NEUROMUSCULAR BLOCKING AGENTS

PART IV. THE SYNTHESIS AND STUDY OF *N*- AND *S*-ALKYL VARIANTS OF DIHEXASULPHONIUM AND DIHEXAZONIUM TRIETHIODIDES

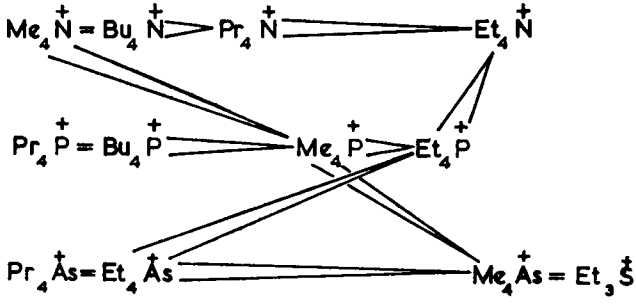
BY FIONA MACLEOD CAREY†, D. EDWARDS*, J. J. LEWIS† AND J. B. STENLAKE*

*From * The School of Pharmacy, The Royal College of Science and Technology, Glasgow and † The Department of Materia Medica and Therapeutics, University of Glasgow*

Received May 28, 1959

NSN- and *NNN*-Tris-onium compounds related to dihexasulphonium and dihexazonium in which *N*-alkyl substituents are varied have been synthesised. All compounds tested qualitatively resembled tubocurarine in their action. Stepwise replacement of Et by Me in dihexasulphonium tri-ethiodide decreases potency and some C 10-like effects appear in compounds XA and XB. Potency also falls when Et groups are replaced successively by *n*-Pr in both dihexasulphonium tri-ethiodide and dihexazonium tri-ethiodide, compounds XIF and XIG being the least active of the series and approximately equipotent with dihexasulphonium trimethiodide (XA). Replacement of one Et group by *n*-Bu at each quaternary ammonium centre of dihexazonium, as in compound XIC increases potency.

THE influence of alkyl substituents on the curariform activity of simple tetra-alkyl mono-quaternary ammonium salts has been studied on frog sartorius and gastrocnemius muscle preparations by Külz[1], Marshall[2], Peiser[3] and Ing and Wright[4]. Quantitative investigation[5] has shown that the effect of small alkyl substituents on potency varies (decreasing in the direction of arrows) according to the nature of the central onium atom as follows.



Alkyltrimethylammonium compounds[1,4] fall into the same order as the tetra-alkylammonium salts, ethyltrimethylammonium being the least active, and butyl-, hexyl- and octyl-trimethylammonium compounds equipotent with tetramethylammonium. Maximum potency on the cat anterior tibialis muscle[6] is also reached in this series with BuN^+Me_3, but activity rapidly falls off with larger alkyl groups (Table I).

The studies of Ariëns and his collaborators[7–9] on avian muscle and on the basis of cumulative concentration-response curves obtained on the frog

rectus abdominis muscle show that increase of alkyl chain length also brings a gradual change from a depolarising neuromuscular block to one which is not competitive. Külz[1] records a steady increase from $n = 2$ to $n = 8$ in the activity of the alkyltriethylammonium compounds $Et_3N^+C_nH_{2n+1}$ on the frog gastrocnemius mucle. Rossum and Ariëns[9], however, report a gradual change from competititive to non-competitive action with increasing alkyl chain length, and note the positive correlation of non-competitive affinity, surface activity and fat-solubility.

TABLE I

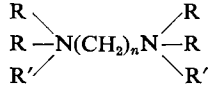
RELATIVE CURARISING ACTIVITY OF ALKYLTRIMETHYLAMMONIUM COMPOUNDS ($R\overset{+}{N}Me_3$) ON CAT

Alkyl group	Relative activity*	Alkyl group	Relative activity*
C_2H_5-	0·8	$CH_3(CH_2)_9-$	0·3
$CH_3(CH_2)_3-$	10·0	$CH_3(CH_2)_{11}-$	0·15
$CH_3(CH_2)_5-$	5·0	$CH_3(CH_2)_{13}-$	0·05
$CH_3(CH_2)_7-$	0·3	$CH_3(CH_2)_{15}-$	0·0

* The activity of $Bu\overset{+}{N}Me_3$ (10) is taken as a standard for comparison.

Ing and Wright[5] have also shown that *N*-methyl-1,2,3,4-tetrahydroquinoline methiodide is more active than the corresponding ethiodide, but that this trend is reversed when the nitrogen is part of a hetero-aromatic system. Thus, pyridine and quinoline ethiodides are more potent than the corresponding methiodides, but strychnine, morphine and nicotine ethiodides, in which the nitrogen-containing ring is saturated, have weaker curariform properties than their methiodides[5,10].

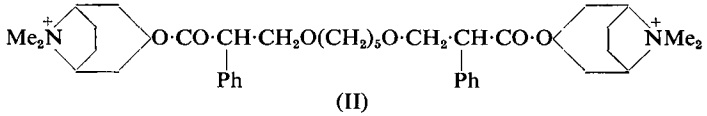
In the polymethylene bistrimethyl- and polymethylene bistriethylammonium series variations in the relative potencies with alkyl group size are evident. The relative order of activity is also dependent on the test preparations. In general, methonium compounds are more potent than their ethonium analogues both on the rat phrenic nerve-diaphragm preparation and in rabbit head drop tests[11]. Thus Barlow, Roberts and Reid[12] have shown that decamethylenebis(dimethylethylammonium)iodide (I; $n = 10$; R = Me; R′ = Et) has about 1/8th of the activity of decamethonium (I; $n = 10$; R = R′ = Me) on the rat phrenic nerve-diaphragm and about 1/15th on the quadriceps of the spinal rabbit. Further substitution of methyl by ethyl groups does not affect the potency on rat diaphragm preparations, but increases it on the rabbit quadriceps upon which decamethylenebis(triethylammonium) (I; $n = 10$; R = R′ = Et) has about 1/4th of the activity of decamethonium (C 10).



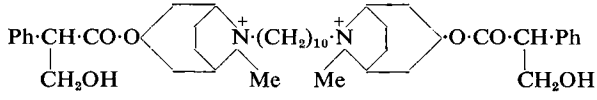
(I)

Thesleff and Unna[13] have shown, however, that decaethonium has weak tubocurarine(TC)-like activity, so that replacement of *N*-methyl by *N*-ethyl substituents not only reduces activity, but changes its character. In this respect, the decamethylenebis (alkyldimethylammonium) salts

(I, R = Me, R′ = alkyl) of Rossum and Ariëns[9] are of special interest. Intrinsic activity falls with increasing alkyl chain length, and the depolarising action of C 10 changes through decamethylenebis (dimethylpropyl ammonium) (I, R = Me; R′ = Pr) which shows mixed competitive and depolarising actions, to compounds with extended alkyl chains such as decamethylenebis(dimethylheptylammonium) (I, R = Me, R′ = $CH_3(CH_2)_6$—) which are almost pure non-competitive antagonists. The different modes of action of TC and decamethonium on the cat, chick, frog, in man and in certain other species are well established, but whether this is due to the incorporation of the terminal onium groups of the former into relatively large heterocyclic nuclei or is dependent more upon the presence of additional ring substituents or other factors is not clear. The bisatropinium compounds (II) and (III)[14,15] appear however to exhibit TC-like rather than C 10-like activity, as

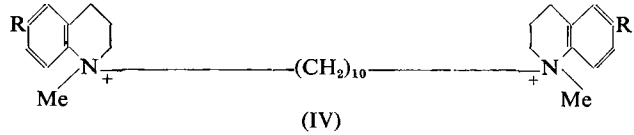


(II)

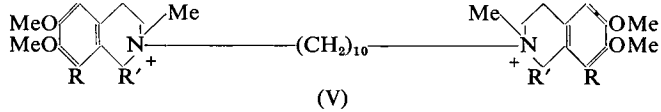


(III)

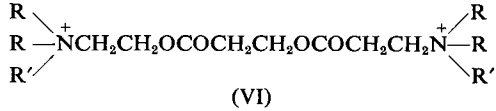
measured by the production of flaccid paralysis in chicks in compounds (III) and (VIII)[16]. Their potencies are 0·5 and 2·0 respectively compared with TC (1·0) by the rabbit head drop method. Similarly the decamethylenebis-1,2,3,4-tetrahydroquinolinium methiodides (IV; R = H)[17] and (IV; R = OMe)[17], and the decamethylenebis-1,2,3,4-tetrahydroisoquinolinium methiodides (V; R = R′ = H)[18], (V; R = OMe; R′ = H)[18] and (V; R = H, R′ = 3,4-dimethoxybenzyl; laudexium)[19] are antagonised by neostigmine and therefore appear to be TC-like in type. The relative species sensitivities to laudexium which are in the order rabbit > cat > man > mouse > rat suggest that it possesses some resemblance to



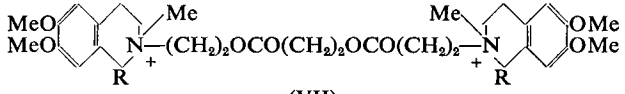
(IV)



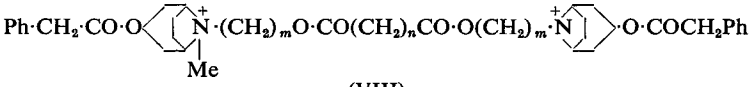
(V)



(VI)



(VII)



(VIII)

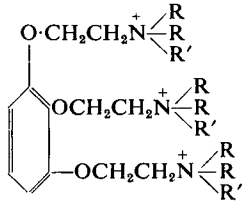
C 10. The depolarising action of suxamethonium (VI; R = R′ = Me) is similarly modified on replacement of the methonium groups by tetrahydroisoquinolinium (VII; R = 3,4-dimethoxybenzyl)[20] and suitably substituted tropinium (VIII; m = 2–3; n = 1–6) groups[16]. Successive replacement of *N*-methyl by *N*-ethyl substituents in suxamethonium lowers the potency as measured in rabbit head drop experiments (Table II)[21], though there is no evidence reported of any change in the type of action.

TABLE II

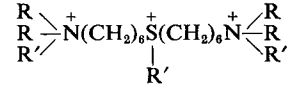
POTENCY OF SUXAMETHONIUM ANALOGUES (VI)[21]

R	R′	Rabbit head drop dose mg./kg.
Me	Me	0·2
Me	Et	0·8
Et	Me	20·0
Et	Et	12·0

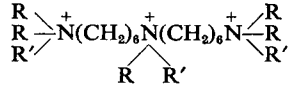
Successive replacement of *N*-ethyl by *N*-methyl groups in the tris-onium compound gallamine (IX; R = R′ = Et) leads to a decrease in curariform potency, though this becomes significant only in the dimethylethylaminoethoxy compound (IX; R = Me; R′ = Et) and the trimethylaminoethoxy compound (IX; R = R′ = Me). There is, however, no departure from the typical TC-like activity of gallamine[23]. In part I of this series[24] we compared the neuromuscular blocking activity of the *NSN*-trisethonium compound, dihexasulphonium tri-ethiodide (X; R = R′ = Et) with that of its methonium analogue (X; R = R′ = Me). The former showed a TC-like neuromuscular blocking action on the cat gastrocnemius muscle, the frog rectus abdominis muscle, the rat diaphragm preparation, in the chick and in rabbit head drop experiments. The latter although qualitatively similar was more recently found to be much less potent (Zoha



(IX)



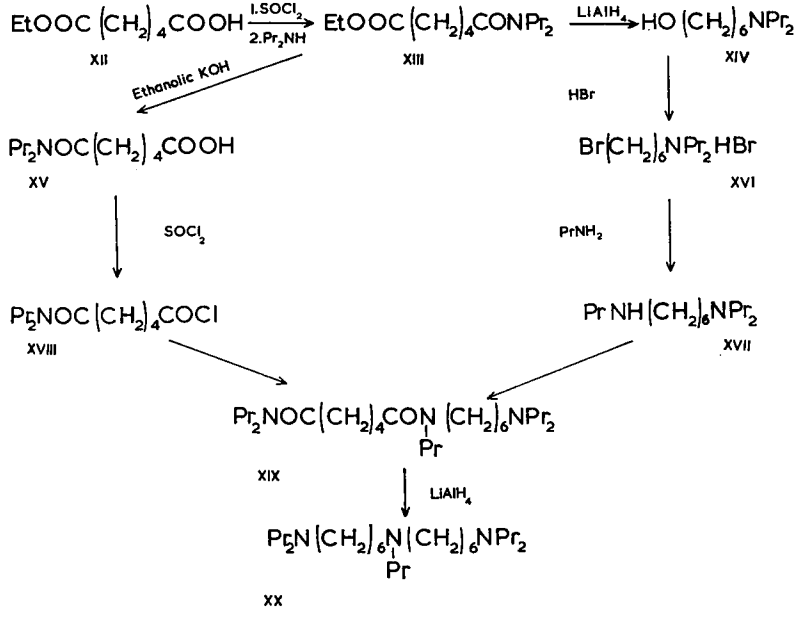
(X)



(XI)

unpublished observations). We have now prepared and examined the related *NSN*-tris-onium compounds described in Table III in order to study the influence of alkyl substituents upon potency and mode of neuromuscular blocking action in this series.

The quaternary compounds 7-ethyl-7-thioniatridecylenebis (dimethylethylammonium) tri-iodide (X; R = Me, R′ = Et), 7-*n*-butyl-7-thioniatridecylenebis (dimethyl-*n*-butylammonium) tri-iodide (X; R = Me, R′ = *n*Bu), 7-methyl-7-thioniatridecylenebis (diethylmethylammonium) tri-iodide (X; R = Et, R′ = Me), and 7-*n*-propyl-7-thioniatridecylenebis (diethyl-*n*-propylammonium) tri-iodide (X; R = Et, R′ = *n*-Pr) were prepared by alkylation of bis-6-dimethylaminohexyl sulphide[24] and bis-6-diethylaminohexyl sulphide[25] as appropriate. In the preparation of *NNN*-tris-onium compounds, an alternative method has been devised for the synthesis of bis-6-diethylaminohexyl ethylamine in improved



yields. This is illustrated by the scheme outlined above for the preparation of the analagous bis-6-di-*n*-propylaminohexyl-*n*-propylamine (XX).

Ethyl *NN*-di-*n*-propyladipamate (XIII), obtained from ethyl hydrogen adipate (XII) by the reactions indicated, was reduced with lithium aluminium hydride to 6-hydroxyhexyldi-*n*-propylamine (XIV), and also hydrolysed to give *NN*-di-*n*-propyladipamic acid (XV). Treatment of

6-hydroxyhexyldi-*n*-propylamine with hydrobromic acid under reflux yielded 6-bromohexyldi-*n*-propylamine hydrobromide (XVI), which with excess *n*-propylamine gave 6-*n*-propylaminohexyldi-*n*-propylamine (XVII). The use of the hydrobromide (XVI) in this reaction rather than the corresponding base[26] prevents cyclisation of the latter and leads to the increased yields.

NN-Di-*n*-propyladipamic acid darkened rapidly when heated with thionyl chloride, but the acid chloride (*NN*-di-*n*-propyladipamoyl chloride, XVIII) was readily obtained by refluxing with thionyl chloride in benzene for exactly seven minutes. Condensation of the product with 6-*n*-propyl-aminohexyldi-*n*-propylamine gave *N*-di-*n*-propylaminohexyl-*NNN*-tri-*n*-propyladipamide (XIV), which on reduction with lithium aluminium hydride yielded bis-6-di-*n*-propylaminohexyl-*n*-propylamine (XX). Quaternisation of the latter with the appropriate alkyl halides gave 7-methyl-7-*n*-propyl-7-azoniatridecylenebis (di-*n*-propylmethylammonium) tri-iodide (XI; R = Pr; R′ = Me), 7-ethyl-7-*n*-propyl-7-azoniatridecylenebis (di-*n*-propylethylammonium) tri-iodide (XI; R = Pr; R′ = Et) and 7,7-di-*n*-propyl-7-azoniatridecylenebis (tri-*n*-propylammonium) tri-iodide (XI; R = R′ = Pr). 7-Methyl-7-ethyl-7-azoniatridecylenebis (diethylmethylammonium) tri-iodide (XI; R = Et; R′ = Me), 7-*n*-propyl-7-ethyl-7-azoniatridecylenebis (diethyl-*n*-propylammonium) tri-iodide (X; R = Et; R′ = Pr) and 7-*n*-butyl-7-ethyl-7-azoniatridecylenebis (diethyl-*n*-butylammonium) tri-iodide (XI; R = Et; R′ = Bu) were obtained from bis-6-diethylaminohexylethylamine[25].

Experimental

Melting points are uncorrected. We are indebted to Miss M. Buchanan for the microanalyses.

NSN-*Tris-onium compounds* (X) were prepared from either bis-6-dimethylaminohexyl sulphide[24] or bis-6-diethylaminohexyl sulphide[25] by refluxing with the appropriate alkyl halide in ethanol, evaporation of the solvent and crystallisation. Reflux times, crystallisation solvents and yields are indicated for each compound in that order, in parentheses.

7-*Ethyl*-7-*thioniatridecylenebis* (*dimethylethylammonium*) *tri-iodide* (35 min.; ethanol; 61 per cent), m.p. 137 to 137·5°. Found: N, 3·6; I, 50·1. $C_{22}H_{51}N_2SI_2$ requires N, 3·7; I, 50·3 per cent.

7-n-*Butyl*-7-*thioniatridecylenebis* (*dimethyl*-n-*butylammonium*) *tri-iodide* (40 min.; ethanol-acetone-ether; 51 per cent), m.p. 131 to 131·5°. Found: N, 3·4; I, 45·35. $C_{28}H_{63}N_2SI_3$ requires N, 3·3; I, 45·3 per cent.

7-*Methyl*-7-*thioniatridecylenebis* (*diethylmethylammonium*) *tri-iodide* (20 min.; ethanol; 94 per cent), m.p. 135 to 136°. Found: N, 3·6; I, 49·6. $C_{23}H_{53}N_2SI_3$ requires N, 3·6; I, 49·4 per cent.

7-n-*Propyl*-7-*thioniatridecylenebis* (*diethyl*-n-*propylammonium*) *tri-iodide* (45 min.; ethanol-ether; 52 per cent), m.p. 125·5 to 126°. Found: N, 3·3; I, 44·3. $C_{29}H_{65}N_2SI_3$ requires N, 3·3; I, 44·5 per cent.

Ethyl NN-*di*-n-*propyladipamate* was prepared from ethyl hydrogen adipate (85 g.) by the method described for the preparation of ethyl *NN*-diethyladipamate[25]. *Ethyl* NN-*di*-n-*propyladipamate* was obtained

as a yellow oil, b.p. 144 to 146°/0·35 mm., n_D^{22} 1·4550 (110 g. 87·6 per cent). Found: N, 5·5. $C_{14}H_{27}O_3N$ requires N, 5·4 per cent.

6-*Hydroxyhexyldi*-n-*propylamine* was prepared from ethyl *NN*-di-*n*-propyladipamate (75·5 g.) by lithium aluminium hydride reduction as described for the preparation of 6-hydroxyhexyldiethylamine[25]. 6-*Hydroxyhexyldi*-n-*propylamine* was obtained as a colourless oil, b.p. 115 to 117°/0·65 mm., n_D^{22} 1·4533 (56·1 g., 95 per cent). Found: equiv. (titration) 200·5; N, 6·9. $C_{12}H_{27}ON$ requires equiv. 201·3; N, 7·0 per cent.

6-n-*Propylaminohexyldi*-n-*propylamine.* 6-Hydroxyhexyldi-*n*-propylamine (55·3 g.) in hydrobromic acid (48 per cent; 30 ml.) was refluxed for 5 hours and evaporated to a thick syrup under reduced pressure. Water (50 ml.) was added and the liquid again evaporated. This procedure was repeated twice after the addition of ethanol (50 ml.). The residual crude 6-bromohexyldi-*n*-propylamine hydrobromide in ethanol (75 ml.) was added slowly (30 min.) to a gently refluxing mixture of *n*-propylamine (100 g.) in ethanol (100 ml.). Refluxing was continued for a further 1½ hours. The excess *n*-propylamine and ethanol were removed by distillation, leaving a solid crystalline mass, which was basified and extracted with ether. Evaporation of the ether and fractionation gave 6-*n*-*propylamino-hexyldi*-n-*propylamine* as a colourless oil, b.p. 115 to 117°/0·45 mm., n_D^{22} 1·4463 (47 g., 70·6 per cent). Found: equiv. (titration) 121·6, N, 11·4. $C_{15}H_{34}N_2$ requires equiv. 121·2, N, 11·6 per cent.

NN-*Di*-n-*propyladipamic acid.* Ethyl *NN*-di-*n*-propyladipamate (27·8 g.) was refluxed for 30 min. with a slight excess of ethanolic potassium hydroxide (130 ml. of 0·9363N) and the bulk of the ethanol removed by distillation. The residual liquid in water (40 ml.) was acidified by addition of hydrochloric acid (50 per cent, 100 ml.), extracted with benzene, dried (Na_2SO_4) and the solvent distilled off. NN-*Di*-n-*propyladipamic acid* was obtained as a yellow viscous oil, b.p. 198°/0·5 mm., n_D^{25} 1·4723, (22·75 g., 91·9 per cent). Found: equiv. (titration) 227·5; N, 6·0. $C_{12}H_{23}O_3N$ requires equiv. 229·3; N, 6·1 per cent.

Bis-6-*di*-n-*propylaminohexyl*-n-*propylamine.* *NN*-Di-*n*-propyladipamic acid (11·6 g.) was dissolved in benzene (10 ml.), refluxed with excess thionyl chloride (6 ml.) for 7 min., and the solute evaporated under reduced pressure; benzene (10 ml.) was added and again evaporated. The acid chloride in benzene (30 ml.) was added slowly (15 min.) to a stirred refluxing solution of excess 6-*n*-propylaminohexyldi-*n*-propylamine (22·4 g.) in benzene (100 ml.). The mixture was refluxed for a further 30 min. and then extracted with hydrochloric acid (10 per cent). The acid extract was basified and extracted with ether, dried (Na_2SO_4) and the solvent removed by distillation. Most of the excess 6-*n*-propylaminohexyldi-*n*-propylamine was recovered by distillation, and the crude *N*-di-*n*-propylaminohexyl-*NN'N'*-tri-*n*-propyladipamide reduced by means of lithium aluminium hydride in ether. Fractional distillation of the product gave *bis*-6-*di*-n-*propylamino-hexyl*-n-*propylamine* as a pale yellow oil, b.p. 211°/0·65 mm., n_D^{21} 1·4582 (16·7 g., 77·5 per cent). Found: equiv. (titration) 142·3; N, 9·8. $C_{27}H_{59}N_3$ requires equiv. 141·9; N, 9·9 per cent.

NN-*Diethyladipamic acid* was prepared from ethyl *NN*-diethyladipamate (100 g.) by the method described for the preparation of *NN*-di-*n*-propyladipamic acid.

NN-*Diethyladipamic acid* was obtained as a yellow viscous oil (83·96 g., 95·7 per cent), b.p. 182°/0·5 mm., $n_D^{20.5}$ 1·4733. Found: equiv. (titration) 201·9; N, 6·7 $C_{10}H_{19}O_3N$ requires equiv. 201·3; N, 7·0 per cent.

Bis-6-diethylaminohexylethylamine was prepared from *NN*-diethyladipamic acid (19·79 g.) and excess 6-diethylaminohexylethylamine (27·8 g.) by the method described for the preparation of bis-6-di-*n*-propylaminohexylethylamine. Bis-6-diethylaminohexylethylamine was obtained as pale yellow oil, b.p. 173 to 176°/0·75 mm., n_D^{25} 1·4588 (19·53 g., 55·9 per cent).

NNN-*Tris-onium compounds* (XI) were prepared from either bis-6-di-*n*-propyl-aminohexyl-*n*-propylamine or bis-6-diethylaminohexylethylamine by refluxing with the appropriate alkyl halide in ethanol, evaporation of the solvent and crystallisation. Reflux time, crystallisation solvent and yields are indicated for each compound in that order, in parenthesis.

7-*Methyl*-7-n-*propyl*-7-*azoniatridecylenebis*(*di*-n-*propylmethylammonium*) *tri-iodide* (10 min.; ethanol; 94 per cent), m.p. 239°. Found: N, 4·9; I, 44·7. $C_{30}H_{68}N_3I_3$ requires N, 4·9; I, 44·7 per cent.

7-*Ethyl*-7-n-*propyl*-7-*azoniatridecylenebis*(*di*-n-*propylethylammonium*)*tri-iodide* (35 min., ethanol-acetone-ether; 66 per cent), m.p. 221°. Found: N, 4·7; I, 42·6. $C_{33}H_{74}N_3I_3$ requires N, 4·7; I, 42·6 per cent.

7,7-*Di*-n-*propyl*-7-*azoniatridecylenebis*(*tri*-n-*propylammonium*)*tri-iodide* (45 min., acetone-ether, 12 per cent), m.p. 206 to 207°. Found: N, 4·5; I, 40·3. $C_{36}H_{80}N_3I_3$ requires N, 4·5; I, 40·7 per cent.

7-*Ethyl*-7-*methyl*-7-*azoniatridecylenebis*(*diethylmethylammonium*)*tri-iodide* (5 min., methanol, 88 per cent), m.p. 227·5 to 228·5°. Found: N, 5·3; I, 48·6. $C_{25}H_{58}N_3I_3$ requires N, 5·4; I, 48·7 per cent.

7-*Ethyl*-7-n-*propyl*-7-*azoniatridecylenebis*(*diethyl*-n-*propylammonium*)*tri-iodide* (30 min., ethanol-acetone-ether, 43 per cent), m.p. 220°. Found: N, 4·9; I, 43·7. $C_{31}H_{70}N_3I_3$ requires N, 4·9; I, 44·0 per cent.

7-*Ethyl*-7-n-*butyl*-7-*azoniatridecylenebis*(*diethyl*-n-*butylammonium*)*tri-iodide* (45 min., acetone-ether, 61 per cent), m.p. 178°. Found: N, 4·6; I, 42·0. $C_{34}H_{73}N_3I_3$ requires N, 4·6; I, 42·0 per cent.

PHARMACOLOGY

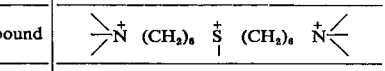
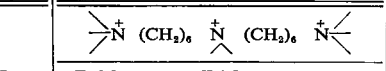
Methods and Results

The methods and materials used have been described elsewhere[24,26]

Neuromuscular blocking activity. To investigate neuromuscular blocking potency in the cat gastrocnemius muscle-sciatic nerve preparation, drugs were administered into the cannulated external jugular vein. None of the compounds tested caused muscular fasciculation or twitching or induced an initial increase in twitch height. XIE was the least potent on this preparation and doses of 0·2 to 0·8 mg./kg. caused a reduction in twitch amplitude, a dose of 0·6 mg./kg. being adequate to reduce it by about 50 per cent. XIC was the most potent member of the series; doses of 0·05 to 0·2 mg./kg. reduced twitch amplitude and 0·1 mg./kg. was

TABLE III

THE INFLUENCE OF CHANGES IN ALKYL GROUP SIZE UPON NEUROMUSCULAR BLOCKING ACTIVITY IN THE CAT, RABBIT, MOUSE, AND FROG

Compound				Cat	Rabbit	Mouse	Frog
				TC = 100	TC = 100	TC = 100	TC = 100
XA	Me_3	Me	Me_3	20	14	5	—
XB	Me_2Et	Et	Me_2Et	26	27	24	14
XC	Me_2Bu	Bu	Me_2Bu	44	33	24	10
XD	$MeEt_2$	Me	$MeEt_2$	87	46	42	16
XE	Et_3	Et	Et_3	95	30	25	25
XF	Et_2Pr	Pr	Et_2Pr	46	51	54	16
							
XIA	Et_2Me	EtMe	Et_2Me	50	52	16	29
XIB	Et_2Pr	EtPr	Et_2Pr	88	69	48	16
XIC	Et_2Bu	EtBu	Et_2Bu	120	155	63	47
XID	Et_3	EtEt	Et_3	100	21	17	50
XIE	$MePr_2$	MePr	$MePr_2$	15	18	11	5
XIF	$EtPr_2$	EtPr	$EtPr_2$	31	46	17	15
XIG	Pr_3	PrPr	Pr_3	20	21	3	13

Pr = *n*-propyl. Bu = *n*-butyl

TABLE IV

THE DURATION OF EFFECT IN THE CAT, AVERAGE DOSES REQUIRED TO PRODUCE RESPIRATORY PARALYSIS IN THE CAT AND PARALYSIS IN THE CHICK IN COMPOUNDS XA TO XF AND XIA TO XIG

Compound	Mean respiratory paralysing dose (mg./kg.)	Respiratory paralysing potency (TC = 100)	Chich paralysis		Paralysing potency (cat) (TC=100)	Duration of effect (cat) in min.
			Dose (mg./kg.)	Potency (chick) (TC=100)		
XA	>2·28	<20	33	50	20	15–25
XB	1·43	30	16	100	26	20–40
XC	1·05	41	20	80	44	10–35
XD	0·70	61	14	115	87	15–45
XE	1·07	45	—	—	95	20–30
XF	0·76	57	19	29	46	30–45
XIA	0·76	57	22	73	50	20–30
XIB	0·48	90	16	100	88	20–30
XIC	0·32	135	12	133	120	20–30
XID	0·85	56	—	—	100	20–30
XIE	>2·82	<20	19	84	15	20–30
XIF	0·81	53	19	84	31	20–30
XIG	1·93	23	33	50	20	15–30

sufficient to cause a 50 per cent reduction. The approximate potencies of these and the other compounds tested are shown in Tables III and IV. The least potent compounds are XB, XIG, XIF and XIE. XD, XIC and XIB are the most potent and XC, XF, XIA occupy intermediate positions. These figures are compared with those of Edwards and others, for XA (1/5 as potent as TC) and XE and XID (equipotent with TC)[26]. The duration of effect varied not only with the dose but with the animal. The ranges of duration of activity (min.) in the cat for an approximate 50 per cent reduction in initial twitch amplitude were as follows; TC,

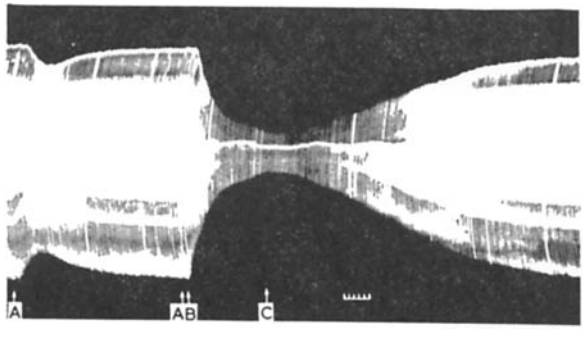


FIG. 1. Ether-potentiation of the effects of XI C on the gastrocnemius muscle-sciatic nerve preparation of the cat.
At A. 0·025 mg./kg. XI C into the external jugular vein.
At B. Ether administration commenced.
At C. Ether administration ceased.
Time = 30 seconds.

15 to 30; XD, 15 to 45; XB, 20 to 40; XC, 10 to 35; XF, 30 to 45; XIA, 20 to 30; XIB, 20 to 30; XIC, 20 to 30; XIG, 15 to 30; XIF, 20 to 30; XIE, 20 to 30. By comparison XA lasted for 15 to 25; XE, 20 to 30 and XID, 20 to 30.

In virtually all respects these compounds behaved like TC. There appears to be no marked qualitative differences from one another or from TC but after XB an indirect tetanus was fairly well maintained. Block was rapidly reversed by edrophonium and more slowly by neostigmine. Potentiation by ether was seen in all; this effect was most striking when XIC was used (Fig. 1).

Rabbit head drop. Comparisons of potency were made with tubocurarine and the experiments were repeated in rabbits which had been given neostigmine (0·1 mg./kg.) by subcutaneous injection 15 minutes earlier. The results are shown in columns 1 and 2 of Table V. Column 3 shows the ratio of the two head drop doses. For all, save compound XF, the ratio is greater than unity indicating a TC-like mode of action.

Acute toxicity in mice. Using 5 or 6 dose levels for each drug and giving each dose to a group of 5 albino mice (average wt. 36 g.) an approximate median lethal dose was estimated. The results are summarised in Table VI. There is fairly good agreement between the toxicity to mice and the paralysing potency estimated on the rabbit and cat.

Paralysing activity in mice. Mice were given an intraperitoneal injection of the drug and placed upon the upper part of a fine wire mesh screen inclined at an angle of 50° to the horizontal. Groups of 5 albino mice (average wt. 36 g.) were used at each dose and 5 or 6 different doses used for each compound. The number of mice in each group which were unable to maintain their position on the screen at each dose was

TABLE V

THE HEAD DROP DOSES (H.D.D.) IN RABBITS OF COMPOUNDS XA TO XF AND XIA TO XIG, AND THE EFFECT UPON THESE OF PRE-TREATMENT WITH NEOSTIGMINE (0·1 mg./kg.)

	1	2	3	
Compound	H.D.D. ± S.E.	H.D.D. ± S.E. after neostigmine	Ratio 2/1	Potency (TC = 100)
XA	2·17 ± 0·059	2·76 ± 0·138	1·22 ($P < 0·01$)	14
XB	1·16 ± 0·047	1·62 ± 0·082	1·40 ($P < 0·01$)	27
XC	0·93 ± 0·071	1·25 ± 0·108	1·34 ($P < 0·05$)	33
XD	0·67 ± 0·031	1·00 ± 0·050	1·50 ($P < 0·01$)	46
XE	0·36 ± 0·017	0·59 ± 0·071	1·64 ($P < 0·01$)	30
XF	0·59 ± 0·035	0·59 ± 0·032	1·00	51
XIA	0·60 ± 0·045	1·51 ± 0·058	2·5 ($P < 0·01$)	52
XIB	0·46 ± 0·024	0·86 ± 0·057	1·9 ($P < 0·01$)	69
XIC	0·20 ± 0·012	0·41 ± 0·036	2·1 ($P < 0·01$)	155
XID	0·51 ± 0·043	0·81 ± 0·057	1·56 ($P < 0·01$)	21
XIE	1·70 ± 0·044	2·98 ± 0·405	1·73 ($P < 0·01$)	18
XIF	0·75 ± 0·052	1·41 ± 0·111	1·36 ($P < 0·01$)	46
XIG	1·47 ± 0·103	2·81 ± 0·179	1·90 ($P < 0·01$)	21

counted after a 30-minute observation period, and from this an approximate 50 per cent paralysing dose (PD 50) was calculated. The results obtained are summarised in Table VI. Once again there is fair agreement between the relative potencies as estimated in mouse, rabbit and cat.

Effects upon blood pressure and respiration in the cat. No depressor effect was observed on the blood pressure. To estimate the average dose required to paralyse respiration, a solution containing 0·1 mg./kg./ml. was infused at a rate of 0·75 ml./min. into the external jugular vein of pentobarbitone-anaesthetised cats using a Palmer's slow injection apparatus. The approximate respiratory paralysing doses are shown in Table IV.

Ganglion blocking activity. Ganglion blocking activity was investigated by noting the effects of up to 2 mg./kg. intravenously of the drug upon the response of the nictitating membrane of the pentobarbitone-anaesthetised cat, to stimulation of the preganglionic fibres of the cervical sympathetic. Only XD (2 mg./kg.) caused any depression of the response but this was much less than that caused by an equal dose of TC.

Chick paralysis. In each case 5 mg./kg. doses were given intraperitoneally to groups of five two-day old chicks and the dose repeated at 30 second intervals until paralysis was seen. With all compounds, a

flaccid TC-like paralysis was observed. The average paralysing doses for each drug are shown in Table IV. When tested on chicks XA caused paralysis which was spastic at first, then becoming flaccid. Dihexasulphonium and dihexazonium have been found to cause a flaccid paralysis (Zoha, unpublished observations).

Frog rectus abdominis muscle. The muscle was set up in a 6 ml. bath containing oxygenated frog Ringer's solution at room temperature. At the doses used (up to 10 μg./ml.) no direct stimulant actions were observed

TABLE VI

THE APPROXIMATE MEDIAN LETHAL DOSE AND PD 50 IN MICE OF COMPOUNDS XA TO XF AND XIA TO XIG

Compound	Approximate median lethal dose (mg./kg.)	PD 50 (mg./kg.)	Potency TC = 100
XA	11·4	7·5	5
XB	4·6	1·6	24
XC	3·5	1·6	24
XD	1·8	0·9	42
XE	1·2	0·8	25
XF	2·6	0·7	54
XIA	2·6	2·4	16
XIB	1·9	0·8	48
XIC	1·4	0·6	63
XID	2·3	1·2	17
XIE	12·7	3·4	11
XIF	5·8	2·2	17
XIG	20	15	3

with any of the compounds tested. All of the compounds (2 to 10 μg./ml.) antagonised contractions produced by acetylcholine (0·3 to 1·0 μg./ml.). The potencies in terms of TC are shown in Table III. XIC was about half as potent as TC but the others showed from 1/20th to 1/4th of the potency.

DISCUSSION

Our findings appear to confirm the observations of Thesleff and Unna[13] and Ariëns and de Groot[22] in the decamethonium series that larger alkyl onium-group substituents favour TC-like activity. Thus compounds XA and XB, in which the terminal onium groups are $Me_3\overset{+}{N}-$ and $Me_2Et\overset{+}{N}-$ respectively show some evidence of decamethonium-like properties, but the remaining members of the present series show purely TC-like activity. (Table VII).

The increase in potency on successive replacement of Me by Et (compounds X A,B,D,E and XI A,D; Table III) in the onium groups of both dihexasulphonium and dihexazonium parallels the influence of similar replacements in the gallamine series (IX)[23], and is in contrast to the decrease in potency which has been observed in the decamethonium[12]

TABLE VII

QUALITATIVE PROPERTIES OF COMPOUNDS XA TO XF AND XIA TO XIG ON THE CAT, CHICK AND FROG RECTUS ABDOMINIS MUSCLE

Compound	Effect on block of:—					Effect of block upon tetanus	Chick paralysis	Effect on frog rectus abdom inis	Effect upon nictitating membrane	Effect upon cat blood pressure
	Neo-stigmine	Edro-phonium	Ether	Adrenaline	Tetanus					
XA	A	A	Prolongs	Transient antagonism	Transient decurarisa-tion	Contraction not sustained	Spastic then flaccid	No direct action. Inhibits ACH con-tractions	0	0
XB	A	A	do.	do.	do.	Contraction fairly well sustained	Flaccid	do.	0	Slight rise
XC	A	A	do.	do.	do.	Contraction not sustained	do.	do.	0	do.
XD	A	A	do.	do.	do.	do.	do.	do.	Slight block	0
XE	A	A	do.	do.	do.	do.	do.	do.	0	Slight rise
XF	A	A	do.	do.	do.	do.	do.	do.	0	do.
XIA	A	A	do.	do.	do.	do.	do.	do.	0	do.
XIB	A	A	do.	do.	do.	do.	do.	do.	0	do.
XIC	A	A	do.	do.	do.	do.	do.	do.	0	do.
XID	A	A	do.	do.	do.	do.	do.	do.	0	0
XIE	A	A	do.	do.	do.	do.	do.	do.	0	Slight rise
XIF	A	A	do.	do.	do.	do.	do.	do.	0	do.
XIG	A	A	do.	do.	do.	do.	do.	do.	0	do.

A = antagonism.
0 = no effect.

and suxamethonium series[21,22]. Thesleff and Unna[13] have also shown in mice and in the chicken sciatic nerve-gastrocnemius muscle preparation that potency increases when Me is replaced by Et in both pentamethonium (XII, $n = 5$, R = Me) and hexamethonium (XII $n = 6$, R = Me),

$$R_3\overset{+}{N}\cdot(CH_2)_n\cdot\overset{+}{N}R_3$$

(XII)

although the actual potencies of compounds of this type are low. No change, however, occurs in the type of action which remains purely TC-like.

It is evident, therefore, that the influence of *N*- or *S*-alkyl substituents cannot be divorced entirely from considerations of inter-onium group spacing. This is shown more clearly by comparison of molecular models of gallamine (IX), hexamethonium, dihexasulphonium and dihexazonium, which reveal almost identical interonium group distances (approximately 9Å; Fig. 2A and B) in all four substances. In reaching this conclusion, it is assumed that no unusual folding of these flexible molecules occurs at the instant of binding at the receptor site, and that the staggered orientation of the positively charged ethoxytriethylammonium groups in gallamine (Fig. 2A) represents their most probable distribution as a result of the natural repulsion of like charges. The structure of tubocurarine (XIII), however, is such that although the molecule cannot be regarded as completely rigid, the spacing of the nitrogen atoms is fixed by considerations of restricted rotation. Moreover, whilst the exact conformation cannot be predicted from models, there is considerable folding of the molecule, which results in reduction of the inter-onium group distance as compared with other estimates of 13 to 15Å based (*a*) on models[14] (*b*) on the number of chain units which separate them (9 carbon and 1 oxygen[11]) and (*c*) on comparison with decamethonium[27]. It is difficult to reconcile the estimate that the terminal groups in decamethonium are only 10Å apart[27] with present views that the hydrocarbon chain is fully extended, giving an *NN* distance of about 15Å. Measurements of inter-onium group distance on models of tubocurarine suggest that it is probably about 9 to 10 Å, so that in this respect the molecule approximates to the structures of hexamethonium (XII, $n = 6$, R = Me), gallamine (IX), dihexasulphonium (X) and dihexazonium (XI). The hexamethonium-like ganglion block caused by tubocurarine could conceivably be explained on the same basis, but the lack of ganglion-blocking activity in (X) (XI) presents an obvious difficulty.

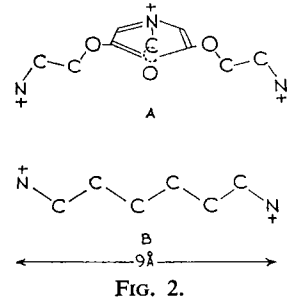


FIG. 2.

Considering now the replacement of Et by larger alkyl substituents in dihexasulphonium and dihexazonium, we have observed that the introduction of a single *n*-propyl group at each quaternary centre as in XF and

XIB leads to a reduction in potency. Potency falls again when a second *n*-propyl substituent is introduced (XIF) and still further when all Et groups have been replaced by *n*-propyl (XIG). Introduction of a single *n*-butyl substituent at each quaternary centre on the contrary enhances potency. These results can be explained in terms of competitive reaction at a receptor surface which presents a repeating pattern of appropriately spaced (9Å) anionic centres, each anionic receptor being in turn associated

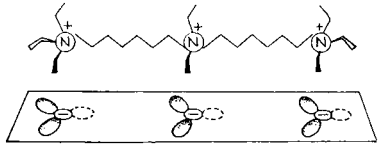


FIG. 3.

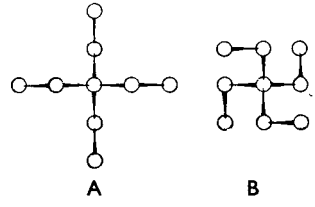


FIG. 4.

with a small number (two or three) of non-ionic satellite receptors at the neuromuscular synapse which ideally are complementary in size and shape to the ethyl substituents (Fig. 3).

Wait and Powell[28] have established the conformation of the tetraethylammonium ion to be that which in projection forms a nordic cross (Fig. 4B), which is in contrast to that of the tetra-*n*-propylammonium (Fig. 4A)[29]. On the assumption then that the swastika-like conformation is ‹‹ in the alkyltriethylammonium groups of dihexasulphonium and

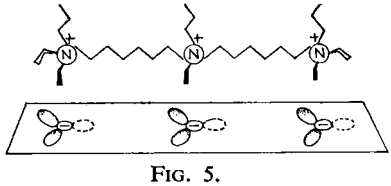


FIG. 5.

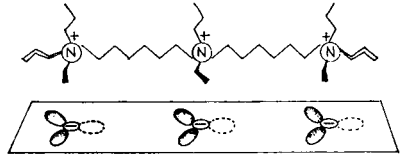


FIG. 6.

dihexazonium, considerable shielding of the charge on the nitrogen atom can be predicted. The competitive nature of the block shown by these compounds, as evidenced for example by reversibility with edrophonium and neostigmine, would probably be fostered by such shielding, which prevents irreversible binding at the receptor surface. The absence of this effect in compounds XA and XB in which Et groups have been replaced by Me groups would permit closer approach to and hence firmer binding with receptors thereby explaining the appearance of decamethonium-like properties which we have observed with these two compounds.

The reduction of potency when a single *n*-propyl substituent is introduced at each quaternary centre in compounds XF and XIB results not only from the partial impairment of fit with satellite receptors at the centre onium group (Fig. 5) but also from the restriction imposed by the *n*-propyl groups on the probability of the molecule reaching a correct orientation with the receptor surface.

The introduction of additional *n*-propyl substituents in compounds XIF and XIG would lead not only to a further impairment of fit with satellite receptors (Fig. 6), but also restrict approach to the receptor surface.

The apparent anomaly of increased activity in the *n*-butyl substituted compound XIC parallels observations on simple monoquaternary compounds[4]. It can be explained in terms of the present hypothesis on the assumption that the surface activity of the *n*-butyl substituents leads to

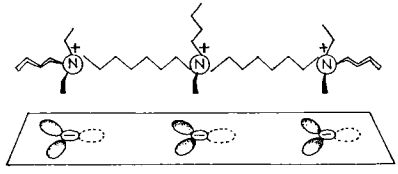


FIG. 7.

the possibility of their alignment as shown in Figure 7, and this more than compensates for any reduction in the probability of the molecule reaching its ideal orientation at the receptor surface.

The hypothesis can be tested further by comparison of equivalent compounds in the dihexasulphonium and dihexazonium series. Replacement of quaternary nitrogen by tertiary sulphur will reduce the goodness of fit with both anionic and satellite receptors by virtue of the increased ionic radius of sulphur (1·04Å) as compared with nitrogen (0·7Å). This acts in several ways (Fig. 8), (*a*) by fractionally increasing interonium

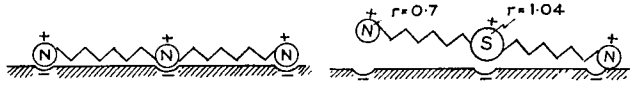


FIG. 8.

group chain length, (*b*) by decreasing the charge density on the central onium group, hence reducing its capacity to bind ionically and (*c*) increasing onium group-receptor distance for one of the two quaternary nitrogens in the molecule. Both (*a*) and (*c*), however, could be negated by limited bending of the hydrocarbon chain. The reduction from two to one in the number of alkyl substituents available on the central onium group for combination with satellite receptors could also contribute to a fall in potency.

Examination of our results reveals only slight reduction of potency when dihexasulphonium triethiodide is compared with dihexazonium triethiodide on the cat, which is of doubtful significance. In man, however, dihexazonium is significantly more potent (Levy, personal communication). Comparison of the azonium compound XIB with its sulphonium analogue also reveals that the latter is significantly less potent in cat, rabbit and mouse.

References

1. Külz, *Pflüg. Arch., ges. Physiol.*, 1922, **195**, 623.
2. Marshall, *Trans. Roy. Soc. Edinb.*, 1914, **50**, 481.
3. Peiser, *Pharmakologische Untersuchungen uber der Wirkung von Tetra-methyl, -äthyl und -propylammonium salzen*, Dissertation, Konigsberg, 1920 (quoted by Ing and Wright[b]).
4. Ing and Wright, *Proc. Roy. Soc.*, 1931, **109B**, 337.
5. Ing and Wright, *ibid.*, 1933, **114B**, 48.
6. Dallemagne and Philippot, *Arch. int. Pharmacodyn.*, 1951, **87**, 127.
7. Ariëns, Simonis and De Groot, *ibid.*, 1955, **100**, 298.
8. Ariëns, Rossum and Simonis, *Arzneimit.-Forsch.*, 1956, **6**, 611.
9. Rossum and Ariëns, *Arch. int. Pharmacodyn.*, 1959, **118**, 393.
10. Trendelenberg, *Heffters Handb. exp. Pharmak.*, Berlin, 1923, **1**, 564.
11. Barlow and Ing, *Brit. J. Pharmacol.*, 1948, **3**, 298.
12. Barlow, Roberts and Reid, *J. Pharm. Pharmacol.*, 1953, **5**, 35.
13. Thesleff and Unna, *J. Pharmacol.*, 1954, **3**, 99.
14. Kimura, Unna and Pfeiffer, *J. Pharmacol.*, 1949, **95**, 149.
15. Kimura and Unna, *ibid.*, 1950, **98**, 286.
16. Haining, Johnson and Smith, *Nature, Lond.*, 1959, **183**, 542.
17. Collier and Taylor, *ibid.*, 1949, **164**, 491.
18. Taylor and Collier, *ibid.*, 1950, **165**, 602.
19. Taylor and Collier, *ibid.*, 1951, **167**, 692.
20. Collier, Gladych, Macauley and Taylor, *ibid.*, 1958, **182**, 1424.
21. Bovet, Bovet-Nitti, Guarino, Longo and Marotta, *Rend. ist. super. Sanita*, 1949, **12**, 106.
22. Ariëns and De Groot, *Arch. int. Pharmacodyn.*, 1954, **99**, 193.
23. Riker and Wescoe, *Ann. N.Y. Acad. Sci.*, 1951, **54**, 373.
24. Edwards, Lewis, Stenlake and Zoha, *J. Pharm. Pharmacol.*, 1957, **9**, 1004.
25. Edwards and Stenlake, *ibid.*, 1955, **7**, 852.
26. Edwards, Lewis, Stenlake and Zoha, *ibid.*, 1958, **10**, suppl., *106T*.
27. Paton and Zaimis, *Brit. J. Pharmacol.*, 1949, **4**, 381.
28. Wait and Powell, *J. chem. Soc.*, 1958, 1872.
29. Zalkin, *Acta Cryst.*, 1957, **10**, 557.