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.

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[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**, 51 (1942).

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

TABLE I

PROPERTIES OF QUATERNARY AMMONIUM SALTS DERIVED FROM CYCLIC AMINES

No.	Compound	C. K. D.[a] 1000 pts. H_2O *Staph. aureus*	*E. typhosa*	M. p., °C.	Soly. parts H_2O[b]	Formula	Halogen, % Calcd.	Found
	Pyridinium salts							
1	Cetyl nitrate	84	48	67–69	5	$C_{21}H_{38}O_3N_2$	7.65[c]	7.84[c]
2	Cetyl sulfate	72	48	115–118	5	$C_{42}H_{76}O_4N_2S$	4.55[d]	4.79[d]
3	Cetyl methosulfate	90	56	82–84	5	$C_{22}H_{41}O_4NS$	3.38[e]	3.41[e]
	Pyridinium bromides							
4	Cetyl	72	44	56–59	10	$C_{21}H_{38}NBr$	19.9	18.8
5	1-(1′-Piperidinocarbonyl)-pentadecyl	36	32	88–90	10	$C_{26}H_{45}O_2N_2Br$	17.1	17.0
6	α-Carbomethoxypentadecyl	48	16	Hyg.	10	$C_{22}H_{38}O_2NBr$	18.7	18.8
	Pyridinium chlorides							
7	*n*-Octyl	0.03	0.06	Oil	..	$C_{13}H_{24}ONCl$	15.2[e]	15.1
8	Lauryl	19	5	86–87	..	$C_{17}H_{30}NCl$	12.5	11.8
9	Myristyl	69	42	75–76	..	$C_{19}H_{36}ONCl$	10.6[e]	10.6
10	Cetyl[f]	80	48	80–83	5	$C_{21}H_{40}ONCl$	9.9[e]	9.9
11	Stearyl	43	26	86–87	..	$C_{23}H_{44}ONCl$	9.2[e]	9.2
12	3-Dodecoxy-2-hydroxypropyl	16	28	80–84	5	$C_{20}H_{36}ONCl$	10.1	10.0
	Piperidinium bromides							
13	Laurylmethyl	12	12	203–204	8	$C_{18}H_{38}NBr$	22.9	22.9
14	Cetylmethyl	60	48	187–190	5	$C_{22}H_{46}NBr$	19.8	19.5
15	Stearylmethyl	6	6	185–190	1000	$C_{24}H_{50}NBr$	18.5	18.2
16	Cetylethyl	60	48	74	5	$C_{23}H_{48}NBr$	21.2	21.1
17	β-Bromoethylcetyl	32	8	150–155	1500	$C_{23}H_{47}NBr_2$	16.4[g]	16.4[g]
18	Stearylcrotyl	7	7	67–70	1000	$C_{27}H_{54}NBr$	16.9	17.1
19	Dilauryl	5	6	179–181	70	$C_{29}H_{60}NBr$	15.90	15.95
20	Dicetyl	5	Inact.	180–183	1000	$C_{37}H_{76}NBr$	13.0	13.0
21	α-Carbomethoxyundecylmethyl	5	Inact.	123–125	10	$C_{19}H_{38}O_2NBr$	20.4	20.0
22	α-Carbomethoxypentadecylmethyl	72	28	158–159	10	$C_{23}H_{46}O_2NBr$	17.8	17.5
23	3-Dodecoxy-2-hydroxypropylmethylpiperidinium chloride	20	36	95–98	5	$C_{21}H_{44}O_2NCl$	9.41	9.35
	Bromides of other quaternaries							
24	Cetyl-2-picolinium	64	40	117–120	5	$C_{22}H_{41}NBr$	20.05	19.9
25	Cetyl-2,4-lutidinium	68	32	62–65	8	$C_{23}H_{44}NBr$	19.4	19.2
26	Cetylquinolinium	48	16	104–106	1000	$C_{25}H_{40}NBr$	18.4	18.3
27	Cetylmethyl-2-pipicolinium	60	24	170–175	5	$C_{22}H_{46}NBr$	19.1	18.9
28	Cetylethyl-2-pipicolinium	32	28	165–175	5	$C_{23}H_{50}NBr$	19.1	18.9
29	Cetylmethylmorpholinium	40	28	178–182	5	$C_{21}H_{44}ONBr$[h]	19.7	19.9
30	Cetylethylmorpholinium	46	6	65	125	$C_{22}H_{46}ONBr$	19.0	18.6

[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 technique 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, %. [d] Sulfur, %. [e] Monohydrate. [f] "Ceepryn" trademark reg. U. S. Pat. Off. [g] Ionizable bromine only, %.

both the pyridinium series (Table I, nos. 7–12) and the N-methylpiperidinium series (Table I, nos. 13–15, 23). Compounds 24–30, Table I, show that nuclear methylation of the amine has a small tendency to lower the germicidal activity of the compound, as does also the change from pyridinium to quinolinium or from piperidinium to morpholinium compounds. In the series of compounds 16–20, Table I, it appears that in the cetylalkylpiperidinium series increasing the molecular weight of the second alkyl group lowers the germicidal activity.

The series of compounds 10 and 1–4, Table I, demonstrates that in the cyclic compounds as in the aliphatic approximately the same activity is obtained in the chloride, bromide, nitrate, sulfate and methosulfate of a quaternary ammonium compound.

Compounds Nos. 5, 6, 12 and 21–23, Table I, show that the introduction of hydroxy, alkoxy, or amide and ester groups into the high molecular weight alkyl group results in compounds of lower germicidal activity than the unsubstituted derivatives.

Summary

A number of N-alkylated quaternary ammonium salts were prepared from cyclic amines, including pyridine, picoline, lutidine, quinoline, piperidine, pipicoline and morpholine. In the

series from the unsaturated cyclic amines the peak of germicidal activity was observed in the cetylpyridinium salts. In the saturated series the peak was observed in cetylmethylpiperidinium bromide.

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The Papilionaceous Alkaloids. I. *Lupinus Macounii*, Rydb.[1]

BY LÉO MARION

Until relatively recently the alkaloids of the leguminous sub-family Papilionaceae had received but little attention. Although in the course of the last fifteen years these alkaloids have formed the subject of several publications, many plants in this group have never been investigated. It is the author's purpose to undertake the study of the alkaloids contained in a number of these plants.

The first plant investigated, which forms the subject of this paper, is *Lupinus Macounii*, Rydb. It was described by Rydberg[2] and the type was collected by John Macoun in the Cypress Hills of Saskatchewan.[3] The plant was made available by Dr. R. H. F. Manske, whose generosity is here gratefully acknowledged.

L. Macounii contains three alkaloids, the major one of which is identical with rhombinine previously isolated from *Thermopsis rhombifolia*.[4] The other two alkaloids appear to be new. One is an oily base ($C_{16}H_{30}O_2N_2$) which differs from rhombinine by containing eight hydrogen atoms more. It is proposed to designate it as hydrorhombinine because it is identical with the product of the catalytic hydrogenation of rhombinine. It was isolated as its perchlorate. The third (alkaloid P1) is crystalline but present in small quantity only. Until the isolation of more of this alkaloid makes it possible to characterize it better, it will be designated by a number.

Experimental

The dried and ground plant material (4900 g.) was extracted in soxhlets with methanol and the solvent largely distilled from the combined extract which was then diluted with water, acidified with hydrochloric acid and kept on the steam-bath overnight. The mixture was cooled, filtered and the insoluble cake warmed again with dilute acid and filtered after cooling. The combined aqueous acid solution was thoroughly extracted with ether, basified with ammonia and repeatedly extracted with chloroform. The basic material recovered from the chloroform extract was dissolved in dilute hydrochloric acid, the solution filtered through charcoal, extracted with ether, basified with ammonia and repeatedly extracted with chloroform. From the combined chloroform extract, after removal of the solvent by distillation, the crude alkaloid was obtained as a thick gum. It was redissolved in dilute hydrochloric acid, the solution thoroughly extracted with ether, basified with ammonia, repeatedly extracted with ether (extract A) and then with chloroform (extract B). The ether extract A was dried over potassium hydroxide pellets and distilled to dryness. The basic residue consisted of a thick oil (9.5 g.) which was fractionated *in vacuo*. Two fractions were obtained: fraction I, b. p. 145–150° (0.3 mm.), a thick, colorless oil, wt. 3.34 g.; fraction II, b. p. 160–170° (0.3 mm.), a thick oil, wt. 2.99 g. and a residue.

Isolation of Rhombinine.—The oily fraction II could not be induced to crystallize. It was dissolved in methanol and the solution made just acid to congo by the cautious addition of 65% perchloric acid. The perchlorate which crystallized immediately was filtered and recrystallized twice from boiling methanol from which it separated as colorless needles melting at 315.[5] Admixture with rhombinine perchlorate failed to depress the melting point. A small quantity of this perchlorate was dissolved in hot water, the solution basified with ammonia, cooled and extracted repeatedly with ether. The combined ether extract was dried over pellets of potassium hydroxide and distilled to dryness on the steam-bath. The residual oil was dissolved in hot methanol and added to a solution of picric acid in methanol. On cooling a picrate separated as an oil which gradually crystallized on standing. After recrystallization from methanol–acetone, it was obtained as pale-yellow leaflets melting at 254° either alone or after admixture with rhombinine picrate.[4]

Isolation of Hydrorhombinine.—The oil obtained as fraction I (b. p. 145–150° (0.3 mm.)) was dissolved in a small volume of methanol and the solution neutralized with 65% perchloric acid. A perchlorate separated which after several crystallizations from methanol still melted over a range. It was dissolved in boiling ethyl acetate and the solution on cooling deposited a crop of crystals of rhombinine perchlorate. The mother liquor was evaporated to dryness, the residue stirred with water containing an excess of ammonia and the resulting solution extracted several times with chloroform. The combined extract was evaporated to dryness and the residue distilled *in vacuo*. The bulk distilled at 140° (0.2 mm.) as a thick, colorless oil which was dissolved in methanol and reconverted to perchlorate. The crystalline perchlorate, after several recrystallizations from methanol, consisted of small stout, colorless prisms melting at 213°, $[\alpha]_D$ −40.9° (c = 0.9 in water). Found: C, 50.67, 50.51; H, 8.10, 8.07; N, 7.38, 7.47. Calcd. for $C_{16}H_{30}O_2N_2 \cdot HClO_4$: C, 50.19; H, 8.10; N, 7.32. The base did not yield a crystalline picrate.

Isolation of Alkaloid P1.—The original mother liquor from which impure hydrorhombinine perchlorate had been obtained was diluted with water and heated on the steam-bath until the methanol had evaporated. The residual aqueous liquor was basified with ammonia and thoroughly extracted with ether. The base recovered from the ether was fractionated *in vacuo*. A small forerun distilled at 110° (0.15 mm.) while the bulk was obtained as a colorless oil, b. p. 120–122° (0.1 mm.), which crystallized on standing. It was crystallized twice from a mixture of absolute ether and petroleum ether from which it separated as small, colorless plates which sintered at 123° and melted at 126°. No perchlorate or picrate of this base could be obtained

(1) Published as *Natl. Research Council, Bull.*, No. 1356.

(2) Rydberg, *Bull. Torrey Botan. Club*, **34**, 42 (1907).

(3) It was suggested by Dr. H. A. Senn, botanist, Central Experimental Farm, Ottawa, that the plant may be identical with, or closely related to *Lupinus argenteus*, Pursh.

(4) R. H. F. Manske and L. Marion, *Can. J. Research*, **B21**, 144 (1943).

(5) All melting points given are corrected.