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Neuromuscular blocking agents: branched-chain tetra-onium compounds

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The compounds tri(6-dimethylaminohexyl)amine, tri(6-diethylaminohexyl)amine and tri(6-dipropylaminohexyl)amine have been synthesised and eight tetra-onium derivatives prepared from these bases have been tested for neuromuscular blocking activity on the cat, hen, frog, rat and mouse. Two of the compounds possessed ganglion blocking activity and this was much weaker than in hexamethonium. All the compounds had tubocurarine-like properties, the most active being 7-ethyl-7-(6-triethylhexylammonium)-7-azoniatridecamethylenebis(triethylammonium) tetra-iodide. The results of the pharmacological tests give little support for a one-point theory of receptor attachment.

RECENT discoveries of potent neuromuscular blocking activity in certain mono- and bis(quaternary ammonium) steroids has served to cast some doubt upon the validity of the classical concept of a two-point theory of receptor attachment (Barlow & Ing, 1948; Paton & Zaimis, 1949) and to re-emphasise the possibility of one-point drug-receptor interaction, together with the importance of other physico-chemical factors (e.g. adumbration and water/fat solubility ratios) known to influence this type of pharmacological activity (Cavallito & Gray, 1960). Thus certain 3,17-bis(quaternary ammonium) androstanes (May & Baker, 1963; Martin-Smith & Sugrue, 1964) in which the quaternary heads lie on opposite sides of the steroid nucleus, possess potent neuromuscular blocking properties while qualitatively similar, weaker, non-depolarising effects are observed among several monoquaternary steroids (Martin-Smith & Sugrue, 1964; Blanpin & Bretaudeau, 1961; Ross & Lewis, 1965, personal communication). It is interesting to note that to explain the activity of a series of tetra(dimethylaminomethyl)methane tetramethobromide derivatives possessing neuromuscular blocking properties, a one-point receptor-site attachment has also been suggested (Kensler, Langemann & Zirkle, 1954). Additionally, using a series of bis-choline ether salts, evidence has been obtained to support a theory of a one-point receptor attachment for ganglion blocking activity (Fakstorp, Pedersen, Poulsen & Schilling, 1957; Fakstorp & Pedersen, 1957).

The present investigation, having a bearing on the problem of a one-point attachment, concerned a series of branched-chain tetra-onium compounds where one of the four quaternary nitrogen atoms was separated from each of the other three by a chain of six methylene groups (I). The structure of these compounds would permit drug-receptor interaction to be maintained either by a single (one-point) onium head or alternatively by a two-point attachment involving the central onium head and one of the terminal ones, or by two of the terminal onium heads.

In addition to their possible neuromuscular blocking potential, suggested by their structural similarity to several poly-onium derivatives of established activity, the present series of compounds was also tested for ganglion

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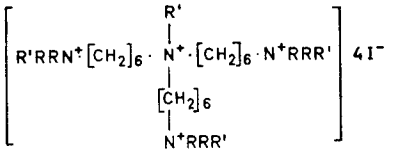
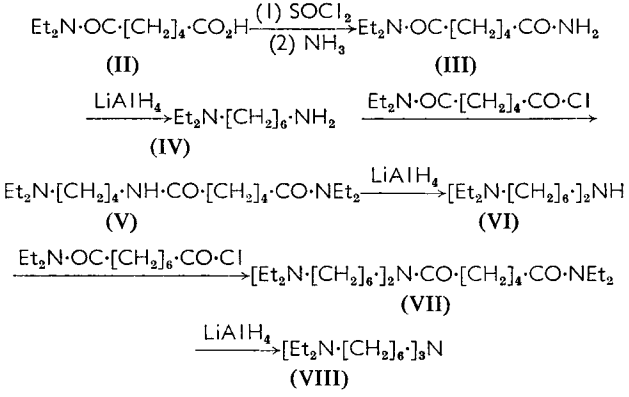


(I)

blocking properties, since they resembled hexamethonium in that a hexamethylene chain separated the central nitrogen atom from each of the terminal ones. In the course of both investigations the well-known influence on biological activity brought about by altering alkyl groups attached to the onium centres was also examined.



CHEMICAL

The compounds described in the experimental section were synthesised as shown in the flow sheet and the final bases (VIII) quaternised with the appropriate alkyl halides to yield the tetra-onium compounds (I)—see also Table 1.

EXPERIMENTAL

Methyl NN-*dimethyladipamate* was prepared from methyl hydrogen adipate (241 g) by the method described for the preparation of ethyl *NN*-diethyladipamate (Edwards & Stenlake, 1955), except that the solution of the acid chloride was added to the solution of dimethylamine. The *product* was obtained as a straw-coloured oil, b.p. 119–121°/0·3 mm, $n_D^{22\cdot5}$ 1·4588 (275 g, 97·6%). Found C, 57·9; H, 8·85; N, 7·7; $C_9H_{17}NO_3$ requires C, 57·75; H, 9·2; N, 7·5%.

Methyl NN-*dipropyladipamate*, prepared from methyl hydrogen adipate (124·5 g) by the above method, was obtained as a yellow oil, b.p. 137°/0·5 mm, n_D^{11} 1·4605 (177·5 g, 93·9%). Found: C, 63·5; H, 10·2; N, 5·9. $C_{13}H_{25}NO_3$ requires C, 64·2; H, 10·4; N, 5·8%.

NN-*Dimethyladipamic acid*, prepared from methyl *NN*-dimethyladipamate (187 g) by the method described for the preparation of *NN*-diethyladipamic acid (Carey, Edwards, Lewis & Stenlake, 1959), was

obtained as a colourless oil, b.p. 164°/0·25 mm, which solidified to a greyish-white hygroscopic solid, m.p. 45° (138 g, 79·7%). Carey, Furst, Lewis and Stenlake (1964) report b.p. 178°/0·05 mm. Found: C, 54·8; H, 8·9; equiv. 172·7. Calc. for $C_8H_{15}NO_3$: C, 55·5; H, 8·7%; equiv. 173·2.

NN-*Dipropyladipamic acid*, prepared from methyl *NN*-dipropyladipamate (177·5 g) by the above method, was obtained as a yellow viscous oil, b.p. 186°/0·2 mm, $n_D^{13·5}$ 1·4763 (158·2 g, 94·6%). Carey, Edwards, Lewis & Stenlake, 1959, found b.p. 198°/0·5 mm, n_D^{25} 1·4723).

NN-*Diethylhexamethylenediamine*. *NN*-Diethyladipamic acid (41·8 g) (Carey & others, 1959), in benzene (30 ml), was refluxed with excess of thionyl chloride (20 ml) for 15 min. After removal of the solvent and excess of reagent, the acid chloride in benzene (20 ml), was added slowly (45 min) to a stirred excess of strong ammonia solution (80 ml, sp.gr. 0·880), cooled to 0°. After standing for 18 hr, the mixture was evaporated to dryness under reduced pressure, the residue extracted with ethanol, and the extract filtered and evaporated. Extraction of this residue with hot benzene gave crude *NN*-diethyladipamide as a brown solid (38 g). The amide, in hot benzene (40 ml) was added slowly (25 min) to a stirred refluxing suspension of lithium aluminium hydride (16 g) in ether (800 ml) and the refluxing continued for 22 hr. The mixture was worked up and extracted in the usual manner to yield *NN*-diethylhexamethylenediamine as a colourless oil, b.p. 155°/15 mm, n_D^{19} 1·4545 (16·6 g, 46·4%). Breslow & Houser (1945) report b.p. 103–105°/10 mm. Found: C, 69·5; H, 13·8. Calc. for $C_{10}H_{24}N_2$: C, 69·8; H, 13·95%.

NN-*Dimethylhexamethylenediamine*, prepared from *NN*-dimethyladipamic acid (88·5 g) by the above method, was obtained as a colourless oil, b.p. 89–91°/13 mm, n_D^{16} 1·4440 (30·4 g, 42%). Short, Biermacher, Dunnigan & Leth (1963) report b.p. 108-109°/32 mm, n_D^{25} 1·4423. Found: C, 66·6; H, 14·0. Calc. for $C_8H_{20}N_2$: C, 66·5; H, 13·9%.

NN-*Dipropylhexamethylenediamine*, prepared from *NN*-dipropyladipamic acid (70 g) by the above method, was obtained as a colourless oil, b.p. 140–143°/15 mm, n_D^{20} 1·4545 (28·2 g, 46·1%). French, Ugnade, Poe & Eilers (1945) report b.p. 97–99°/1 mm. Found: C, 72·3; H, 14·1; N, 13·7. Calc. for $C_{12}H_{28}N_2$: C, 72·1; H, 14·0; N, 14·0%.

Di(6-diethylaminohexyl)amine. *NN*-Diethyladipamic acid (28·7 g), in benzene (21 ml), was refluxed with excess of thionyl chloride (14 ml) for 20 min, and the solvent and excess of reagent evaporated off under reduced pressure. An excess of *NN*-diethylhexamethylenediamine (24·5 g), in benzene (50 ml), was added slowly (30 min) to a stirred solution of the acid chloride in benzene (50 ml) and the mixture refluxed for 30 min. After cooling, water (50 ml) was added, the mixture basified by the addition of an excess of sodium hydroxide solution and then extracted with benzene. The benzene extracts, after drying (Na_2SO_4) and removal of solvent and most of the unchanged *NN*-diethylhexamethylenediamine, yielded crude *N*-diethylaminohexyl-*N'N'*-diethyladipamide (22 g), which was reduced in the normal manner with lithium aluminium hydride in ether. Fractional distillation of the product gave di(6-*diethylaminohexyl*) *amine* as a colourless oil, b.p. 182–184°/1 mm, $n_D^{21·5}$ 1·4620 (14·4 g,

30·8%). Found: C, 73·4; H, 13·7; N, 12·8. $C_{20}H_{45}N_3$ requires C, 73·3; H, 13·7; N, 12·8%.

Di(6-*dimethylaminohexyl*)*amine*, prepared from *NN*-dimethyladipamic acid (17·7 g) and excess of *NN*-dimethylhexamethylenediamine (29 g) by the above method, was obtained as a colourless oil, b.p. 141–145°/0·3 mm, n_D^{20} 1·4595 (11·6 g, 41·8%). Found: C. 70·5 H, 13·7. $C_{16}H_{33}N_3$ requires C, 70·8; H, 13·6%.

Di(6-*dipropylaminohexyl*)*amine*, prepared from *NN*-dipropyladipamic acid (22·3 g) and excess of *NN*-dipropylhexamethylenediamine (39 g) by the above method, was obtained as a colourless oil, b.p. 190–195°/0·3 mm, n_D^{17} 1·4628 (22·7 g, 60·8%). Found: C, 74·5; H,13·7; N, 11·1. $C_{24}H_{53}N_3$ requires C, 75·0; H, 13·8; N, 10·9%.

Tri(6-*diethylaminohexyl*)*amine* was prepared from *NN*-diethyladipamic acid (8·6 g) and di (6-diethylaminohexyl)amine (14 g) by a similar method to that described above for the preparation of the latter compound. Fractional distillation of the reduction product yielded *tri*(6-*diethylaminohexyl*)*amine* as a colourless oil, b.p. 215–220°/0·7 mm, $n_D^{12·5}$ 1·4678 (7·7 g, 37·3%). Found: C, 74·95; H, 13·65; N, 11·5; equiv. 120·3. $C_{30}H_{66}N_4$ requires C, 74·6; H, 13·7; N, 11·6%; equiv. 120·7.

Tri(6-*dimethylaminohexyl*)*amine*, prepared from *NN*-dimethyladipamic acid (7·5 g) and di(6-dimethylaminohexyl)amine (11·5 g) by the above method, was obtained as a colourless oil, b.p. 173–176°/0·2 mm, n_D^{18} 1·4656 (3.5 g 20·3%). Found: C, 71·95; H, 13·4, $C_{24}H_{54}N_4$ requires C, 72·4; H, 13·6%.

Tri(6-*dipropylaminohexyl*)*amine*, prepared from *NN*-dipropyladipamic acid (13·2 g) and di(6-dipropylaminohexyl)amine (22·2 g) by the above method, was obtained as a colourless oil, b.p. 250–260° (bath)/0·1 mm, $n_D^{15·5}$ 1·4668 (14·8 g, 45·3%). Found: C, 76·0; H,14·1; N, 9·8. $C_{36}H_{78}N_4$ requires C, 76·3; H, 13·8; N, 9·9%.

Tetra-onium compounds were prepared from either tri(6-diethylaminohexyl)amine, tri(6-dimethylaminohexyl)amine or tri(6-dipropylaminohexyl)amine by refluxing with the appropriate alkyl halide in ethanol, evaporating off the solvent and crystallising the product. Reflux time, crystallisation solvent and yields are indicated for each compound in that order, in parenthesis.

7-(6-*Diethylhexylmethylammonium*)-7-*methyl*-7-*azoniatridecamethylene-bis*(*diethylmethylammonium*) *tetraiodide*. (25 min; ethanol; 97%), m.p. 262°. Found: I, 48·5; N, 5·5; $C_{34}H_{78}I_4N_4$ requires I, 48·3; N, 5·3%.

7-*Ethyl*-7-(6-*triethylhexylammonium*)-7-*azoniatridecamethylenebis*(*triethylammonium*) *tetraiodide*. (50 min; ethanol-ether; 81%), m.p. 266°. Found: I, 45·7; N, 5·1. $C_{38}H_{86}I_4N_4$ requires I, 45·85; N, 5·1%.

7-(6-*Diethylhexylpropylammonium*)-7-*propyl*-7-*azoniatridecamethylene-bis*(*diethylpropylammonium*) *tetraiodide*. (1 hr; acetone-ethanol-ether; 60%), m.p. 167°. Found: C, 43·2; H, 7·9; I, 43·25; N, 4·8. $C_{42}H_{94}I_4N_4$ requires C, 43·4; H, 8·1; I, 43·6; N, 4·8%.

7-(6-*Hexyltrimethylammonium*)-7-*methyl*-7-*azoniatridecamethylenebis*(*trimethylammonium*) *tetraiodide*. (5 min, ethanol; 70%), m.p. 227° (hygro-

scopic). Found: C, 34·1; H, 7·1; I, 51·9, N, 5·25. $C_{28}H_{66}I_4N_4$ requires C, 34·8; H, 6·9; I, 52·5; N, 5·2%.

7-Ethyl-7-(6-ethylhexyldimethylammonium)-7-azoniatridecamethylenebis-(ethyldimethylammonium) tetraiodide. (35 min, ethanol; 74%), m.p. 228°. Found: C, 36·7; H, 7·5; I, 49·6; N, 5·4. $C_{32}H_{74}I_4N_4$ requires C, 37·6; H, 7·3; I, 49·6; N, 5·5%.

7-(6-Hexyldimethylpropylammonium)-7-propyl-7-azoniatridecamethylenebis(dimethylpropylammonium) tetraidodide. (1 hr; acetone-ethanol-ether; 84%), m.p. 184°. Found: C, 39·7; H, 7·7; I, 46·8. $C_{36}H_{82}I_4N_4$ requires C, 40·1; H, 7·7; I, 47·0%.

7-(6-Hexylmethyldipropylammonium)-7-methyl-7-azoniatridecamethylenebis(methyldipropylammonium) tetraiodide. (18 hr without refluxing; ethanol; 95%), m.p. 220°. Found: C, 42·6; H, 8·5; I, 44·4; N, 4·8. $C_{40}H_{90}I_4N_4$ requires C, 42·3; H, 8·0; I, 44·7; N, 4·9%.

7-Ethyl-7-(6-ethylhexyldipropylammonium)-7-azoniatridecamethylenebis-(ethyldipropylammonium) tetraiodide. (40 min; acetone-ethanol-ether; 24%), m.p. 197°. Found: C, 43·9; H, 8·1; I, 42·1; N, 4·8. $C_{44}H_{98}I_4N_4$ requires C, 44·4; H, 8·3; I, 42·6; N, 4·7%.

PHARMACOLOGICAL

Methods. The evaluation of neuromuscular blocking activity was made using conventional techniques previously described, including (Edwards, Lewis, Stenlake & Zoha, 1957, 1958; Edwards, Lewis, Stenlake & Stothers, 1961) the cat and hen gastrocnemius muscle-sciatic-nerve, the rat phrenic-nerve diaphragm, the frog rectus abdominis muscle and the mouse inclined-screen method (Thomson, 1946). Sympathetic ganglion-blocking activity was estimated using the nictitating membrane preparation of the cat and parasympathetic ganglion blockade by the guinea-pig ileum peristaltic reflex experiment (Trendelenburg) (Feldberg & Lin, 1949). Toxicity measurements on mice employed the inclined-screen method.

Results. Table 1 shows the comparative molar potencies of the compounds compared with tubocurarine (TC) on the frog, the rat, the mouse, the cat and the hen.

All eight compounds possessed varying degrees of muscle relaxant activity of the non-depolarising variety, there being no evidence of any depolarising properties. Even the methyl analogue (compound IA) caused no contracture of the hen gastrocnemius muscle when used in doses producing approximately 85% inhibition of twitch height. None of the compounds showed significant effects on the blood pressure of the urethane/pentobarbitone anaesthetised rat. Weak ganglion blocking activity existed in only two of the compounds investigated (compounds IB and IC). The all-methyl derivative (compound IA) was not active in this respect, and the active compounds were approximately 2·5–13% as active as hexamethonium on a molar basis.

The therapeutic ratio (LD50/PD50) for all the compounds tested was higher than that for tubocurarine and variation in the response of different species to any one compound was marked. The general order of increasing sensitivity to the compounds was rat, mouse, frog, cat, hen.

Discussion

The compounds investigated bore some structural and pharmacological resemblance to those of Kensler, Zirkle, Matallana & Condouris (1954) (IX, n = 2,3 or 4 and R = Me or Et). In keeping with these and numerous other observations conducted on linear tri- and tetra-onium compounds (Carey & others, 1959; Edwards, Lewis, McPhail, Muir & Stenlake, 1960), the all-ethyl compound (IE) (Table 1) was found to be the most active in the majority of species examined. Its high potency in the hen again emphasises the marked variation between mammalian and avian species (Blaber & Bowan, 1962).

$$[(RRRN^{+}\cdot[CH_2]_n\cdot)_3CH]3Br^{-} \qquad \text{(IX)}$$

Replacement of ethyl groups by methyl or propyl groups led to a reduction in neuromuscular activity in all species except rodents (Table 1),

TABLE 1. PHARMACOLOGICAL ACTIVITY

$$[(RRR'N^{+}\cdot[CH_2]_6\cdot)_3N^{+}R']4I^{-}$$

Compound	Substituents		Relative molar potencies (TC = 100)					Type of activity
	R	R'	Cat	Hen	Frog	Rat	Mouse	
IA	Me	Me	14	63	18	0·42	9·1	TC-like
IB	Me	Et	30	190	34	0·45	17	TC-like
IC	Et	Me	13	45	31	1·0	5·9	TC-like
ID	Me	Pr	26	78	15	0·71	14	TC-like
IE	Et	Et	79	815	35	0·67	12	TC-like
IF	Pr	Me	27	98	12	0·46	14	TC-like
IG	Et	Pr	66	310	22	0·87	49	TC-like
IH	Pr	Et	51	272	11	0·19	16	TC-like

but none of the compounds, not even the trimethyl compound (IA), showed decamethonium-like properties. This lack of depolarising activity in related compounds, where ethyl groups have replaced methyl groups, has been attributed (Carey & others, 1959) to the considerable shielding of the charge on the nitrogen atom which would prevent the close approach to the receptor required for this type of activity (Paton, 1961).

Using Courtauld atomic models the maximum distance measured between the central nitrogen atom and any other nitrogen atom is about 9Å and between terminal nitrogen atoms, with a tetrahedral arrangement of groups attached to the central nitrogen atom, about 14·7 Å Since it seems unlikely that the effect on internitrogen distance of alkyl substituents in the onium heads in the present series will be as pronounced as that in some bis-quaternary compounds (Elworthy, 1963, 1964), and since the molecules are not rigid structures, two-point attachment could be made on anionic receptors at any distance up to 9 Å by means of a central nitrogen atom and one of the terminal ones, or up to 14·7 Å by any two of the terminal ones. Although the distance of 9 Å approaches the mean distance of 9·5 Å calculated by Elworthy (1963) for decamethonium, the structure of these molecules being as they are, it cannot be assumed that drug-receptor interaction is occurring at a central nitrogen atom and one of the terminal ones.

The virtual absence of ganglion blocking activity in these compounds is

in agreement with a similar lack of this activity in related compounds (Edwards & others 1958; Carey & others, 1959).

It is interesting to note that the most potent member was the diethylmethyl derivative (IC); this is unlike hexamethonium analogues where the ethyldimethyl derivative was found to be the most potent (Wien, Mason, Edge & Langston, 1952).

The present results give little supporting evidence for a one-point theory of receptor attachment. Neither the all-methyl (compound IA) nor the all-ethyl (compound IE) derivative shows properties resembling respectively those of the classic mono-quaternary compounds tetramethylammonium or tetraethylammonium. Although the adumbrating effect of the remainder of the molecule must be taken into account, it is difficult with a one-point theory of receptor attachment to explain the absence of ganglion blockage in compound IE and of ganglion stimulating properties in compound IA.

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