282. *Chemical Constitution and Surface-active Properties. 2-Alkylpyridines and 2-Alkyl-1-methylpyridinium Iodides.*

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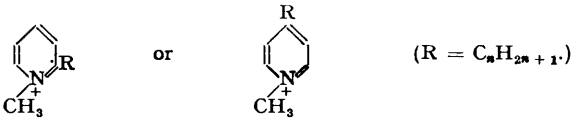
The hydrochlorides and methiodides of a number of 2-alkylpyridines have been examined for their effect on aqueous surface tension and for their antibacterial power. Maximum activity in both respects was exhibited by the salts of 2-pentadecylpyridine.

Extensive investigations have been carried out on the properties of quaternary ammonium germicides containing a long alkyl group attached directly to nitrogen, for example, cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC). Few data are available, however, on compounds in which the alkyl group is connected to the nitrogen atom through a ring system, as in the alkylpyridinium salts yielding cations of the type :



($R = C_nH_{2n+1}$.)

The discovery by Tschitschibabin (*Bull. Soc. chim.*, 1936, **3**, 1607; 1938, **5**, 429) of a general method for the preparation of the higher homologues of pyridine made available a series of bases having valuable physical and biochemical properties. The method, involving the interaction of 2- or 4-methylpyridine with alkyl halides in the presence of sodamide was applied by Knight and Shaw (*J.*, 1938, 682) to the preparation of 2-alkylpyridines having side-chains of 13—19 carbon atoms. The authors indicated that the salts of these bases with strong acids resembled soaps in producing foaming solutions in water and acting as emulsifying agents. Subsequently Barkovsky (*Ann. Chim.*, 1944, **19**, 487) prepared further members of the series and reported on some of their properties, especially their high antibacterial activity (for the 2-*n*-alkyl series, results were given for $n = 12$ and 19). Values found for the effect on aqueous surface tension of one member of the series ($n = 19$) indicated that the hydrochlorides possess marked surface activity. Comparative values for the wetting power of a few of the alkylpyridinium salts are also given in U.S.P. 2,247,266.

In order to make a systematic comparison of their properties, eight 2-*n*-alkylpyridines have now been prepared with side-chains containing from 8 to 19 carbon atoms. Their salts have been examined for their effect on aqueous surface tension and their antibacterial power. Since Barkovsky had reported (*loc. cit.*) that 2-nonadecylpyridine could be readily converted into the methiodide, the bases were refluxed with methyl iodide at atmospheric pressure. Although the long alkyl group adjacent to the nitrogen atom might be expected to have a sterically hindering effect, all the bases reacted readily under these conditions. It is noteworthy that, whereas those containing a side-chain of more than 11 carbon atoms gave stable crystalline derivatives, the products obtained from the lower members were not stable : thus, 2-undecylpyridine gave a solid which darkened and decomposed on storage. Although moderately soluble in hot water, the methiodides are sparingly soluble at room temperature (of the order of 0·01% w/v at 20° for the higher members). It is also of interest that aqueous solutions of the higher alkylpyridine methiodides give only a slight immediate precipitate on treatment with silver nitrate : silver iodide is only slowly precipitated; the process is more rapid in the presence of nitric acid.

Adam and Shute (*Trans. Faraday Soc.*, 1938, **34**, 758) have pointed out that with dilute solutions of such compounds as cetylpyridinium chloride a very slow fall in surface tension takes place and the final values are often not reached for a week. Further, the final tension is to some extent independent of the concentration of the solution down to high dilutions.

It seems probable that the same effect should apply to the 2-alkylpyridinium salts, but this could not easily be allowed for when using the given technique : the above authors showed that the ageing effect applies to the surface only and not to the solution as a whole; if the solution is kept for a week and a fresh surface is formed, the same slow fall occurs. Thus the results reported here for the more dilute solutions are not to be regarded as absolute values. However,

since the *relative* effect is under examination, the objection is less serious, particularly since Adam and Shute also showed that there is a critical concentration for each compound at which the final value is reached almost immediately, and the value is dependent on chain length.

Of the five methiodides examined, that of 2-pentadecylpyridine showed the greatest effect; although at concentrations of M/5000 and M/10,000 the two higher homologues gave a greater depression, the minimum value obtained was 41 dynes/cm. for both these compounds, as compared with the 33 dynes/cm. given by the other methiodides.

The values obtained for the surface tensions of the bases in hydrochloric acid are in general lower at corresponding concentrations than for the methiodides. A notable feature of the behaviour of the higher members (from tridecylpyridine) is the persistence of the minimum value to very great dilutions; this is particularly evident in the case of pentadecylpyridine, which again gave the greatest effect. In this connection it is of interest that Adam and Shute (*loc. cit.*) also reported that in the presence of inorganic salts the final values were reached far more rapidly (see also Hill and Hunter, *Nature*, 1946, **158**, 585). This may account for the much greater persistence of the minimium value on dilution in the case of the acid solutions of the bases, as compared with the methiodides, the presence of the acid causing the final value to be reached more quickly.

An interesting point is the difference between the behaviour of the higher members and that of octyl-, nonyl-, and decyl-pyridines. These lower homologues fail to show the persistence of the minimum value on dilution, while undecylpyridine shows a transitional type of behaviour between these two groups.

Bacteriological Results.—Series I : Of the 2-*n*-alkylpyridine bases, hydrochlorides, and methiodides examined (see Table I), only the methiodides of the higher members (alkyl = tridecyl, pentadecyl, heptadecyl, and nonadecyl) showed outstanding bacteriostatic activity, and of these pentadecylpyridine methiodide gave considerably the greatest effect. In the presence of 10% serum the antibacterial activity was very markedly lowered.

The hydrochlorides of the higher members had no bacteriostatic effect against *Staph. aureus* or *Escherichia coli* at 1 : 5000, the highest concentration used. It is not surprising that these salts should be so much less active than the methiodides, since at the dilutions used the hydrochlorides must be very largely hydrolysed, leaving only a small proportion in the form of the active cation. Nevertheless, it is noteworthy that the free bases themselves (lower members) showed a moderate activity against *Staph. aureus*. In the presence of 10% of serum this fell to a figure of the order of 1 : 6000 which was common throughout the series of active compounds.

Series II : In a comparison of 2-pentadecylpyridinium methiodide, cetyltrimethylammonium bromide, and phenol, the variation between the values for each substance was very large (this is not uncommon : see Lawrence, " Quaternary Ammonium Germicides," 1950). Nevertheless the following general conclusions can be drawn : pentadecylpyridine methiodide is considerably more active than cetyltrimethylammonium bromide against *Staph. aureus* and also against *E. coli*, which is far more resistant. In general the activity of both compounds is much reduced in the presence of serum.

Series III, A and B : These series comprised tests of the bactericidal (as opposed to the bacteriostatic) properties. The values are to some extent dependent on experimental conditions (*e.g.*, composition of media and strain of test organism), and the comparison with literature values should be made with this reservation. In the comparison of the alkylpyridinium salts (series A), the differences appear rather smaller than in the bacteriostatic tests, but again the three higher methiodides show relatively high activity, of the same order as that of cetylpyridinium chloride. The comparison (series B) indicates a greater potency for pentadecylpyridine methiodide than for cetyltrimethylammonium bromide; the difference in activity between the two compounds is much smaller than under the conditions of the bacteriostatic tests (Table II) where the period of exposure was 24 hours.

Attempts have frequently been made to relate surface activity and antibacterial power (see, for example, Lawrence, *op. cit.*; Glassman, *Bact. Rev.*, 1948, **12**, No. 2). It is generally accepted that the action of antibacterial compounds is not to be explained solely in terms of their effect on surface tension : thus solutions of compounds of differing chemical types having comparable surface tensions differ widely in their antibacterial power. However, when comparing the members of a single series it is interesting that for the 2-alkylpyridinium salts the point of maximum activity coincides for surface activity and antibacterial power. 2-Pentadecylpyridine can be regarded as containing a chain of 16 carbon atoms attached to the quaternary nitrogen atom (since the side-chain is linked to nitrogen through one of the carbon atoms of the ring); in this respect it is analogous to cetylpyridinium chloride.

Experimental.

Preparation of 2-n-Alkylpyridines.—2-*n*-Octyl-, -nonyl-, decyl-, -undecyl-, -tridecyl-, -pentadecyl-, -heptadecyl-, and -nonadecyl-pyridine were prepared by Knight and Shaw's method (*loc. cit.*). 2-Nonyl- and 2-decylpyridine appear to be new :

2-*n*-Nonylpyridine, b. p. 128°/3·5 mm., gives a *picrate*, m. p. 55° (Found : C, 54·9; H, 6·3; N, 12·6. $C_{20}H_{26}O_7N_4$ requires C, 55·3; H, 6·0; N, 12·9%).

2-*n*-Decylpyridine, b. p. 138°/3 mm., gives a *picrate*, m. p. 67° (Found : C, 56·35; H, 6·2; N, 13·0. $C_{21}H_{28}O_7N_4$ requires C, 56·3; H, 6·25; N, 12·5%).

Methiodides.—The base was refluxed with excess of methyl iodide for 2 hours and the excess of iodide distilled off *in vacuo*. The residue was twice extracted with boiling ether, and recrystallised from ethyl acetate. The following appear to be new :

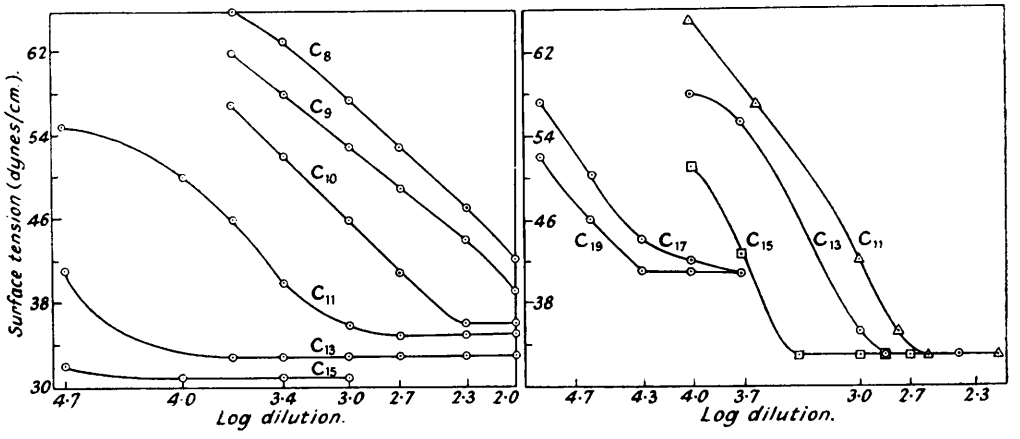
2-n-*Tridecyl-* (Found : I, 31·4. $C_{19}H_{34}NI$ requires I, 31·5%), 2-n-*pentadecyl-* (Found : I, 28·9. $C_{21}H_{38}NI$ requires I, 29·5%), and 2-n-*heptadecyl-pyridine methiodide* (Found : I, 28·2. $C_{23}H_{42}NI$ requires I, 27·7%). These and 2-*n*-nonadecylpyridine methiodide (Found : I, 26·9. Calc. for $C_{25}H_{46}NI$: I, 26·1%) softened at *ca.* 87°, except that 2-tridecylpyridine methiodide softened at 77°.

Fig. 1.

2-Alkylpyridines in 0·5N-HCl *at* 25°.

Fig. 2.

2-Alkylpyridine methiodides in water at 25°.



Surface Tension.—Determinations were carried out at 25° with the du Noüy tensimeter, readings being taken after 15 minutes' equilibration. In addition to measurements on aqueous solutions of the methiodides (Fig. 2), readings were taken for solutions of the bases (or their hydrochlorides) in 0·5N-hydrochloric acid (Fig. 1). In this way the comparison could be extended to the bases which did not give stable methiodides. To avoid complications caused by variation in the degree of hydrolysis on

Table I.

Highest dilution giving bacteriostatic effects of 2-alkylpyridine derivatives.

Each figure given should be multiplied by 1/1000.

50% Inhibition after 18 hrs. at 37° in 2% glucose broth.

		Staph. aureus 4163		*Staph. aureus* 2301		*E. coli* 86		Complete inhibition. *Staph. aureus* 4163.	Complete inhibition. *E. coli* 86.
Alkyl.	Deriv-ative.		with 10% of serum.		with 10% of serum.		with 10% of serum.		
Octyl	Base	1/12	1/6	1/12	1/6	—	—	1/8	—
Nonyl	Base	1/100	,,	>1/32	,,	—	—	1/64	—
Decyl	Base	1/24	,,	,,	,,	—	—	1/16	—
Undecyl ...	HCl	1/150	,,	,,	,,	—	—	1/100	—
Tridecyl ...	HCl	>1/5	>1/5	—	—	>1/5	>1/5	—	>1/5
,, ...	MeI	1/1200	1/7	—	—	1/30	,,	1/800	1/5
Pentadecyl	HCl	>1/5	>1/5	—	—	>1/5	,,	—	>1/5
,,	MeI	1/6000	1/7	—	—	1/60	,,	1/3200	1/5
Heptadecyl	HCl	>1/5	>1/5	—	—	>1/5	,,	—	>1/5
,,	MeI	1/2400	1/15	—	—	1/15	,,	1/1600	1/10
Nonadecyl	HCl	>1/5	>1/5	—	—	>1/5	,,	—	>1/5
,,	MeI	1/1200	1/15	—	—	1/15	,,	1/800	1/10

dilution, the original solutions were diluted with acid of the same concentration. No curves were drawn for heptadecyl- or nonadecyl-pyridine in acid solution, since the compounds gave values of 37—38 and 35—36 dynes/cm. respectively at all concentrations down to M/100,000.

Bacteriological.—Series I. The bacteriostatic effects of the compounds on *Staph. aureus* and *E. coli* are listed in Table I.

Because of their low solubility in water at room temperature, the compounds were dissolved in a few drops of alcohol and diluted with water to give colloidal solutions or fine suspensions. These were used for serial dilutions in 2% glucose broth at pH 6·8; for such dilutions one loopful of test organism was used as inoculum, and incubation was at 37°.

Series II. Since 2-pentadecylpyridine methiodide (PPM) showed considerably the greatest activity, it was compared with phenol and cetyltrimethylammonium bromide (CTAB). Table II shows concentrations necessary for complete inhibition of growth for 24 hours at 37° in glucose broth at pH 6·8.

TABLE II.

2-*n*-Pentadecylpyridine methiodide	*Staph. aureus* 4163.						*E. coli*.					
				with 10% of serum.						with 10% of serum.		
	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.
(PPM) ...	1/3200	1/51,000	1/25,600	1/7	>1/16	1/64	1/60	1/32	1/64	>1/5	—	1/2
CTAB	—	1/800	1/3200	—	>1/16	1/80	—	>1/2	>1/2	—	<1/2	>1/2
Phenol	—	5	3	—	5	5	—	2·5	2·5	—	5	2·5

Series IIIA. The critical killing dilution (highest dilution of germicide that will kill in 10 minutes but not in 5) was determined for all the compounds, together with that of phenol, which is included together with a recorded value (Quisno and Foter, *J. Bact.*, 1946, **52**, 111) for cetylpyridinium chloride (CPC) for comparison. Bacto-Difco peptone was used in place of Armour special peptone, and Lemco meat extract in place of Liebig beef extract. With the exception of the value for phenol, figures given are accurate only within 30%, since usually two-fold serial dilutions were used.

TABLE III.

Critical killing dilutions for 2-n-*alkylpyridine derivatives.*

[Each figure given should be multiplied by 1/1000 (except for phenol).]

Alkyl.	Derivative.	*Staph. aureus* 4163.		*Staph. aureus* 2301	
			with 10% of serum.		with 10% of serum.
Octyl	Base	>1/1	>1/1	>1/1	>1/1
Nonyl	Base	,,	,,	,,	,,
Decyl	Base	,,	,,	,,	,,
Undecyl	HCl	,,	,,	,,	,,
Tridecyl	HCl	>1/10	>1/10	>1/10	>1/10
,, ...	MeI	,,	,,	,,	,,
Pentadecyl	HCl	>1/10	>1/10	>1/10	>1/10
,, ...	MeI	1/80	,,	1/80	1/20
Heptadecyl	HCl	>1/10	,,	>1/10	>1/10
,, ...	MeI	1/80	,,	1/240	1/40
Nonadecyl	HCl	>1/10	,,	1/40	>1/10
,, ...	MeI	1/80	,,	1/120	1/30
CPC *		1/83	1/12·5	—	—
Phenol		1/96	1/72	—	—

* Average of 5 *Staph.* strains.

Series IIIB. The following table shows finally a further comparison of the critical killing dilutions of PPM, CTAB, and phenol, made by Hoogerheide's method (*J. Bact.*, 1945, **49**, 277), against *Staph. aureus*. The suspension of *Staph. aureus* was diluted 10 times in phosphate buffer at pH 8 containing the germicide.

Critical killing dilutions—using Staph. aureus.

(Figures to be multiplied by 1/1000.)

	I.	II.	III.
PPM	1/80	1/50	1/64—1/80
CTAB	—	>1/16	1/25
Phenol	—	12·5	12·5

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