SYNTHESIS AND PROPERTIES OF ISOCYURONIUM BROMIDE: A NEW MYORELAXATION DRUG

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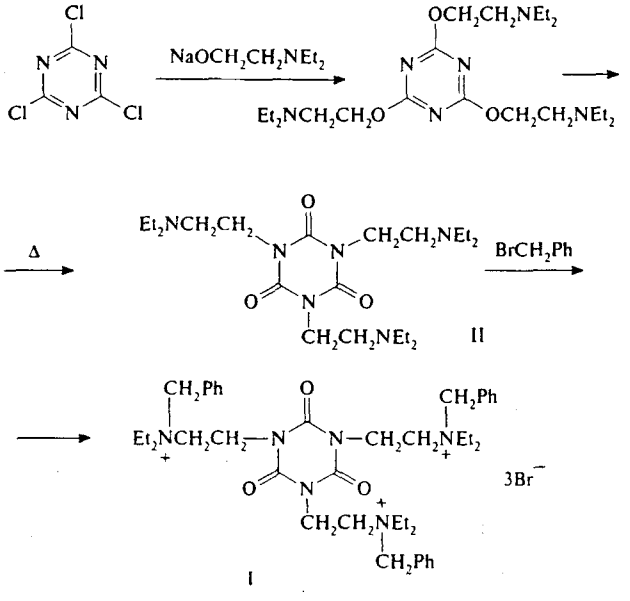
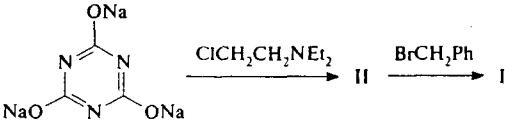
Previously we have demonstrated [1] that trihalide substituents of *tris* [(2-diethylbenzylammonio)ethyl] isocyanurates exhibit a high curarelike activity. The results of pharmacological investigations indicate that 1,3,5-*tris* [(2-benzyldiethylammonio)ethyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione tribromide (I) named "isocyuronium bromide" can be used in medicine as a myorelaxation drug. This compound was synthesized by way of interaction between cyanuric chloride and sodium 2-(diethylamino)ethylate, followed by thermal isomerization of the resulting cyanurate to isocyanurate (II) and quaternization of the latter ester by benzyl bromide.



However, using this pathway for the synthesis of I has a number of disadvantages hindering its commercial implementation. In particular, reaction used for the obtaining of 2-ethylaminoethanol alcoholate involves metallic sodium or sodium hydride, which renders the process dangerous with respect to explosion or self-ignition. The target compound is obtained at a comparatively low yield (32%). Moreover, the quaternization of amino groups proceeding in a heterogeneous medium implies the presence of intermediate quaternization products (mono- and diammonium salts) besides the target product. As a result, product I contains only about 95% of the main compound, which does not satisfy the pharmacopeial requirements.

The purpose of this work was to develop a method of synthesis that can be used in the commercial production of compound I for medicinal purposes.

We have proposed a new reaction pathway patented in [2], according to which compound I is obtained using a two-stage process. In the first stage, a commercially available trisodium salt of cyanuric acid is treated with 2-chloroethyldiethylamine in a boiling isopropyl alcohol. In the second stage, the reaction product II is treated with benzyl bromide in acetonitrile (the latter medium provides homogeneity of the reaction mass).



This scheme allows all disadvantages of the previous process to be removed: neither sodium nor its hydride is used and sufficiently compound I is obtained with a high yield on the basis of commercially available (domestic) raw materials. The initial compound is a trisodium salt of cyanuric acid, which is readily obtained by boiling cyanuric acid with sodium hydroxide in water. The second reagent is 2-chloroethyldiethylamine hydrochloride – a commercial compound that is widely used in the pharmaceutical industry for introducing diethylaminoethyl radicals in the molecules of various drugs [3].

Implementation of the above scheme increases the yield of compound I from 30 to 70% and renders the process tech-

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nologically acceptable and safe from the standpoint of fire risk. The new method was used to obtain an experimental batch of I (several kilograms) and refine characteristics of the commercial product.

We have studied the stability of compound I in various media. It was found that a two-year storage of the product exposed to the light in an oxygen atmosphere did not detrimentally affect the purity of compound I as judged by TLC. Neither mineral nor organic acids react with compound I, but aqueous alkaline solutions decompose the drug.

The stability of compound I was further assessed by the method of radiochromatography using a specially prepared tritium-labeled sample. Neither storage of an 0.3% aqueous solution of compound I for 4 months at room temperature nor boiling of this solution for 2 h led to any detectable changes.

EXPERIMENTAL CHEMICAL PART

1,3,5-*Tris*[(2-benzyldiethylammonio)ethyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (II). To a suspension of 1.52 g (0.0078 mole) of thoroughly triturated sodium cyanurate in anhydrous isopropyl alcohol heated to boiling was added dropwise with stirring 3.2 g (0.025 mole) of 2-chloroethyldiethylamine and the reaction mixture was boiled for 10 h. The precipitate was separated by filtration, the filtrate was evaporated, and the residue was dissolved in 50 ml of ethyl acetate. The solution was washed with water (4 × 5 ml) and dried over sodium sulfate. Finally, the solvent was distilled off to obtain 2.6 g (78%) of compound II in the form of a colorless oil.

The IR spectrum of compound II exhibits the following absorption bands (ν_{max}, cm^{-1}): 2955, 2920, 2865 (C–H), 1690 (C–O), 1440, 760 (triazine cycle); R_f, 0.58 (Al_2O_3, Brockman's activity II; ethyl acetate). Found (%): C, 58.53; H, 10.02; N, 19.63. For $C_{21}H_{42}N_6O_3$ anal. calcd. (%): C, 59.15; H, 9.96; N, 19.72.

1,3,5-*Tris*[(2-benzyldiethylammonio)ethyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione tribromide (I). To 1.02 g (0.0024 mole) of compound II was added 1.5 g (0.0088 mole) benzyl bromide in 30 ml of anhydrous acetonitrile and the mixture was boiled for 5 h. Then 0.5 g of activated charcoal was added and the boiling was continued for 1 h. The charcoal was filtered, the filtrate evaporated, and the residue mixed with absolute diethyl ether (~30 ml). The mixture was triturated, the solvent was decanted from a solid residue formed during this treatment, and the residue dried in vacuum to yield 2.12 g (91%) of compound I.

The IR spectrum of compound I displays the following absorption bands (ν_{max}, cm^{-1}): 3069, 3038 (PhH), 2978 (C–H), 1690 (C–O), 1586 (C–C in benzene ring), 1472, 759 (triazine cycle). The UV spectrum of I measured in 0.05% aqueous solution in the range 220 – 300 nm has the absorption maxima at 263 ± 2 and 269 ± 2 nm and a shoulder in the region of 256 – 300 nm. The ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 1.43 (t, 18H, $CH_2\underline{CH_3}$), 3.62 (q, 12H, $\underline{CH_2}CH_3$), 3.75 (m, 6H, $NCH_2\underline{CH_2}N$), 4.63 (m, 6H, $N\underline{CH_2}CH_2N^+$), 4.83 (s, 6H, $\underline{CH_2}Ph$), 7.3 – 7.7 (m, 15H, Ph); R_f, 0.26 (TLC on silica gel; chloroform – methanol – water, 65 : 25 : 4).

The content of compound I in the reaction product is 98 – 99% as determined by the method of nonaqueous potentiometric titration [4]. Found (%): C, 52.94; H, 6.85; N, 8.45. For $C_{42}H_{63}Br_3N_6O_3$ anal. calcd. (%): C, 53.67; H, 6.71; N, 8.95.

EXPERIMENTAL PHARMACOLOGICAL PART

The curarelike properties of compound I were studied in comparison with the action of nondepolarizing myorelaxants d-tubocurarine chloride (Burroughs Wellcome & CO., London, England), pipecuronium bromide (Gedeon Richter, Budapest, Hungary), pancuronium bromide (Lasapharma S. P. A., Italy), and a depolarizing myorelaxant suxamethonium chloride (Unique Pharmaceutical Labs, Bombay, India).

The curarelike activity of test preparations was assessed in the experiments on intact rabbits weighing 2.5 – 3.2 kg and cats weighing 2.5 – 3.5 kg. The animals were kept in a vivarium and fed with a standard ration. The drugs were introduced by single intravenous injections in a range of doses from immobilizing (with retained external respiration) to producing apnea; each dose was tested in a group of 6 – 10 animals. In the experiments on rabbits, the myorelaxation effect was assessed by the symptom of head inclination in combination with weakness in extremities; in cats, the effect was assessed by the symptom of turning. These manifestations were used to determine an average effective dose (ED_{50}, mg / kg) and the duration of the curarelike effect produced by this dose (Et_{50}, min). A special series of tests was performed to determine the apnea dose (ApD_{50}) and the time required for the complete glottis opening with the possibility of trachea intubation (Et^{int}) for the intravenous injection of myorelaxants at doses close to ApD_{50}. The experimental data were processed using the Litchfield – Wilcoxon method.

We have also studied the mechanism of the blocking action of compound I on the myoneural conduction (NMC). For this purpose, we have performed a comparative electromyographic study of the effects produced by our new myorelaxant, d-tubocurarine chloride, suxamethonium chloride, and acetylcholine on the NMC of gastrocnemius muscles in rabbits narcotized with urethane (600 – 800 mg / kg, i.p.). The NMC state was evaluated by the parameters of electromyograms measured in the gastrocnemius muscle in response to the motor nerve stimulation by rectangular electric pulses (pulse width, 1 msec; frequency range, 10 – 100 cpsec; pulse train duration, 1 sec; interval between trains, 2 – 3 sec). The lowest frequency (10 cps), following the series of tetanizing frequencies, was used to reveal the post-tetanic reaction. The nerve was excited using a universal electronic stimulator (Nihon Kohden, Japan). The peripheral segment of the motor nerve was excited by immersed bipolar platinum electrodes.

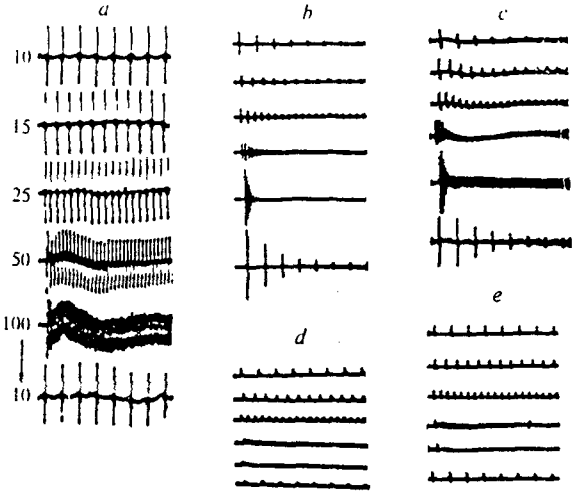

Fig. 1. Electromyograms showing a change in the functional lability of axoneural synapses in the gastrocnemius muscles of rabbit under the action of various drugs: *a*) initial electromyograms (figures at the curves indicate the frequency of motor nerve stimulation in cycles per second); *b*) compound I (0.06 mg/kg, i.v.); *c*) d-tubocurarine chloride (0.15 mg/kg, i.v.); *d*) acetylcholine chloride (10 mg/kg, i.v.); *e*) suxamethonium chloride (0.3 mg/kg, i.v.).

The electromyograms were recorded by a four-channel electromyograph Disa (Denmark) with the aid of silver plate electrodes implanted into the gastrocnemius muscle. The myorelaxants were injected to animals intravenously at equieffective doses producing the head inclination symptom in intact animals; acetylcholine was injected into the femoral artery at a dose of 10 mg/kg.

A special series of electromyographic experiments on rabbits narcotized with urethane was undertaken for studying the interaction of compound I with the acetylcholinesterase inhibitor prozerin.

In order to refine the localization site and mechanism of action of compound I (in comparison with those of d-tubocurarine chloride) on the H-choline receptor complex of axomuscular synapse, special experiments were also performed on an isolated nervous-muscular preparation of the tailor's muscle of lake frog. The effect of myorelaxants on the end-plate postsynaptic membrane was studied by the intracellular microelectrode technique using a Nikoh Kohden set of equipment.

The amplitude and decay halftime ($\tau_{0.5}$) of the end-plate current (EPC) were determined using a conventional two-electrode scheme of potential measurements across the end-plate membrane. The measurements were performed during a rhythmic stimulation of the motor nerve in the nervous-muscular preparation by overthreshold pulses with a duration of 40 μsec and a frequency of 0.33 Hz. The glass microelectrodes filled with a 3 M KCl solution were embedded into the end-plate region at a distance of 100 μm from each other. The potential microelectrode had a resistance of the order of 10 – 25 mΩ, while the current electrode was selected so as to have a resistance below 10 mΩ. The end-plate focus was localized by the electrophysiological technique. The EPC amplitude (I_{EPC}) and the response decay halftime were calculated on a computer. In order to prove the competitive character of the blocking action of the myorelaxant and to exclude the possible presynaptic effects, we have studied the influence of compound I on the EPC induced by introduction of an exogenic agonist (carbamylcholine chloride or carbachol) with a pipette into the end-plate region. The muscle contractions and fibrillations in response to the carbachol application were blocked by tetrodoxin at a concentration of 0.3 μM. The time of carbachol application was 80 sec; the level of membrane potential corresponded to that in the rest.

The channel-blocking effects of compound I were studied by plotting the current – voltage and kinetic end-plate characteristics (for the myorelaxant concentrations 2 – 20 μM). These characteristics were obtained by measuring EPC at fixed membrane potentials varied with a step of 20 mV in the interval from – 130 to – 30 mV. The time of maintaining the potential at each fixed level was 3 sec. By the end of this period, a single EPC signal was recorded and characterized by amplitude and decay halftime.

RESULTS AND DISCUSSION

A comparative analysis of data on the curarelike activity of isocyuronium bromide (I) and the known clinical myorelaxation drugs (see Table 1) showed that the activity of compound I was 2 – 2.5 times that of d-tubocurarine chloride, but lower by a factor of 5 – 6 and 12 – 13 than the activity of pancuronium bromide and pipecuronium bromide, respectively. The sequence of clinical relaxation of the muscles of various groups was the same for all preparations. This sequence was characteristic of the action of nondepo-

TABLE 1. Characteristics of Curarelike Activity of Isocyuronium Bromide, d-Tubocurarine Chloride, Pipecuronium Bromide, and Pancuronium Bromide

Preparation	Test animals	ED_{50}, mg/kg	Et_{50}, min	ApD_{50}, mg/kg	Et^{int}, sec
Compound I	Rabbits	0.053 (0.048 – 0.069)	8.1 ± 1.5	0.083 (0.07 – 0.1)	30 – 60
	Cats	0.145 (0.1 – 0.2)	9.1 ± 1.9	0.34 (0.26 – 0.45)	30 – 60
d-Tubocurarine chloride	Rabbits	0.12 (0.1 – 0.13)	15.8 ± 2.1	0.17 (0.16 – 0.18)	60 – 100
	Cats	0.4 (0.37 – 0.43)	–	–	–
Pipecuronium bromide	Rabbits	0.004 (0.003 – 0.006)	12.1 ± 1.9	0.009 (0.007 – 0.01)	210 – 270
	Cats	0.012 (0.011 – 0.013)	20.0 ± 3.7	0.015 (0.012 – 0.018)	210 – 270
Pancuronium bromide	Rabbits	0.009 (0.008 – 0.011)	10.0 ± 0.8	0.013 (0.01 – 0.015)	80 – 120
	Cats	0.03 (0.027 – 0.034)	15.0 ± 1.0	0.051 (0.048 – 0.054)	80 – 120

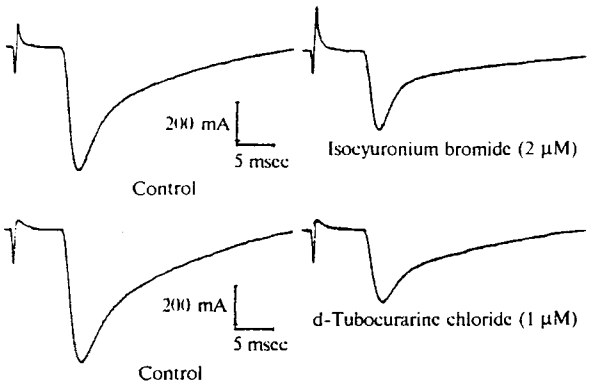

Fig. 2. EPC curves measured in the axoneural synapse of frogs showing changes induced by injecting equieffective doses of isocyuronium bromide and d-tubocurarine chloride.

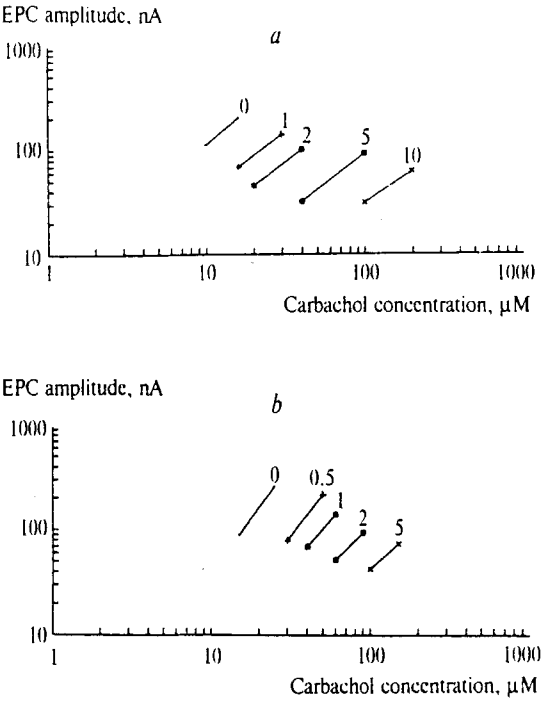

Fig. 3. Concentration – response curves for the carbachol activity measured in experiments on the axoneural synapse of frogs in the presence of (*a*) isocyuronium bromide and (*b*) d-tubocurarine chloride; values at the curves indicate drug concentrations in μM.

larizing myorelaxants: the relaxation is first developed in muscles of the neck and extremities, then involves the body muscles, and finally, the muscles of respiratory system. As for the rate of development of the clinical myorelaxation, compound I was superior to both d-tubocurarine chloride and pancuronium and pipecuronium bromides. Indeed, the complete glottis opening (making free trachea intubation possible) in the cats and rabbits tested took place 30 – 60 sec after injection of compound I at a dose of 1 – 1.5 ApD_{50}. The same effect upon injection of the equieffective doses of d-tubocurarine chloride was observed for Et_{int} = 60 – 100 sec, while the corresponding values for pancuronium bromide and pipecuronium bromide were 80 – 120 and 210 – 270 sec, respectively. The duration of relaxation was approximately the same for all the myorelaxation agents studied.

A comparative analysis of the influence of isocyuronium bromide and drugs having depolarizing (suxamethonium chloride, acetylcholine chloride) or nondepolarizing (d-tubocurarine chloride) character of action on the electromyograms (Fig. 1) showed that suppressive action of the new drug on the myoneural conduction is like that of d-tubocurarine chloride.

The nondepolarizing character of action of compound I was also confirmed by the results of electromyographic experiments involving prozerin. It was found that a single prozerin injection (0.1 mg/kg, i.v.) at the level of the axomuscular blocking (induced by the apnea dose of compound I suppressing NMC in the diaphragm and gastrocnemius muscle by 72.6 ± 5.6% and 91.5 ± 2.4%, respectively) rapidly restored NMC in the synapse of diaphragm and gastrocnemius muscle virtually to the initial level.

The process of decurarization in test animals repeatedly injected with compound I at the apnea dose (to a total of 5 – 20 ApD_{50}) required 3 – 5 prozerin doses (0.1 mg/kg) for reliable restoration of the NMC level).

Microelectrophysiological investigation of the effects of compound I and d-tubocurarine chloride on the H-choline receptor complex showed that both myorelaxation drugs are characterized by similar concentration dependences of the EPC amplitude (I_{EPC}) and decay halftime ($\tau_{0.5}$), differing only by the average effective concentrations (MIC_{50}) equal to 2 and 1 μM for compound I and d-tubocurarine chloride, respectively (Fig. 2). The equieffective concentrations of both drugs reduced the $\tau_{0.5}$ value to approximately the same extent, which implies their ability (despite competition) to block the ion channels of the H-choline receptor complex [5 – 7]. The two compounds are also characterized by approximately equal degrees of manifestation and relative weights of the non-competitive components in their axomuscular blocking effects.

The competitive activity components of compound I and d-tubocurarine chloride were studied by introduction of carbachol with a pipette into the end-plate region. Figure 3 shows the corresponding "dose – response" plots. As is seen, both compound I and d-tubocurarine chloride produce a concentration-dependent parallel shift of the equilibrium response segments to the right along the agonist concentration axis. The equilibrium dissociation constants determined from the Shield curves plotted for each myorelaxant were K = 0.78 and 0.43 μM for compound I and d-tubocurarine chloride, respectively. These values indicate (like the MIC_{50} data) that the competitive activity of compound I is approximately half that of d-tubocurarine chloride.

Study of the channel-blocking effect of compound I by measuring the EPC kinetics and current – voltage curves showed that the EPC decay observed for a membrane potential of 30 – 50 mV is described by a two-exponent function. This gives strong evidence that the agent studied is capable of blocking the open ion channels [6, 7].

The comparative microelectrophysiological investigation of the blocking action of compound I and d-tubocurarine chloride on the H-choline receptor complex of the axoneural synapse showed that the two substances have principally identical mechanisms of action. The new myorelaxant, like d-tubocurarine chloride, is capable of competing with acetylcholine for the receptor recognition sites and blocking the pen ion conduction channels of the H-choline receptor complex.

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