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

BY R. S. SHELTON, M. G. VAN CAMPEN, C. H. TILFORD, H. C. LANG, L. NISONGER, F. J. BANDELIN[1] AND H. L. RUBENKOENIG

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).

TABLE I

PROPERTIES OF ALIPHATIC QUATERNARY AMMONIUM SALTS CONTAINING AMIDE AND ESTER GROUPS

| 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 |
|---|---|---|---|---|---|---|---|
| Carbamylmethylcetyldimethyl- chloride | 8 | 8 | 107–110 | ... | $C_{20}H_{40}ON_2Cl$ | 9.81 | 7.45 |
| Carbethoxymethyldimethyllauryl- chloride | 12 | 24 | 45–55 | 10 | $C_{18}H_{38}O_2NCl$ | 11.4 | 11.2 |
| -iodide | 8 | 16 | Oil | 10 | $C_{18}H_{38}O_2NI$ | .. | .. |
| -nitrate | 18 | 28 | Oil | 10 | $C_{18}H_{38}O_5N_2$ | .. | .. |
| -salicylate | 8 | 12 | Hygro. | 6 | $C_{25}H_{43}O_5N$ | .. | .. |
| Carbethoxymethylcetyldimethyl- chloride | 36 | 16 | 72–74 | 100 | $C_{22}H_{46}O_2NCl$ | 9.44 | 9.10 |
| -bromide | 36 | 20 | 61–63 | 100 | $C_{22}H_{46}O_2NBr$ | 18.4 | 17.1 |
| -iodide | 48 | 20 | 47–53 | 40 | $C_{22}H_{46}O_2NI$ | 26.2 | 26.1 |
| -nitrate | 24 | 16 | 59–62 | 40 | $C_{22}H_{46}O_5N_2$ | .. | .. |
| β-Acetoxyethyldimethyllauryl- chloride | 5 | 28 | 163–165 | 5 | $C_{18}H_{38}O_2NCl$ | 10.6 | 10.5 |
| -bromide | 8 | 12 | 63–66 | 4 | $C_{18}H_{38}O_2NBr$ | 21.05 | 21.2 |
| -iodide | 5 | 6 | 86–88 | 20 | $C_{18}H_{38}O_2NI$ | 29.7 | 30.4 |
| -nitrate | 5 | 12 | Oil | 10 | $C_{18}H_{38}O_5N_2$ | .. | .. |
| -laurate | 5 | 4 | 65–70 | 200 | $C_{30}H_{61}O_4N$ | .. | .. |
| β-Acetoxyethylcetyldimethyl- bromide | 36 | 12 | 70–75 | 50 | $C_{22}H_{46}O_2NBr$ | 18.3 | 18.3 |
| -iodide | 42 | 10 | 89–90 | 80 | $C_{22}H_{46}O_2NI$ | 26.3 | 25.6 |
| -nitrate | 38 | 7 | 54–55 | 40 | $C_{22}H_{46}O_5N_2$ | .. | .. |
| -*p*-hydroxybenzoate | 24 | 8 | 78–81 | 30 | $C_{29}H_{51}O_5N$ | .. | .. |
| -laurate | 5 | Inact. at 4 | 59–61 | Slt. | $C_{34}H_{69}O_4N$ | 2.81[c] | 2.98[c] |
| β-Acetoxyethylcetyldiethyl- bromide | 60 | 12 | 69–72 | 10 | $C_{24}H_{50}O_2NBr$ | 17.3 | 18.2 |
| β-Acetoxyethylcetyl-di-*n*-butyl- bromide | 52 | 16 | 52–54 | 4 | $C_{28}H_{58}O_2NBr$ | 15.4 | 15.3 |

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

**β-Acetoxyethylcetyldiethylammonium Bromide.**—Equimolecular amounts of cetyl bromide and β-diethylaminoethyl acetate were heated together for eighteen hours at 70°.

**β-Acetoxyethylcetyldibutylammonium Chloride.**—Equimolecular amounts of cetyl bromide and β-dibutylaminoethylacetate were heated together for two weeks at 70°.

The remaining quaternary ammonium iodides in the table, the laurates and the salicylate were readily obtained by metathesis between the quaternary ammonium chloride or bromide and the corresponding potassium salt in absolute alcohol or in acetone. The nitrates and the *p*-hydroxybenzoate were obtained by treating the quaternary ammonium bromide or chloride with the required silver salt.

## Discussion

In considering the figures given for bactericidal activity throughout this series of papers it should be borne in mind that for the most part the critical killing dilution given for any one compound is based on a single series of dilutions tested on the same day against the same bacterial suspension. Due to variations in viability among individual bacterial suspensions made from the same parent culture occasional discrepancies appear. Such discrepant results were rechecked only when they seriously obscured an understanding of the general trend of the data.

It is apparent from the data in the table that the antibacterial activities of the halides and the nitrate of any one quaternary ammonium radical do not vary significantly. However, in each case where tried, the laurate is of considerably lower potency and solubility than the halides and nitrate.

In the first of this series of papers[2] the critical killing dilutions of lauryltrimethylammonium bromide against *Staph. aureus* and *E. typhosa* were reported to be 1:4000 and 1:9000, respectively, and the corresponding values for cetyltrimethylammonium bromide were 1:80,000 and 1:40,000. Comparison of these values with those given in Table I for the carbamylmethyl, β-acetoxyethyl and carbethoxymethyl derivatives of these compounds leads to the following conclusions: In the cetyl compound, substituting a carbamylmethyl group for one of the original methyl groups results in an 80–90% loss of germicidal activity. Substituting a carbethoxymethyl group or a β-acetoxyethyl group produces a compound only about half as active against *Staph. aureus* and somewhat less than half as active against *E. typhosa*. The loss in activity against *E. typhosa* is somewhat greater in the case of the β-acetoxyethyl derivative than with the carbethoxymethyl derivative.

In the lauryl compound the substitution of an N-carbethoxymethyl group gives about twice as great activity against both organisms. Substitution with an N-β-acetoxyethyl group results in a smaller increase in activity against *E. typhosa* and no significant change in the activity against *Staph. aureus*. In the last two compounds of Table I it appears that the activity against *Staph. aureus* of the β-acetoxyethylcetyl derivatives can be somewhat increased by lengthening the short chain N-alkyl radicals, without, however, any

improvement in the activity against *E. typhosa*. The activity of these compounds against *E. typhosa* is considerably below that already reported for cetyltriethylammonium bromide and cetyltri-*n*-butylammonium bromide.


## Summary

N-Carbamylmethyl, N-carbethoxymethyl and N-β-acetoxyethyl derivatives of several quaternary ammonium compounds containing one N-cetyl or N-lauryl group and three low molecular weight N-alkyl radicals were prepared. The germicidal activity of these compounds was studied, and in some cases the effect on the activity of different anions was tested.

In the N-lauryl group of compounds the germicidal activity was improved by the substitution of a carbethoxymethyl or β-acetoxyethyl group for an original N-methyl radical. In the N-cetyl group the reverse was true. The halide salts and the nitrate all gave about the same activity. Organic anions usually lowered the activity of the compound.


CINCINNATI, OHIO RECEIVED NOVEMBER 18, 1945

---

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

# Quaternary Ammonium Salts as Germicides. III. Quaternary Ammonium Salts Derived from Cyclic Amines

BY R. S. SHELTON, M. G. VAN CAMPEN, C. H. TILFORD, H. C. LANG, L. NISONGER, F. J. BANDELIN[1] AND H. L. RUBENKOENIG

In a study of the effect of structure upon the germicidal activity of this class of quaternary ammonium salts, a number of N-alkylated quaternary ammonium derivatives of several cyclic amines were prepared. Kolloff, Wyss, Himelick and Mantele[2] prepared and tested the chlorides, bromides and iodides of N-lauryl, N-myristyl and N-cetyl quaternary ammonium derivatives of pyridine, 2-picoline and 4-picoline. A few such compounds were made in this Laboratory and the results are reported in this paper. These data check those given by Kolloff, *et al.*, fairly closely, except that here the N-cetyl derivatives are found to have a somewhat higher germicidal activity, and the marked rise in melting point remarked by Kolloff on remelting a sample was not observed in the samples prepared for the present investigation. Cetyl bromide or cetyl chloride prepared from cetyl alcohol was used in the preparation of the cetyl derivatives, and it was observed that cetyl alcohol from different sources consistently gave products of different germicidal power, even though the samples of cetyl alcohol responded almost identically to the usual tests for purity.

The properties of the cyclic quaternary ammonium salts, in general, resemble those of the aliphatic compounds described in earlier papers,[3] except that they are less stable in alkaline solutions. Some of the properties of the compounds prepared are summarized in Table I.

## Experimental

The preparation of most of these compounds is easily carried out by one or another of the methods described previously for the preparation of the aliphatic quaternary ammonium salts. The alkylpyridinium and quinolinium salts were prepared by heating the alkyl halide with a 10 to 30% excess of the amine at temperatures from 60 to 130°. The excess of amine speeds up the reaction considerably and usually eight to sixteen hours heating is sufficient to carry the reaction to 95% of completion, the alkyl bromides requiring much less time than the chlorides. It is often advisable to employ a relatively low reaction temperature since at the higher temperatures there is a tendency for a tertiary amine hydrohalide to be formed, especially when the amine contains nuclear substituents, as in the picolines and lutidines. When the reaction is complete one or two recrystallizations usually give a pure product.

The dialkylpiperidinium or morpholinium salts were generally prepared in essentially the same way. It was usually convenient to allow the lauryl, cetyl or other alkyl halide of high molecular weight to react, first, with a bimolecular quantity of the cyclic amine at 20–60° without a solvent. At the completion of this reaction, half of the amine used is present as the alkyl derivative and half has reacted with the halogen acid set free during the alkylation, forming the hydrohalide. When complete reaction had occurred, the amine hydrohalide was removed by filtration and the substituted amine obtained by distillation of the filtrate under reduced pressure. Often the filtrate contained so few impurities that purification by distillation was not essential before use in the next step of the synthesis. This alkylamine was then allowed to react with the low molecular weight alkyl halide under as mild conditions as possible and usually without a solvent. When necessary, alcohol is the best solvent for reaction, but often must be removed afterward to make crystallization of the product possible.

In order to obtain α-carbomethoxyalkyl derivatives, the alkyl bromide, used in the reactions described above, was replaced by the methyl ester of the required alpha-bromo fatty acid.

1-(1′-Piperidinocarbonyl)-pentadecylpyridinium bromide was synthesized by reaction between pyridine and α-bromopalmitic piperidide.

The quaternary ammonium sulfate and the nitrate were obtained by metathesis between the quaternary ammonium bromide and silver sulfate or nitrate. The methosulfate was prepared by refluxing an alcoholic solution of the quaternary ammonium chloride and dimethyl sulfate.

## Discussion

The germicidal activity is shown to be greatest in the compounds containing an N-cetyl group in

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

(2) Kolloff, Wyss, Himelick and Mantele, *J. Am. Pharm. Assn.*, **31**, 54 (1942).

(3) R. S. Shelton, *et al.*, THIS JOURNAL, **68**, 753 (1946); **68**, 755 (1946).