THE BACTERICIDAL ACTIVITY OF PHENOLS IN AQUEOUS SOLUTIONS OF SOAP

PART III. THE BACTERICIDAL ACTIVITY OF CHLOROXYLENOL IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE

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IN a previous paper[1] it was shown that the sparingly-water-soluble compound 5-chloro-2-hydroxydiphenylmethane (benzylchlorophenol) may be solubilised by aqueous solutions of potassium laurate when the latter are sufficiently concentrated to be micellar. The bactericidal activity of such solutions was found to be related to the concentration of the benzylchlorophenol in the micelles, and not to its overall concentration in the system as a whole.

The present communication deals with the solubility and bactericidal activity in aqueous solutions of potassium laurate of 2-chloro-5-hydroxy-1:3-dimethylbenzene (chloroxylenol), a phenol of somewhat greater water-solubility than benzylchlorophenol. This compound was selected for further study because, although more water-soluble than benzylchlorophenol, it is not so soluble that appreciable concentrations obtain in the aqueous phase when it is dissolved in aqueous solutions of potassium laurate. Compounds which are markedly soluble in water were avoided, since the bactericidal activity of systems containing such compounds dissolved in aqueous potassium laurate solutions, must be the summation of the activity of the aqueous phase and of the activity possessed by the micelles containing the compound. Such a condition would introduce difficulties in the interpretation of bactericidal measurements.

1. *The Solubility of Chloroxylenol in Aqueous Solutions of Potassium Laurate.*

EXPERIMENTAL

The solubility of chloroxylenol in aqueous solutions of potassium laurate was determined by the method described in an earlier paper[1]. Essentially, it consisted of diluting a series of solutions of chloroxylenol and potassium laurate with water, until crystals of chloroxylenol deposited on standing at 20° C. for about twenty-four hours.

For convenience, we prepared initially a concentrated aqueous solution of 0·3 M potassium laurate saturated with chloroxylenol. This solution could be diluted several-fold with water without deposition of crystals of chloroxylenol. The addition of water beyond a certain critical volume, however, did result in the deposition of chloroxylenol crystals on standing. A solution containing slightly less than this critical volume of water was used as the basic working solution throughout the experiments. Its composition was as follows:—

Chloroxylenol ..	..	10·20g. or 0.265 M
Potassium Laurate	..	31·931 g. or 0·134 M
Distilled water to	..	1,000·00 ml.

The relative molar proportions of chloroxylenol and potassium laurate were approximately 1 : 2.

The addition of a volume of 0·1 M potassium laurate solution to the working solution, reduced the molar proportion of chloroxylenol to potassium laurate in the system, and increased the volume of water that could be added before the chloroxylenol crystallised out. Systems containing gradually increasing volumes of 0·1 M potassium laurate per unit volume of working solution were prepared until one was obtained which could be diluted with an infinite volume of water without crystallisation of chloroxylenol occurring on standing.

The composition of the systems prepared and examined is shown in Table I, together with the concentration of potassium laurate at which crystallisation occurred. The solubility curve of chloroxylenol in potassium laurate is shown in Figure 1, where the solubility is expressed as mols of chloroxylenol solubilised per mol. of potassium laurate at different concentrations of the latter.

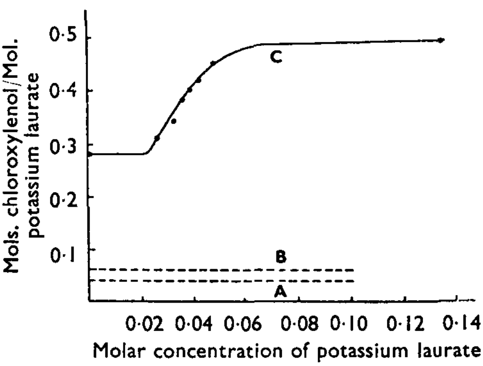

FIG. 1. The solubility of chloroxylenol in potassium laurate solution. The composition of the solutions examined for bactericidal activity is represented by points along lines A and B.

A. $\frac{\text{0·046 mol. chloroxylenol}}{\text{mol. potassium laurate}}$

B. $\frac{\text{0·061 mol. chloroxylenol}}{\text{mol. potassium laurate}}$

C. Solubility of chloroxylenol.

The diagram shows that the solubility of chloroxylenol per mol. of potassium laurate remains constant for concentrations of potassium laurate up to about 0·02 M. As the concentration of the latter is increased from this value to about 0·04 M the relative solubility of chloroxylenol increases very rapidly. A further increase in the potassium laurate concentration from about 0·04 M to about 0·06 M produces a much less rapid increase in the solubility of the chloroxylenol. In excess of about 0·06 M potassium laurate there is negligible increase in the solubility of chloroxylenol per molecule of potassium laurate, even for a five-fold increase in the concentration of the latter.

Discussion

The overall sigmoidal shape of the solubility curve for chloroxylenol in potassium laurate closely resembles that described in a previous paper for the solubility of benzylchlorophenol in potassium laurate[1], except that the inflection in the curve is less marked. The increase in solubility of chloroxylenol with increase in the concentration of potassium laurate occurs over very approximately the same potassium laurate concentration range as did the increase in solubility of benzylchlorophenol. The solubility curve is similar to that obtained by McBain, Merrill and Vinograd[2] for the solubilisation of a water-insoluble dye by solutions of

TABLE I

THE SOLUBILITY OF CHLOROXYLENOL IN SOLUTIONS OF POTASSIUM LAURATE

Solutions, — 6	Volume of working solution, ml.	Weight of chloroxylenol in working solution, g.	Weight of potassium laurate in working solution, g.	Volume of 0·1 M potassium laurate added, ml.	Weight of potassium laurate added, g.	Total weight of potassium laurate in final solution, g.	Mols. chloroxylenol/mol. soap	Maximum volume of water that may be added before crystals deposit, ml	Total volume of final solution, ml.	Molar concentration of potassium laurate at end-point
A	1·0	0·0102	0·0319	nil	nil	0·0319	0·485	trace	1·0	0·134
B	1·0	0·0102	0·0319	0·10	0·0024	0·0343	0·451	2·0	3·1	0·046
C	1·0	0·0102	0·0319	0·20	0·0048	0·0367	0·416	2·5	3·7	0·042
D	1·0	0·0102	0·0319	0·30	0·0071	0·0390	0·397	3·0	4·3	0·038
E	1·0	0·0102	0·0319	0·40	0·0095	0·0404	0·377	3·5	4·9	0·035
F	1·0	0·0102	0·0319	0·60	0·0143	0·0462	0·336	4·5	6·1	0·032
G	1·0	0·0102	0·0319	0·80	0·0191	0·0510	0·030	6·5	8·3	0·026
H	1·0	0·0102	0·0319	1·00	0·0238	0·0556	0·277	∞	∞	$1/\infty = 0$

sodium lauryl sulphonate. It represents the formation of micellar material in aqueous solutions of potassium laurate. As the amount of micellar material per unit volume increases with increase in the concentration of the potassium laurate, so the solubility of chloroxylenol per molecule of potassium laurate increases until the micelles attain their maximal size. This occurs between 0·05 and 0·06 M potassium laurate. Further increase in the concentration of potassium laurate produces no further increase in the size of the micelles, with the result that there is no further increase in the solubility of chloroxylenol per molecule of potassium laurate. This is in agreement with the observations of McBain *et al.*[2].

It is interesting to note that the apparent concentration of chloroxylenol in the aqueous phase of saturated systems containing slightly less than the critical concentration of potassium laurate is greater than its published water-solubility (1 in 3,000). A similar anomalous solubility was noted with benzylchlorophenol in solutions of potassium laurate of similar concentration, and has been observed by other workers employing different systems[3,4].

2. *The Bactericidal Activity Against* Bacterium coli *of Chloroxylenol in Aqueous Solutions of Potassium Laurate*

EXPERIMENTAL

Two series of solutions containing chloroxylenol and potassium laurate were prepared. Each series contained an arbitrary proportion of chloroxylenol to potassium laurate, and was prepared by taking a fixed volume of a concentrated solution containing the arbitrary proportion of chloroxylenol to potassium laurate and distributing it into stoppered glass bottles. Increasing volumes of carbon dioxide free water were then added to each of the containers of a series, so that the solutions thus produced consisted of increasing dilutions of the concentrated parent solution. The composition of the individual solutions may be represented by points along two horizontal lines drawn on the solubility curve at the 0·046 and 0·061 molecules of chloroxylenol/molecule of potassium laurate levels respectively (Fig. 1), and is shown in Table II.

TABLE II

MEAN DEATH-TIMES OF *Bacterium coli* IN SOLUTIONS OF CHLOROXYLENOL IN AQUEOUS POTASSIUM LAURATE

Concentration of potassium laurate	Mean death-times of *Bacterium coli* when $\frac{\text{mol. chloroxylenol}}{\text{mol. potassium laurate}} = 0·061$	Mean death-times of *Bacterium coli* when $\frac{\text{mol. chloroxylenol}}{\text{mol. potassium laurate}} = 0·046$
0·0113 M	213·0 minutes	—
0·0130 M	55·8 ,,	>240·0 minutes
0·0146 M	35·0 ,,	—
0·0162 M	7·2 ,,	16·3 ,,
0·0194 M	3·3 ,,	5·3 ,,
0·0227 M	2·6 ,,	—
0·0259 M	1·9 ,,	3·6 ,,
0·0292 M	3·0 ,,	—
0·0324 M	4·2 ,,	7·8 ,,
0·0389 M	4·3 ,,	11·3 ,,
0·0438 M	4·3 ,,	—
0·0454 M	—	11·6 ,,
0·0518 M	5·6 ,,	15·0 ,,
0·0648 M	6·2 ,,	10·0 ,,
0·0778 M	5·6 ,,	6·0 ,,
0·0907 M	3·2 ,,	4·6 ,,
0·1037 M	3·3 ,,	—

The bactericidal activity of the solutions against *Bacterium coli* was determined by adding 0·2 ml. of a 24-hour culture of the organisms to 5·0 ml. of the solution being assayed. Immediately after mixing, small volumes of the reaction mixture were transferred to sterile glass tubes maintained at 20°C, where the reaction proceeded for a known time, after which it was quenched by the addition of sterile broth. Details of the method were given in our previous paper[5].

The death-times of *Bacterium coli* in the different solutions are recorded in Table II. They are shown superimposed on the solubility curve for chloroxylenol in potassium laurate in Figure 2. The upper line represents the death-times of *Bacterium coli* in solutions containing 0·046 mol. chloroxylenol/mol. potassium laurate, and the lower line the death-times in solutions containing 0·061 mol. chloroxylenol/mol. potassium laurate. The general shape of the death-time curves closely resembles that obtained with solutions containing benzylchlorophenol solubilised by potassium laurate.

The weakest solutions examined in each series contained about 0·01 M potassium laurate and had low activity. As the concentration of the solutions was increased with respect to both the chloroxylenol and the potassium laurate, the death-times of the organisms fell sharply, reaching a minimum in solutions containing about 0·025 M potassium laurate. As the concentration of the potassium laurate was increased beyond about 0·025 M and that of the chloroxylenol increased by the same proportion, the death-times began to increase again in both series of solutions. These increases in the death-times continued with increase in the concentration of both components of the solutions, until the soap concentration approximated 0·05 M, when a minimum of bactericidal activity was observed. A second increase in activity with increase in soap concentration was observed in solutions containing potassium laurate in excess of about 0·05 M.

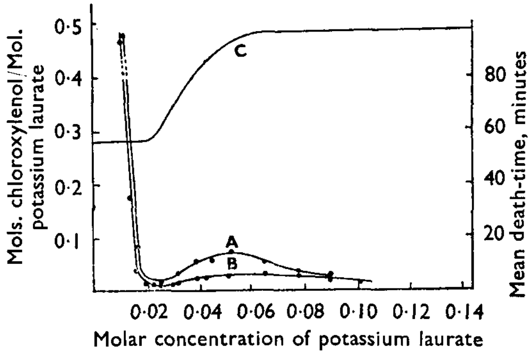

FIG. 2. The bactericidal activity against *Bact. coli* of solutions with constant chloroxylenol-potassium laurate ratio and increasing potassium laurate concentration, and the relation of the activity to the solubility of chloroxylenol.

A. $\frac{\text{0·046 mol. chloroxylenol}}{\text{mol. potassium laurate}}$

B. $\frac{\text{0·061 mol. chloroxylenol}}{\text{mol. potassium laurate}}$

C. Solubility of chloroxylenol.

DISCUSSION

The experiments showed that the changes in bactericidal activity that are associated with changes in the concentration of chloroxylenol and potassium laurate in aqueous systems closely resemble those previously reported in systems containing benzylchlorophenol and potassium laurate[5].

The very rapid decrease in the death-times that was observed as the concentration of both the chloroxylenol and the potassium laurate was increased by the same proportion, over the range 0·01 to 0·02 M potassium laurate, can be attributed to the increase in the concentration of both components of the system.

When the concentration of the potassium laurate reaches 0·02 M micelles begin to form in the solution. That is, as the concentration of potassium laurate is increased from below 0·02 M to above 0·02 M, a transition takes place from a true solution to a colloidal solution containing micelles. This change in the physical state of the solution is accompanied by an increase in the death-times, even though the concentration of both the chloroxylenol and the potassium laurate has been increased. The increase in the death-times continued until the potassium laurate concentration was about 0·05 M and parallelled that observed in the experiments with benzylchlorophenol[5]. It was shown in the latter experiments that as the concentration of potassium laurate was increased from about 0·02 M to about 0·04 M and that of benzylchlorophenol by the same proportion, the concentration of soap in the micellar state increased much more rapidly than did the concentration of the benzylchlorophenol. That is, as the concentration of both soap and benzylchlorophenol increased over the soap range 0·02 M to 0·04 M, the concentration of benzylchlorophenol in the micelles decreased.

TABLE III

THE RELATION BETWEEN THE PERCENTAGE SATURATION OF POTASSIUM LAURATE MICELLES BY CHLOROXYLENOL AND THEIR BACTERICIDAL ACTIVITY AS INDICATED BY THE DEATH-TIME OF *Bacterium coli*

Mol. chloroxylenol / Mol. potassium laurate	Molar concentration of potassium laurate	Mols. chloroxylenol / Mol. pot. laurate to saturate solution	Percentage saturation of micelles	Log. of percentage saturation of micelles	Death-time of *Bacterium coli*	Log. of death-time of *Bacterium coli*
0·0457	0·026	0·304	$\frac{0{\cdot}0457 \times 100}{0{\cdot}304} = 15{\cdot}03$	1·1770	3·6 min.	0·5563
0·0457	0·032	0·354	$\frac{0{\cdot}0457 \times 100}{0{\cdot}354} = 12{\cdot}91$	1·1109	7·8 ,,	0·8921
0.0457	0·039	0·405	$\frac{0{\cdot}0457 \times 100}{0{\cdot}405} = 11{\cdot}28$	1·0525	11·3 ,,	1·0531
0·0457	0·045	0·440	$\frac{0{\cdot}0457 \times 100}{0{\cdot}440} = 10{\cdot}39$	1·0165	11·6 ,,	1·0645
0·0457	0·052	0·476	$\frac{0{\cdot}0457 \times 100}{0{\cdot}476} = 9.60$	0·9823	15·0 ,,	1·1761
0·0609	0·026	0·304	$\frac{0{\cdot}0609 \times 100}{0{\cdot}304} = 20{\cdot}00$	1·3017	1·9 ,,	0·2787
0·0609	0·029	0·335	$\frac{0{\cdot}0609 \times 100}{0{\cdot}335} = 18{\cdot}18$	1·2596	3·0 ,,	0·4771
0·0609	0·032	0·354	$\frac{0{\cdot}0609 \times 100}{0{\cdot}354} = 17{\cdot}20$	1·2356	4·2 ,,	0·6232
0·0609	0·039	0·405	$\frac{0{\cdot}0609 \times 100}{0{\cdot}405} = 15{\cdot}04$	1·1772	4·3 ,,	0·6335
0·0609	0·044	0·436	$\frac{0{\cdot}0609 \times 100}{0{\cdot}436} = 13{\cdot}97$	1·1451	4·3 ,,	0·6335
0·0609	0·052	0·476	$\frac{0{\cdot}0609 \times 100}{0{\cdot}476} = 12{\cdot}79$	1·1070	5·6 ,,	0·7482

In order to determine whether the same explanation applied in the case of the present experiments with chloroxylenol and potassium laurate, the percentage saturation of the micelles by chloroxylenol has been calculated for both series of solutions for the potassium laurate range 0·02 M to 0·05 M. The values are shown in Table III, where the death-times of *Bacterium coli* in the systems is also recorded. Reference to Figure 3 will show that the death-time of *Bacterium coli* in the solutions is related to the logarithm of the percentage saturation of the micelles by chloroxylenol. It is independent of the concentration of chloroxylenol in the systems as a whole.

The second increase in activity observed as the concentration of the potassium laurate is increased from about 0·05 M cannot be due to an increase in the concentration of the chloroxylenol in the micelles, since at this concentration they reach their maximal size. A similar phenomenon was reported in the experiments with benzylchlorophenol, and we offered the suggestion that this second increase in activity was due to an increase in the number of micelles per bacterium introduced into the system. Preliminary experiments have indicated the possible validity of this explanation and experiments are in progress to substantiate it.

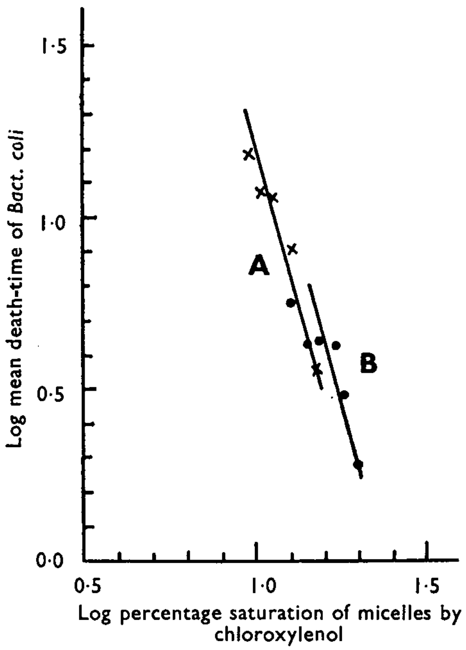

FIG. 3. Relation between the percentage saturation of micelles by chloroxylenol and the log mean death-time of *Bact. coli*.

A. $\frac{0\cdot046 \text{ mol. chloroxylenol}}{\text{mol. potassium laurate}}$

B. $\frac{0\cdot061 \text{ mol. chloroxylenol}}{\text{mol. potassium laurate}}$

SUMMARY

1. The slightly water-soluble compound, chloroxylenol, is solubilised by aqueous solutions of potassium laurate.

2. It is solubilised by internal solution in the micelles which form in potassium laurate solution.

3. The bactericidal activity of systems containing chloroxylenol solubilised by potassium laurate is independent of the concentration of chloroxylenol in the system.

4. The bactericidal activity of the system is a function of the concentration of chloroxylenol in the soap micelles.

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