SYNTHESIS OF PHOSPHONIUM AND AMMONIUM DERIVATIVES OF BENZO-CROWN ETHERS AND THEIR CHOLINOLYTIC ACTIVITY

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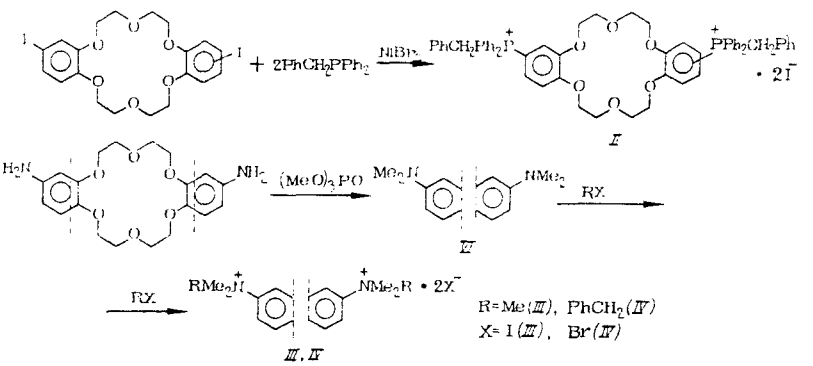
Molecules of compounds blocking the nervous-muscular transmission (the myorelaxants), generally have two positively charged groups (the onium centers) and a nonpolar fragment separating them, which interact, respectively, with the "anionic" and "hydrophobic" sections of the choline receptor of the postsynaptic membrane [5, 6]. Thus it is not to be excluded that the action of the myorelaxants is dependent on the surface activity of their molecules at the water–membrane phase interface [2, 6]. It is therefore of interest to extend the study of the structural-functional dependences in the series of myorelaxants in view of recently obtained data on the high membranotropicity and surface activity of macrocyclic complexones [4, 10].

A synthesis was carried out in the present work of phosphonium and ammonium derivatives of benzo-crown ethers and their curare-like properties were studied.

The synthesis of 4',4"(5")-bis(diphenylbenzylphosphonium)-dibenzo-18-crown-6 diiodide (II) was carried in a single step by the reaction of 4',4'(5")diiododibenzo-18-crown-6 with diphenylbenzylphosphine in the presence of catalytic amounts of nickel dibramide.

The bromodiphenylbenzylphosphonium derivatives of benzo-15-crown-5 (V) and dibenzo-18-crown-6 (I) were synthesized in a similar way and were described in [3].

The bistrialkylammonium derivatives of dibenzo-18-crown-6 (III, IV) were synthesized according to a scheme which includes the methylation of 4',4"-diaminodibenzo-18-crown-6 by trimethyl phosphate, followed by the quaternization of the nitrogen atoms of bis(dimethylamino)-dibenzo-18-crown-6 (VI) thus formed by methyl iodide or benzyl bromide.



Compounds I-V obtained have a crystalline structure, are soluble in polar organic solvents, while bromides I, IV are readily soluble in water. The composition and structure of the compounds were confirmed by the elemental analysis, IR and ^{1}H and ^{31}P NMR spectroscopy data.

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TABLE 1. Characteristics of Diphosphonium and Diammonium Derivatives of Dibenzo-18-crown-6 I-IV

Compound	Yield, %	mp, °C	Empirical formula
I[a]	65	80—100	$C_{58}H_{56}Br_2O_6P_2$
II[b]	70	120—125	$C_{58}H_{56}I_2O_6P_2$
III[c]	95	147, dec.	$C_{28}H_{40}I_2N_2O_6$
IV[d]	75	108, dec.	$C_{44}H_{48}Br_2N_2O_6$

Notes. [a]Mixture of cis, trans-isomers (δ_p, 22.5 ppm [3]).
[b]Mixture of cis, trans-isomers (δ_p, 22.0 ppm).
[c]cis-isomer.
[d]trans-isomer.

TABLE 2. Acute Toxicity (LD_{50}) of Compounds I-V and d-Tubocurarine in Various Modes of Administration (the confidence intervals determined with a 95% probability are given in brackets)

Compound	Mode of administration LD_{50}		
	i/v	i/p	i/g
I, II	0.64 (0.33—1.2)	6.4 (4.4—9.3)	200
III	9.5	34 (20—41)	—
IV	4.2 (3.7—4.5)	10.4	—
V	12 (10—15)	69 (52—92)	200
d-Tubocurarine	0.14	—	—

EXPERIMENTAL (CHEMICAL)

4',4"(5")-Bis(diphenylbenzylphosphonium)dibenzo-18-crown-6 diiodide (II). A mixture of 5.8 mmoles of diiodiddibenzo-18-crown-6, 18 mmoles of diphenylbenzylphosphine and 2.9 mmoles of nickel dibromides was stirred for 3 h at 195°C. After cooling, the reaction mixture was dissolved in 200 ml of dichloroethane, washed with aqueous ammonia, and evaporated. The product was separated by reprecipitation with ether from a solution in chloroform.

4',4"-Bis(dimethylamino)-dibenzo-18-crown-6 (VI). A 15 mmole portion of trimethylphosphate was added at 175°C to a solution of 9.2 mmoles of diaminodibenzo-18-crown-6 in 40 ml of o-dichloro-benzene. The reaction mixture was boiled with stirring for 2 h, then cooled, and after adding a solution of 1.52 g of sodium hydroxide in 7 ml of water, was boiled for another 1 h. After cooling, the organic layer was separated, the solvent was evaporated, and the residue was dried under a vacuum of 0.05 mm Hg. The product was extracted with boiling hexane, mp 97-101°C, yield, 30%. $C_{24}H_{34}N_2O_6$. PMR spectrum ($DCDl_3$, ppm): 5.87-6.83 m, C_4H_3; 3.55-4.22 m, OCH_2; 2.78 s, CH_3.

4',4"-Bis(trimethylammonium)dibenzo-18-crown-6 diiodide (III). A solution of 1 mmole of compound VI and 2.5 mmoles of methyl iodide in 10 ml of acetonitrile was boiled for 9 h. The crystals that separated out were filtered off, washed with ether and dried.

4',4"-Bis(Dimethylbenzylammonium)dibenzo-18-crown-6 dibromide (IV) was obtained in a similar way from 1.45 mmole of compound VI and 3.62 mmoles of freshly distilled benzyl bromide.

EXPERIMENTAL (BIOLOGICAL)

The curare-like action of the compounds was studied on the phrenic-diaphragmal preparation of rats. In the experiments rats of both sexes were used, each weighing 250 ± 50 g. The preparation was placed in a 10 ml dish filled with a Liley solution. The experiments were carried out at 30°C, pH 7.8, under aeration conditions using carbogen (5% CO_2 + 95% O_2). The nerve was placed in a suction electrode. The irritation was effected by bundles of pulses (20 Hz) at a supramaximal amplitude once in 10 sec. The time of the incubation of the preparation with myorelaxants was not less than 10 min.

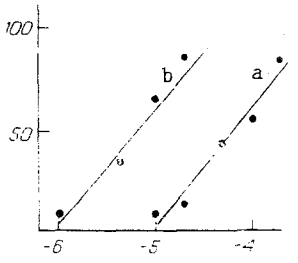

Fig. 1

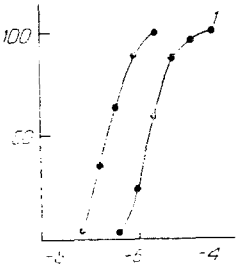

Fig. 2

Fig. 1. Curare-like action of compound IV (a) and d-tubocurarine (b) in experiments on a phrenic-diaphragmal preparation of a rat. Here and in Fig. 2: on the abscissa) log of molar concentration of the myorelaxant; on the ordinate) decrease in the amplitude of the induced contractions of the diaphragmal muscle, in % of the index in the control.

Fig. 2. Dependence of the amplitude of induced contractions of lilac intestine of a guinea pig on the methylfurmethide concentration. The data for curve 1 were recorded in the presence of compound I in a physiological solution (10^{-6} M).

The influence of the compounds on the contraction of smooth muscles was studied on an isolated section of the small intestive of a guinea pig. In the experiments animals of both sexes were used each weighing 350 ± 50 g. The preparation was placed in a 10 ml dish filled with a Krebs-Henseleit solution. The experiments were carried out at the temperature of 36°C at pH 7.8 under aeration conditions using carbogen. The contraction of the small intestine section was induced by the muscarine agonist methylfurmethide. For recording the muscle contraction, a US-2 sensor ("Stathem", USA) and a R-612 polygraph ("Beckman", USA) were used.

The curare-like activity of the compounds was evaluated from their ability to decrease the contraction amplitude by 50% in the course of 3 min of the action of the myorelaxant (EC_{50}). The EC_{50} value was calculated using a linear regression equation. The dissociation constant (K_d) was calculated using the Gaddum equation:

$$K_d = [B] \cdot (OK - 1),$$

where [B] is the concentration of the antagonist, OK is the ratio of ED_{50} of methylfurmethide in the presence of the antogonist and EC_{50} of methylfurmethide in the control [8].

The influence of the compounds on the acetylcholine induced ionic currents was evaluated in experiments on isolated identifiable neurons of a mollusk. Limn. stagnalis. The experiments were carried out on a fixed membrane potential, using the intracellular perfusion method. The composition of the medium and other experimental conditions were described previously in [7].

The toxicity of the compounds was evaluated from the LD_{50} value determined on nonpedigree white mice under various modes of administration. The LD_{50} was calculated according to [9].

All the compounds studied (Table 2) are toxic at intravenous (i/v) and intraperitoneal (i/p) administration, and are considerably less toxic (up 200 mg/kg) during their intragastric (i/g) administration. A characteristic indication of toxic action is "paralysis" of respiration, after which short-term clonic spasms develop, leading to the death of the anumal. A slow i/v administration of the compounds to rabbits causes a head "inclination" symptom, which is characteristic for compounds with myorelaxing activity.

In experiments with a phrenic-diphragmal preparation, compound IV was approximately one order of magnitude inferior in its curare-like activity to d-turbocurarine (Fig. 1). The ED_{50} for d-tubocurarine and compound IV are $6.5 \cdot 10^{-4}$ and $6.3 \cdot 10^{-5}$ M, respectively. The blockade of the nervous-muscular transmission by compound IV is intensified with increase in the frequency of irritations. A pessimal reaction of the muscle during the tetanization of the of the motoric nerve (50 Hz, 5 sec) and a pronounced posttetanic weakening of the blockade

are observed. These symptoms indicate an antidepolarizing action mechanism of compound IV in experiments using a phrenic-diaphragmal preparation [1].

The interaction of charged crown-ether derivatives with m-choline receptors was studied on an isolated section of small intestine of a guinea pig. It was shown with this subject that compound I induces a parallel shift to the right of the "concentration-contractive response" curve when the selective agonist of m-choline receptors, methylfurmethide, is acting on the preparation (Fig. 2). The K_d value of compound I is $8.8 \cdot 10^{-8}$.

In experiments on isolated identifiable neurons of mollusck Limn. stagnalic it was found that compounds I-V in the concentration of 10^{-5} M do not change the conductivity of a nonactivated neuronal membrane, but reversibly suppress acetylcholine (10^{-4} M) induced ionic currents by 80% (compound I) and 95% (compound V). The myorelaxant of a nondepolarizing type of action, d-tubocurarine suppresses the membrane currents by 66% under these conditions.

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SYNTHESIS AND CURARE-LIKE ACTIVITY OF β-(TRIALKYLAMMONIUM)ETHYL ESTERS OF THIOCYANURIC ACID*

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It is known that some full esters of thiocyanuric acid that contain a quaternary nitrogen atom in the ester group (analogs of cholinic esters) have curare-like activity that is increased when a benzyl radical is present as a substituent at the quaternary nitrogen atom [4]. In continuation of these investigations we have synthesized and studied a group of mixed esters of thiocyanuric acid containing 1, 2, or 3 (β-dialkylbenzyl-ammonium)ethyl groups (compounds of types I-III). (see Scheme 1)

It was expected to find out the influence of the number of onium centers on the development of the curare-like effect by the investigations. Isolated literature data give evidence in favor of the presence of three onium groups in the structures of related myorelaxants. Compare, for example, the data for pyrolaxon and its analogs [1] (see Scheme 2.)

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