[CONTRIBUTION FROM THE RESEARCH DIVISION OF ARMOUR AND COMPANY]

The Relation of Structure and Critical Concentration to the Bactericidal Activity of Quaternary Ammonium Salts[1]

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Evidence is presented which indicates that the bactericidal activity of quaternary ammonium salts increases with increased charge density on the nitrogen atom but that other factors such as increased bulk around the nitrogen may act to decrease this activity. Within the series of compounds tested activity reaches a maximum at a carbon chain length of sixteen to eighteen, depending upon the test organism. Bactericidal activity generally appears to increase with decreased critical micelle concentration within the series of compounds tested.

The bactericidal activity of higher aliphatic quaternary ammonium salts is related to specific structural characteristics of these compounds. Length of the higher aliphatic chain,[2] ring substitution of phenyl-containing compounds[2] and the total number of carbon atoms in the aliphatic groups[3] are all significant factors.

The present investigation represents an attempt to evaluate, by refined techniques, the effect upon bactericidal activity of variation of charge density upon the nitrogen atom, variation in bulk around the nitrogen atom, and variation in chain length. In order to minimize bacteriostatic effects a "neutralizer" was employed after exposure of the organism to the quaternary ammonium salt. Comparison of the activity of the compounds studied was on a molar basis.

Bactericidal activity of higher aliphatic quaternary ammonium salts is probably related to their colloidal or surface active properties since the lower homologs do not exhibit this activity. The tendency of these colloidal electrolytes to leave solution is manifest by their ability to form micelles in aqueous solutions. It is reasonable to assume that the bactericidal action of cationic colloidal electrolytes is related to this tendency. Since the critical concentration for micelle formation can be determined with accuracy, its correlation with bactericidal activity will be considered.

Experimental

In general, the quaternary ammonium compounds were prepared from a tertiary amine and the appropriate chloride according to procedures previously described.[4] The phenyl and β-phenylethyl derivatives were synthesized by complete methylation of the proper secondary amines.

Dodecyldimethylphenylammonium Chloride.—In the presence of 6.9 g. (0.1 equivalent) of Na_2CO_3 and 50 cc. of methanol, 26.1 g. (0.1 equivalent) of N-dodecylaniline[5] and 30 g. (excess) of methyl chloride were caused to react in a bomb for 12 hours at 80°. Upon cooling, the contents of the bomb were removed, filtered, and evaporated to dryness. Crystallization at −70° from ethyl acetate yielded 14.0 g. of pure white quaternary ammonium salt.

Dodecyldimethyl-β-phenylethylammonium Chloride.—Dodecyl-β-phenylethylamine, b.p. 168° (0.5 mm.), prepared from dodecyl bromide and β-phenylethylamine, was methylated to the tertiary amine, dodecylmethyl-β-phenylethylamine, b.p. 178° (0.2 mm.), by using formaldehyde and formic acid according to directions described in earlier publications.[4] This product was quaternized with methyl chloride to give an over-all 50% yield of ammonium salt.

Critical concentrations for micelle formation (C.M.C.) were determined by the method and in the equipment described in earlier publications from this Laboratory.[6]

Formulas, analyses and physical constants for the series of salts are given in Table I.

The bactericidal activity was determined by exposing known numbers of organisms to known concentrations of quaternary ammonium salts, then neutralizing the quaternary compounds with sauramin sodium[7] after selected time intervals. The numbers of survivors were determined by plate counts. Results of these tests are given in Table II. The activities are expressed broadly in terms of concentration and, within these broad groups, times required to achieve 99.99% kill (as determined by log of number of survivors *vs.* time plots) further differentiate among compounds.

Results and Discussion

The extent of interaction of a quaternary nitrogen atom and a neighboring phenyl ring may be expressed either in terms of the electron density of the ring or the charge density of the nitrogen. The latter notation is used in this discussion since high germicidal activity is associated with the positive character of cationic colloidal electrolytes. The extent of the interaction of a ring removed by successive methylene groups, and quaternary nitrogen atom has been shown quantitatively by determining the per cent. of *m*-isomer obtained on nitration of certain phenyl-containing quaternary ammonium salts.[8] These percentages parallel the relative bactericidal activities of the series of compounds in which one methyl group has been replaced by a dodecyl group. This relationship is shown in Table III. These data indicate that the higher charge density on the nitrogen atom, the more strongly bactericidal is the compound.

The high bactericidal activity of the trifluoromethylbenzyl derivative (No. 13) supports this hypothesis. The strongly electronegative trifluoromethyl group competes with the positive nitrogen atom for the ring electrons, thereby increasing the positive charge density on the nitrogen atom. The greater activity of the dimethyl-β-phenylethyl compound (No. 11) as compared with its structural isomer, the benzylethylmethyl com-

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[4] A. W. Ralston, D. N. Eggenberger, H. J. Harwood and P. L. DuBrow, *ibid.*, **69**, 2095 (1947).

[5] S. Adler, L. Haskelberg and F. Bergmann, *J. Chem. Soc.*, 976 (1940).

[6] D. N. Eggenberger and H. J. Harwood, THIS JOURNAL, **73**, 3353 (1951).

[7] C. A. Lawrence, *J. Am. Pharm. Assoc., Sci. Ed.*, **37**, 57 (1948).

[8] E. A. Alexander, "Ionic Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 9.

Table I

	Quaternary ammonium chloride	M.p.[a] (cor.), °C.	Elementary analyses, %: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	C.M.C. (N)
(1)	$C_{12}H_{25}N^+(CH_3)_3$	246 dec.	68.27	68.27	12.99	12.95	0.0228
(2)	$C_{12}H_{25}N^+(CH_3)_2(C_2H_5)$	189 dec.	69.15	69.22	13.06	12.87	.0213
(3)	$C_{12}H_{25}N^+(CH_3)(C_2H_5)_2$	155 dec.	69.94	69.98	13.12	12.92	.0199
(4)	$C_{12}H_{25}N^+(C_2H_5)_3$	115 dec.	70.65	70.23	13.18	12.93	.0193
(5)	$C_{13}H_{27}N^+(CH_3)_3$	250 dec.	69.15	69.11	13.06	12.92	.0112
(6)	$C_{14}H_{29}N^+(CH_3)_3$	241 dec.	69.94	69.83	13.12	12.98	.0053
(7)	$C_{16}H_{33}N^+(CH_3)_3$	228 dec.	71.31	71.01	13.23	13.35	.0015
(8)	$C_{18}H_{37}N^+(CH_3)_3$	220 dec.	72.46	72.42	13.32	13.03	.000346
(9)	$C_6H_5—N^+(CH_3)_2(C_{12}H_{25})$	114 dec.	73.69	73.73	11.13	10.93	.00765
(10)	$C_6H_5—CH_2N^+(CH_3)_2(C_{12}H_{25})$	42.5-43[b]	74.18	74.21	11.27	11.19	.0081
(11)	$C_6H_5—CH_2CH_2N^+(CH_3)_2(C_{12}H_{25})$	108-110	74.64	74.87	11.39	11.26	.0041
(12)	$C_6H_5—CH_2N^+(CH_3)(C_2H_5)(C_{12}H_{25})$	102-103.5	74.64	74.79	11.39	11.13	.0077
(13)	$CF_3C_6H_4—CH_2N^+(CH_3)_2(C_{12}H_{25})$[c]	159	64.76	64.86	9.14	8.91	.0032
(14)	$C_6H_5—(CH_2)_3—N^+(CH_3)_2(C_{12}H_{25})$	55	75.05	74.41	11.50	11.53	.00313

[a] Small traces of moisture can cause the melting points of quaternary ammonium salts to vary widely [F. K. Broome, C. W. Hoerr and H. J. Harwood, This Journal, **73**, 3350 (1951)]. [b] Melting point of 3/2 hydrate. Analysis is for the anhydrous compound. [c] *m*-Trifluoromethylphenyl.

Table II

Compound	*M. aureus* Test concn. $M \times 10^4$	*M. aureus* Killing time[a] in min.	*E. Coli* Test concn. $M \times 10^4$	*E. Coli* Killing time[a] in min.
1	7.50	3.6	7.50	1.5
2	7.50	1.8	7.50	2.0
3	7.50	2.2	7.50	1.5
4	7.50	1.8	7.50	2.0
5	2.50	1.3	2.50	15
6	0.750	3.7	0.750	25
7	.750	0.35	.750	2.5
8	.750	.6	.750	0.8
9	1.00	10.6	1.25	12.4
10	1.00	9.5	1.25	11.1
11	1.00	0.73	1.25	9.4
12	1.00	10.0	1.25	12.7
13	1.00	0.37	1.25	6.0
14	1.00	1.6	...	...

[a] Time required for 99.99% kill.

Table III

n	*m*-Isomer, % $[C_6H_5(CH_2)_nN(CH_3)_3]Cl$	Relative killing time[a] $[C_6H_5(CH_2)_n\text{-}N(CH_3)_2C_{12}H_{25}]Cl$
0	100	100
1	88	89
2	19	7
3	5	15

[a] Basis compound 9 = 100.

pound (No. 12), corroborates the hypothesis that the observed differences are due to an electrical effect rather than the bulk effect of an additional methylene group.

The attempt to determine the effect of bulk around the nitrogen atom by substituting ethyl for methyl groups was complicated by the accompanying increase in charge density resulting from the greater electronegativity of ethyl groups. Thus with *M. aureus* the activities were in the following order: triethyl (No. 4) = dimethylethyl (No. 2) > methyldiethyl (No. 3) > trimethyl (No. 1). In general it appears that addition of ethyl groups results in increasing activity although irregularities suggest opposing factors. The benzylmethylethyl compound (No. 12) is less active than the benzyldimethyl (No. 10). In this case the electrical effect of the ethyl groups is probably negligible in the presence of the benzyl substituent. This suggests that increased bulk around the nitrogen decreases activity, but conclusions are withheld until more experimental evidence is available.

The most effective long chain length is in the vicinity of C_{16}, but appears to be specific for the organism involved. On *E. coli* the order of reactivity is: $8 > 7 \gg 6 \gg 5 \gg 1$; whereas with *M. aureus* the order is: $7 > 8 \gg 6 \gg 5 \gg 1$. On a molar basis the C_{18}-salt is not vastly different from the C_{16}-salt with either organism. There seem to be no special or extraordinary properties associated with the odd carbon chain, C_{13}, since the activity of the C_{13}-salt lies between those of C_{12} and C_{14}. That an increase in the length of the long aliphatic chain has a greater effect than an identical increase in one of the shorter chains is obvious from the relative activities of structural isomers (No. 2 and No. 5; No. 3 and No. 6).

It is interesting to note that germicidal activity is high in compounds of lower critical micelle concentration. Consider the pairs of compounds No. 6 and No. 7 and No. 11 and No. 12 in Table I. In the former case more lengthening of the alkyl chain causes the C.M.C. to decrease; whereas, in the latter case an electrical effect leads to a decrease in C.M.C. These same two widely different causes produce a similar increment in bactericidal activity. This suggests the simple relationship that higher bactericidal activity is associated with cationic compounds of lower C.M.C. Obviously, in all cases the relationship must fail at the point where solubility decreases below the effective germicidal concentration.

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