Synthesis and Bactericidal Properties of Some Pyridinium Chlorides with Alkylthiomethyl Hydrophobic Groups

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ABSTRACT

Alkylthiomethyl chlorides were synthesized via the reaction of thioalcohols with paraformaldehyde and hydrogen chloride. The optimum reaction parameters were determined. Alkylthiomethylpyridinium chlorides were synthesized via the reaction of octylthiomethyl or dodecylthiomethyl chlorides with pyridine and its methyl derivatives. The surface active properties and minimum inhibitory concentrations (M.I.C.) toward various strains of microorganisms were determined for the compounds synthesized. It was found that they exhibit better bactericidal activity than commercial cetylpyridinium and cetyltrimethylammonium bromides.

INTRODUCTION

Alkoxymethyl chlorides, often called chloromethylalkyl ethers, have been extensively described in the literature (1,2). They possess high reactivity and are used therefore as intermediates to prepare more complex compounds.

Their sulphur analogs are also very interesting, especially from a practical point of view. It is possible to obtain from them interesting compounds possessing germicidal, fungicidal, bactericidal, anticorrosive and other properties (3).

Therefore, we considered the technical possibility of the synthesis of alkylthiomethyl chlorides with long alkyl chains, and we synthesized appropriate quaternary salts and determined their surface active and bactericidal properties.

$$RSH + HCHO + HCl \rightleftharpoons RSCH_2Cl + H_2O \quad (1)$$

$$RSCH_2Cl + N(R_1)(R_2) \longrightarrow [RSCH_2\overset{\oplus}{N}(R_1)(R_2)]\,Cl^{\ominus} \quad (2)$$

EXPERIMENTAL PROCEDURES

Materials

n-Octyl and n-dodecyl thioalcohols were obtained from Merck (Germany), paraformaldehyde from Fluka AG (Switzerland). Pyridine, 3-methylpyridine, 4-methylpyridine, 3,4-dimethylpyridine (Merck, Germany) were used in the quaternization step.

Alkylthiomethyl Chloride Synthesis

Gaseous hydrogen chloride was introduced with efficient mechanical stirring into a mixture of thioalcohol (0:34 mole) and paraformaldehyde until the solution was saturated (2-3 hr). The reaction was carried out under isothermal conditions at temperatures which varied in different experiments from 0 C up to 60 C. The mole ratio of paraformaldehyde to thioalcohol was varied from 1.0 mole/mole to 1.4 mole/mole. Some experiments were carried out without diluent, while in an other one benzene was used as a solvent. The amount of solvent varied from 0 to 530 ccm/mole of thioalcohol.

In all experiments an emulsion of reaction water in the organic phase was obtained which was then dried with anhydrous sodium sulphate, filtered and evaporated. Hydrogen chloride absorbed in the reaction products was stripped off with dry nitrogen.

The amount of alkylthiomethyl chloride as well as dialkyl thioacetal in the final product was determined according to a method described previously (4), and the yield of alkylthiomethyl chloride (percentage) was calculated.

Warning! Alkylthiomethyl chlorides are potential carcinogens.

Synthesis of Alkylthiomethylpyridinium Chlorides

Alkylthiomethyl chloride was dropped into pyridine or one of its methyl derivatives with efficient mechanical stirring at 80 C. An equimolar ratio of reactants was used. The reaction mixture was stirred at 80 C for 2 hr, and the precipitate was extracted three times with hot n-heptane. The solid residue was twice recrystallized from anhydrous

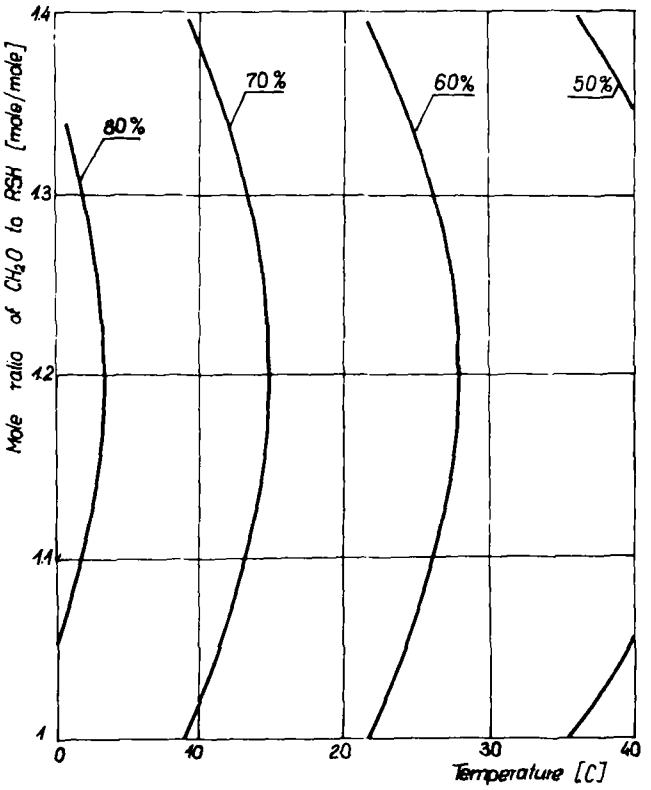

FIG. 1. Effect of temperature and mole ratio of paraformaldehyde to octyl thioalcohol on the content of octylthiomethyl chlorides (reaction run without diluent).

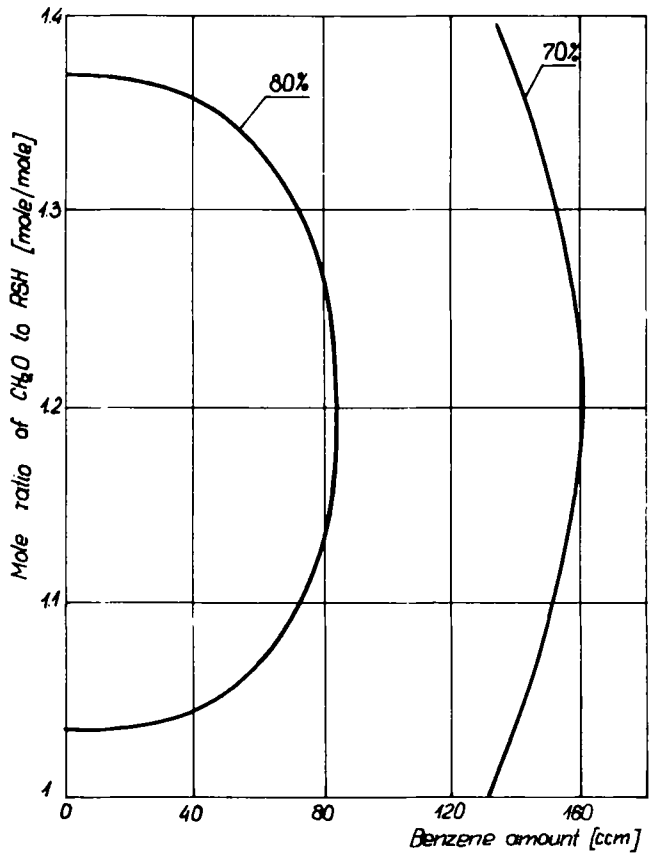

FIG. 2. Effect of mole ratio of paraformaldehyde to octyl thioalcohol and the amount of benzene on the content of octylthiomethyl chloride at 0 C.

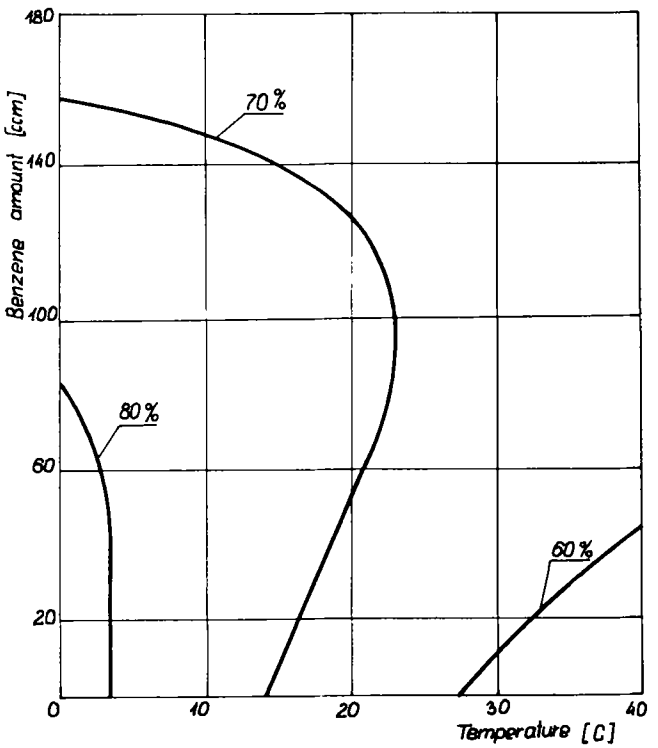

FIG. 3. Effect of temperature and the amount of benzene on the content of octylthiomethyl chloride at a mole ratio of paraformaldehyde to thioalcohol of 1.2:1.

acetone.

Purity of the products was checked by IR spectra and melting points. IR spectra were taken on a Specord IR-71. Two characteristic bands at 3500-3200 cm^{-1} for $\equiv\overset{+}{N}$ - and 2600-2200 cm^{-1} $\equiv\overset{+}{N}H$ were noted.

Melting points (uncorrected) were measured with the Boetius microscopic instrument.

Minimum Inhibitory Concentration of Alkylthiomethylpyridinium Chlorides

Minimum inhibitory concentrations (M.I.C.) for the test compounds were determined by serial dilution (5) against 14 different microbial strains. The following organisms were used: Staphylococcus albus 614, Staphylococcus aureus 209 P, Klebsiella pneumonise 138, Escherichia coli 503, Candida albicans 505, Bacillus subtillis 32, Streptococcus faecalis 8040, Sarcina lutea ATCC 9341, Pseudomonas aeruginosa A-52, Serratia marcescens 6/46, Gaffkya tetragena PZH 2/49, Rhodothorula glutinis 29, Serratia marcescens 76, Streptococcus pyogenes C.R.

Bacterial cultures were stored at 4 C in solid agar medium and the fungi in solid medium of the Sabouraud type. The microbiological media were prepared according to a method described in the literature (6).

RESULTS AND DISCUSSION

Synthesis of Alkylthiomethyl Chlorides

Böhme (7) found that when alkylthiomethyl chlorides are synthesized dialkyl thioacetals are formed as by-products.

$$RSH + HCHO + HCl \rightleftharpoons RSCH_2Cl + H_2O \quad (3)$$

$$RSCH_2Cl + RSH \rightleftharpoons (RS)_2CH_2 + HCl \quad (4)$$

Our thermodynamic calculations (8,9) indicate that in an ideal gas state the equilibrium constants of the reactions 3 and 4, regardless of the length of the alkyl chain, are equal to ca. 10^4 and 5.10^3, respectively. These values confirmed that the reaction equilibrium is shifted toward undesired acetal.

On the basis of 19 experiments, an empirical model was developed that correlates the content of octylthiomethyl chloride (Y) with temperature, mole ratio of paraformaldehyde to thioalcohol and the amount of benzene used as a solvent.

$$Y = 70.90 - 6.737\,x_1 + 1.982x_1^2 - 5.348x_2^2 - 4.474x_3^2 + 8.646x_1 \cdot x_2$$

where

$$x_1 = \frac{\text{temperature [C]}-20}{20}$$

$$x_2 = \frac{\text{mole ration of HCHO to RSH}-1.2}{1.2}$$

$$x_3 = \frac{\text{benzene amount [ccm]}-90}{90}$$

The model derived is statistically valid. Its correlation coefficient is equal to 0.976, and minimum, mean, and maximum relative errors of the approximation of the content of octylthiomethyl chloride amount to 0.00%, 3.09% and 5.78%, respectively. The equation derived can be used to approximate the content and the yield of alkylthiomethyl chloride and to determine the effects of the parameters considered. This is illustrated in Figures 1-3. The curves in these figures are plotted at several fixed values of chloride content.

The results indicate that the content of desired chloride increases with decreasing temperature and with increasing mole ratio of paraformaldehyde to thioalcohol up to ca. 1.2 mole/mole.

The use of bezene as a diluent is undesirable because it causes the reaction equilibrium to be shifted toward thioacetal formation. However, in the synthesis of dodecyl-

TABLE I

Physicochemical and Surface Active Properties of Alkylthiomethylpyridinium Chloride

Compound	Melting point °C	Surface tension at 20 C c = 0.0125% dyne/cm	Surface tension at 20 C c = 0.1% dyne/cm	Foam height[a] H_1 cm	Foam height[a] $\frac{H_1}{H_{10}} \cdot 100$ %	Wetting ability[b] g/dm³
1. Dodecylthiomethylpyridinium chloride	63 - 64	63.2	39.6	14	18	1.6
2. Dodecylthiomethyl-4-methylpyridinium chloride	49 - 50	63.0	43.9	0	o	1.9
3. Dodecylthiomethyl-3,5-dimethylpyridinium chloride	94.5 - 95	61.2	40.2	8.5	6	2.1
4. Octylthiomethyl-3-methylpyridinium chloride	125.5 - 127	63.8	62.5	0	0	3
5. Octylthiomethyl-3,4-dimethylpyridinium chloride	123 - 125	63.5	56.5	0	0	3

[a]By Ross-Miles method at 20 C, distilled water, c = 1 g/dm³; H_1 foam height after 1 min; H_{10} foam height after 10 min.
[b]Concentration of the agent corresponding to sinking time of standard cotton equal to 100 sec.

TABLE II

Minimum Inhibitory Concentration (Incubation Time, 24 hr) [mg/dm³]

Microbic groups	Strain	Compound 1[a]	2	3	4	5	6	7
Cocci	Staphylococcus aureus 209 P	0.18	0.18	0.73	11.72	5.86	1.46	1.46
	Sarcina lutea ATCC 9341	0.0014	0.003	0.006	11.72	2.93	0.73	0.36
	Gaffkya tetragena P ZH 2/49	0.09	0.006	0.01	1.46	1.46	5.86	2.91
	Staphylococcus albus 614	2.91	0.01	0.0001[xxb]	1.46	1.46	1.46	0.73
	Streptococcus faecalis 8040	5.86	2.93	2.93	187.50	93.75	2.93	0.73
	Streptococcus pyogenes CR	5.86	0.01	0.0001[xxb]	0.73	1.46	0.05	1.46
	Mean value	2.48	0.52	0.61	35.77	17.82	2.08	1.28
Rods	Pseudomonas aeruginosa A-52	23.44	187.50	187.50	750	750	250[xc]	250[xc]
	Klebsiella pneumoniae 138	11.72	5.86	5.86	93.75	46.88	46.88	93.75
	Escherichia coli 503	2.91	2.93	5.86	46.88	46.88	5.86	11.72
	Serratia marcescens 6/46	187.50	187.50	187.50	375	375	250[x]	250[xc]
	Serratia marcescens 76	5.86	0.0001[xxb]	0.0001	0.36	0.36	0.36	0.36
	Mean value	46.27	76.76	77.34	253.20	243.82	110.62	121.17
Fungi Yeast-Like fungi	Candida albicans 505	5.86	5.86	2.93	93.75	93.75	2.91	0.73
	Rhodothoruta glutinis 29	5.86	0.05	0.18	5.86	5.86	1.46	1.46
	Mean value	5.86	3.00	1.56	49.81	49.81	2.19	1.10
Bacilli	Bacillus subtilis 32	1.46	0.01	0.01	23.44	11.72	5.86	0.73
	Overall mean value	18.54	28.06	28.11	114.55	102.67	41.13	44.03

[a]1—Dodecylthiomethylpyridinium chloride, 2—Dodecylthiomethyl-4-methylpyridinium chloride, 3—Dodecylthiomethyl-3,5-dimethylpyridinium chloride, 4—Octylthiomethyl-3-methylpyridinium chloride, 5—Octylthiomethyl-3,4-dimethylpyridinium chloride, 6—Cetylpyridinium bromide, 7—Cetyltrimethylammonium bromide.
[b]xx — less than 0.001.
[c]x — M.I.C. ⩾ 250.

thiomethyl chloride, a diluent must be used to render the reaction mixture homogeneous, but the amount of benzene used ought not to exceed 230 ccm/mole of thioalcohol.

Under above conditions it was possible to obtain products containing ca. 80% of desired chloride which was used without acetal separation for the synthesis of pyridinium salts.

Synthesis and Properties of Alkylthiomethylpyridinium Chlorides

The desired reaction product may be contaminated with amine hydrochloride due to the presence of traces of water.

$$2RSCH_2Cl + H_2O \longrightarrow (RS)_2CH_2 + 2HCl + HCHO$$

Removal of amine hydrochloride by crystallization is difficult and, as we stated, the synthesis should be conducted under strictly anhydrous conditions.

Dialkyl thioacetal in alkylthiomethyl chloride is not troublesome, because it can be easily separated from the reaction product by extraction and crystallization. Purification of alkylthiomethyl chloride is thus not necessary.

In the IR spectra of the products obtained after crystallization, the following characteristic bands at 3500-3200, 1580, 1490, 1280, 1200, 1130, 780, 720, 680 cm^{-1} can be observed. In none of the products was the band at 2700-2200 corresponding to ≡NH found, which proved the absence of amine hydrochlorides. The yields were ca. 95%.

Surface active properties of alkylthiomethylpyridinium chlorides are shown in Table I. The results indicate that the quaternary ammonium salts obtained from octylthiomethyl chloride do not show surface activity due to high hydrophilicity. The products containing 12 carbon atoms in the hydrophobic chain show greater surface activity. At a concentration of 0.1% in water, their surface tension is decreased to ca. 40 dyne/cm, and their CMC are ca. 0.1%, but foaming and wetting properties are rather poor.

Minimum inhibitory concentration of the compounds examined against various microorganisms after 24 hr and 48 hr of incubation are shown in Tables II and III. For comparison, M.I.C. values of cetylpyridinium bromide (compound 6) and cetyltrimethylammonium bromide (compound 7) were also determined.

TABLE III

Minimum Inhibitory Concentration (Incubation Time, 48 hr) [mg/dm^3]

Microbic groups	Strain	Compound						
		1[a]	2	3	4	5	6	7
Cocci	Staphylococcus aureus 209 P	0.36	0.18	0.73	11.72	11.72	1.46	2.91
	Sarcina lutea ATCC 9341	0.09	0.0003	0.01	11.72	11.72	0.73	0.36
	Gaffkya tetragena P ZH 2/49	0.09	0.02	0.05	2.93	1.46	5.86	2.93
	Staphylococcus albus 614	5.86	0.05	0.01	2.93	2.93	5.86	0.73
	Streptococcus faecalis 8040	5.86	2.93	5.86	187.50	93.75	2.93	0.73
	Streptococcus pyogenes CR	5.86	0.05	0.02	2.93	2.93	0.18	1.46
	Mean value	3.02	0.54	1.20	36.62	20.75	2.83	1.52
Rods	Pseudomonas aeruginosa A-52	187.50	187.50	187.50	750	750	250[xb]	250[xb]
	Klebsiella pneumoniae 138	11.72	5.86	5.86	93.75	46.88	46.88	93.75
	Escherichia coli 503	2.91	2.93	5.86	46.88	46.88	5.86	11.72
	Serratia marcescens 6/46	187.50	187.50	187.50	375	750	250[xb]	250[xb]
	Serratia marcescens 76	5.86	0.0007	0.0001	5.86	2.93	0.73	0.36
	Mean value	79.10	76.76	77.34	254.30	319.34	110.69	121.17
Fungi Yeast-Like fungi	Candida albicans 505	5.86	5.86	5.86	187.50	187.50	5.86	0.73
	Rhodothoruta glutinis 29	11.72	0.05	0.36	5.86	23.44	1.46	1.46
	Mean value	8.79	3.00	3.11	96.68	105.47	3.66	1.10
Bacilli	Bacillus subtilis 32	1.46	0.02	0.09	23.44	11.72	11.72	0.73
	Overall mean value	30.90	28.07	28.55	122.00	138.85	42.11	44.13

[a] 1—Dodecylthiomethylpyridinium chloride, 2—Dodecylthiomethyl-4-methylpyridinium chloride, 3—Dodecylthiomethyl-3,5-dimethylpyridinium chloride, 4—Octylthiomethyl-3-methylpyridinium chloride, 5—Octylthiomethyl-3,4-dimethylpyridinium chloride, 6—Cetylpyridinium bromide, 7—Cetyltrimethylammonium bromide.

[b] x — M.I.C. $\geqslant$ 250.

Results indicate that alkylthiomethylpyridinium chlorides containing 12 carbon atoms in the alkyl chain show good bactericidal activity. They are more active than derivatives containing only 8 carbon atoms in the hydrophobic chain. Antimicrobial activity thus coincides with surface activity of the examined compounds. The structure of the amine used does not influence significantly the bactericidal activity, and the differences observed in M.I.C. values are not statistically valid.

In comparison with commercial quaternary ammonium bromides (compound 6 and 7), quaternary salts with an alkylthiomethyl group containing 12 carbon atoms in the alkyl chain show higher activity. The differences in M.I.C. are statistically valid.

Activities of the salt synthesized against various microorganisms are significantly different. They are the most effective against cocci and show good activity against Bacillus subtilis and fungi. Their activity against bacilli is rather poor, although better than that of the commercial bromides.

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