3-[2-(m-Hydroxyphenyl)-2-hydroxyethyl]sydnoneimine Hydrochloride (XII). To a solution of 3.79 g (20 mmole) of the hydrochloride (XIX) in 10 ml of water was added 5 ml of a 15% solution of NaOH and 2.68 g (0.02 mole) of formaldehyde bisulfite, cooled to 8-10°C, and 1.3 g (0.02 mole) of KCl in 10 ml of water added dropwise. The mixture was kept for 3 h, acidified to pH 7.0, extracted with ethyl acetate, the extract evaporated, the residue dissolved in absolute ethanol, and ethereal hydrogen chloride added to give 2.65 g of N-[2-(m-hydroxyphenyl)-2-hydroxyethyl]aminoacetonitrile hydrochloride (XX). Nitrogen oxides were passed through a solution of the nitrile (XX) in 30 ml of ethanol for 2 h at 0-5°C, and the solution was acidifed with ethereal hydrogen chloride to give (XII).

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QUATERNARY AMMONIUM SALTS OF DIMETHYLAMINOALKYL 2-CHLORO- AND 2,4-DICHLOROBENZOATES AND THEIR REACTION WITH CHOLINESTERASES

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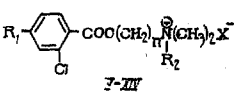
Quaternary ammonium salts (QAS) derivative of dialkylaminoalkyl esters of carboxylic acids, structurally analogous to acetylcholine, display anticholinesterase properties, and have been investigated as short-acting myorelaxants [1-3].

We have synthesized the esters of dimethylaminoalcohols and 2-chloro- and 2,4-dichlorobenzoic acid, and their QAS, and studied their anticholinesterase activity with respect to several types of cholinesterase (CE).

The compounds, of general formula (V-XIV, Table 1),



where $R_1 = H$ or Cl, $R_2 = H$ or alkyl, X=Cl, Br, I

were synthesized in the usual way, by reacting the chlorides of the appropriate acids with the dimethylaminoalcohols to give the ester hydrochlorides (I-IV, Table 1). These ester hydrochlorides were then converted to the free bases, and then to the QAS (V-XIV, Table 1) by reacting the esters with alkyl halides in dry acetone [1, 2].

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TABLE 1. Dimethylaminoalkyl 2-Chloro- and 2,4-Dichlorobenzoates (I-IV) and QAS (V-XIV)

Compound	R_1	R_2	X^-	n	mp, °C	Found, %		Molecular formula	Calculated, %		R_f
						N	X^-		N	X	
I	H	H	Cl	2	118—119	5,56	27,08*	$C_{11}H_{15}Cl_2NO_2$	5,3	26,89*	—
II	H	H	Cl	3	68	5,05	25,82*	$C_{12}H_{17}Cl_2NO_2$	5,03	25,54*	—
III	Cl	H	Cl	2	148—149	5,02	35,3*	$C_{11}H_{14}Cl_3NO_2$	4,96	35,55*	—
IV	Cl	H	Cl	3	129	4,27	33,74*	$C_{12}H_{16}Cl_3NO_2$	4,48	34,09*	—
V	H	CH_3	I	2	168—169	—	34,19	$C_{12}H_{17}ClINO_2$	—	34,4	0,21
VI	H	CH_3	I	3	162	—	32,85	$C_{13}H_{19}ClINO_2$	—	33,09	0,26
VII	Cl	CH_3	I	2	204—205	—	31,1	$C_{12}H_{16}Cl_2INO_2$	—	31,42	0,24
VIII	Cl	C_2H_5	I	2	131—132	—	29,9	$C_{13}H_{18}Cl_2INO_2$	—	30,36	0,33
IX	Cl	C_3H_7	I	2	161—163	—	29,02	$C_{14}H_{20}Cl_2INO_2$	—	29,39	0,40
X	Cl	C_2H_5	Br	2	158—159	—	21,39	$C_{13}H_{18}BrCl_2NO_2$	—	21,54	0,13
XI	Cl	CH_3	I	3	196—197	—	30,1	$C_{13}H_{18}Cl_2INO_2$	—	30,36	0,26
XII	Cl	C_2H_5	I	3	154—155	—	29,1	$C_{14}H_{20}Cl_2INO_2$	—	29,39	0,35
XIII	Cl	C_3H_7	I	3	188—189	—	27,98	$C_{15}H_{11}Cl_2INO_2$	—	28,43	0,42
XIV	Cl	C_2H_5	Br	3	180	—	20,53	$C_{14}H_{20}OBrCl_2NO_2$	—	20,75	0,15

*Total chlorides.

The structures of the compounds were confirmed by their elemental analyses, positive hydroxamic acid reaction for ester groups, and IR spectroscopy. The IR spectra displayed typical absorption at 760 cm^{-1} (C—Cl), 1720-1730 cm^{-1} (C=O), and 1130 cm^{-1} (C—O—). The spectra of the ester hydrochlorides, unlike those of the QAS, showed absorption at 2300-2600 cm^{-1} (ammonium band).

The purities and identities of the compounds were established by TLC. The chromatographic mobilities of the QAS were found to be linearly dependent on the length of the alkyl group R_2. See, for example, the R_f values of (VII-IX) and (XI-XIII) (Table 1).

EXPERIMENTAL CHEMISTRY

IR spectra were obtained on a UR-20 instrument (East Germany) as a suspension in vaseline oil. Brockman grade III alumina was used in TLC, solvent system dichloroethane—ethanol (4:1), development with iodine vapor.

Dimethylaminoalkyl 2-Chloro- and 2,4-Dichlorobenzoate Hydrochlorides (I-IV). To a benzene solution of 0.01 mole of the acid chloride, obtained from the acid and thionyl chloride, was added dropwise with ice-cooling and constant stirring a benzene solution of 0.02 mole of the dimethylaminoalcohol. The solid which separated was filtered off after 30 min, and recrystallized from a mixture of absolute alcohol and dry ether. Yields quantitative.

(2-Chlorobenzoyloxyalkyl)trialkylammonium and (2,4-Dichlorobenzoyloxyalkyl)ammonium Salts (V-XIV). The hydrochloride (0.01 mole) was dissolved in water, ammonia solution added until the pH reached 10, the free base which separated was extracted with ether, and the ether extract dried over anhydrous sodium sulfate. The ether was distilled off, the residue dissolved in acetone, 0.05 mole of the alkyl halide added, and the mixture boiled for 30 min. The precipitated QAS was filtered and recrystallized from a mixture of absolute alcohol and dry ether.

EXPERIMENTAL BIOLOGY

CE from various sources differ considerably in their substrate-inhibitory specificity [3]. We have examined the reaction of the QAS with acetyl-CE from human erythrocytes, butyryl-CE from horse serum, frog brain CE, and CE from the visual ganglia of the Pacific squid.

The enzymes employed were a commercial water-soluble preparation of acetyl-CE (EC 3.1.1.7) from human blood erythrocytes and butyryl-CE (EC 3.1.1.8) from horse blood serum from the Perm Scientific-Research Institute vaccines and sera, with specific activities of 1.2 and 9.6 A.U., together with aqueous homogenates of the brain of the frog *Rana temporaria* (45 mg/ml) and the optical ganglia of the squid *Todorodes pacificus* (3mg/ml). The substrate used was acetylcholine iodide (Chemapol, Czech SSR). The catalytic activities of the enzymes were measured at pH 7.5 and 25°C by the method of Ellman [4, 5]. The efficiencies of the reversible inhibitors were calculated from the values of the generalized inhibitory constant $\bar{K}_i$ [5], which in the case of combined inhibition is composed of the competitive (K_i) and noncompetitive (K_i') inhibitory constants in accordance with the equation $1/\bar{K}_i = 1/K_i = 1/K_i'$ [5].

TABLE 2. Anticholinesterase Efficiency of the Test Compounds

Compound	Human erythrocyte acetyl-CE		Horse serum butryl-CE		Frog brain CE		Squid optical ganglion CE	
	type of inhibition	pK	type of inhibition	pK	type of inhibition	pK	type of inhibition	pK
III	C	5,06	K	5,05	C	4,13	C	4,07
VII	C	5,76	C	5,16	C	3,92	C	4,53
IX	C	5,32	C (K)	5,29	C	4,03	C	4,29
IV	C	5,00	C	4,91	C	4,01	C	3,62
XI	C	4,14	C (K)	5,24	C	3,98	C	3,94
XII	C	4,69	C	5,11	C (K)	4,22	C (K)	4,27
XIII	C (N)	4,89	C	5,00	C	4,71	C	4,55
V	C (K)	5,17	K	5,43	C	3,86	C (K)	4,15
VI	C (K)	4,38	C	4,74	C	3,90	C	4,01

Note. Type of inhibition: K) competitive; C) combined; N) noncompetitive.

In competitive inhibition, when $1/K_i' \to 0$, $\overline{K}_i = K_i$ [5]. Table 2 gives the values of $p\overline{K}_i = -\log \overline{K}_i$, which is more convenient for analyzing the results obtained.

RESULTS AND DISCUSSION

For all the test compounds (III-VI, IX, XI-XIII), it was not possible to measure their rates of enzymatic hydrolysis in the presence of the four CE, even at enzyme concentrations 10 times greater than those at which the rate of hydrolysis of acetylcholine were determined. The nondependence of the extent of inhibition by these compounds of CE activity on time of incubation with the inhibitor is evidence of the reversible nature of the inhibition. The aminoesters (III-VI, IX, XI-XIII) were examined as reversible inhibitors of the hydrolysis of acetylcholine in the presence of the CE (Table 2).

In the series of "homocholines" (IV, XI-XIII), changing the structure of the alkyl group in the ammonium moiety [H(IV)—CH_3 (XI)—C_2H_5 (XII) — C_3H_7 (XIII)] resulted in a 5-10-fold increase in antienzyme efficiency with respect to frog and squid CE, and acetyl-CE was inhibited by the trimethylammonium derivative (XI) seven times less strongly than by the dimethylamine analog (IV), which interacts with the enzyme at pH 7.5 in the protonated form. When the length of the polymethylene chain was reduced, only in the interaction of the trimethylammonium compounds (VII) and (XI) with acetyl-CE was there a greater than 40-fold reduction in effectiveness. These "stringent" requirements for the structure of the effector are in full agreement with the literature report [3] of the high specificity of acetyl-CE for compounds with the choline structure.

As would be expected, the differences in the anticholinesterase activities of the monochlorobenzoic (V and VI) and dichlorobenzoic acid (VII and XI) derivatives were slight, indicating that the steric factor plays a decisive part in the reaction of these compounds with CE.

Examination of the results obtained provides an approach to the practical problem of finding specific inhibitors, i.e., those which inhibit the activity of one enzyme much more effectively than those of the others. Thus, (VII) was a more powerful inhibitor of acetyl-CE than of butyryl-CE, squid, and frog CE by facotrs of 4, 17, and 70, respectively. Compound (XI) was a specific inhibitor for butyryl-CE, its activity being 13-20 times greater than for the other CE.

Generally speaking, these esters were 10-20 times less effective as inhibitors of frog and squid CE than of mammalian CE. This is in agreement with our earlier findings [5].

Hence, these aminoesters are not substrates for the CE examined, but are rather reversible inhibitors of these enzymes, (VII) being a specific inhibitor of acetyl-CE, and (XI) a specific inhibitor of butyryl-CE.

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SYNTHESIS AND TUBERCULOSTATIC ACTIVITY OF 3- AND 5-SUBSTITUTED 2-ARYLFURANS

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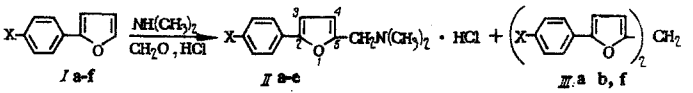
As a continuation of our studies on the synthesis and biological activity of arylfuran derivatives [1], we carried out the aminomethylation of 2-arylfurans (Ia-d) and bromination of 2-aryl-5-methylfurans (IVa, b).

The aminomethylation of Ia-d was accomplished through the use of the Mannich condensation reaction. When diethylamine and formaldehyde were reacted with Ia-d, an aminomethyl group was introduced in the open α position of the furan ring, and 2-aryl-5-dimethylaminomethylfuran chlorohydrates (IIa-d) were formed at a 42%-86% yield.

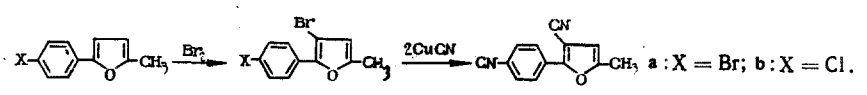


Ia-f IIa-c IIIa b, f

a: X = Cl; b: X = CH_3; c: X = OCH_3; d: X = H; e: X = $NHCOCH_3$; f: X = NO_2.

Aminomethylation was accomplished by boiling the components of the mixture for 5 to 10 h in isoamyl alcohol. The reaction proceeded only at 4 < pH < 7. Arylfurans with electron donor groups or a substituent chlorine atom in the benzene ring were successfully introduced in the Mannich reaction. The attempt to aminomethylate Ie led to the formation of bis[2-(p-nitrophenyl)furyl-5]methane (IIIe). Analogous compounds (IIIa, b) were obtained as side products in the Mannich reaction with Ia, b. The quantity of IIIa, b produced depended upon the acidity of the reaction mixture and increased with rise in pH. The formation of these kinds of compounds has been previously observed in the radical arylation of 2-hydroxymethylfuran [2]. The structure of compounds IIa-d and IIIb, e was confirmed by EPR spectra (Table 1).

A study of the IVa,b bromination indicated that, just as in our earlier study of 2-aryl-5-bromofuran bromination [3], the bromine atom goes into position 3 of the furan ring, adjacent to the carbon atom carrying the aryl residue, which results in the formation of 2-aryl-3-bromo-5-methylfurans (Va, b):



IVa, b Va, b VI

a: X = Br; b: X = Cl.

The structure of Va, b was confirmed by PMR spectroscopy. Spin-spin coupling between the furan ring proton and the methyl group was observed in the PMR spectra of these compounds:

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