462. *The Mechanism of the Antibacterial Action of Phenols and Salicylaldehydes. Part III.*[1] *Substituted Benzaldehydes.*

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The bactericidal activity of some substituted benzaldehydes against *Ps. aeruginosa* increases in the order of increasing partition coefficient for the system cyclohexane–0·05M-aqueous sodium borate. The bactericidal activities are *not* enhanced by trace metals, but depend on pH and partly on the reactivity of the carbonyl group. The bactericidal process may involve condensation of the formyl group with amino-groups in the bacterial cell wall. Substituted benzyl alcohols and benzoic acids are inactive under our test conditions.

In view of the high bactericidal activities of salicylaldehydes relative to their partition coefficients,[2] it is important to determine the extent to which this activity is due to the formyl group. The bactericidal activities of a number of substituted benzaldehydes other than salicylaldehydes have therefore been determined, and as shown in Table 1, these

Table 1.

(*a*) Inverse molar concentrations (*i.e.*, M/*x*) of substituted benzaldehydes killing *Ps. aeruginosa* in 40 min., and
(*b*) Partition coefficients for the system cyclohexane–0·05M-aqueous sodium borate. Results in parentheses were obtained with oleyl alcohol.

Subst.	None	4-OMe	4-F	2-OMe	2-Me	4-Me	4-Cl	2-OEt	2-Cl	4-Br	2-Br
(*a*)	51	68	75	112	193	221	374	394	729	799	959
(*b*)	18·8(28)	11·2(27)	18	19(36)	27	56	79	79	108	116	149

[1] Part II, Burton, Clarke, and Gray, *J.*, 1964, 1314.
[2] Clarke, Cowen, Gray, and Osborne, *J.*, 1963, 168.

increase with increasing partition coefficient for the system cyclohexane–0·05M-aqueous sodium borate. For only three of these benzaldehydes could partition coefficients be obtained with oleyl alcohol as the organic phase, but the similarity between these partition coefficients and those obtained using cyclohexane suggests that hydrogen bonding between solute and solvent is not an important factor, as it is in the case of phenols.[1] These facts suggest that the relative ease of penetration of the cell by various benzaldehydes other than salicylaldehydes is governed by the same factors which determine their relative solubilities in a hydrocarbon solvent.

Further, we have shown [2] that trace metals considerably enhance the bactericidal properties of salicylaldehydes. It was therefore suggested that chelation with metal ions facilitates the bactericidal action of these compounds, and explains (*a*) the high bactericidal activities relative to the partition coefficients, and (*b*) the absence of a simple relationship between these quantities. Methylation of salicylaldehyde destroys its chelating properties and, as anticipated, the bactericidal activity of 2-methoxybenzaldehyde was not enhanced by trace metals (Table 2).

TABLE 2.

Effect of metal ions on the bactericidal activity of 2-methoxybenzaldehyde (M/5000 in 0·05M-aqueous sodium borate) against *Ps. aeruginosa*. (*a*) 2-Methoxybenzaldehyde + buffer, (*b*) metal salt (M/2500) + buffer, and (*c*) 2-methoxybenzaldehyde + buffer + metal salt.

Metal ion		Ca^{2+}	Zn^{2+}	Fe^{2+}	Ni^{2+}
No. of viable organisms *	(*a*)	591	591	800	800
	(*b*)	131	121	174	145
	(*c*)	162	263	370	180

* These figures were obtained after diluting the test solution a hundred thousand times, as described in Part I.[2]

However, the bactericidal activities of the substituted benzaldehydes depend on pH. By use of the buffer solutions indicated in the Experimental section, 2-methoxybenzaldehyde was found to be considerably more active against *Ps. aeruginosa* at pH 9—10 than at pH 6—8. The increased sensitivity of *Ps. aeruginosa* to the aldehyde with increasing pH may be solely due to the influence of pH on the resistance of the bacteria, but condensation reactions of aldehydes are also catalysed under alkaline conditions. It is possible that a reaction involving the formyl group may play a part in the bactericidal action of benzaldehydes, and if this is so, the reactivity of the formyl group should be important. For reactions involving the formation of oximes and Schiff bases, the order of reactivity for 4-substituted benzaldehydes is $NO_2 > \text{halogeno} > H > NMe_2$.[3] Table 3 shows the relative bactericidal activities of a number of substituted benzaldehydes as

TABLE 3.

(*a*) Relative bactericidal activities of 4-substituted benzaldehydes (equimolar in 0·05M aqueous sodium borate) against *Ps. aeruginosa*, and
(*b*) Partition coefficients for the system cyclohexane–0·05M-aqueous sodium borate.

Substituent		Control	NMe_2	F	Cl	Br	I	NO_2
(*a*) No. of viable organisms after 1 hr.*	(i)	342	440	438	389	154	24	284
	(ii)	32	47	47	28	24	3	22
(*b*)		—	9·8	18	79	116	216	3·5

* These figures were obtained after diluting the test solution (i) 10,000 times, (ii) 100,000 times, as described in Part I.[2]

given by viable count experiments; as indicated by the partition coefficients the dominating factor is clearly lipid solubility, but it is possible that low lipid solubility is to some extent counteracted by a high reactivity of the formyl group in the case of 4-nitrobenzaldehyde.

[3] Ingold, "Structure and Mechanism in Organic Chemistry," Bell, London, 1953, p. 679.

That the increased sensitivity of *Ps. aeruginosa* to the benzaldehydes at higher pH values might be due to the formation of the corresponding benzyl alcohol or benzoic acid was eliminated by testing pure specimens of such compounds. Using the modified Rideal–Walker test, no bactericidal activity was shown after 60 minutes by solutions, in 0·05M-aqueous sodium borate, of benzoic acid (M/24), of 2-fluorobenzoic acid (M/28), 4-nitrobenzoic acid (M/42), 2-bromobenzyl alcohol (M/187), 2-ethoxybenzyl alcohol (M/152), 2-fluorobenzyl alcohol (M/125), and 4-methoxybenzyl alcohol (M/69).

It seems certain, therefore, that the formyl group is the reactive centre involved in the bactericidal process for the benzaldehydes. Although lipid solubility is obviously the factor which determines the bactericidal efficiency of a benzaldehyde, the reactivity of the formyl group may also be important. This may imply that the toxic process involves condensation of the aldehyde with the amino-groups of certain cell components, and considerable evidence does exist [4] for such reactions.

Experimental

The test organism was *Ps. aeruginosa* (formerly *Ps. pyocyanea*, N.C.T.C. strain 1999). Details of bacteriological techniques and the method used to determine partition coefficients are given in Part I.[2]

The *o*- and *p*-halogenobenzaldehydes are known compounds, and were prepared by slight modifications of the method described by Clarke.[5] Particularly good overall yields were obtained for *o*- and *p*-fluorobenzaldehyde (49 and 68% respectively), prepared from the corresponding fluorotoluenes.

2-Ethoxy-, b. p. 138—140°/15 mm., 2-bromo-, m. p. 80°, and 4-fluoro-benzyl alcohol, b. p. 96—99°/15 mm., were obtained by reduction of the corresponding benzaldehyde with sodium borohydride in isopropyl alcohol. The other compounds tested were commercial products.

Effect of pH *on the Bactericidal Activity of 2-methoxybenzaldehyde.*—Solutions of 2-methoxybenzaldehyde (1 g.) in a buffer solution (500 ml.) of known pH were used as the stock solutions in a modified Rideal–Walker test.[2] The buffer solutions used were those described by Britton,[6] and gave pH values of 6·0, 7·0, 8·0, 9·0, and 10·0. A solution in a borate buffer (pH 9·2) was also tested. The results were reproducible to within ±10%.

Effect of Metal Ions on the Bactericidal Activity of 2-Methoxybenzaldehyde.—Viable count determinations were carried out by using solutions of 2-methoxybenzaldehyde in 0·05M-aqueous sodium borate with and without added trace metals, as described for 5-chlorosalicylaldehyde.[2]

Bactericidal Activity and the Reactivity of the Formyl Group.—Viable count determinations [2] were carried out by using equimolar solutions of the 4-substituted benzaldehyde in 0·05M-aqueous sodium borate. The results are shown in Table 3, together with the partition coefficients for the system cyclohexane–0·05M-aqueous sodium borate.

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[4] Goodman and Gilman, "Pharmacological Basis of Therapeutics," Macmillan, London, 1947, p. 835; Dakin, *J. Biol. Chem.*, 1929, **84**, 675; Yuki, *J. Pharm. Soc. Japan*, 1961, **81**, 267; Stacey and Barker, "Polysaccharides of Micro-organisms," Clarendon Press, Oxford, 1960, p. 46; Gullard and Mead, *J.*, 1935, 210.

[5] Clarke, *J.*, 1957, 3807.

[6] Britton, "Hydrogen Ions," Chapman and Hall, London, 1955, p. 359.