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Neuromuscular blocking agents: alkyl and heterocyclic analogues of simple linear trisonium compounds

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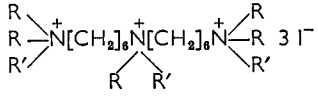
A further series of linear hexamethylene-*NNN*-trisonium compounds has been synthesised and tested for neuromuscular blocking activity. In the alkyl-substituted derivatives, at least one Et is required for significant potency in mammals. Apart from the Et_2Bu^n and $EtBu^n_2$ compounds, larger alkyl substituents lower potency. High potency in mammals requires at least two Et groups on the terminal nitrogens, if the third group is not Me. Only weak activity was shown by the morpholino-deriatives but the fowl was highly sensitive towards the piperidino- and tetrahydropapaverino-compounds. The results are discussed.

THE effect of varying alkyl substitution upon the neuromuscular blocking activity in the linear hexamethylene-*NNN*-trisonium compounds (I) has been described earlier (Edwards, Lewis, Stenlake & Zoha, 1958; Carey, Edwards, Lewis & Stenlake, 1959; Edwards, Lewis, McPhail, Muir and Stenlake, 1960; Edwards, Stenlake, Lewis & Stothers, 1961). Further compounds of this type have now been synthesised with the object

(I) 

of completing the series and investigating the effect on the neuromuscular blocking potency of this basic structure produced by progressively substituting methyl, ethyl, n-propyl and n-butyl groups at R and R′.

It is well established that bis-onium compounds, in which the onium ion forms part of a heterocyclic system, are tubocurarine-like rather than decamethonium-like (Stenlake, 1963). Tris-onium compounds of structure (I) in which the terminal onium groups incorporate piperidinium, morpholinium and tetrahydropapaverinium substituents have now been prepared, and their potencies recorded.

Chemical

Two routes to the intermediate bis-6-dialkylaminohexylalkylamines (II) have been described earlier (Edwards, Lewis, Stenlake & Zoha, 1958;

$R_2N{\cdot}[CH_2]_6{\cdot}N(R'){\cdot}[CH_2]_6{\cdot}NR_2$ (II) $R_2N{\cdot}[CH_2]_6{\cdot}Br$ (III) $R_2N{\cdot}[CH_2]_6{\cdot}NHR'$ (IV)

$R_2N{\cdot}CO{\cdot}[CH_2]_2COCl$ (V) $R_2N{\cdot}CO[CH_2]_4{\cdot}CO{\cdot}N(R'){\cdot}[CH_2]_6{\cdot}NR_2$ (VI)

Carey, Edwards, Lewis & Stenlake, 1959). The method described

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in the latter publication was adopted in the present work, though an alternative route to the diamines (IV) through the corresponding *NNN'* - trialkyladipamides (VII) has been examined.

$R_2N{\cdot}CO[CH_2]_4CONHR'$ (VII) $R_2N{\cdot}CO{\cdot}[CH_2]_4{\cdot}COOH$ (VIII)

The adipamic acids (VIII) (R = Me, Bu^n, piperidyl, morpholinyl, or tetrahydropapaverinyl) were prepared from ethyl hydrogen adipate by methods similar to those previously described (Carey & others, 1959). The adipamic acids are all freely soluble in cold water and can be isolated only by keeping the volume of hydrolysate to a minimum and extracting continuously with an organic solvent. Traces of contaminating adipic acid can be removed by making use of its insolubility in benzene.

In contrast to *NN*-diethyl- and *NN*-dipropyladipamic acids, both the *NN*-dimethyl and *NN*-dibutyl compounds partly disproportionated to adipic acid and the corresponding bis-amide on vacuum distillation. This occurred when an air-leak was used to promote even boiling but with an orange stick in place of an air-leak, distillation was accomplished without disproportionation. Similar observations on the disproportionation of *NN*-diethyldiglycollamic acid have been reported by Edwards, Lewis, McPhail, Muir & Stenlake (1960) and on *N*-substituted adipamic and succinamic acids by Prelog (1930).

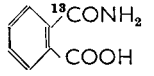
When *NN*-dibutyladipamic acid was heated (1 hr) at 250° either under nitrogen or in presence of air, both conditions led to the same amount of disproportionation. Reaction temperature also appears to be unimportant, since the distillation temperature of *NN*-dimethyladipamic acid (178°/0·05 mm) and *NN*-dibutyladipamic acid (198°/0·03 mm) do not differ markedly from those of *NN*-diethyladipamic acid (182°/0·05 mm) and *NN*-dipropyladipamic acid (198°/0·5 mm) which do not disproportionate.

NN-Dibutyladipamic acid was not formed when an equimolecular mixture of adipic acid and *NNN'N'*-tetrabutyladipamide was heated at 150° for 1½ hr either under nitrogen, or with air being drawn through the mixture. The reaction therefore is not reversible. The mechanism is probably one of intramolecular catalysis, several analogous cases being well-established.

Leach & Lindley (1953) observed the hydrolysis of the terminal amide links in glycyl-L-asparagine (IX, R = H) and L-leucyl-L-asparagine (IX, $R = (CH_3)_2CH{\cdot}CH_2$) in aqueous solution between pH 1·2 and 3·5. Both reactions were of first order in $\overset{+}{N}H_3{\cdot}CHR{\cdot}CONH{\cdot}CH(CH_2{\cdot}CONH_2){\cdot}COOH$ and were independent of the external hydrogen ion concentration in this pH range, indicating that the undissociated carboxyl group is the reacting species. Together with the small negative entropies of activation this is consistent with an intramolecular reaction involving proton transfer from the unionised carboxyl to the terminal amide link. A six-membered hydrogen-bonded ring structure was postulated for the peptides in solution. Phthalamic acid (X) is likewise hydrolysed 10^5 times faster than benzamide

at pH 3 and the rate of reaction is independent of pH between pH 1·3 and

$NH_2{\cdot}CHR{\cdot}CONH{\cdot}CH(CH_2{\cdot}CONH_2){\cdot}COOH$ (IX)



(X)

2·6 (Bender 1957). Hydrolysis of a ^{13}C-labelled phthalamic acid (as X) with $H_2{}^{18}O$ (Bender, Chow & Chloupek, 1958) yields phthalic acid which, on decarboxylation, forms both $^{13}C^{16}O^{18}O$ and $^{12}C^{16}O^{16}O$. The carboxyl carbonyl group thus behaves as a bifunctional catalyst, simultaneously attacking the carbonyl atom of the amide and donating a proton to the departing ammonia molecule with the formation of the anhydride. A similar mechanism also appears to operate in the hydrolysis of *NN*-dimethylaminomaleamic acid (Dahlgren & Simmerman, 1963).

The infra-red spectra of all four adipamic acids in carbon tetrachloride show evidence of intramolecular hydrogen bonding (submaxima at 2,650 cm^{-1}) which would appear to favour anhydride formation by a mechanism similar to that postulated by Bender (1957) for the phthalamic acids. No satisfactory explanation of the stability of *NN*-diethyl and *NN*-dipropyl adipamic acids relative to that of the *NN*-dimethyl and *NN*-dibutyladipamic acids can be advanced on the evidence available at present.

The adipamic acids (VIII) were converted to the corresponding diamides in the usual way by reacting the acid chloride with the appropriate base. Reduction of the diamides with lithium aluminium hydride in ether or ether-tetrahydrofuran gave the required diamines (IV). 6-Dimethylaminohexylmethylamine, 6-di-n-butylaminohexyl-n-butylamine, 6-(1′-piperidino) hexylethylamine, 6-(1′-morpholino)hexylethylamine and 6-(2′ tetrahydropapaverinyl)hexylethylamine, obtained in this way, were each condensed with the corresponding adipamic acid chloride (V) and the product reduced with lithium aluminium hydride to yield bis(6-dimethylaminohexyl)methylamine, bis(6-di-n-butylaminohexyl)n-butylamine, bis-(6-morpholinohexyl)ethylamine and bis(6,2′-tetrahydropapaverinylhexyl)-ethylamine respectively. The quaternary ammonium salts (Ia–Im) shown in Table 1 were obtained in the usual way. Trichloroacetic acid which has been reported (Vilsmeier, 1958) to give highly crystalline double salts of the type $R_4N^+Cl_3C{\cdot}CO{\cdot}O^-Cl_3C{\cdot}COOH$ from hygroscopic mono- and bis-quaternary halides gave only oily products with the hygroscopic piperidinium and morpholinium halides examined.

Experimental

Ethyl NN-*dimethyladipamate.* Ethyl hydrogen adipate (40 g) was refluxed (1½ hr) at 90–100° with excess thionyl chloride (50 ml). Excess thionyl chloride was removed in the usual way. The crude acid chloride in dry ether (50 ml) was slowly added to a stirred solution of anhydrous dimethylamine (35 g) in dry ether (300 ml) at 0°. The reaction mixture was then refluxed for one hr. The precipitated dimethylamine hydrochloride was filtered off, washed with more ether and the filtrate and

washings were extracted with water, then with aqueous sodium carbonate and finally dried (Na_2SO_4). Removal of the ether and distillation gave the required product as a pale-yellow oil (36·5 g, 79%) b.p. 128°/1·5 mm, n_D^{24} 1·4560. (Andrews, Bergel & Morrison, 1953, found b.p. 102–106°/0·25 mm, n_D^{20} 1·4573). Found: N, 7·0. Calc. for $C_{10}H_{19}NO_3$: N, 7·0%.

The following amide esters were similarly prepared from ethyl hydrogen adipate, except that the acid chloride was added to the reaction vessel at room temperature and, during the extraction procedure, the ethereal solution was first washed with dilute hydrochloric acid to remove excess of the secondary amine.

Ethyl NN-*di-n-butyladipamate*, yellow oil (52 g, 93%), b.p. 146°/0·1 mm, n_D^{16} 1·4580. Johnson (1958), quotes b.p. 136–138°/0·15 mm, n_D^{16} 1·4569. Found: N, 4·9. Calc. for $C_{16}H_{31}NO_3$: N, 4·9%.

Ethyl N-*adipoylpiperidine*, yellow oil (60·5 g, 78%), b.p. 166–167°/0·2 mm. Avison (1951) gives b.p. 148–152°/0·5 mm. Found: N, 6·0. Calc. for $C_{13}H_{23}NO_3$: N, 5·8%.

Ethyl N-*adipoylmorpholine*, pale yellow oil (52 g, 71%), b.p. 177–183°/0·1 mm. Found: N, 5·8. $C_{12}H_{21}NO_4$ requires N, 5·8%.

6-*Hydroxyhexyldimethylamine.* Ethyl *NN*-dimethyladipamate (21 g) in dry ether (50 ml) was added slowly to a stirred, refluxing suspension of lithium aluminium hydride (5 g) in dry ether (120 ml) and the solution was refluxed for 5 hr. The reaction mixture was cooled in an ice-bath and brine added cautiously to decompose the complex and excess lithium aluminium hydride. Sodium hydroxide (20% solution, 100 ml) was added with stirring to produce a gel from which the ethereal supernatant was decanted. The gel was extracted with more ether (2 × 200 ml) and the combined ether extracts dried (Na_2SO_4). Evaporation of the solvent yielded a mobile liquid which was distilled to give the product as a colourless oil (10 g, 66%), b.p. 70°/5 mm, n_D^{20} 1·4485. Andrews & others, 1953, give b.p. 114–116°/12 mm, $n_D^{20·5}$ 1·4482. Found: N, 9·5; titration equivalent 148·3. Calc. for $C_8H_{19}NO$: 9·7%; equiv. 145·2.

The *hydrobromide*, recrystallised from ethanol-ether in white leaflets, m.p. 85–88°. Found: N, 6·0; Br, 35·4. $C_8H_{20}BrNO$ requires N, 6·2; Br, 35·8%.

The *methiodide.* Methyl iodide (1 ml) was slowly added to the base (0·6 g) in ether (4 ml). Recrystallisation of the dense white precipitate (ethanol-ether) gave a quantitative yield of white leaflets, m.p. 126°. Found: N, 4·9; I, 44·4. $C_9H_{22}INO$ requires N, 4·9; I, 44·25%.

NN-*Dimethyladipamic acid.* Ethyl *NN*-dimethyladipamate (12·5 g) was refluxed (1 hr) with ethanolic potassium hydroxide (approx. 2/3 N; 135 ml), the solution cooled and just neutralised with dilute hydrochloric acid. Ethanol was removed under reduced pressure and benzene (2 × 50 ml) added and similarly evaporated to remove the last traces of ethanol. The residual potassium salt was acidified with hydrochloric acid (20 ml, 25%) and the precipitated potassium chloride filtered off. Continuous extraction of the filtrate with ether gave the required *acid* as a viscous grey oil which was not distilled (8·2 g, 76%). Found: N, 7·9; titration equivalent 175·6. $C_8H_{15}NO_3$ requires N, 8·1; equiv. 173·2. Subsequent

distillation of the acid gave a yellow oil (b.p. 178°/0·05 mm, equiv. 172·2) which crystallised as rosettes of thick white needles after several months.

NNN′-*Trimethyladipamide.* Thionyl chloride (8 ml) in benzene (30 ml) was added to *NN*-dimethyladipamic acid (10 g) suspended in benzene (70 ml) and the excess reagent and the solvent were removed almost immediately below 50° under reduced pressure: benzene (20 ml) was added and removed in the same way. The sparingly soluble crude *NN*-dimethyladipamoyl chloride suspended in benzene (50 ml) was stirred at 0° and dry methylamine passed in for 2 hr until the uptake was complete. The precipitated methylamine hydrochloride was filtered off, washed with benzene and the combined benzene extracts evaporated to leave a yellow oil (10·5 g, 98%) which crystallised rapidly. The *product* crystallised from dry acetone-ether in fine colourless needles, m.p. 54–56° which were collected under nitrogen. Alternatively the oil distilled, b.p. 218–220°/0·2 mm. Found: N, 14·8. $C_9H_{18}N_2O_2$ requires N, 15·0%. The diamide was extremely hygroscopic and almost insoluble in ether.

6-*Dimethylaminohexylmethylamine.* *NNN′*-trimethyladipamide (6·4 g) in dry tetrahydrofuran (30 ml) was added over 25 min to a stirred refluxing suspension of excess lithium aluminium hydride (5 g) in tetrahydrofuran (60 ml). Refluxing was continued for 5 hr and the excess reagent was decomposed with water. The supernatant tetrahydrofuran was decanted and the gel extracted with several further volumes of tetrahydrofuran (250 ml). The combined extracts were dried (Na_2SO_4), the solvent removed and the product distilled to give 6-*dimethylaminohexylmethylamine*, as a colourless, mobile oil (3·0 g, 55%), b.p. 78°/0·2 mm. Found: N, 17·4; titration equivalent 79·2. $C_9H_{22}N_2$ requires N, 17·7%; titration equivalent 79·1.

Bis(6-*dimethylaminohexyl*)*methylamine.* *NN*-Dimethyladipamic acid (2·9 g) was treated with thionyl chloride (1·8 ml) as previously described, and a suspension of the acid chloride in benzene (18 ml) was added slowly to a stirred, refluxing solution of 6-dimethylaminohexylmethylamine (5·5 g) in benzene (35 ml). After refluxing for a further 30 min, the reaction mixture was extracted with dilute hydrochloric acid (2 × 25 ml), the acid solution basified with sodium hydroxide (35 ml, 20%), and extracted with benzene (4 × 50 ml). The benzene solution was dried (Na_2SO_4) and the brown mobile oil (6·8 g) which remained after evaporation of the solvent gave, on distillation, some 6-dimethylaminohexylmethylamine (*ca.* 0·75 g), b.p. 58–60°/0·1 mm. The crude undistilled *N*-dimethylaminohexyl-*NN′N′*-trimethyladipamide in ether was reduced with lithium aluminium hydride (1·5 g) and the product extracted as described for 6-hydroxyhexyldimethylamine. Fractional distillation gave a fore-run of the diamine (1·8 g), and *bis*(6-*dimethylaminohexyl*)*methylamine*, as a pale-yellow oil (2·3 g, 48%), b.p. 130°/0·07 mm, n_D^{20} 1·4533. Found: N, 15·0; titration equivalent 95·3. $C_{17}H_{39}N_3$ requires N, 14·7%; titration equivalent 95·2.

NN-*Di-n-butyladipamic acid.* The acid was prepared by saponification of ethyl *NN*-di-n-butyladipamate (50 g), as described for the preparation of *NN*-dimethyladipamic acid. Continuous extraction of the aqueous

acid solution with ether, followed by distillation gave the required *product*, as a very viscous, yellow oil (39·2 g., 85%) b.p. 198°/0·03 mm, $n_D^{16.5}$ 1·4720. Found: N, 5·2; titration equivalent 257·8. $C_{14}H_{27}NO_3$ requires N, 5·4%; titration equivalent 257·4.

NNN'-*Tri-n-butyladipamide.* Crude *NN*-di-n-butyladipamoyl chloride was prepared by refluxing the acid (28 g) and thionyl chloride (16·5 ml) in benzene (25 ml) at 90–95° for 7 min: it was isolated as described for *NN*-dimethyladipamoyl chloride. The acid chloride in benzene (75 ml) was then added to an ice-cold solution of n-butylamine (25 ml) in benzene (100 ml) and the solution refluxed for 1 hr during which a further 5 ml of n-butylamine and 50 ml of benzene were added. The reaction mixture was filtered and washed with dilute hydrochloric acid (20 ml) and water (10 ml) and dried (Na_2SO_4). Evaporation of the solvent left a brown viscous oil which was fractionally distilled, rejecting the first fraction. The required *product* was a viscous brown oil (26·5 g, 78%), b.p. 214°/0·04 mm. Found: N, 9·0. $C_{18}H_{36}N_2O_2$ requires N, 9·0%.

6-*Di-n-butylaminohexyl-n-butylamine* was obtained as a pale-yellow oil (14·3 g, 79%), $n_D^{23.5}$ 1·4502, by the reduction of the diamide (20 g) in ether with lithium aluminium hydride (5 g) as described under the preparation of 6-hydroxyhexyldimethylamine. b.p. 132°/0·08 mm. Found: N, 9·8; titration equivalent 143·1. $C_{18}H_{40}N_2$ requires N, 9·9%; titration equivalent 142·3.

Bis(6-*di-n-butylaminohexyl*)*n-butylamine.* *NN*-Di-n-butyladipamoyl chloride in benzene (25 ml), prepared from the acid (6·9 g) as previously described, was added (15 min) to a stirred, refluxing solution of 6-di-n-butylaminohexyl-n-butylamine (15 g) in benzene (50 ml). The mixture was refluxed for 30 min. The solution gelled on cooling and was, therefore, reheated on the water-bath and extracted as described for the preparation of bis(6-dimethylaminohexyl)methylamine. On distillation, 6-di-n-butylaminohexyl-n-butylamine (5·6 g, b.p. 124°/0·03 mm) was recovered, but almost no residue was left in the distillation flask.

In a further experiment, evaporation of the reaction mixture left a viscous residue which was dried *in vacuo* and reduced with lithium aluminium hydride (3·5 g) in ether. The product was fractionally distilled and yielded the required *base* only (5·2 g), b.p. 214–218°/0·05 mm, with a fore-run (1·0 g), b.p. 130–214°/0·05 mm. (Total yield 47%.) Found: N, 8·6; titration equivalent 162·4; fore-run, equivalent 165·0. $C_{32}H_{69}N_3$ requires N, 8·5%; titration equivalent 165·3.

N-*Adipoylpiperidine.* Ethyl *N*-adipoylpiperidine (17 g) was refluxed with ethanolic potassium hydroxide (250 ml, approx. 2/3 N) for 3 hr. The solution was neutralised and the ethanol removed under reduced pressure: benzene (2 × 25 ml) was then added and likewise removed. The residue was acidified with hydrochloric acid (15 ml) and water (10 ml), filtered, and extracted with chloroform (100 ml). After drying (Na_2SO_4) and evaporation of the chloroform, a golden-yellow oil was obtained which crystallised spontaneously in rosettes of long needles. These were dissolved in a small volume of benzene and filtered to remove any adipic acid. Evaporation of the benzene and recrystallisation (charcoal) from

chloroform-ether-light petroleum (b.p. 40–60°) gave the pure *product* in colourless rosettes (14·5 g, 96%), m.p. 81–83°. Found: N, 6·7; titration equivalent 212·9. $C_{11}H_{19}NO_3$ requires N, 6·6%; titration equivalent 213·3.

N-*Adipoylmorpholine* was prepared similarly from ethyl *N*-adipoylmorpholine (20 g). Recrystallisation (chloroform-ether-light petroleum, b.p. 40–60°) gave rosettes of white needles (14 g, 79%), m.p. 63–65°. Found: N, 6·65; titration equivalent 215·9. $C_{10}H_{17}NO_4$ requires N, 6·5%; titration equivalent 215·2.

N-*Ethyladipamoylpiperidine.* *N*-Adipoylpiperidine (13 g) in benzene (50 ml) was treated with thionyl chloride (10 ml) at 70–80° for 10 min, and the solvent and excess reagent, plus two additional volumes of benzene were successively removed under reduced pressure. Anhydrous ethylamine (15 ml) in benzene (20 ml) was added with stirring to the crude acid chloride in benzene (80 ml) at 0°. The reaction mixture was allowed to stand for several hr, filtered and evaporated, leaving a viscous black oil. A solution of this oil in chloroform (100 ml) was washed with water (2 × 15 ml) and dried (Na_2CO_3). Fractional distillation, as for *NNN'*-tri-n-butyladipamide, gave N-*ethyladipamoylpiperidine* (10 g, 68%), b.p. 236–242°/0·13 mm, as a dark viscous oil which solidified very slowly on standing. Found: N, 11·1. $C_{13}H_{24}N_2O_2$ requires N, 11·7%.

N-*Ethyladipamoylmorpholine* was prepared similarly from *N*-adipoylmorpholine (20·5 g), but the acid was treated with thionyl chloride at 60–75° for 20 min. Fractional distillation gave a light-brown oil (16°8 g, 73%), b.p. 234°/0·1 mm, which crystallised on standing, m.p. 61–64° with softening at 56°. Found: N, 11·6. $C_{12}H_{22}N_2O_3$ requires N, 11·6%.

6-*Piperidinohexylethylamine.* *N*-Ethyladipamoylpiperidine (22 g) in a mixture of ether and tetrahydrofuran (100 ml, approx. 3:1) was slowly added to a refluxing suspension of lithium aluminium hydride in a similar mixture (100 ml) and refluxed for 5 hr. Excess reagent was decomposed by the successive addition of water (8 ml), sodium hydroxide (6 ml, 20%) and water (28 ml) giving a granular precipitate which was readily extracted with ether. Fractional distillation gave 6-*piperidinohexylethylamine* as a mobile, colourless oil (14·25 g, 73%), b.p. 112–118°/0·07 mm, n_D^{21} 1·4685. Found: N, 12·7; titration equivalent 109·3. $C_{13}H_{28}N_2$ requires N, 13·5%; titration equivalent 106·2.

Reineckate. This crystallised from aqueous acetone in fine, pink platelets which were dried *in vacuo* below 50°. The decomposition point was *ca.* 186°. Found: N, 22·3; titration equivalent 438. $C_{21}H_{44}Cr_2N_{14}O_2S_8$ requires N, 22·2%; titration equivalent 442·7.

6-*Morpholinohexylethylamine* was prepared similarly to 6-piperidinohexylethylamine from *N*-ethyladipamoylmorpholine (16·7 g). The *product* was obtained as a colourless oil (10·2 g, 69%), b.p. 110–114°/0·1 mm, $n_D^{17.5}$ 1·4680. Found: N, 13·5; titration equivalent 108·6. $C_{12}H_{26}N_2O$ requires N, 13·1%; titration equivalent 107·2.

Bis(6-*piperidinohexyl*)*ethylamine.* The acid chloride prepared from *N*-adipoylpiperidine (7 g) as previously described, was rapidly added in benzene (35 ml) to a stirred solution of 6-piperidinohexylethylamine

(13 g) in benzene (50 ml). After refluxing for 45 min, the precipitated 6-piperidinohexylethylamine hydrochloride was filtered off, and the filtrate evaporated to give a viscous dark oil. This was dissolved in chloroform (200 ml), washed with water (2 × 20 ml) and dried (Na_2SO_4). Recovered 6-piperidinohexylethylamine base weighed 4·2 g.

Evaporation of the main chloroform solution yielded crude *N*-6-piperidinohexyl-*N*-ethyladipamoylpiperidine (12·5 g) which was further dried in a vacuum desiccator before reducing with lithium aluminium hydride (3·5 g) in ether as described under 6-piperidinohexylethylamine. Fractional distillation gave a fore-run of the required product and 6-hydroxyhexyl-1′-piperidine (1 g), b.p. 129–222°/0·05 mm, and then the required *product* only (8·5 g, 68%), b.p. 226–232°/0·05 mm. Found: N, 11·0; titration equivalent 127·1. $C_{24}H_{49}N_3$ requires N, 11·1%; titration equivalent 126·6.

Bis(6-morpholinohexyl)ethylamine was prepared from *N*-adipoylmorpholine (5·5 g) and 6-morpholinohexylethylamine (10 g) by the method described for the synthesis of bis(6-piperidinohexyl)ethylamine. Fractional distillation gave a fore-run (1·1 g), b.p. 142–176°/0·03 mm, followed by the *product* (6·2 g) as a pale-yellow oil, b.p. 223–226°/0·03 mm. Found: N, 11·1; titration equivalent 127·2. $C_{22}H_{45}N_3O_2$ requires N, 11·0%; titration equivalent 127·9. The fore-run was identical (infra-red spectrum) with the required product (total yield of triamine 74%). 6-Morpholinohexylethylamine (2·3 g, b.p. 111–112°/0·1 mm) was recovered from the hydrochloride precipitated during the condensation reaction.

Tetrahydropapaverine was obtained from the hydriodide by the extraction method of Pyman (1909), and from papaverine by catalytic hydrogenation (Craig & Tarbell, 1948).

Ethyl N-*adipoyltetrahydropapaverine.* Ethyl adipoyl chloride prepared from ethyl hydrogen adipate (7·5 g), was added in benzene (25 ml) to a stirred solution of tetrahydropapaverine (14·5 g) and triethylamine (10 ml) in benzene (75 ml); the mixture gently refluxed for 45 min. Precipitated triethylamine hydrochloride was filtered off and the filtrate washed with water (20 ml), sodium carbonate (20 ml, 10%) and dried (Na_2SO_4). Evaporation of the solvent gave the *product* as a viscous yellow oil (18 g., 84%) which would not solidify on prolonged drying *in vacuo*. Found: N, 2·9. $C_{28}H_{38}NO_7$ requires N, 2·8%.

N-*Adipoyltetrahydropapaverine* was obtained as a viscous oil by the saponification of the ester (17·5 g) as described for *N*-adipoylpiperidine. The product was contaminated with a little adipic acid which was removed by dissolving in benzene and filtering. The successive addition and evaporation of several volumes of dry ether followed by drying over potassium hydroxide gave a flaky, yellow powder (15·5 g, 94%), m.p. 38–42° (decomp.). Found: N, 3·0; titration equivalent 468·9. $C_{26}H_{34}NO_7$ requires N, 3·0%; titration equivalent 472·6.

N-*Ethyladipamoyltetrahydropapaverine.* The crude acid chloride, prepared by heating *N*-adipoyltetrahydropapaverine (5 g) with thionyl chloride dissolved in benzene (40 ml) was added to a stirred solution of anhydrous ethylamine (4 ml) in benzene (30 ml) at room temperature. The solution

was stirred for 2 hr, filtered, washed with water (10 ml) and sodium carbonate (10 ml, 20%) and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave N-*ethyladipamoyltetrahydropapaverine* as an oil (4·75 g, 90%) which was solidified by drying *in vacuo* over potassium hydroxide pellets for several hr. Found: N, 5·2. $C_{28}H_{39}N_2O_6$ requires N, 5·6%.

6,2′-*Tetrahydropapaverinylhexylethylamine* was obtained as a dark-yellow oil (3 g, 71%) by the reduction with lithium aluminium hydride (1·5 g) of *N*-ethyladipamoyltetrahydropapaverine (4·5 g) in ether-tetrahydrofuran (100 ml, 1 : 1). A paper chromatogram (Whatman's No. 1), ascending method, using butanol-acetic acid-water (4 : 1 : 5) gave an Rf value of 0·56 to 0·61. An aliquot portion (1·5 g) of the product in ether-methanol (80 : 1) chromatographed on alumina (8 × 2 cm) gave 6,2′-*Tetrahydropapaverinylhexylethylamine*, Rf 0·56–0·61. Found: N, 5·8; titration equivalent 242·8. $C_{28}H_{43}N_2O_5$ requires N, 5·9%; titration equivalent 236·8.

The *Reineckate* was purified by precipitation from acetone-ethanol-water as a pink powder. Found: N, 16·9. $C_{36}H_{59}Cr_2N_{14}O_6S_8$ requires N, 17·1%.

Bis(6,2′-*tetrahydropapaverinylhexyl*)*ethylamine*. The crude acid chloride, prepared from *N*-adipoyltetrahydropapaverine (6·5 g), was added in benzene (50 ml) to a stirred solution of 6,2′-tetrahydropapaverinylhexylethylamine (5·5 g) and triethylamine (2 ml) in benzene (30 ml), then refluxed for 1 hr. The mixture was allowed to stand overnight, the precipitated triethylamine hydrochloride filtered off and more benzene (100 ml) added to the filtrate which was washed with water (10 ml) and sodium carbonate (10 ml, 20%) and then dried (Na_2SO_4). Removal of the solvent gave a hygroscopic powder (11 g) which on reduction with lithium aluminium hydride (3 g) in a mixture of ether and tetrahydrofuran (100 ml, 1 : 1) gave the crude triamine, as a viscous yellow oil (8 g, 76%). Paper chromatography (Whatman's No. 1) using butanol-acetic acid-water (4 : 1 : 5), showed extensive tailing, with no definite spot discernible. An aliquot portion (6 g) of the oil in ether-methanol (80 : 1) was chromatographed on alumina (15 × 2 cm), and the eluate collected in fractions of 150, 150, 75, 75 and 75 ml. Fractions one and two yielded *bis*(6,2′-*tetrahydropapaverinylhexyl*)*ethylamine* as a pale-yellow oil (3 g and 1·5 g resp.), Rf 0·7, λmax (in ethanol) 284 mμ (ε 10,600). Found: N, 4·8; titration equivalent 298·9. $C_{54}H_{79}N_3O_8$ requires N, 4·7%; titration equivalent 300·7. The third fraction gave a yellow oil (1 g) which deposited a yellow solid on standing; the infra-red spectrum of this showed a hydroxyl band at 3,200 to 3,700 cm^{-1}. The fourth and fifth fractions gave 6-hydroxylhexyl-2′-tetrahydropapaverine as a yellow solid (0·4 g), νmax. 3,200 to 3,700 cm^{-1} (hydroxyl), λmax. 284 mμ (ε 6,540), and Rf 0·85 to 0·89 in butanol-acetic acid-water (4 : 1 : 5). Found: N, 3·1; titration equivalent 435. $C_{26}H_{38}NO_5$ requires N, 3·15%; titration equivalent 444·6.

NNN-*Trisonium compounds* were prepared from either bis-6-dimethylaminohexylmethylamine, bis-6-di-*n*-butylaminohexyl-*n*-butylamine, bis-6-piperidinohexylethylamine, bis-6-morpholinohexylethylamine, or

bis-6,2′-tetrahydropapaverinylhexylethylamine by treating with the appropriate alkyl halide in ethanol, evaporating the solvent and crystallising the product. Reaction conditions and time, crystallisation solvent and yields are indicated for each compound in that order, in parenthesis.

7,7-Dimethyl-7-azoniatridecylenebis(trimethylammonium)tri-iodide (room temperature, 24 hr, water-ethanol-ether, 84%), m.p. 246–248° (decomp.). Found: N, 5·8; I, 53·9. $C_{20}H_{48}I_3N_3$ requires N, 5·9; I, 53·5%.

7-Ethyl-7-methyl-7-azoniatridecylenebis(dimethylethylammonium)tri-iodide (room temperature, 4 days, ethanol-methanol, 61%), m.p. 188–190°. Found: N, 5·6; I, 50·8. $C_{23}H_{54}I_3N_3$ requires N, 5·6; I, 50·6%.

7-Methyl-7-n-butyl-7-azoniatridecylenebis(di-n-butylmethylammonium)-tri-iodide (room temperature 24 hr, refluxed 20 min, ethanol-ethyl acetate-water, 86%), m.p. 176–180° (decomp.). Found: N, 4·5; I, 41·3. $C_{35}H_{78}I_3N_3$ requires N, 4·6; I, 41·3%.

7-Ethyl-7-n-butyl-7-azoniatridecylenebis(di-n-butylethylammonium)tri-iodide (refluxed without solvent 15 min, precipitation from n-propanol-ethyl acetate, 88%), m.p. 163–166° (decomp.). Found: N, 4·3; I, 39·6 $C_{38}H_{84}I_3N_3$ requires N, 4·4; I, 39·5%.

7-n-Butyl-7-n-propyl-7-azoniatridecylenebis(di-n-butyl-n-propylammonium)tri-iodide (refluxed without solvent 80 min, precipitation from n-propanol-ethyl acetate-ether), m.p. 143–146° (decomp.). Found: N, 4·1; I, 37·8. $C_{41}H_{90}I_3N_3$ requires N, 4·2; I, 37·9%.

7,7-Di-n-butyl-7-azoniatridecylenebis(tri-n-butylammonium)tri-iodide (refluxed without solvent 80 min, precipitation from ethanol-acetone-ether), m.p. 140–142° (decomp.). Found: N, 4·0; I, 37·0. $C_{44}H_{96}I_3N_3$ requires N, 4·0; I, 36·3%.

7-Ethyl-7-methyl-7-azoniatridecylenebis(N-methylpiperidinium)tri-iodide (room temperature 24 hr, methanol-light petroleum (b.p. 40–60°), 91%), m.p. 199–211°. Found: N, 5·0; I, 47·2. $C_{27}H_{58}I_3N_3$ requires N, 5·2; I, 47·3%.

7,7-Diethyl-7-azoniatridecylenebis(N-*ethylpiperidinium*)*tri-iodide* (room temperature without solvent 24 hr, ethanol-ether, 76%), m.p. 226° (decomp.) with darkening at 214°. Found: N, 4·9; I, 44·6. $C_{30}H_{64}I_3N_3$ requires N, 5·0; I, 44·9%.

Trichloroacetate. Trichloroacetic acid (1 g) in water (1 ml) added dropwise to a vigorously stirred solution of the semi-solid ethiodide (0·12 g) in water (1 ml) precipitated a yellow oil. The supernatant liquid was decanted and the oil triturated with a dilute solution of trichloroacetic acid (5%) giving a fine white powder which was filtered and dried *in vacuo*. The *trichloroacetate* was slightly hygroscopic and was not recrystallised. Found: N, 2·9; titration equivalent 488·9. $C_{42}H_{67}Cl_{18}N_3O_{12}$ requires N, 2·9%; titration equivalent 481.

7-Ethyl-7-methyl-7-azoniatridecylenebis(N-*methylmorpholinium*)*tri-iodide* (room temperature, 24 hr, triturated with dry ether, 57%). The *product* was a very hygroscopic yellow powder (1·2 g, 57%) with no definite m.p. Found: N, 5·1; I, 47·3. $C_{25}H_{54}I_3N_3O_2$ requires N, 5·2; I, 47·0%.

Trichloroacetate. A semi-solid sample of the methiodide (0·5 g) treated with a solution of trichloroacetic acid gave the trichloroacetate

as a yellow powder after prolonged trituration. The *product* was washed with water and dried *in vacuo*. Found: N, 3·0; titration equivalent 459·1. $C_{37}H_{57}Cl_{18}N_3O_{14}$ requires N, 3·0%; titration equivalent 468·7.

7,7-*Diethyl*-7-*azoniatridecylenebis*(N-*ethylmorpholinium*)*tri-iodide* (room temperature, 24 hr, precipitation with methanol-ethanol-ether, 63%), m.p. 197–200°. Found: N, 4·7; I, 44·9. $C_{28}H_{60}I_3N_3O_2$ requires N, 4·9; I, 44·7%.

7-*Ethyl*-7-*methyl*-7-*azoniatridecylenebis*(N-*methyltetrahydropapaverinium*)-*tri-iodide* (room temperature in ether 24 hr, washed with dry ether, 81%). The melting-point of the methiodide was indefinite. Gladych & Taylor (1962) have also observed that the quaternary salts of other tetrahydropapaverine derivatives melt over a wide range. Found: N, 3·0; I, 28·8. $C_{57}H_{88}I_3N_3O_8$ requires N, 3·2; I, 28·7%.

Pharmacological

METHODS

The methods were as described previously (Edwards, Stenlake, Lewis & Stothers, 1961). All drugs and control solutions were injected in 0·9% sodium chloride solution.

Neuromuscular blockade. The compounds were compared with tubocurarine on the gastrocnemius muscle-sciatic nerve preparation of the pentobarbitone-anaesthetised cat and fowl, on the rabbit by the head-drop assay method; on the mouse by the inclined-screen method; on the frog isolated rectus abdominis muscle, in terms of inhibition of acetylcholine-induced contractures, and on the three-day old chick.

The duration of paralysis after doses causing 40 to 60% inhibition of the gastrocnemius-sciatic preparation in the cat was also estimated.

Sympathetic ganglion block was assessed on the nictitating membrane preparation of the cat, by comparing the drug-induced reduction in the response to preganglionic tetanic stimulation of the cervical sympathetic nerve, with that due to tubocurarine. Doses were four or five times those causing 50% inhibition of twitch height in the gastrocnemius-sciatic preparation.

Respiration and blood pressure. The dose required to paralyse respiration was estimated on the pentobarbitone-anaesthetised cat. Effects on blood pressure were also noted.

RESULTS

Qualitative tests indicated that the compounds possessed no depolarising activity.

In Table 1, the molar potencies are shown as percentages of the molar potency of tubocurarine, and in Fig. 1, the compounds are arranged along the abscissa in order of increasing neuromuscular blocking potency in the cat. For comparison, Table 1 and Fig. 1 contain compounds previously described (Carey & others, 1959).

The highest sensitivity to the compounds when compared with tubocurarine was shown by fowls. Neuromuscular blocking potency was less in the mouse and on the frog rectus abdominis muscle, than in the cat and the rabbit preparations.

TABLE 1. INFLUENCE OF ALKYL ONIUM SUBSTITUENTS UPON NEUROMUSCULAR BLOCKING POTENCY (TUBOCURARINE = 100)

Compound	$R_3R'\overset{+}{N}\cdot[CH_2]_6\cdot\overset{+}{N}\cdot[CH_2]_6\cdot NR_2R'$ (R, R′)			Cat	Rabbit	Mouse	Chick (fowl)	Frog
Ia	Me_3	Me_2	Me_3	6	6	6	20(20)	5
Ib	Me_2Et	MeEt	Me_2Et	22	15	13	86(65)	10
Ic	Et_2Me	EtMe	Et_2Me	50	52	16	*(321)	29
Id	Et_3	Et_2	Et_3	99	78	64	495(495)	52
Ie	Et_2Pr	EtPr	Et_2Pr	97	76	53	144(*)	18
If	Et_2Bu	EtBu	Et_2Bu	139	179	73	210(353)	54
Ig	Pr_2Me	PrMe	Pr_2Me	16	20	12	86(37)	5
Ih	Pr_2Et	PrEt	Pr_2Et	35	46	19	125(157)	17
Ii	Pr_3	Pr_2	Pr_3	24	25	4	298(422)	16
Ij	Bu_2Me	BuMe	Bu_2Me	33	23	8	132(128)	25
Ik	Bu_2Et	BuEt	Bu_2Et	82	56	29	191(197)	38
Il	Bu_2Pr	BuPr	Bu_2Pr	33	19	6	155(155)	20
Im	Bu_3	Bu_2	Bu_3	33	20	5	150(161)	53

* Insufficient material to test.

Respiratory paralysing potency closely paralleled neuromuscular blocking potency for the whole series.

Significant sympathetic ganglion blockade was noted only in compound Ia (Me_3), which was about half as potent as tubocurarine. Compound Ib was about one-tenth as potent as tubocurarine.

The duration of paralysis produced by doses causing 40–60% inhibition

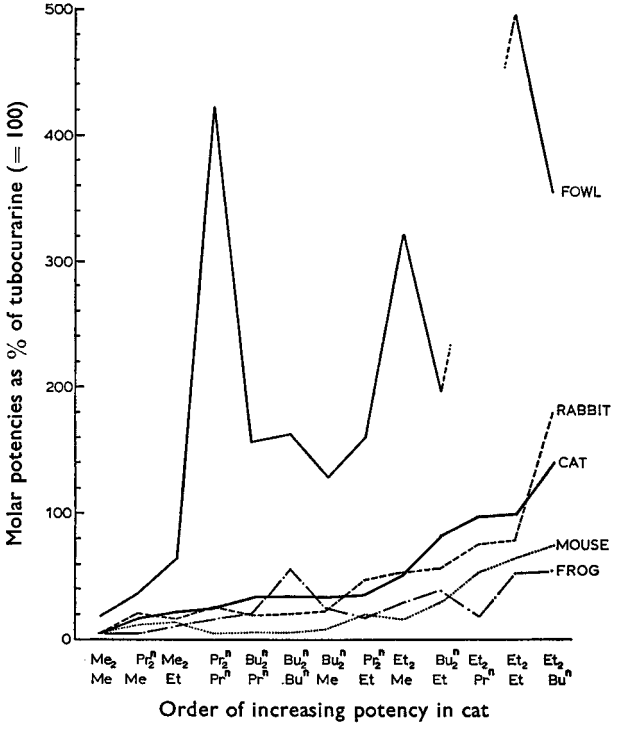
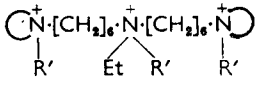


FIG. 1. Comparative neuromuscular blocking potencies of compounds in Table 1. The groups on the abscissa are $R_{(2)}$ (top row) and R′ (bottom row) of the formula.

of the gastrocnemius-sciatic preparation of the cat was in all instances within the limits of 15–29 min found with tubocurarine.

Compounds with heterocyclic substituents. The neuromuscular blocking potencies are shown in Table 2. The most notable features are the low potencies of the morpholino-derivatives, especially in the mammalian species, and the high susceptibility of the fowl to the piperidino- and the tetrahydropapaverino-compounds.

TABLE 2. INFLUENCE OF TERMINAL HETEROCYCLIC SUBSTITUENTS ON NEUROMUSCULAR BLOCKING POTENCY (TUBOCURARINE = 100)



Compound	Alkyl substituent R′	Heterocyclic groups	Cat	Rabbit	Mouse	Fowl	Frog
In	Me	piperidine	47	30	26	360	7
Io	Et	piperidine	99	53	51	450	10
Ip	Me	morpholine	8	*	7	20	*
Iq	Et	morpholine	10	7	6	43	33
Ir	Me	tetrahydropapaverine	103	61	51	400	107

* Insufficient material to test.

Only the morpholino-compounds showed ganglion blocking activity. Respiratory paralysing potency was similar to muscle relaxant potency in all the heterocyclic compounds (Tables 2 and 3).

Discussion

In the *NNN*-trisonium salts investigated, only the alkyl substituents on the onium atoms have been varied, thus allowing an assessment of the effects of alkyl substitution alone.

Compound Ia (Table 1) has tubocurarine-like properties and is an example of a long chain methonium derivative with no depolarising activity. Its significant ganglion blocking activity may therefore indicate that its configuration at the receptor surface in terms of N^+ to N^+ distance, is closer to that of hexamethonium than to that of decamethonium. It also emphasises that for depolarizing activity the dimensions of the molecules must be rigidly defined. The ganglion blocking potency of the Me_3 compound (Ia), and also the Me_2Et compound (Ib) (the other compounds being inactive in this respect), confirms the views of Fakstorp, Pedersen, Poulsen & Schilling (1957) that, for ganglion blockade, more than one methyl substituent on the onium nitrogen is advantageous. Unlike Fakstorp & others (1957), we have found the fully methyl-substituted compound to be a more potent ganglion blocking agent than the dimethylethyl derivative. No general inferences can be made, however, since the two series of compounds are not analogous.

It seems most useful to explain the range of antagonistic potency shown in terms of goodness of fit at the receptor site, the extent of shielding of

TABLE 3. INFLUENCE OF TERMINAL SUBSTITUENTS ON DURATION OF PARALYSIS, RESPIRATORY PARALYSING POTENCY AND SYMPATHETIC GANGLION BLOCK

Compound	Sympathetic ganglion block (cat)*	Respiratory paralysing potency in cat (TC = 100)	Duration of paralysis† (min)	
			Cat	Fowl
Ia	27	7	17–23	26–36
Ib	6	21	15–20	31–36
Ic	0	57	20–41	—
Id	0	59	15–18	—
Ie	0	99	18–29	—
If	0	156	24–26	—
Ig	0	29	17–29	—
Ih	0	60	15–29	—
Ii	0	27	23–29	—
Ij	0	24	15–23	—
Ik	0	78	15–23	—
Il	0	26	18–23	28–37
Im	0	29	20–23	—
In	8	53	15–22	20–27
Io	0	88	15–19	20–28
Ip	29	10	17–18	43–53
Iq	30	10	15–22	37–50
Ir	3	91	20–23	40–50
TC	53	100	15–29	19–28

* Per cent inhibition caused by 4 to 5 times the dose required for 50% inhibition of twitch height in the gastrocnemius-sciatic preparation.
† Time taken for twitch height to recover to control levels following doses causing 40–60% inhibition of the gastrocnemius-sciatic preparation.

the onium nitrogen and perhaps the magnitude of the dissociation and association rate-constants calculated for the drug-receptor reaction (Paton, 1961).

The results suggest that for compounds with alkyl substituents, at least one Et group is required for significant tubocurarine-like muscle relaxant potency in mammals. Larger alkyl substituents, apart from compounds If (Et_2Bu^n) and Ik ($EtBu^n_2$), reduce potency, possibly because they hinder the approach of the onium nitrogen to the anionic site of the receptor. In the chick, an Et group was not essential for high potency (see Ii, Il, Im in Table 1).

The high potency of the Et_2-substituted compounds (Ic, Id, Ie and If) may reflect a combination of optimal receptor fit and minimal shielding of the charged nitrogen atom. The fall in potency in the Me_3 and Me_2Et derivatives would then be due to a poorer fit on the receptor. Reduced potency among compounds with large alkyl groups (e.g. Ii, Ij, Il and Im) may thus indicate a poor fit on to the receptor with shielding of the charged nitrogen atom. In general, it seems that at least two ethyl substituents on the terminal nitrogen atom are required for high potency (compounds Ie and If) provided that the third substituent is not methyl, when potency falls to about half.

Only a limited series of compounds containing heterocyclic substituents was available. When the terminal onium groups were incorporated into a piperidine ring, potency was increased over the Me_3, compound (Ia),

but the corresponding morpholine derivatives were of very low neuromuscular blocking potency. This effect has been ascribed to charge delocalization caused by the oxygen function (Mason & Wien, 1955).

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