XIII did not readily add bromine. The methyl alcoholic filtrate of this experiment yielded 18.5% of IV when diluted with water, melting point and mixed melting point with a sample of this compound described in another experiment, 57°. This product also turned pink upon exposure to light.

Acknowledgment.—We wish to express our appreciation to Mrs. M. S. Sherman for carrying out the recorded microanalyses.

Summary

The molecular rearrangement of fluorylidene dimethyl sulfide to fluorene-1-dimethyl sulfide was effected in an alkaline medium. By graded oxidation the rearrangement product was converted into fluorene-1-dimethyl sulfone, fluorenone-1-dimethyl sulfone and fluorenone-1-carboxylic acid. The above-named acid and its ethyl ester were characterized by comparison with authentic specimens. The $-SCH_3$ group of fluorene-1-dimethyl sulfide was substituted by $-OCH_3$, —Br, —OH and —H. Fluorene-1-methyl bromide in an alcoholic solution of sodium methylate yielded *sym.*-difluorene-1,1′-ethylene and fluorene-1-dimethyl oxide.

BELTSVILLE, MARYLAND RECEIVED JANUARY 24, 1946

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE WM. S. MERRELL COMPANY]

Quaternary Ammonium Salts as Germicides. I. Non-acylated Quaternary Ammonium Salts Derived from Aliphatic Amines[1]

BY R. S. SHELTON, M. G. VAN CAMPEN, C. H. TILFORD, H. C. LANG, L. NISONGER, F. J. BANDELIN[2] AND H. L. RUBENKOENIG

The antibacterial properties of quaternary ammonium compounds were first observed toward the end of the last century among the carbonium dyestuffs, such as auramin,[3] methyl violet[3] and malachite green.[4] These types of compounds are effective chiefly against the Gram-positive organisms. Later Jacobs and Heidelberger[5] studied the antibacterial activity of substituted hexamethylenetetrammonium salts. In the nineteen-twenties Browning, *et al.*, found greater and somewhat less selective bactericidal powers among styryl and anil quinolinium salts,[6] the flavine dyes[7] and quaternary derivatives of pyridine, quinoline, acridine and phenazine.[8] Hartman and Kagi[9] observed antibacterial activity in quaternary ammonium compounds of acylated alkylene diamines. With the disclosure by Domagk[10] of the much improved germicidal activity obtained when a large aliphatic residue was attached to the quaternary nitrogen atom, the study of the use of quaternary ammonium salts as germicides was greatly stimulated. In the past ten years many patents have been issued in the field, but few reports have been published of systematic study of these compounds and of the influence of their chemical structure upon their antibacterial properties. We wish to record here the work done in this Laboratory on this problem.

There was first prepared a series of simple aliphatic quaternary ammonium salts of the general type $R(CH_3)_3NBr$, where R is a straight chain alkyl group of 6 to 18 carbon atoms. These compounds, when pure, are obtained as fine white, nearly odorless, crystals. In general they are nearly insoluble in diethyl ether and benzene, sparingly soluble in acetone, and, except for the stearyl derivative, freely soluble in water and alcohol. They are stable in acid solution and in all but very strongly alkaline solutions. Table I shows the effect upon germicidal activity of variations in the chain length of the R group.

To determine the effect of the anion upon the activity, the following cetyltrimethylammonium salts were prepared: the chloride, bromide, iodide, nitrate, sulfate, methosulfate, secondary orthophosphate, acetate, benzoate, cyanide, laurate, hydrocinnamate, salicylate and fluosilicate. The phosphate, laurate and salicylate gave definitely lower activity; no significant differences in germicidal power were observed among the other salts, except that the halides and the sulfate gave slightly higher activity against *Staph. aureus* than did the other salts. The solubility in water of the series varied from about 1:4 to 1:10, except for the iodide and the laurate, whose solubilities were 1:2000 and 1:250, respectively.

Similar compounds were prepared in which one or more of the N-methyl groups were replaced by other radicals. In Table II is shown the effect of these substitutions upon antibacterial activity.

(1) This series of papers was presented in part at the September, 1939, meeting of the American Chemical Society, in Boston, and at the April, 1940, meeting in Cincinnati.

(2) Present address: Flint, Eaton and Company, Decatur, Illinois.

(3) Stilling, "Anilifarbstoffe als Antiseptica und ihre Anwendung in der Praxis," Trubner, Strassburg, 1890.

(4) See and Moreau, *Bull. gen. Therap.*, **120**, 502 (1891).

(5) Jacobs and Heidelberger, *Proc. Nat. Acad. Sci. U. S.*, **1**, 226 (1915); *J. Biol. Chem.*, **20**, 659 (1915); *J. Exptl. Med.*, **23**, 569 (1916).

(6) Browning, Cohen, Ellingworth and Gulbransen, *Proc. Roy. Soc.*, London, **100B**, 293 (1926).

(7) Browning, Gulbransen, Kennaway and Thornton, *Brit. Med. J.*, **1**, 73 (1926).

(8) Browning, Cohen, Gaunt and Gulbransen, *Proc. Roy. Soc.*, London, **93B**, 329 (1922).

(9) Hartman and Kagi, *Z. angew. Chem.*, **4**, 127 (1928).

(10) Domagk, *Deut. med. Wochschr.*, **61**, 829 (1935).

TABLE I

PROPERTIES OF ALKYLTRIMETHYLAMMONIUM BROMIDES: $R(CH_3)_3NBr$

R	C. K. D.[a] 1000 pts. H_2O *Staph. aureus*	C. K. D.[a] 1000 pts. H_2O *E. typhosa*	M. p., °C.	Soly. parts H_2O[b]	Formula	Bromine, % Calcd.	Bromine, % Found
n-Hexyl	Inact.	0.015	.....	1	$C_9H_{22}NBr$	35.7	36.0
n-Octyl	.03	0.075	198–200	4	$C_{11}H_{26}NBr$	31.5	32.0
Lauryl	4	9	228–230	20	$C_{15}H_{34}NBr$	26.0	26.4
Myristyl	38	36	244–255	5	$C_{17}H_{38}NBr$	23.8	23.6
Cetyl	80	40	237–243	10	$C_{19}H_{42}NBr$	21.9	21.9
Stearyl	64	8	230–240 dec.	1000	$C_{21}H_{46}NBr$	20.4	20.5
Oleyl	46	24	225–230	7500	$C_{21}H_{44}NBr$	20.5	20.6

[a] Critical Killing Dilution—that concentration of the substance which will kill organisms of standard phenolic resistance in ten minutes but not in five, determined at 37° by the technic described for the determination of phenol coefficients in Circular 198 of the U. S. Department of Agriculture. [b] Approximate solubility at room temperature.

TABLE II

EFFECT OF VARIOUS SUBSTITUENTS ON THE ANTIBACTERIAL ACTIVITY OF ALIPHATIC QUATERNARY AMMONIUM SALTS

No.	Ammonium salt	C. K. D.[a] 1000 pts. H_2O *Staph. aureus*	C. K. D.[a] 1000 pts. H_2O *E. typhosa*	M. p., °C.	Soly. parts H_2O[b]	Formula	Halogen, % Calcd.	Halogen, % Found
1	Benzyldimethyllauryl- bromide	32	30	.....	5	$C_{21}H_{38}NBr$	20.8	20.4
2	Benzyldimethyllauryl- cyanide	20	28	.....	..	$C_{22}H_{38}N_2$	..	..
3	Benzyldimethylpentadecyl- bromide	52	36	59–61	5	$C_{24}H_{45}NBr$	18.8	18.6
4	Benzyldiethylpentadecyl- bromide	48	28	76–78	5	$C_{26}H_{49}NBr$	17.5	17.6
5	Benzylcetyldimethyl- bromide	76	32	68–70	20	$C_{25}H_{47}NBr$	18.2	18.1
6	Benzylcetyldimethyl- nitrate	44	8	60–65	20	$C_{25}H_{47}O_3N_2$	6.63[c]	6.02[c]
7	Cetyl-*o*-chlorobenzyldimethyl- bromide	52	8	61–64	5	$C_{25}H_{46}NClBr$	16.9[d]	16.3[d]
8	2-Chloroethyldimethyllauryl- chloride	8	36	165–167	10	$C_{16}H_{35}NCl_2$	11.3[d]	11.1[d]
9	Cetyldimethylethyl- bromide	60	40	178–186	5	$C_{20}H_{44}NBr$	21.2	21.1
10	Cetyltriethyl- bromide	85	52	145–155	5	$C_{22}H_{48}NBr$	19.7	19.5
11	Cetyltriethyl- iodide	100	36	175–177	1000	$C_{22}H_{48}NI$	28.0	27.9
12	*n*-Butylcetyldimethyl- bromide	90	40	83–84	6	$C_{22}H_{48}NBr$	19.7	19.6
13	Cetyldi-*n*-butylmethyl- bromide	28	16	50–52	10	$C_{25}H_{54}NBr$	18.0	17.8
14	Cetyl-tri-*n*-butyl- bromide	48	16	68–70	10	$C_{28}H_{60}NBr$	16.3	16.1
15	Benzylcetylmethylpropyl- iodide (*l*)[e,f]	28	6	99–100	..	$C_{27}H_{51}NI$	24.6	24.4
16	Benzylcetylmethylpropyl- iodide (*dl*)[e]	28	6	83–85	..	$C_{27}H_{51}NI$	24.6	24.3
17	2,3-Dihydroxypropyldimethyllauryl- chloride	5	20	.....	8	$C_{17}H_{38}O_2NCl$	11.0	10.7
18	Cetyl-2,3-dihydroxypropyldimethyl- chloride	60	40	*ca.* 110	6	$C_{21}H_{46}O_2NCl$	9.37	9.15
19	Cetyldiethyl-2-hydroxyethyl- bromide	8	12	117–121	5	$C_{22}H_{48}ONBr$	...	...
20	3-Dodecoxy-2-hydroxypropyltrimethyl- chloride	29	28	90–93	5	$C_{18}H_{40}O_2NCl$	11.05	11.15

[a] See ref. *a*, Table I. [b] Approximate solubility at room temperature. [c] Nitrogen, %. [d] Ionizable halogen only, %. [e] Supplied by courtesy of Dr. Wallace R. Brode, Department of Chemistry, Ohio State University, Columbus, Ohio. [f] $[\alpha]^{25}D$ 5.90°.

Experimental

Since these quaternary ammonium salts were made by means of well-known reactions it is not thought necessary to give the details of the preparation of each compound. The reactions used were those of alkyl halides with primary and secondary amines to give the hydrohalides of secondary and tertiary amines, and of alkyl halides with tertiary amines to give quaternary ammonium halides. Temperature and solvent had some effect on the speed of the reaction. The reaction was carried out either with no solvent or in the presence of alcohol. In most cases satisfactory yields were obtained by letting the reaction mixture stand several days at room temperature, by refluxing the mixture several hours, or by heating to 80–125° in a closed vessel. When, as was usually the case, the starting materials were a long chain alkyl halide and a substituted amine, a slight to 100% excess of amine was used. When a tertiary amine containing a long chain alkyl radical was treated with a low molecular weight alkyl halide it was usually found advantageous to use a 25–75% excess of alkyl halide.

The three different halides prepared were formed by treating the appropriate tertiary amines with an alkyl chloride, bromide or iodide according to the anion desired. Cetyltrimethylammonium acetate, benzoate, nitrate, hydrocinnamate, salicylate and sulfate were prepared by treating cetyltrimethylammonium bromide in alcoholic solution with the appropriate silver salts. Cetyltrimethylammonium methosulfate was obtained by the reaction of cetyldimethylamine with dimethyl sulfate. The fluosilicate was prepared by refluxing the quaternary ammonium bromide with fluosilicic acid in alcoholic solution and crystallizing the product. The laurate and the cyanide were synthesized by treating cetyltrimethylammonium bromide with potassium laurate or potassium cyanide in alcohol. The phosphate was made by treating the bromide with potassium hydroxide in alcoholic solution, filtering to remove the potassium bromide formed, and neutralizing the filtrate with phosphoric acid.

Discussion

From the data in Table I it is apparent that in the alkyltrimethylammonium bromide series very little germicidal activity is obtained when the long

chain alkyl group contains less than eight carbon atoms. As the chain is lengthened in this group the germicidal activity increases substantially, reaching a maximum with cetyltrimethylammonium bromide. With the substitution of the stearyl group the activity and the solubility fall off sharply, and this effect is not appreciably influenced by replacement by the unsaturated oleyl group. The lower members of the series are more active against *E. typhosa* than against *Staph. aureus*, whereas this relation is reversed as the chain length of the alkyl group is increased.

Comparison of compounds 1–6 in Table II with those in Table I indicates that in the lower members of the series replacement of one of the methyl groups by a benzyl radical enhances the activity somewhat, but this effect dwindles as the chain length of the large alkyl residue is increased, and benzylcetyldimethylammonium bromide is no more active than cetyltrimethylammonium bromide. Compounds 7 and 8 show no significant change in activity due to chlorine substitution in one of the alkyl radicals. In the data for compounds 9–14 it is apparent that replacement of the methyl groups in cetyltrimethylammonium bromide by ethyl groups has no effect on the activity, while replacement with more than one butyl group tends to lower the activity. It will be noted in the case of compounds 15 and 16 of the table that the racemic and levo isomers had equal antibacterial activities. The data for compounds 17 to 20 show that the introduction of hydroxyl groups tends to diminish the germicidal activity of this series of quaternary ammonium salts.

Summary

A series of quaternary ammonium salts derived from aliphatic amines, and containing one long chain alkyl radical and three short chain radicals, has been prepared and the germicidal powers of the compounds have been studied. In the alkyltrimethylammonium bromide series the maximum antibacterial activity is found in cetyltrimethylammonium bromide.

In a series of cetyltrimethylammonium compounds the anion was found, in general, to have little influence on the germicidal activity.

Substitution of N-benzyl, N-butyl or N-ethyl groups for N-methyl groups in the cetyl series of compounds either did not affect or reduce the germicidal activity. Introduction of hydroxy groups gave similar results.

CINCINNATI, OHIO RECEIVED OCTOBER 18, 1945

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE WM. S. MERRELL COMPANY]

Quaternary Ammonium Salts as Germicides. II. Acetoxy and Carbethoxy Derivatives of Aliphatic Quaternary Ammonium Salts

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In a further exploration of the influence of chemical structure upon the antibacterial activity of quaternary ammonium salts some of the compounds described in an earlier paper[2] were modified by the substitution of a carbethoxymethyl, a β-acetoxyethyl, or in one case a carbamylmethyl group for one of the low molecular weight N-alkyl residues. The products are of the type $RR_1(R_2)_2NX$, where R = cetyl or lauryl, R_1 = carbethoxymethyl, β-acetoxyethyl or carbamylmethyl, R_2 = methyl, ethyl or *n*-butyl, and X = a monovalent anion. With several of the compounds the effect of changing the anion was also studied. Properties of the compounds prepared are given in Table I.

Experimental

All these compounds were purified readily by recrystallization from acetone or by solution in a small amount of alcohol and precipitation by the addition of ether.

Carbamylmethylcetyldimethylammonium Chloride.—A methanol solution of equimolecular amounts of cetyldimethylamine and chloroacetamide was heated at 60° for ten days.

Carbethoxymethyldimethyllaurylammonium Chloride.—An equimolecular mixture of dimethyllaurylamine and ethyl chloroacetate was allowed to stand sixty hours at room temperature.

Carbethoxymethylcetyldimethylammonium Chloride, Bromide and Iodide.—Cetyldimethylamine was mixed in equimolecular amounts with the required ethyl haloacetate. With the chloroacetate the reaction was completed after four hours at 70°, with the bromoacetate after two hours at room temperature, and with the iodoacetate after standing overnight at room temperature.

β-Acetoxyethyldimethyllaurylammonium Chloride.—Treatment of dimethyllaurylamine with β-chloroethyl acetate resulted in the formation of dimethyllaurylamine hydrochloride. One mole of dimethyllaurylamine was heated with two moles of ethylene dichloride for one hundred hours at 100°. The β-chloroethyldimethyllaurylammonium chloride produced in this reaction was heated one hour on the steam-bath in 50% alcohol solution with an equimolecular amount of potassium acetate. Conversion to the β-acetoxy derivative was 95% complete.

β-Acetoxyethyldimethyllaurylammonium Bromide.—Dimethyllaurylamine was heated with a small excess of β-bromoethyl acetate for eight hours at 60°.

β-Acetoxyethylcetyldimethylammonium Bromide.—Equimolecular amounts of cetyldimethylamine and β-bromoethyl acetate were heated together for twenty-four hours at 70°.

(1) Present address: Flint, Eaton and Company, Decatur, Illinois.

(2) R. S. Shelton, *et al.*, THIS JOURNAL, **68**, 753 (1946).