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Short Communications

Antibacterial Effect of Some Amines in the Vapour State

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Cyclopentadrin (1-cyclopentyl-2-methyl-amino-propane), a strongly basic water-insoluble amine, utilized in inhalers to produce local decongestion of the mucosa, was found to exert also a marked antibacterial effect in its vapour state.[1] It was suggested that the antibacterial action was due to its strongly basic properties and its lipophilic nature, facilitating entry into the bacterial membrane. Although it has been suggested that volatile alkylamines generated as decomposition products of dithiocarbamates might be involved in their fungicidal action (see Horsfall [2]), the antimicrobial action of amines in the gaseous phase does not seem to have been further investigated. In the present paper, data from tests with thirteen commercially available aliphatic and alicyclic amines, all with boiling points above 30° (listed in Table 2) are given.

Staphylococcus aureus was used as the test organism. In all the experiments reported here, one strain (No. 8532, from the National Collection of Type Cultures, London) designated as No. 3 in the previous paper,[1] has been used, although other strains were also used in comparative tests.

Tests were performed in small 3 ml glass tubes, containing a cup of aluminium foil supported by a glass bead at the bottom of the tube. With this arrangement, a second, inner aluminium cup containing the bacteria was kept clear from all contact with the liquid amine. 0.05 ml of amine was pipetted into the bottom of the outer tube, and the inner cup containing the bacteria was introduced at zero time. The tubes were tightly closed with rubber stoppers and the experiments performed in a water-bath at 25°C. Before introduction of the bacteria, the system was brought into equilibrium at 25°. The bacteria were introduced with Agla-syringe in a 0.010 ml sample containing viable organisms in the order of 10^6 from a 16 hour-shake broth-culture at 37°, standardized to give an extinction value of about 0.6. After exposure to the amine atmosphere for various time intervals, each cup was dropped into a 10 ml saline solution. The viable count was obtained by plating 1 ml of three to four serial dilutions of the saline solution on to a glucose, yeast and meat extract agar and counting colonies after incubation at 37° for 24 h. Each series was run in four to eight replicates and experiments were repeated two or three times.

The mean numbers of survivors and death-rate of *Staph. aureus* after exposure to each of the different amines were determined. None of the amines provoked a logarithmic order of death, which is a frequently reported course for antibacterial agents (see Sykes [3]) but the variation of death-rate proceeded mainly in accordance with one of the three representative patterns given in Table 1. Diethylamine as well as propyl, isopropyl-, *n*-, *sec*- and *tert*-butylamine (boilingpoints 35°–78°, see Table 2) provoked an initially fast death-rate which gradually decreased with time, whereas triethylamine, like tripropyl-, dipropyl-, and diisopropylamine (boilingpoints 84°–110°) gave an accelerating phase up to a maximum followed by a phase of somewhat lower though almost constant death-rate. The remaining three amines (boilingpoints 180°–205°), represented by cyclopentadrin, started with a long period of very slow death-rate, followed by a relatively high rate. The different patterns of death-rate, however, are most probable due to different concentrations of the amines in the gas phase, an unavoidable situation when different amines having differing boilingpoints are subjected to similar experimental conditions with respect to pressure and temperature. For the first group of amines the

Table 1. Mean numbers of survivors and variation of death-rate with time for *Staphylococcus aureus* exposed to saturated atmosphere of three different amines at 25°. For each amine data are given from two experiments (1,2). Death-rate $K = (1/t) \log_{10} (n_0/n)$ where n_0 and n are the numbers of colonyforming bacteria at the beginning and end of the time interval t min.

Time min	Diethylamine				Triethylamine				Propylhexedrin			
	Survivors, No.		K		Survivors, No.		K		Survivors, No.		K	
	1	2	1	2	1	2	1	2	1	2	1	2
0	2 610 000	2 830 000			1 740 000	2 140 000			3 540 000	4 740 000		
0.5	24 700	16 400	4.05	4.49	1 280 000	1 450 000	0.267	0.338				
1	7 000	2 850	2.52	2.99	400 000	390 000	0.638	0.739	3 510 000	3 990 000	0.004	0.074
2	90	77	2.23	2.28	383 000	384 000	0.378	0.332	3 300 000	3 080 000	0.015	0.094
4	< 10	< 10	(1.60)	(1.61)	37 800	174 000	0.415	0.272	3 370 000	3 020 000	0.005	0.049
8						960		0.294	3 260 000	2 210 000	0.005	0.041
16					< 10	10	(0.390)	0.333	3 230 000	2 100 000	0.003	0.022
32									1 800	5 060	0.134	0.124
64									< 10	< 10	(0.102)	(0.104)

Table 2. Relative antibacterial activity of various amines ($Ct_{99.9}$) calculated as a theoretical value in terms of mmoles of compounds required to achieve 99.9 % kill in 1 min at 25°. C = mmoles amine per litre air at 25° extrapolated from vapour pressure data in the literature. $t_{99.9}$ = exposure time (min) required to bring about 99.9 % kill, obtained as experimental data.

Amine	B.p. °C	C	$t_{99.9}$	$Ct_{99.9}$
Propylhexedrin	205	0.029	28	0.8
Cyclopentadrin	184	0.039	26	1.0
Octyl-	180	0.054	12	0.6
Butyl-	78	5.92	1.85	11.0
Propyl-	49	16.9	0.75	12.7
Sec-butyl-	63	8.07	2.13	17.2
Isopropyl-	35	32.3	1	32.3
Tert-butyl-	46	18.9	2	37.8
Dipropyl-	110	1.07	4	4.3
Diisopropyl	84	4.21	2	8.4
Diethyl-	55	13.4	1.1	14.7
Tripropyl-	156	0.27	88	24.0
Triethyl-	90	2.31	6.5	15.0

concentrations varied in a range from 32.3 to 5.2 mmoles per litre air, in the second and third from 4.21 to 0.27, and from 0.054 to 0.029 mmoles, respectively (see Table 2). Possibly, a lag phase even for the first group of amines, present in high concentrations in the vapour phase, could have been detected if a shortening of the test-period could have been experimentally possible.

Under the test conditions used, it is apparently impossible to compare directly the effect of the different amines on the basis of death-rate. It would, however, be expedient to express the relative antibacterial activity of the various amines in terms of mmoles required to bring about 99.9 % kill in one minute at 25° (*cf.* Phillips [4]). A comparison between the effects of the different amines lends support to the suggestion that the lipophilic properties play a decisive role in the activity of these compounds (see Table 2). Not only the two alicyclic water-insoluble amines but also octylamine, the least water-soluble of the three primary amines in the series of directly-comparable amines tested, gave a $Ct_{99.9}$-value of 1 or < 1, *i.e.* they were about twenty times more effective than butyl- and propylamines and approximately 300 times as effective as the well-known gaseous disinfectant ethylene oxide.[4,5] Entirely in accordance with this, the infinitely soluble *sec*-butyl-, isopropyl- and *tert*-butylamines gave high $Ct_{99.9}$-values. In the same way, the effect of the secondary amines seems to be correlated to the water-solubility. Dipropylamine is slightly soluble in water and was several times more effective than the readily water-soluble diethylamine. Regarding the tertiary amines, however, even factors other than the degree of watersolubility seem to govern their antibacterial activity, as tripropylamine, though only very slightly soluble in water, gave one of the highest $Ct_{99.9}$-values of all series.

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1. Norkrans, B. and Bolmstedt, J. *Svensk Farm. Tidskr.* **66** (1962) 885.
2. Horsfall, J. G. *Principles of fungicidal action*, Wattham, Mass. 1956.
3. Sykes, G. *Disinfection and sterilization*, London 1958.
4. Phillips, Ch. R. *Am. J. Hyg.* **50** (1949) 280.
5. Kaye, S. *Am. J. Hyg.* **50** (1949) 289.

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Die Umsetzung von Hydroxypyridinen mit Schwefeldichlorid

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Auf Grund der bekannten Reaktionen des Schwefeldichlorids mit Phenolen [1] bzw. mit Säureamiden [2] konnte man bei der Umsetzung mit Hydroxypyridinen bzw. Pyridonen verschiedene isomere Produkte erwarten, die im folgenden für das 2-Pyridon formuliert sind.

Die Umsetzungen in siedendem Benzol (mit oder ohne Zusatz von Triäthylamin als säurebindendem Mittel) führten zu folgenden Ergebnissen:

2-Pyridon: 2,2′-Dihydroxy-5,5′-dipyridylsulfid (I).