	(24,47) (24,35)		$C_{30}H_{34}Br_2N_2S_2$	55,70	5,26	4,33	(24,80)	
$p\text{-}NO_2C_6H_4CH_2Br$	Benzene	V			7,74 7,56	(15,23) (15,50)	12,13 12,47	$C_{23}H_{26}Br_2N_3O_2S_2$			8,08	(15,40)	12,30
$C_6H_5SO_3CH_3$	DMF	VI	48,80	4,45	7,20 7,63	(21,32) (21,49)	8,38 8,37	$C_{29}H_{32}Br_2N_4S_2O_4$	48,90	4,34	7,60	(21,75)	8,68
$C_6H_5SO_3C_2H_5$	Benzene	V	58,02 58,20	5,96 6 20	6,05 6,30		20,12 20,15	$C_{23}H_{28}N_2S_2O_3$	58,00	5,88	5,88		20,07
	Benzene	V*	47,13 47,26	5,50 5,72	6,08 6,09	27,81 27,88	14,03 14,25	$C_{18}H_{25}IN_2S_2$	47,00	5,43	6,09	27,60	13,90

*After reaction of IV with $C_6H_5SO_3C_2H_5$, the $C_6H_5SO_3^-$ ion was displaced by the I^- anion.

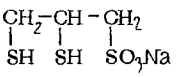
Tests were conducted further on a biological object. Experiments on isolated rat phrenic-diaphragm showed that sodium sulfite in equimolecular concentration removes a 100% neuromuscular block caused by compound KhGM-1, and transfer of nerve impulses is completely restored.

The reaction products of KhGM-1 with sodium sulfide (compounds VII and VIII) proved practically inactive at concentrations equal to that of the KhGM-1 on a molar basis.

Further, since the strongest S-nucleophilic agents are aliphatic mercaptides, some physiologically harmless compounds containing the mercapto group were tested, for example, cysteine (IX) and also unithiol (X):



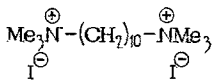
IX



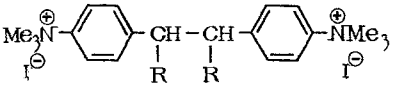
X

Both of these compounds proved to be active KhGM-1 antagonists, just like sodium sulfite.

At the same time, as control experiments showed, none of these S-nucleophilic agents affected neuromuscular block caused by myorelaxants not containing the disulfide bond, such as, for example, tubocurarine, ditilin (I), decamethonium (XI), paramion (XII, R = C_2H_5), and also (XII, R = H). The latter compound is a structural analog of KhGM-1, and differs only in the presence of a C—C bond between the phenyl rings instead of an S—S bond.



XI



XII

Thus, on the model, diquaternary myorelaxant containing an S—S bond plus S-nucleophilic agent, it has been possible to demonstrate in principle a new route for controlling the action of a pharmacological material. This has been done by a directed selection of such a myorelaxant and such an agent that their interaction leads to removal of the curare-like effect by direct inactivation of the miorelaxant molecule.

The proposed method can be used in principle in making any physiologically active compound containing a labile disulfide bond, if products free of undesired physiological activity are formed when the bond is broken.

EXPERIMENTAL

4,4'-Bis-(dimethylaminophenyl) Disulfide (IV).* To a solution of 180 g (1.87 mole) of dimethylaniline in 250 ml of hexane was dropped in, with stirring and ice-cooling, a solution of 50.5 g (0.374 mole) of sulfur monochloride in 250 ml of hexane, at such a rate that the temperature of the reaction mixture did not exceed 15°. Then the solvent was stripped off, the residue was extracted with dilute hydrochloric acid, the solution was neutralized, the precipitate which fell was washed on the filter with water, and it was crystallized twice from ethanol. Yield, 49 g (44%), mp 117-118°.

4,4'-Bis-(dimethylaminophenyl) Disulfide Monomethiodide. A solution of 1 g (0.00328 mole) of IV and 2.8 g (0.0098 mole) of methyl iodide in 10 ml of dry benzene was kept for seven days. The crystals which fell out were washed with benzene and then with ether, and were recrystallized from ethanol. Yield 0.77 g (52.7%), mp 148-149°.

4,4'-Bis-(dimethylaminophenyl) Disulfide Monomethosulfate. This was prepared analogously to the methiodide. Yield 56%, mp 146-147°.

Quaternary Salt from 4,4'-bis-(dimethylaminophenyl) Disulfide and Methyl Benzenesulfonate. This was also prepared similarly to the methiodide. Yield, 30%; mp 153-154°.

*Product IV was prepared in 12% yield by the method of Merz [2]. By appropriately changing the method, we prepared it in 44% yield.

4,4'-Bis-(dimethylaminophenyl) Disulfide Monoethiodide. A solution of 6.08 g (0.02 mole) of IV and 7.44 g (0.04 mole) of ethyl benzenesulfonate in 40 ml of dry benzene was kept for 25 h at 80°. Then the solvent was stripped off, the residue was dissolved in 10 ml of methanol, an excess of ether was added, the precipitate which fell was dissolved in methanol, and 1 g of sodium iodide was added. The crystals which fell from the solution were separed and crystallized from ethanol. Yield 1.45 g (47%) mp 162-163°.

Monoquaternary Salt from 4,4'-Bis-(dimethylaminophenyl) Disulfide and Benzyl Iodide. A solution of 3 g (0.237 mole) of benzyl chloride and 5 g (0.033 mole) of sodium iodide in 20 ml of acetone was kept for 1 h at 55°, an excess of ether was added, the mixture was filtered, the filtrate was evaporated, the residue (benzyl iodide) was dissolved in 30 ml of dry benzene, and 3.04 g (0.01 mole) of IV was added. The solution so obtained was kept for 3 days at 15-20°, the precipitate which separated was removed, and it was crystallized from propanol. Yield 0.94 g (18%), mp 102-103°.

Monoquaternary Salt from p-Nitrobenzyl Bromide and 4,4'-Bis-(dimethylaminophenyl) Disulfide. A solution of 1.52 g (0.005 mole) of IV and 2.16 g (0.01 mole) of p-nitrobenzyl bromide in 20 ml of dry benzene was kept for 3 days at 15-20°. The precipitate was separated and crystallized from ethanol. Yield 0.26 g (10%), mp 139-140°.

4,4'-Bis-(dimethylaminophenyl) Disulfide Dimethiodide. A solution of 5 g (0.0164 mole) of IV and 18.5 g (0.13 mole) of methyl iodide in 250 ml of dry acetone was kept for seven days at 15-20°, and the precipitate was crystallized from n-propyl alcohol. Yield 3 g (33%), mp 151-152°.

4,4'-Bis-(dimethylaminophenyl) Disulfide Bismethosulfate. A. Prepared like the dimethiodide, it was obtained in 28% yield; mp 204-205°.

B. A solution of 0.5 g (0.00116 mole) of V ($R = CH_3$, $X^- = CH_3SO_4^-$) and 0.292 g (0.0023 mole) of dimethyl sulfate in 100 ml of acetone was kept for seven days at 15-20°. The precipitate was crystallized from ethanol. Yield 0.15 g (23%); mp 204-205°.

Diquaternary Salt from Benzyl Iodide and 4,4'-Bis-(dimethylaminophenyl) Disulfide. A solution of 6.35 g (0.05 mole) of benzyl chloride and 9 g (0.06 mole) of sodium iodide in 30 ml of acetone was heated at 60° for one-half hour, the precipitate of sodium chloride was filtered off, and 3.04 g (0.01 g mole) of IV was added to the filtrate. The precipitate which fell after 2 h was separated and crystallized from methanol. Yield 5.8 g (78%), mp 126-127°.

Diquaternary Salt from Benzyl Bromide and 4,4'-Bis-(dimethylaminophenyl) Disulfide. A mixture of 3.8 g (0.03 mole) of benzyl chloride, 6.15 g (0.06 mole) of sodium bromide, and 30 ml of methanol was kept at 65-70° for 2 h, then it was filtered, the filtrate was evaporated, and 1.52 g (0.005 mole) of IV was added to the residue, plus 10 ml of acetone; the solution obtained was kept at 15-20° for four days. The precipitate was crystallized from ethanol. Yield 0.35 g (11%), mp 111-112°.

Diquaternary from p-Nitrobenzyl Bromide and 4,4'-Bis-(dimethylaminophenyl) Disulfide. A solution of 3.04 g (0.01 mole) of IV and 13 g (0.06 mole) of p-nitrobenzyl bromide in 20 ml of dimethylformamide was kept at 15-20° for 14 h, 100 ml of ether was added, and the precipitate was crystallized from methanol. Yield 128 g (17.5%); mp 125-127°.

A. Cleavage of KhGM-1 with Sodium Sulfite to Form VII and VIII. To a solution of 1 g (0.0018 mole) of KhGM-1 in 5 ml of water was added a solution of 0.68 g (0.0054 mole) of sodium sulfite in 5 ml of water. After 10 min the precipitate which formed was filtered off and was washed with water and with methanol. Yield of VII, 0.23 g (52.5%), mp 226°. Found %: C 43, 31, 43, 68; H 4.98, 4.94; N 5.76, 5.94; S 26.24, 26.36. $C_9H_{13}NS_2O_3$. Calculated %: C 43.70; H 5.26; N 5.67; S 25.90.

The aqueous filtrate from VII was evaporated, the residue was boiled in n-propyl alcohol, and the hot solution was filtered. A white crystalline product separated from the filtrate; this was removed, washed, and crystallized from propanol. Yield of VIII, 0.22 g (44%); mp 190-191°. Found %: S 23.35, 23.24. $C_{10}H_{17}NS_2O_4$. Calculated %: S 23.00.

A mixed mp of VIII with a known sample gave no depression.

B. Cleavage of 4,4'-Bis-(dimethylaminophenyl) Disulfide Dimethiodide (VI; $R = CH_3$, $X^- = I^-$) with Sodium Sulfite to Form VII and p-Dimethylaminophenylmercaptan Methiodide (VIIIa). An experiment performed similarly to the one above (see A) led to obtaining VII (0.25 g, 59%) and VIIIa (0.31 g, 62%); mp of VIIIa, 167-168°. Found %: N 4.86, 5.11; S 10.70, 10.74. $C_9H_{14}NSI$. Calculated %: N 4.75, S 10.83.

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