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ANTIBACTERIAL ACTIVITIES OF SOME BIS*ISO*QUINOLINIUM SALTS

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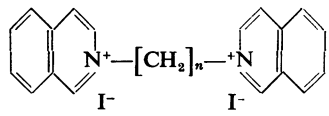
Buttle (private communication) observed that decamethylene bis*iso*quinolinium bromide (Compound No. 7, Collier and Taylor, 1949), which had been prepared in a research programme on neuromuscular blocking agents, possessed considerable antibacterial activity. It was therefore interesting to inquire how far the antibacterial activities of bis*iso*quinolinium salts were influenced by alterations in chemical structure, particularly by those factors that had been found to affect neuromuscular blocking activity. The effect of reduction of the heterocyclic nucleus was examined by comparing Compounds 7 and 8 and that of introduction of methoxyl groups by comparing Compounds 8, 15, and 20 (Taylor and Collier, 1950, 1951). In order to examine the effect of variations in length of the polymethylene chain we prepared a series of polymethylene bis*iso*quinolinium salts of general formula (I), where $n=8$–14,

(I) 

16, 18, or 20. Compound 7, rather than Compounds 8, 15, or 20, was chosen as the basis for this series, since it was the least toxic and was derived from most readily accessible starting material.

METHODS

In vitro observations on the inhibitory activities of the *iso*quinolinium compounds and of cetyltrimethylammonium bromide (CETAB) were made with the following strains of bacteria: *Streptococcus pyogenes* (NCTC 8195); *Str. faecalis* (M75); *Staphylococcus aureus* (Oxford H); *Vibrio cholerae* (48210); *Salmonella typhi* (NCTC 5758); *Shigella shigae* (NCTC 4837); *Sh. flexneri* (NCTC 4832); *Pseudomonas pyocyanea* (NCTC 8099); and *Mycobacterium phlei* (NCTC 525). The strain of *Bacterium coli* used was one isolated at Ware from human faeces in 1947.

Drugs were serially diluted by 1 in 2 in 1% peptone (Difco)-water containing 0.5% dextrose and adjusted to *p*H 7.2. After autoclaving, the dilutions were inoculated with a washed suspension of a 22-hour culture of the bacterial species concerned and adjusted to give approximately 1,000 organisms per culture tube (5 ml.). Cultures were incubated at 37° C. and growth read by eye at 24 hours and subsequently. Results were expressed as the minimal inhibitory concentration (M.I.C.), which was the least concentration in which no growth was visible at 24 hours or, with *Myco. phlei*, at 5 days.

RESULTS

Effect of Variation of End-group on Activity

The *in vitro* activities of four decamethylene compounds and of CETAB against a variety of bacteria are expressed in Table I. Whereas the bis*iso*quinolinium compounds possess appreciable activity in all species except *Ps. pyocyanea*, none is as active as CETAB. It is difficult to draw systematic conclusions from these results, but two trends are apparent: (*a*) In two species of *Shigella* the methoxylated compounds (15 and 20) were appreciably less active than those without such groups (7 and 8); (*b*) in *Str. pyogenes* and in *Myco. phlei* the three bistetrahydro*iso*quinolinium compounds (8, 15, and 20) were more active than the bis*iso*quinolinium compound (7).

Effect of Chain-length on Activity

The relationship of chain-length to activity is indicated in Fig. 1. In each bacterial species examined, increase in chain-length up to the octadecamethylene compound was accompanied by a steady enhancement of antibacterial activity. The eicosane compound exhibited no more and sometimes less activity than the octadecamethylene. A very similar trend against streptococci, staphylococci, and *B. coli* was found in the methonium series by Paton and Zaimis (1949).

The question arose as to whether the higher members of the bis*iso*quinolinium series should be investigated for *in vivo* antibacterial activity. Preliminary toxicity tests in mice of each member

TABLE I

ANTIBACTERIAL ACTIVITIES OF DECAMETHYLENE BIS*ISO*QUINOLINIUM DERIVATIVES
$\{R^+—[CH_2]_{10}—R^+\}2X^-$

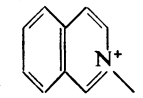
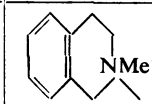
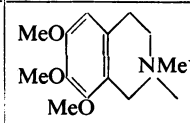
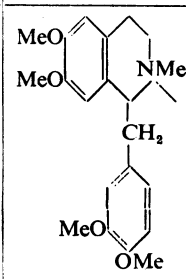
Ser. No.	Structure of End-group R	M.I.C in μg. per ml. against									
		Str. pyogenes	*Str. faecalis*	*Staph. aureus*	*V. cholerae*	*S. typhi*	*Sh. shigae*	*Sh. flexneri*	*B. coli*	*Ps. pyocyanea*	*Myco. phlei*
7		125	160	1.26	160	40	10	20	320	>200	>200
8		25	80	2.5	320	40	40	80	160	>200	12.5
15		12.5	125	0.78	>1,000	250	500	500	1,000	>200	3.55
20		25	50	1.56	625	312	200	312	>200	>200	12.5
	CETAB	0.63	1.12	0.195	25	25	3.125	—	100	>200	1.56

TABLE II

POLYMETHYLENE BIS*ISO*QUINOLINIUM IODIDES

Value of *n*	Reaction Time (hr.)	Reaction Solvent	M.p. (d= decomp.)	Cryst. Form.	Crystn. Solvent	Found, %*				Formula	Required, %			
						C	H	N	I		C	H	N	I
8	30	Benzene	258–259° (d)	Yellow rosettes or clusters	MeOH	50.0	5.05	4.4	40.6	$C_{26}H_{30}N_2I_2$	50.0	4.85	4.5	40.7
9	72	,,	211–212°	Yellow needles	EtOH	50.9	5.1	4.4	39.7	$C_{27}H_{32}N_2I_2$	50.8	5.1	4.4	39.8
11	80	,,	183–184°	Yellow granules	EtOH	52.6	5.4	4.0	37.9	$C_{29}H_{36}N_2I_2$	52.25	5.45	4.2	38.1
12	30	,,	206–207° (d)	Lemon yellow microcrystals	MeOH-EtOH	53.3	5.6	3.95	36.9	$C_{30}H_{38}N_2I_2$	52.9	5.6	4.1	37.35
13	120	,,	185–187°	Yellow granules	EtOH	53.8	5.95	3.9	36.2	$C_{31}H_{40}N_2I_2$	53.6	5.8	4.0	36.6
14	48	,,	170–171°	,, ,,	EtOH	54.3	6.0	3.7	35.65	$C_{32}H_{42}N_2I_2$	54.2	6.0	3.95	35.9
16	48	Alcohol	145.5–146.5°	Yellow nodules or rosettes of tiny needles	EtOH-Et_2O	55.2	6.3	3.7	34.2	$C_{34}H_{46}N_2I_2$	55.4	6.3	3.8	34.5
18	72	,,	138–140°†	Yellow powder‡	EtOH-Et_2O	56.8	6.35	3.6	33.0	$C_{36}H_{50}N_2I_2$	56.5	6.6	3.7	33.25
20	120	,,	143.5–144.5°	Yellow needles§	EtOH-Et_2O	57.4	7.2	3.7	31.45	$C_{38}H_{54}N_2I_2$	57.6	6.9	3.5	32.1

* Dried at 100° *in vacuo*. †After drying at 100° *in vacuo*. The air-dried salt sinters at *ca.* 75–77°, hardens and again softens at *ca.* 116–118°, again hardens and melts at 138–140°. ‡ Contains solvent of crystallization—lost 3.65% by weight on drying *in vacuo* at 100°. § Contains solvent of crystallization—lost 6.0% by weight on drying *in vacuo* at 100°.

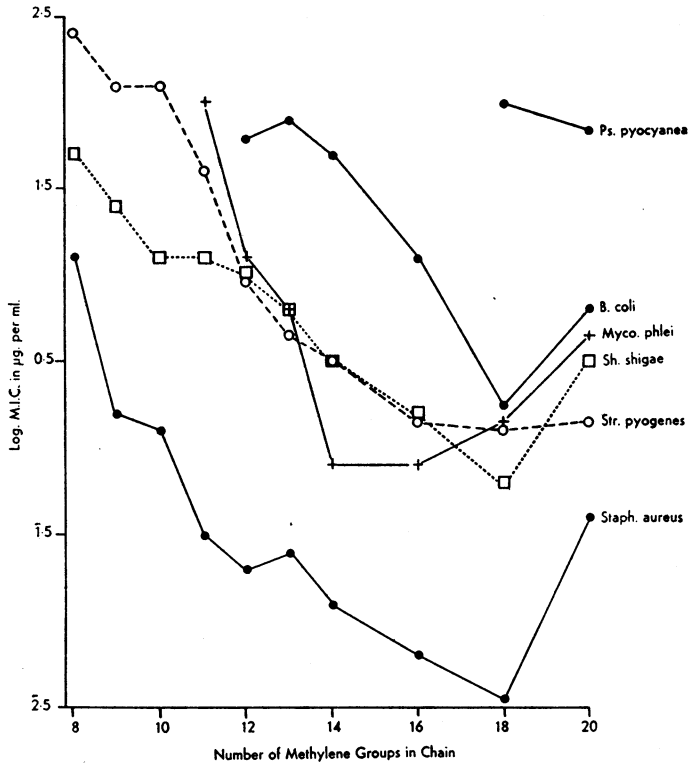


FIG. 1.—Effect of chain-length on activities of polymethylene bis*iso*quinolinium compounds against six species of bacteria. Log M.I.C. (μg. per ml.) plotted against the number of methylene groups in the polymethylene chain.

of the series, however, showed that all compounds possessed considerable toxicity and that there was a steady increase in toxicity with increase in chain-length. For example, while mice tolerated subcutaneous doses of 40 mg. per kg. of the octa- and nona-methylene compounds, they tolerated 5 but not 10 mg. of the octadecamethylene and eicosane compounds. The high toxicity of these compounds, increasing with increased antibacterial activity, suggested that *in vivo* antibacterial tests would be fruitless.

CHEMICAL SECTION

(Microanalyses are by Drs. Weiler and Strauss, Oxford. All melting points are uncorrected.)

The polymethylene glycols required in this work have all been described in the literature (Chuit, 1926; Chuit and Hausser, 1929; Ashton and Smith, 1934; Shiina, 1937; Bouveault and Blanc, 1903). The m.p. of tetradecamethylene glycol was found to be 86.5–87° C., whereas Chuit (1926) recorded an m.p. of 85° C. (Found: C, 73.0; H, 13.1. Calc. for $C_{14}H_{30}O_2$: C, 73.0; H, 13.15%). An apparently unrecorded intermediate in the synthesis of eicosane diol is 1:18-*dicyano-octadecane*, which crystallized from light petroleum (b.p. 40–60° C.) in long flat needles, m.p. 66–67° C. (Found: C, 78.9; H, 11.7; N, 9.3. $C_{20}H_{36}N_2$ requires C, 78.9; H, 11.9; N, 9.2%). Treatment of the glycols with excess hydriodic acid (sp. gr., 1.94) yielded the corresponding polymethylene di-iodides, all of which were known (Ashton and Smith, 1934; Shiina, 1937; v. Braun and Danziger, 1912; v. Braun, 1909; Ziegler and Weber, 1937) with the exception of the following, to which no references could be found in the literature: *tridecamethylene di-iodide*, small needles, m.p. 39–40° C., from alcohol (Found: C, 35.9; H, 6.2; I, 57.7. $C_{13}H_{26}I_2$ requires C, 35.8; H, 6.0; I, 58.3%), and *tetradecamethylene di-iodide*, plates, m.p. 49–50° C., from alcohol (Found: C, 37.4; H, 6.1; I, 56.2. $C_{14}H_{28}I_2$ requires C, 37.3; H, 6.3; I, 56.4%). *Hexadecamethylene di-iodide*, plates, m.p. 55–56° C., from alcohol (Found: C, 40.5; H, 6.8; I, 52.9. $C_{16}H_{32}I_2$ requires C, 40.2; H, 6.7; I, 53.1%), is mentioned by Ziegler and Weber (1937), but no physical properties are given.

The *polymethylene bisisoquinolinium salts* were prepared by boiling the appropriate polymethylene di-iodide with 50% excess of *iso*quinoline in either benzene or alcohol solution for from 30–120 hours; the reaction mixture was cooled and filtered, and the product recrystallized. With the hexadecamethylene and octadecamethylene derivatives, it was necessary to add ether to the alcoholic reaction mixture in order to precipitate the crude quaternary salt.

The following decamethylene derivatives have already been described in connection with other work (Taylor, 1951, 1952): the bis*iso*quinolinium dibromide (Cpd. 7), the bis-(1:2:3:4-tetrahydro-2-methyl*iso*quinolinium) di-iodide (Cpd. 8), the bis (1:2:3:4-tetrahydro-6 : 7 : 8-trimethoxy-2-methyl*iso*quinolinium) di-iodide (Cpd. 15), and the bis [1-(3′:4′-dimethoxybenzyl)-1:2:3:4-tetrahydro-6:7-dimethoxy-2-methyl*iso*quinolinium] dimethosulphate (Cpd. 20). The properties of the other salts are given in Table II.

SUMMARY

1. A series of polymethylene bis*iso*quinolinium salts, with 8–14, 16, 18, and 20 methylene groups, has been prepared. These were examined *in vitro* for inhibitory activity against 6 species of bacteria.

2. In all species inhibitory activity increased with increase in chain-length up to the octadecamethylene compound. The toxicity to mice showed a similar trend.

3. The inhibitory activities of four decamethylene bis*iso*quinolinium compounds that had previously been prepared were examined *in vitro* in ten species of bacteria. In two species of *Shigella* the methoxylated compounds were appreciably less active than those without such groups. In *Str. pyogenes* and *Myco. phlei* the three tetrahydro compounds were more active than the unreduced compound.

4. The antibacterial activities of the compounds examined do not appear sufficient, in view of their relatively high toxicities, to encourage prospects of their being useful against bacterial infections in man. The antibacterial activity of Compound 20, however, may be of some practical importance in maintaining sterile solutions of this drug, which has been found an effective muscle-relaxant in man (Bodman, Morton, and Wylie, 1952).

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