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A note on a series of decamethylenebis[(4-substituted amino)quinaldinium] salts with potent antibacterial properties

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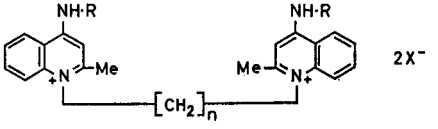
A number of decamethylenebis[4-(substituted amino)quinaldinium acetates] have been prepared and certain of these showed markedly increased antibacterial activity compared with the unsubstituted compound. Local therapeutic activity, assessed by mouse protection tests, reached a maximum in the C_3–C_6 range of the *n*-alkylamino-homologues, but increased activity was associated with increased toxicity.

THE antibacterial properties of certain bis-quaternary heterocyclic compounds including the polymethylenebis(4-aminoquinaldinium) salts have been described in a number of publications reviewed by D'Arcy & Taylor (1961).



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A number of derivatives of decamethylenebis(4-aminoquinaldinium) salts (I; n=10, R=H: dequalinium B.P.) have been prepared, together with a series of 4-alkylamino- and 4-cycloalkylamino-compounds. The synthesis and antimicrobial properties of these compounds are now described.

Materials and methods

CHEMISTRY

4-Alkylaminoquinaldines were prepared by reacting together 4-chloroquinaldine or 4-phenoxyquinaldine with an alkylamine in refluxing phenol. Details of hitherto unreported alkyl- and cycloalkyl-aminoquinaldines are given in Table 1.

The decamethylene bis-quaternary di-iodides (I; n=10, X=I) were made by refluxing 2 moles of base with decamethylene di-iodide in ethyl methyl ketone or in methyl isobutyl carbinol. The di-iodides were converted into water-soluble diacetates by reaction with silver acetate in methanol. These analysed generally as hydrated salts.

The bis-quaternary acetamido salt (compound 4) was prepared directly by acetylating dequalinium [decamethylenebis(4-aminoquinaldinium acetate): compound 1]. The salt was isolated as a di-iodide, the diacetate being obtained from it in the usual way.

Examples of these reactions are given in the Experimental section, and details of the salts are given in Table 2.

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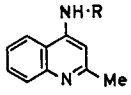
TABLE 1. 4-ALKYL- AND 4-CYCLOALKYL-AMINOQUINALDINES[1]



R	M.p. °C	Crystallization[2] solvent	Analyses					
			Found %			Required %		
			C	H	N	C	H	N
CH_3CH_3	180–181·5	E–W	77·6	7·7	14·65	77·4	7·6	15·0
$CH(CH_3)_2$	161–162·5	B	78·1	8·2	13·7	78·0	8·05	14·0
$CH_2{\cdot}CH{:}CH_2$[3] ..	145·5–146·5	B–P	78·9	7·0	13·85	78·7	7·1	14·1
$[CH_2]_3{\cdot}CH_3$	94–96	B–P	78·4	8·7	13·0	78·5	8·5	13·1
$[CH_2]_4{\cdot}CH_3$	106–107	B–P (b.p. 100–120°)	78·7	8·7	12·4	78·9	8·8	12·3
	130–132	B–P (b.p. 100–120°)	79·95	7·9	11·95	79·6	8·0	12·4
$CH(CH_3){\cdot}CH_2{\cdot}$ $CH(CH_3)_2$	122–124	E–W	78·9	9·2	12·0	79·3	9·15	11·6
	150	B–P	79·9	8·1	11·2	79·95	8·4	11·7
$[CH_2]_6{\cdot}CH_3$	118–119	P (b.p. 100–120°)	79·6	8·8	11·05	79·6	9·4	10·9
$[CH_2]_7{\cdot}CH_3$	79·5–80·5	P (b.p. 60–80°)	79·9	9·7	10·0	79·95	9·7	10·4
$[CH_2]_3{\cdot}CH(CH_3){\cdot}CH_2{\cdot}$ $C(CH_3)_3$[4]	118–120	B–P	70·7	9·55	9·1	71·2	10·1	8·75

[1] Prepared from 4-chloroquinaldine unless otherwise stated.
[2] E = Ethanol, W = Water, B = Benzene, P = Light petroleum, b.p. 40–60° unless stated otherwise.
[3] Prepared from 4-phenoxyquinaldine.
[4] Dihydrate.

BACTERIOSTATIC AND BACTERICIDAL EVALUATION

The *in vitro* bacteriostatic activity of the compounds was determined using a conventional tube dilution technique (Caldwell, Cox, D'Arcy & Rowe, 1961) against *Staphylococcus aureus* CN 491, *Escherichia coli* AH, *Proteus vulgaris* LH 14 and *Pseudomonas aeruginosa* NCTC 8203.

Bactericidal activity was assessed by a quantitative pour plate technique fulfilling the requirements of the British Standard method (1960). Samples were inactivated by dilution in suramin sodium 0·2% w/v (Antrypol, I.C.I. Ltd.).

CHEMOTHERAPEUTIC ACTIVITY

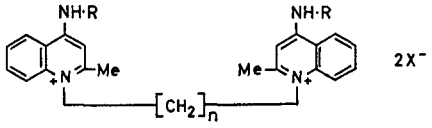
Protective tests were made in mice infected with *Staphylococcus aureus* 663. Cultures of this strain were grown overnight in Todd-Hewitt broth (37°). Culture suspensions in 2·5% hog mucin were injected intraperitoneally into groups of 10 mice (about 10^8 cells/mouse; minimal lethal dose, MLD × 10). Suspensions of the drugs in 10% gum acacia were given by the same route within 30 min of administering the culture. The numbers of mice surviving over 24 hr and 7 days were recorded.

Results

BACTERIOSTATIC AND BACTERICIDAL PROPERTIES

The effects of substitution in the 4-amino-group of dequalinium (compound 1) are summarized in Table 3. Introduction of an acetamido-group has little effect on bacteriostatic activity compared with the parent

TABLE 2. DECAMETHYLENEBIS[4-(SUBSTITUTED AMINO)QUINALDINIUM] SALTS



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(n = 10; X = I or $CH_3 \cdot CO_2 \cdot 2H_2O$)

Comp. No.	R	Salt	Cryst. solvent[1]	M.p. °C	Analyses							
					Found %				Required %			
					C	H	I	N	C	H	I	N
1	CH_3	Iodide	M–E	262–4	51·7	6·2	33·9	7·6	52·0	6·0	34·4	7·6
		Acetate[2]	E–A	180–2	67·3	8·4	—	9·2	67·6	8·5	—	8·8
2	$CH_2 \cdot CH_3$	Iodide	M–E	246	53·6	6·5	33·3	7·05	53·3	6·3	33·1	7·3
		Acetate	E–A	159–160	69·1	8·4	—	8·3	68·45	8·7	—	8·4
3	$CO \cdot CH_3$	Iodide	E	224–5	51·4	5·4	31·55	6·9	51·4	5·6	31·95	7·05
		Acetate[2]	E	>360	69·0	7·85	—	8·3	69·3	7·65	—	8·3
4	$[CH_2]_2 \cdot CH_3$	Iodide[4]	E	142–4	53·0	6·8	31·95	6·8	53·2	6·7	31·2	6·9
		Acetate	Er–EA	111–2	68·3	9·1	—	7·7	69·1	9·0	—	8·1
5	$CH_2 \cdot CH : CH_2$	Iodide[4]	M–E	134	53·25	6·4	31·2	7·1	53·5	6·2	31·4	6·9
		Acetate	M–A	130–2	69·3	8·55	—	8·0	69·5	8·5	—	8·1
6	$[CH_2]_3 \cdot CH_3$	Iodide	E	210–1	55·6	6·85	30·85	6·85	55·5	6·85	30·85	6·8
		Acetate[5]	A–EA–Er	117–8			—	7·8			—	7·75
7	$[CH_2]_4 \cdot CH_3$	Iodide	E	224–5	56·3	7·1	29·9	6·5	56·6	6·9	29·9	6·6
		Acetate[6]	A	88–90	68·2	9·65	—	7·6	68·7	9·4	—	7·3
8		Iodide	M–E	252–3	56·2	6·5	30·4	6·9	56·7	6·7	30·0	6·6
		Acetate	A–Er	80–81	67·6	8·8	—	7·2	67·5	9·0	—	7·2
9	$[CH_2]_5 \cdot CH_3$	Iodide	E	175–7	57·45	7·5	28·8	6·3	57·4	7·3	28·9	6·4
		Acetate	A–Er	80·5–81	70·7	9·6	—	7·25	71·1	9·6	—	7·2
10		Iodide	M	300–1	57·1	6·7	—	6·4	57·7	6·9	29·0	6·4
		Acetate	A	122–4	71·25	9·4	—	7·8	71·3	9·1	—	7·2
11	$[CH_2]_6 \cdot CH_3$	Iodide	M–E	198–200	58·05	7·7	28·05	6·7	58·3	7·6	28·0	6·2
		Acetate	M–A–Er	112–4	71·6	10·6	—	7·15	71·5	9·7	—	6·9
12	$CH(CH_3) \cdot CH_2 \cdot CH\ CH_3)_2$	Iodide	E–Er	231–2	57·5	7·5	28·1	6·2	57·4	7·3	28·9	6·4
		Acetate	A–Er	65–7	71·0	10·5	—	7·2	71·1	9·6	—	7·2
13	$[CH_2]_7 \cdot CH_3$	Iodide	M–E	168–171	58·9	7·6	27·35	6·1	59·1	7·8	27·1	6·0
		Acetate	A	92–3	71·7	9·7	—	7·0	71·9	9·9	—	6·7
14												

[1] M = Methanol, E = Ethanol, A = Acetone, Er = Ether, EA = Ethyl acetate.
[2] All acetates are dihydrates unless otherwise stated.
[3] Anhydrous.
[4] Monohydrate.
[5] Trihydrate.
[6] Tetrahydrate.

compound, whilst substitution by an allyl, cyclopentyl or cyclohexyl group results in slight increase in activity against *Staphylococcus aureus* and some improved activity against *Escherichia coli* and *Proteus vulgaris*.

The most significant changes in activity are associated with substitution by a straight chain alkyl group. From Fig. 1 it is seen that an increase in the number of substituent carbon atoms up to C_4 (n-butyl) results in improved antistaphylococcal activity whilst optimal activity against *Escherichia coli* is reached at C_5 (n-pentyl) and against *Proteus vulgaris* peak activity is shown at C_7 (n-heptyl).

The series of decamethylenebis[4-(substituted amino)quinaldinium] compounds were compared for their bactericidal action against *Staphylococcus aureus* and *Escherichia coli* and from the results, summarized in Table 3, it is generally apparent that increased activity is associated with increase in the size of the substituent group.

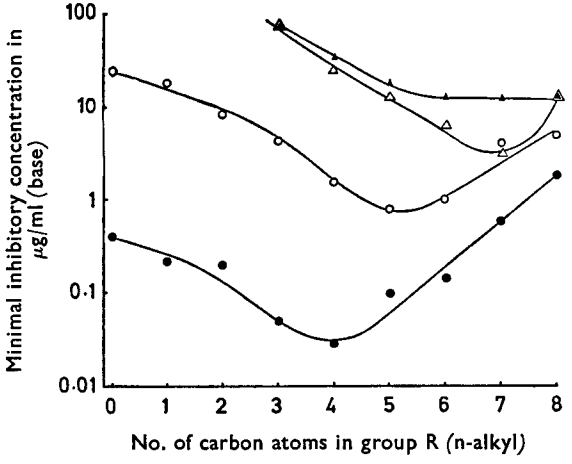

FIG. 1. Bacteriostatic activity of decamethylenebis[(4-n-alkylamino)quinaldinium acetates] against *Staphylococcus aureus* CN491 (●—●), *Escherichia coli* AH (○—○), *Proteus vulgaris* LH14 (△—△) and *Pseudomonas aeruginosa* NCTC8203 (▲—▲) after 24 hr incubation at 37°.

LOCAL CHEMOTHERAPEUTIC ACTIVITY

The activity of the decamethylenebis(4-alkylaminoquinaldinium) salts has been compared with dequalinium (compound 1) in intraperitoneal protection tests in mice against *Staphylococcus aureus*. The results, summarized in Table 4, showed that maximum therapeutic activity occurred when the n-alkyl substituent contained three to six carbon atoms (compounds, 5, 8, 10). An associated increase in toxicity is found with increased chain length. The ratio of the curative dose to the equivalent

TABLE 3. BACTERIOSTATIC AND BACTERICIDAL ACTION OF DECAMETHYLENEBIS[4-(SUBSTITUTED AMINO)QUINALDINIUM ACETATES]

Comp. No.	Minimal Inhibitory Concentration μg/ml (base) at 24 hr				Bactericidal activity* Mean % reduction on on viable count	
	Staph. aureus CN491	*E. coli* AH	*Ps. aeruginosa* 8203	*Pr. vulgaris* LH14	*Staph. aureus* CN491 10^{-6}M	*E. coli* AH 10^{-5}M
1 (Dequalinium)	0·3	25	>100	100	15·87	99·87
2	0·22	17·7	>100	>100	21·43	94·98
3	0·19	8·8	>100	>100	53·47	91·89
4	0·17	50	>100	>100	59·01	99·92
5	0·05	4·4	75	75	0	98·36
6	0·04	12·5	100	100	7·14	69·8
7	0·03	1·5	35	25	65·44	99·79
8	0·1	0·8	17·7	12·5	91·88	99·96
9	0·4	1·1	>100	35	45·87	99·09
10	0·16	1·0	12·5	6·2	97·52	99·99
11	0·17	1·5	>100	25	0	99·96
12	0·6	4·4	12·5	3·1	97·10	99·99
13	0·15	0·6	35	4·4	—	—
14	1·8	4·4	12·5	12·5	96·34	99·99

* After 15 min contact at 20° C.

toxic dose indicates that the optimum response, in the series of 4 n-alkylamino-compounds occurred when the n-alkyl substituent contained six carbon atoms.

TABLE 4. PROTECTION OF MICE BY DECAMETHYLENEBIS[4-(SUBSTITUTED AMINO)-QUINALDINIUM ACETATES] AND POLYMETHYLENEBIS(4-AMINOQUINALDINIUM ACETATES). Compounds administered intraperitoneally 30 min after intraperitoneal inoculation of culture

Compound No.	Formula I	*Staphylococcus aureus* 663		
		ED75 (7 days) mg/kg	LD75* mg/kg	ED75/LD75 ratio
1 (Dequalinium)	R = H; $n = 10$	4·0	9·3	0·43
2	R = CH_3; n = 10	2·4	5·1	0·46
5	R = C_3H_7; n = 10	1·7	5·9	0·29
8	R = C_5H_{11}; n = 10	1·0	4·2	0·24
10	R = C_6H_{13}; n = 10	2·2	4·6	0·48
14	R = C_9H_{17}; n = 10	>10 (Toxic)	<0·6	—
—	R = H $n = 14$	>10 (Toxic)	—	—
—	R = H $n = 16$	>10 ,,	—	—
—	R = H $n = 18$	>10 ,,	—	—

* LD75 assessed in infected animals.

Discussion

The group of decamethylenebis[4-(substituted amino)quinaldinium] salts show significant changes in the peak of bacteriostatic activity against different bacterial species which are associated with an increase in the size of the alkyl substituent. There is little difference in activity between straight-chain and branched-chain alkyl-substituted compounds (cf. compounds 10 and 13) when the substituents are of approximately similar size. The obvious effects of allyl substitution (compound 6) are not apparent in bacteriostatic results but considerable reduction in bactericidal activity is shown and similar effects are seen with the cycloalkyl compounds (9, 11). These cycloalkyl compounds are significantly less active than the corresponding n-alkyl compounds (8, 10).

The activity of the dequalinium salts in protecting mice locally against intraperitoneal infection with *Staphylococcus aureus* was described by Babbs, Collier, Austin, Potter & Taylor (1956). The ability of the decamethylenebis(4-n-alkylaminoquinaldinium) salts to protect mice in similar experiments runs parallel with the bacteriostatic activity of these compounds.

Experimental

4-*Heptylaminoquinaldine.* A mixture of 4-chloroquinaldine (10·0 g) heptylamine (6·5 g) and phenol (10·0 g) was heated in an oil bath at 180°. After an initial exothermic reaction, which occurred when the mixture was at about 140°, the heating was continued for 4 hr. When cool, the mixture was poured into sodium hydroxide solution (20%: 200 ml) to precipitate an orange gum, which solidified. This was separated by filtration, washed, dried and crystallized from light petroleum (b.p. 100–120°) to give the base as a pink solid (7·5 g), m.p. 115–120°. After two recrystallizations, pale pink rods, m.p. 118°–119°, were obtained.

Decamethylenebis(4-heptylaminoquinaldinium iodide). 4-Heptylaminoquinaldine (2·85 g) and decamethylene di-iodide (2·0 g) in ethyl methyl ketone (20 ml) were refluxed for 96 hr. The mixture was cooled, filtered and the residue was washed successively with ethyl methyl ketone, acetone, warm water, acetone and ether to leave the *quaternary salt* as a pale purple solid (2·68 g), m.p. 190–192°. After three recrystallizations, with decolorization by charcoal, from a mixture of methanol and ethanol, buff coloured plates, m.p. 198–200°, were formed.

Decamethylenebis(4-heptylaminoquinaldinium acetate). The above di-iodide (3·63 g), dissolved in warm methanol (100 ml), was stirred with silver acetate (1·34 g) for 2 hr. The precipitated silver iodide (1·90 g) was removed and, after being clarified by filtration through diatomaceous earth, the solution was evaporated under reduced pressure. On addition of dry ether the residual oil slowly gave a pale pink solid (2·79 g), m.p. 107–108° (decomp.). This was purified by being dissolved in a mixture of acetone (100 ml) and methanol (10 ml) and treatment with charcoal. The solution was concentrated to 50 ml, dry ether (50 ml) was added and the oil which was obtained was triturated with dry ether to give the *bis-quaternary diacetate* as a pink solid (2·06 g), m.p. 111–112° (decomp.), raised to m.p. 112–114° (decomp.) on subsequent similar treatment.

Decamethylenebis(4-acetamidoquinaldinium iodide). Decamethylenebis-(4-aminoquinaldinium acetate) (10 g), acetic acid (50 ml) and acetic anhydride (50 ml) were heated under reflux for 26 hr. The solution was concentrated to a small volume and was treated with a solution of sodium iodide (10 g) in water. A dark oil, which slowly solidified, precipitated. It was separated from the aqueous solution and was extracted with hot ethanol (350 ml). The hot extract was boiled with charcoal, filtered, concentrated and allowed to cool. The *bis-quaternary di-iodide* separated as a yellow solid (5·3 g), m.p. 224° (decomp.), which was twice recrystallized from ethanol to give dark yellow plates, m.p. 224–225° (decomp.).

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