256. *The Mechanism of the Antibacterial Action of Phenols and Salicylaldehydes. Part II.*[1] *Substituted Phenols.*

By D. E. Burton, K. Clarke, and G. W. Gray.

A comparison is made of the bactericidal activities of a number of substituted phenols against *Ps. aeruginosa* with their partition coefficients between 0·05M-aqueous sodium borate and certain organic phases. A relationship exists between the bactericidal activities and the partition coefficients only for organic phases which can form hydrogen bonds with the hydroxyl group of the solute, and it seems probable that penetration of the cell by the phenol molecules is assisted by hydrogen-bonding. It is therefore important to choose the correct type of solvent for measurements of partition coefficients when comparisons are to be made with biological data.

Several workers [2] have shown that the order of the bactericidal efficiencies of phenols is the same as the order of their partition coefficients between an aqueous phase and various organic phases. In all instances the organic phase, *e.g.*, oleyl alcohol or olive oil, was chosen to simulate the types of substance which may occur in bacterial cell membranes.[3] In Part I [1] we showed that for a number of substituted phenols such a relationship existed between the germicidal activities against *Ps. aeruginosa* and the partition coefficients between aqueous sodium borate and oleyl alcohol. However, experimental difficulties with salicylaldehydes led to the use of cyclohexane as the organic phase, on the assumption [4] that, although the magnitude of the partition coefficients would be altered, the order would remain unchanged. However, the results in Table 1 show clearly that, for phenols, both the magnitude and the order change drastically on replacing the oleyl alcohol by cyclohexane.

Mecke [5] has confirmed that solute–solvent interactions occur between phenol and a number of solvents with proton-attracting properties. Oleyl alcohol belongs to this class, whereas cyclohexane does not. To estimate the possible importance of this solute–solvent interaction, we measured the partition coefficients of a number of phenols between 0·05M-aqueous sodium borate and (i) methyl decanoate (a proton-attracting solvent) and

[1] Part I, Clarke, Cowen, Gray, and Osborne, *J.*, 1963, 168.
[2] Richardson and Reid, *J. Amer. Chem. Soc.*, 1940, **62**, 413; Fogg and Lodge, *Trans. Faraday Soc.*, 1945, **41**, 361; Albert, Hampton, Selbie, and Simon, *Brit. J. Exptl. Path.*, 1950, **31**, 425.
[3] Albert and Hampton, *J.*, 1954, 505.
[4] Collander, *Acta Physiol. Scand.*, 1947, **13**, 363.
[5] Mecke, *Discuss. Faraday Soc.*, 1950, **9**, 161.

(ii) n-dodecane (an inert solvent of comparable molecular size). The partition coefficients using cyclohexane and dodecane are low and of the same order of magnitude, and, if these are plotted against the killing concentrations given in Table 1, it is clear from the random

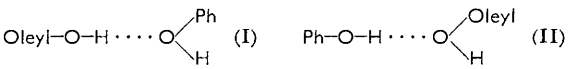
TABLE 1.

Killing concentrations (M/x) at 40 min., and partition coefficients for phenols between 0·05M-aqueous sodium borate and (a) oleyl alcohol, (b) methyl decanoate, (c) cyclohexane, and (d) dodecane.

Subst.	x	(a)	(b)	(c)	(d)	Subst.	x	(a)	(b)	(c)	(d)
None	12	17	16	0·1	0·2	2-Me	50	65	85	1·1	1·0
2-F	13	8	10	0·2	—	3-Cl	50	58	91	0·2	—
3-F	18	27	36	0·1	—	4-Cl	56	104	150	0·2	0·2
2-Cl	19	17	22	1·2	0·8	4-OMe ...	62	10	—	—	—
3-OMe ...	22	14	—	—	—	2,4-Br_2 ...	83	103	—	—	—
2-OMe ...	30	14	—	—	—	3-Br	91	104	131	0·3	—
2-I	32	61	—	—	2·9	4-Br	95	168	—	—	—
4-F	34	31	—	—	—	3-I	169	168	258	0·8	—
2-Br	<43	23	30	1·8	—	4-I	203	389	—	1·0	—
3-Me	40	61	67	0·5	0·5	4-Cl-3-Me	217	288	450	1·4	2·3
4-Me	40	63	—	—	—	2,4-I_2	266	349	—	—	—

distribution of the points that quite different factors control the solubility of the phenol in a hydrocarbon and the process whereby the phenol kills the organism.

The partition coefficients for oleyl alcohol and methyl decanoate are much larger than those for the hydrocarbon solvents, suggesting that hydrogen-bonding between the solute (phenol) and the organic solvent may assist the passage of the solute from the aqueous phase into the organic solvent. In the case of oleyl alcohol two types of bonding might be envisaged:



Bearing in mind the relative acidities of alcohols and phenols, bonding as in (II) should be the more important. For methyl decanoate, hydrogen-bonding between the phenolic hydroxyl group and the oxygen of the carbonyl group is the only possibility. Therefore, since the partition coefficients with methyl decanoate are higher than those with oleyl alcohol, it appears that hydrogen-bonding as in (I) does not supplement that of type (II).

For hydrocarbon solvents, the partition coefficients for *o*-substituted phenols are greater than those for the *m*- and *p*-isomers. However, when methyl decanoate or oleyl alcohol is used as the organic phase, the partition coefficients obtained for the *o*-halogenophenols are much smaller than those for the *m*- and *p*-isomers, and we suggest that this reversal can be explained in terms of the hydrogen-bonding which can occur in such systems.

The type of hydrogen-bonding in a system can be determined under favourable conditions by infrared spectroscopy. The sharp band associated with a free hydroxyl group occurs at about 3600 $cm.^{-1}$, while a hydroxyl group involved in intermolecular hydrogen bonding gives either a sharp band at about 3450 $cm.^{-1}$ (dimeric) or a broader band between 3200 and 3400 $cm.^{-1}$ (polymeric). West [6] has shown that no self-association of phenols (*i.e.*, intermolecular hydrogen-bonding) occurs in inert solvents at concentrations of 0·02M or less, and consequently, at a suitable concentration, the spectrum of phenol dissolved in dodecane or carbon tetrachloride shows the band at 3607 $cm.^{-1}$ characteristic of a free hydroxyl group (Table 2). However, for an equivalent solution in methyl decanoate, the free-hydroxyl band is absent, and is replaced by a strong band at 3450 $cm.^{-1}$ characteristic of a dimeric, intermolecular hydrogen-bonding between the phenol and the solvent. Bonds of this type will not be broken on dilution, and, as expected, the band at 3450 $cm.^{-1}$ persisted even on fifty-fold dilution of the solution.

[6] West, *J. Amer. Chem. Soc.*, 1959, **81**, 1614.

Similar results were obtained for *m*- and *p*-halogenophenols. During partition experiments, intermolecular hydrogen bonds of this kind may be formed between the phenol and the methyl decanoate or oleyl alcohol, and these interactions should assist the transfer of the phenol from the aqueous phase to the organic phase.

For the *o*-halogenophenols dissolved in dodecane or carbon tetrachloride, a strong band at 3523 cm.$^{-1}$ (intramolecular hydrogen-bonding) was accompanied by a weaker band at 3603 cm.$^{-1}$ (free OH), whereas in methyl decanoate the *o*-halogenophenols gave a single band at about 3440 cm.$^{-1}$ which is probably associated with a single-bridge, intermolecular hydrogen bond involving the solvent. Intermolecular associations are therefore formed by the *o*-halogenophenols in the presence of a suitable solvent, but it is considered that these associations will be less readily formed than for the *m*- and *p*-isomers, because the intramolecular associations must first be broken.

In partition experiments, the phenol molecules in the aqueous phase will be hydrogen-bonded to the water molecules. This association should be extensive for *m*- and *p*-substituted phenols, but much less for *o*-substituted phenols in which intramolecular hydrogen-bonding occurs. When an organic solvent is used which can compete favourably in hydrogen-bonding to the hydroxyl group of the *m*- and *p*-substituted phenols, the transfer of the molecules to the organic phase will be favoured relative to the transfer of the molecules of the *o*-substituted phenols. The partition coefficients for the *o*-isomers will therefore be smaller than those for the *m*- and *p*-isomers. A hydrocarbon solvent cannot however compete in the above manner in attracting the molecules of the *m*- and *p*-substituted phenols away from their associations with water molecules, and when using such solvents all the partition coefficients are lower, but the *o*-isomers, which will be least involved in such intermolecular associations, give the highest values.

In confirmation of the above ideas, it is noted that for the cresols the partition coefficients for oleyl alcohol are the same within about 3%, presumably because intramolecular hydrogen-bonding between the hydroxyl group and the *o*-methyl group is insignificant.

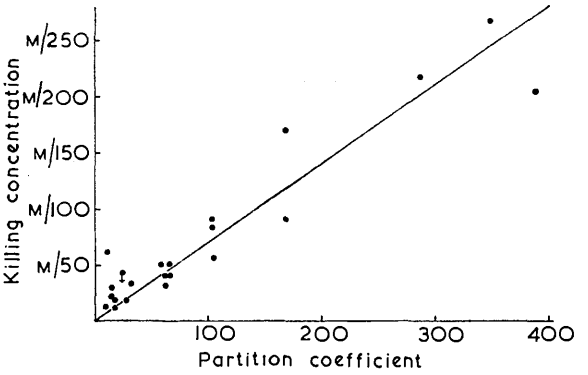
In general, therefore, the results in Table 1 suggest that hydrogen-bonding interactions are responsible for the higher partition coefficients with oleyl alcohol and methyl decanoate, and for the relative partition of the various *o*-, *m*-, and *p*-isomers which have been studied.

The methoxyphenols are, however, anomalous. *o*-Methoxyphenol dissolved in carbon tetrachloride gives a strong band at 3472 cm.$^{-1}$ which is associated with intramolecular hydrogen-bonding between the hydroxyl group and the oxygen of the methoxy-group. At equivalent concentrations in the same solvent, the *m*- and *p*-isomers absorb at 3625—3630 cm.$^{-1}$, characteristic of the free hydroxyl group. One would therefore anticipate that, for oleyl alcohol, the partition coefficients of the *m*- and *p*-methoxyphenols would be greater than that of *o*-methoxyphenol. As shown in Table 1, this is not the case, and no simple explanation for the order of the partition coefficients, $o = m > p$, can be offered.

From the results in Table 1, it is clear that there is a general tendency for the germicidal activity of a phenol to increase as the partition coefficient between aqueous sodium borate and oleyl alcohol or methyl decanoate increases. A plot of killing concentration against partition coefficient for oleyl alcohol confirms this trend (Figure). There is a considerable scatter of the points about a straight line, but, bearing in mind the substantial errors [1] involved in measuring killing concentrations and partition coefficients, the results strongly suggest that, if hydrogen bonding between the phenol molecules and the solvent molecules determines the order of the partition coefficients for methyl decanoate and oleyl alcohol, the same interactions play a part in determining the germicidal activities of the phenols. *p*-Methoxyphenol appears to be the most anomalous compound, having either a higher germicidal activity or a lower partition coefficient than would have been expected.

The apparent importance of hydrogen-bonding in defining the relationship between partition coefficient and bactericidal activity does not in itself prove that hydrogen bonding plays a dominant role in the germicidal mechanism of action of the phenol. However, it

has been shown [7] that it is impossible to establish "drug fastness" of bacteria towards phenol by growing the bacteria in sub-lethal concentrations of phenol. This would suggest that the lethal process does not involve a specific chemical reaction with one particular cell component such as a vital enzyme. In addition, it is thought that phenols denature proteins by breaking the hydrogen bridges between the peptide chains, so rupturing the polymeric association prevalent throughout the system. It may be that phenols make use of their ability to form hydrogen bonds, not only in penetrating the



The relationship between the killing concentrations of phenols and their partition coefficients in the system oleyl alcohol–0·05M-aqueous sodium borate, as given in Table 1.

cell, but also in their killing mechanism which may involve combination with many of the components of the cell.

These findings emphasise the importance of the correct choice of organic solvent for partition coefficient determinations, when comparisons are to be made with biological data. For phenols, the important requirement for the solvent is that it should simulate

TABLE 2.

Infrared absorptions of solutions of phenol.

		Hydroxyl stretching frequencies (cm.$^{-1}$)		
		Free	Dimeric	Polymeric
Solvent		3607	3450	3375
Dodecane or carbon tetrachloride	0·05	Very strong	Absent	Weak
	0·025	Strong	Absent	Very weak
Methyl decanoate	0·05	Absent	Very strong	Weak
	0·01	Absent	Very strong	Doubtful
	0·002	Absent	Medium	Absent

substances present in cell membranes by being able to form hydrogen bonds with the phenol molecules. Partition coefficients obtained for substituted phenols using non-polar solvents cannot be compared with bactericidal activities.

EXPERIMENTAL

The test organism was *Ps. aeruginosa* (formerly *Ps. pyocyanea*, N.C.T.C. strain 1999). Details of bacteriological techniques and of the method used to determine partition coefficients are given in Part I.[1]

The phenols were purchased whenever possible and were carefully purified. The fluoro- and iodo-phenols were synthesised. In the case of *o*-fluorophenol, a good overall yield of 39% was obtained from *o*-anisidine (lit.,[8] 13%). The physical constants of the substituted phenols agreed with those quoted in the literature although *m*-iodophenol was found to have m. p. 42° (lit.,[9] m. p. 40°).

[7] Fogg and Lodge, *Trans. Faraday Soc.*, 1945, **41**, 359.
[8] Bennett, Brooks, and Glasstone, *J.*, 1935, 1822.
[9] Buchan and McCombie, *J.*, 1932, 2858.

Detection of Hydrogen-bonding in Solutions of Phenols.—The infrared absorptions of a number of phenols dissolved in dodecane, carbon tetrachloride, and methyl decanoate were obtained in the range 2000—4000 cm.$^{-1}$ using a Unicam S.P. 100 double-beam recording spectrophotometer equipped with rock-salt optics. A typical set of results is shown in Table 2.

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