reserpine than the females. The presence of yohimbine in crude plant material may distort results obtained by mouse blepharoptotic assays where the goal of the assay is the quantitation or the detection of only reserpine-like alkaloids.

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Synthesis and Pharmacological Effects of Bis-trialkylammonium Alkanol Carbonates

By LEO A. POHORYLES, L. WISLICKI, and SHALOM SAREL

The synthesis and pharmacological effects of a series of bis-trialkylammonium alkanol carbonates of the type I, are described. The compounds were tested in the cat for their effects on electrical excitability of striated muscle, respiration, and blood pressure, and in the frog rectus abdominis. While the ethyl derivative, (I-1), lowers blood pressure without affecting the muscle twitch, the propyl-(I-3) and, to a much higher extent, the butyl derivative, (I-6), depress neuromuscular transmission and also direct muscle excitability. Branching of the alkanol moiety (I-2), or replacement of the carbonate oxygen by sulfur (I-7), or insertion of ethyl for the methyl groups on the quaternary nitrogen (I-4 and I-5), weaken the effect. The block is similar to that caused by depolarizing agents.

IT IS WELL known that the curarizing activity of bis-quaternary ammonium compounds of the structure $R_3\overset{+}{N}—(CH_2)_x—\overset{+}{N}R_3$ is not substantially weakened by inserting in the linear polymethylene chain bivalent radicals like carbonyl and/or oxygen for the methylene groups. Introduction of one or more ester groupings into the chain linking the two ammonium groups provides powerful curare-like neuromuscular blocking agents; thus, choline esters of dicarboxylic acids, particularly that of succinic acid, proved to be valuable clinically. However, these substances were found to be not without drawbacks. These include, for the nondepolarizing drugs, their long action and side effects on heart rate or blood pressure. With suxamethonium, which *in vivo* is usually quickly decomposed by enzymatic hydrolysis, a high incidence of muscle pain (1, 2), the absence of an effective antagonist, and instability within a range of pH's (3) have been troublesome.

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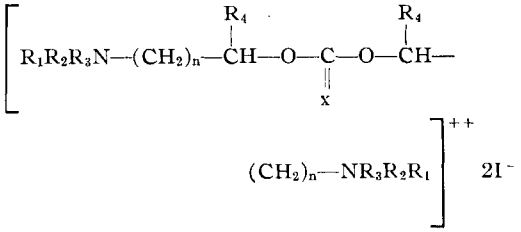
In view of the great stability shown by open chain carbonates toward hydrolysis both in acid and alkaline solutions (4), the synthesis of a series of bis-trialkylammonium alkanol carbonates of the structure I was undertaken.



I

I-1 $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $n = 1$; $x = O$.
I-2 $R_1 = R_2 = R_3 = R_4 = CH_3$; $n = 1$; $x = O$.
I-3 $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $n = 2$; $x = O$.
I-4 $R_1 = R_2 = CH_3$, $R_3 = C_2H_5$, $R_4 = H$, $n = 2$, $x = O$.
I-5 $R_1 = R_2 = C_2H_5$; $R_3 = CH_3$; $R_4 = H$; $n = 2$; $x = O$.
I-6 $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $n = 3$; $x = O$.
I-7 $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $n = 3$; $x = S$.

Differences in structure were designed with a

view toward studying their effect on the pharmacological pattern. As expected, the new quaternary ammonium iodides reported here exhibited remarkable stability in water solutions for a long period of time and could even be sterilized at 80° for 2 hours without chemical decomposition or changes in their pharmacodynamic effect.

EXPERIMENTAL PROCEDURE[1]

Materials.—The sources and purification procedures for the dialkylaminoalkanols used were as follows: commercial dimethylaminoethanol was fractionated and the cut b_{690} 130–133°, n_D^{22} 1.4280 was used. The 3-diethylamino-1-propanol (Eastman Chemical Co.) was fractionally distilled and the cut b_{23} 91–93° was collected. The 3-dimethylamino-1-propanol (Light's Organic Chemicals) was fractionated and the fraction b_{690} 185–190° was used. Thiophosgene (Matheson Coleman and Bell) was used without purification.

1-Dimethylamino-2-propanol was prepared in 30% yield by reacting dimethylamine with propylene oxide following Goldfarb's procedure,[5] b_{690} 116–119° (reported (5) b_{758} 124.5–126°), n_D^{17} 1.4115.

4-Chloro-1-butanol, b_4 62–66°, n_D^{20} 1.4490, was prepared in 50% yield following the method of Starr and Hixon (6).

4-Dimethylamino-1-butanol was prepared by reacting 4-chloro-1-butanol with aqueous dimethylamine at elevated temperature in a sealed system, as described below: a mixture of 4-chloro-1-butanol (109 Gm., 1 mole) and 30% aqueous dimethylamine (90 Gm., 2 moles) was placed in an autoclave and then gradually heated to 150°, causing the pressure to rise to 20 Atm. After 6 hours, heating was stopped and the reaction mixture was continuously extracted with ether during 4 days. The desired dimethylamino-butanol was isolated and purified by its fractionation through an efficient column, giving 56 Gm. (50% yield), of a colorless oil b_{20} 90–93°, n_D^{20} 1.4405 (Avison (7) reported b_{12} 78°, and Breckpot (8) reported n_D^{20} 1.4408).

The synthetic method used in this study involved: (*a*) the phosgenation of the appropriate ω-dialkylaminoalkanol (the resulting bis-dialkylaminoalkyl carbonate being not always isolated), followed by (*b*) quaternization of the corresponding base by means of methyl iodide.

Preparation of Bis-trialkylammonium-alkyl Carbonate Halogenides.—In a typical experiment, a cooled solution of 27 Gm. (0.27 mole) of phosgene in 135 ml. dry toluene was added with stirring to a solution of 4-dimethylamino-1-butanol (58.4 Gm., 0.4 mole) in dry toluene (100 ml.) at 0°, at such a rate that the temperature of the reaction mixture was maintained at 14 to 20°. After the addition was completed, the reaction mixture was stirred for an additional 2 hours, giving a heavy hygroscopic white precipitate (72 Gm.) of the required ester dihydrochloride. It was collected by quick filtration at the water pump, washed with dry ether, and then dissolved in dry ethanol. The free bis-dimethylaminobutyl carbonate was obtained by saturating the above ethanolic solution with dry gaseous ammonia at 0°. The free base, which is very soluble in ethanol, remained in the solvent; whereas the insoluble ammonium chloride separated, giving a white crystalline precipitate. Excess of ammonia was first removed by passing through the reaction mixture a stream of dry nitrogen and then the precipitate of ammonium chloride was removed by filtration. The free base was used in the subsequent step without being isolated.

Bis-trimethylammonium-butyl Carbonate Iodide.—To the resulting solution of bis-dimethylaminobutyl carbonate in ethanol (500 ml.) described above, there was added dropwise, with shaking, methyl iodide (80 Gm.) at such a rate that the temperature of the reacting system was maintained at 25°. After addition was completed, the reaction mixture was heated at 40° for 2 hours and then it was concentrated to half its volume by evaporating the solvent at reduced pressure. On cooling, the required bis-trimethylammonium-butyl carbonate iodide separated as a semisolid product. The purification of the bis-quaternary ammonium salt was effected by the dropwise addition of either *n*-butanol or dry ether to its stirred methanolic solution, providing 25 Gm. of the pure compound as a white powder.

Bis-trimethylammonium-butyl Thiocarbonate Iodide.—A solution of 4-dimethylaminobutanol-1 (20 Gm., 0.17 mole) in 100 ml. dry toluene was reacted with thiophosgene (10 Gm., 0.087 mole) dissolved in 50 ml. toluene in the fashion described above. After removing half of the amount of solvent by distillation at reduced pressure, the thiocarbonate derivative thus formed was not isolated but reacted with an excess of methyl iodide. The desired quaternary ammonium iodide could not be induced to recrystallize from ordinary solvents; it was purified by its repeated dissolution in methanol and precipitation by adding ether.

The melting points and yields of the compounds prepared are given in Table I.

PHARMACOLOGY

The experiments were performed on cats under pentobarbital anesthesia following induction with ether; a total of 34 animals was used. An external jugular vein was cannulated for injections, and a femoral or carotid artery for measurement of blood pressure. Respiration was recorded by means of a tambour connected to a tracheal cannula which was fitted with an adjustable air leak.

To test muscle excitability, an Achilles tendon was separated from the bone and connected to an isometric lever. The ipsilateral sciatic nerve was divided high in the thigh and its distal portion mounted on a pair of shielded silver electrodes, the distal electrode serving as cathode. The anode of a second pair of electrodes was attached to the gastrocnemius near its origin and the cathode placed on its tendon. Supramaximal square wave stimuli were administered with a Fleming stimulator; pulse width of indirect stimuli was 0.15 msec. and that of direct stimuli 1.2 msec. By means of relays, indirect and direct stimuli could be delivered alternately; they were applied either at a rate of 15/min. or in pairs, an indirect stimulus preceding a direct

[1] All m.p.'s were taken on Dr. Tottoli melting point apparatus and are uncorrected.

TABLE I.—PROPERTIES OF SOME BIS-TRIALKYLAMMONIUM-ALKYL CARBONATE DI-IODIDES

No.		M.p., °C.	Yield, %		Calculated C	Calculated H	Calculated I	Calculated N	Found C	Found H	Found I	Found N
1	Bis-trimethylammonium-ethyl carbonate	203–205	50–60	$C_{11}H_{26}I_2N_2O_3$	27.0	5.33	52.0	5.74	27.32	5.49	51.2	6.1
2	Bis-1-trimethylammonium-2-propyl carbonate	242	18	$C_{13}H_{30}I_2N_2O_3$	30.2	5.81	49.22	5.42	30.3	5.88	50.37	5.15
3	Bis-1-trimethylammonium-3-propyl carbonate	166–167	65	$C_{13}H_{30}I_2N_2O_3$	30.2	5.81	49.22	5.42	29.6	5.77	48.9	5.68
4	Bis-1-dimethylethylammonium-3-propyl carbonate	189	40	$C_{15}H_{34}I_2N_2O_3$	33.1	6.25	46.7	5.15	32.0	6.66	45.76	5.07
5	Bis-1-diethylmethylammonium-3-propyl carbonate	197–199	60	$C_{17}H_{38}I_2N_2O_3$	35.66	6.64	44.4	4.49	35.82	7.08	43.33	4.61
6	Bis-1-trimethylammonium-4-butyl carbonate	186	57	$C_{15}H_{34}I_2N_2O_3$	33.1	6.25	46.7	5.15	33.35	6.15	46.0	5.18
7	Bis-1-trimethylammonium-4-butyl thiocarbonate	280 (decompn.)	..	$C_{15}H_{34}I_2N_2O_2S$	32.0	6.10	45.3	5.0	31.80	6.28	46.0	5.3

one by 2 seconds and each pair being separated from the next by 6 seconds.

For preparations of the rectus abdominis muscle, *Rana ridibunda* was used.

RESULTS

The lower homolog in the series, compound I-1, in which the ammonium groups are separated by a linear chain of seven atoms, did not affect muscle excitability, but 0.2–0.5 mg./Kg., given intravenously, reduced blood pressure by 40–55 mg. Hg for a few minutes. Intramuscular injection of 16 mg./Kg. was ineffective (3 observations).

With the next higher homolog in this series, compound I-3, in which the ammonium groups are separated by a linear carbonate chain of nine atoms, inhibition of muscle excitability appeared while the hypotensive action receded (2 observations). As Fig. 1 shows, 0.3 mg./Kg. i.v. depressed neuromuscular transmission and, to a lesser extent, the response to direct stimulation and respiratory movements; 1 mg./Kg. abolished indirect excitability for 75 minutes, halved the response to direct stimulation for 50 minutes, and caused apnea for one-half hour.

The blocking effect on muscle was strongly enhanced in I-6 (16 observations). Sensitivity of the animals to this substance varied greatly: usually

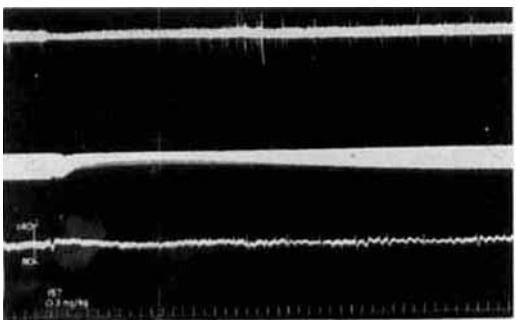


Fig. 1.—Cat, ether-pentobarbital anesthesia. Alternating direct and indirect supramaximal stimulation of gastrocnemius muscle. From top down: respiration, gastrocnemius twitch, blood pressure, time, in minutes, and signal. Effect of 0.3 mg./Kg. I-3 i.v.

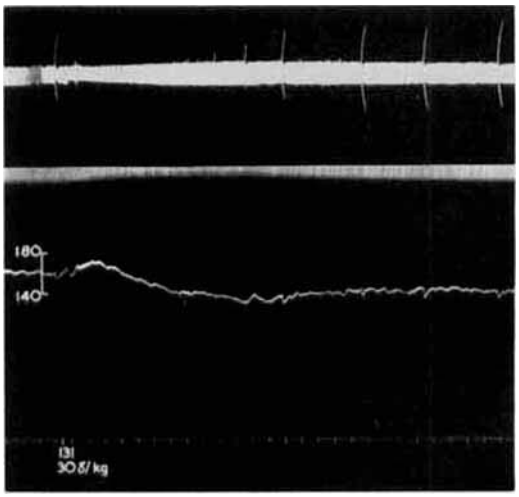


Fig. 2.—Arrangement as in Fig. 1. Effect of 30 mcg./Kg I-6 i.v.

15–30 mcg./Kg. produced only a partial block in the gastrocnemius and very slight depression of respiration (Fig. 2); 40 mcg./Kg. (Fig. 3) caused, after fast injection, potentiation of the twitch response and general fasciculations which lasted for a few minutes; neuromuscular transmission and spontaneous respiration were abolished and direct muscle excitability depressed for longer periods. Recovery of respiration was much quicker than that of the leg muscle.

Neostigmine did not restore neuromuscular transmission though two intravenous injections of 30 mcg./Kg. each, administered within 1 minute, stimulated respiration and speeded up return of excitability slightly.

The onset of the block after substance I-6 was similar to that after depolarizing agents, e.g., suxamethonium, in that it was characterized by augmentation of twitch height and by fasciculations; it also caused in the frog rectus abdominis a contraction whose speed rose with the dose administered without, however, approaching that observed after similar doses of acetylcholine (Fig. 4); tachyphylaxis did not appear with the amounts used.

The thio-derivative of the carbonic acid, I-7, proved to be about 100 times less effective as a

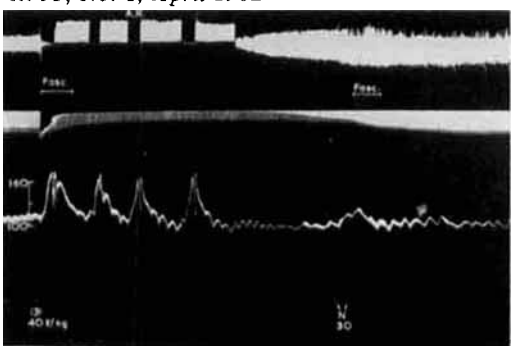


Fig. 3.—Arrangement as in Fig. 1. A.R., artificial respiration; Fasc., general fasciculations; N, 30 mcg./Kg. neostigmine i.v.; effect of 40 mcg./Kg. I-6 i.v.

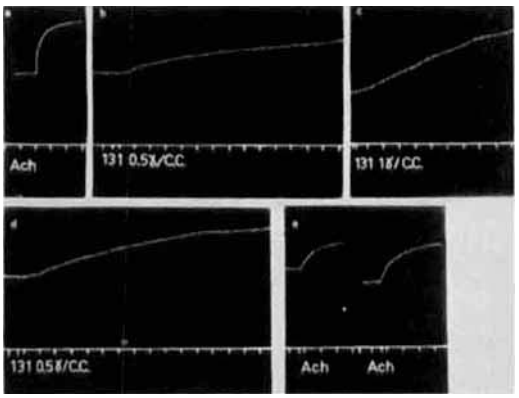


Fig. 4.—Effects of 0.5–1 mcg./ml. I-6 on frog rectus abdominis muscle compared to that of 0.7 mcg./ml. acetylcholine (Ach). Intervals between curves, 5 minutes when the muscle was washed three times. Time in min.

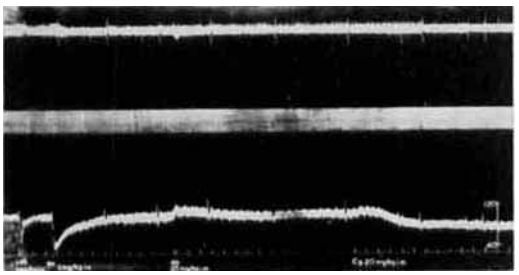


Fig. 5.—Arrangement as in Fig. 1. Effect of 1 and 5 mg./Kg. I-2 i.v. and of 25 mg./Kg. I-2 i.m. compared to that of 20 mg./Kg. hexamethonium i.m.

blocking agent than the parent compound (3 observations). About 3 mg./Kg. were required to produce effects on muscle excitability, respiration, and blood pressure similar to those observed after 15–40 mcg./Kg. of I-6.

Unlike lengthening of the chain in the linear homologs of the series, branching at the carbons linked to the oxygens resulted in compounds with diminished potency. Thus, 1–5 mg./Kg. of I-2, given i.v., produced a brief hypotension, but 25 mg./Kg., injected i.m., did not lower blood pressure (4 observations). In the experiment shown in Fig. 5 these effects are compared with that of hexamethonium. Substitution in the quaternary ammonium group of methyls by ethyls also weakened the biological actions. Thus I-4, which has one ethyl group linked to each ammonium group, had a shorter paralyzing action than the trimethyl analog, and the di-ethyl methyl analog (I-5) was even ten times less effective (3 observations).

DISCUSSION

The weak hypotensive action of I-1 and the absence of this effect in I-3 is in keeping with the observations made by Paton and Zaimis (9) on the cat's superior cervical ganglion.

As far as neuromuscular blocking potency was concerned, Paton and Zaimis' curve shows highest activity for decamethonium (C_{10}) and a steep fall for longer chain substances; for the series reported in this paper, the distance of 11 atoms resulted in a compound which was 10–20 times more powerful in depressing muscle excitability than the one whose chain was shorter by two carbons.

The decreasing tendency in curarizing action resulting either from substituting methyls for ethyls on the quaternary nitrogen or from branching of the polymethylene chain corresponds to that reported by Bovet and co-workers (10).

Augmentation of the twitch and general fasciculations which frequently appear immediately after injection of I-6 indicate that the block caused by this substance is of the depolarizing type. A comparable action is the failure of neostigmine to antagonize the block, which conforms to that described for decamethonium, and the stimulating action on the frog rectus abdominis, which corresponds to that of suxamethonium and other depolarizing agents (9, 11).

Unlike suxamethonium which undergoes partial hydrolysis upon keeping its aqueous solutions at room temperature (12), I-6 was found to be stable for at least 1 year under comparable conditions.

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