Relationship of Physicochemical Properties of Isomeric Trifluoromethylphenols with Their Bactericidal Effect on *Escherichia coli*

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Abstract ☐ The bactericidal activities of isomeric trifluoromethylphenols against *Escherichia coli* were found to be in the order: $m > p > o$. These activities are related to the partition coefficients of the compounds between polar organic phases and aqueous buffer and these, in turn, are related to hydrogen bonding and polar characteristics.

Keyphrases ☐ Trifluoromethylphenols, isomeric—physicochemical properties-bactericidal effect correlation, *Escherichia coli*, partition coefficients, hydrogen bonding, polar characteristics ☐ Bactericidal activity, isomeric trifluoromethylphenols—relationship to physicochemical properties, *Escherichia coli* ☐ Partition coefficients, isomeric trifluoromethylphenols—related to bactericidal activity

Many studies have been made of the bactericidal activities of halogen-substituted phenols (*e.g.*, 1, 2), but these concern only ring-halogenated compounds. In an investigation of the bactericidal effectiveness of phenols substituted with halogen in a side chain, the effects of the three isomeric α,α,α-trifluoromethylphenols on *Escherichia coli* were examined, and these effects were related to certain physicochemical properties of the compounds.

EXPERIMENTAL

Materials—The phenols were prepared from the corresponding amines by diazotization, and approximately 40% yields were obtained. Purification was effected by vacuum distillation, followed in the cases of the *o*- and *p*-isomers by repeated vacuum sublimation. In each case, GC of the purified material on 3% E30 methyl silicone gum gave only a single peak.

The organism used was *E. coli* (NCIB8114), grown and counted on oxoid nutrient agar (pH 7.4) consisting of 0.1% Lab-Lemco, 0.2% yeast extract, 0.5% peptone, and 0.5% sodium chloride, gelled with 1.5% agar. The nutrient agar was made up at six-fifths of these concentrations to allow for dilution by the reaction mixture.

The buffer used for the bactericidal evaluations was McIlvaine's standard buffer solution, prepared from citric acid and disodium hydrogen phosphate, at a total concentration of approximately 0.04

Table I—Bactericidal and Physicochemical Properties of the Trifluoromethylphenols

Property	Meta-	Para-	Ortho-
Percent w/v concentration for 0.1% survival of *E. coli* in 10 min. (*C*)	0.041	0.069	0.110
Partition coefficient, oleyl alcohol–buffer, pH 5	525	393	294
Partition coefficient, methyl oleate–buffer, pH 5	393	306	220
Partition coefficient, cyclohexane–buffer, pH 5	1.45	1.18	2.39
pKa	8.27	8.08	7.74
$\Delta\nu_{OH}$, CCl$_4$ to 10% v/v ether in CCl$_4$ (cm.$^{-1}$)	249	257	269
$\Delta\nu_{OH}/\nu_{OH}$ (free)	0.0690	0.0712	0.0742
Dipole moment	2.78	3.07	3.29

M. The pH of the buffer was adjusted to 5.0, since *o*- and, particularly, *p*-trifluoromethylphenols were found to decompose to the corresponding hydroxybenzoic acid in aqueous solution above pH 5.5.

Methods—Bactericidal evaluation was by the method of viable counts. *E. coli* suspensions were prepared from the washed 24-hr. surface growth. The suspensions were diluted with water to give about 1 × 10⁷ viable cells/ml. (viability of the initial suspensions was 45–75%). The reaction mixture was prepared by adding 5 ml. of buffered phenol solution to 45 ml. of bacterial suspension. Replicate 1-ml. aliquot samples were taken from the mixture at appropriate intervals and immediately diluted appropriately with sterile 0.9% saline to prevent further bactericidal activity. One milliliter of the final dilution was added to 5 ml. of melted nutrient agar in roll-tubes. The tubes were incubated at 37° for 48 hr. and then counted.

The pKa values were determined by automatic titration of 0.01 N solutions against 0.1 N sodium hydroxide solution on a potentiograph[1] calibrated at pH 3.99 and 9.20. It was established spectroscopically that during the time taken (about 1.5 min.) for a titration, no detectable decomposition of the *o*- or *p*-isomers occurred.

Dipole moments were determined[2] in benzene solution at 25°. Results were calculated by the method of Smith (3).

Partition coefficients were obtained by measuring concentrations spectrophotometrically after thorough mixing of the two phases at 25°, following the method of Clarke *et al.* (4). The aqueous phase was McIlvaine's buffer, pH 5.0, and the nonaqueous phases were oleyl alcohol, methyl oleate, and cyclohexane, respectively.

IR spectra were taken[3] at a concentration of about 0.05 M so as to preclude self-association. Variable pathlength cells with KBr windows[4] were used in the determinations. Frequencies are estimated to be accurate to ± 2 cm.$^{-1}$.

RESULTS AND DISCUSSION

The bactericidal and physicochemical properties of the trifluoromethylphenols are given in Table I.

The bactericidal effects of the isomers, based on a 99.9% kill in 10 min., are in the order: $m > p > o$. This is similar to the findings of Pinney and Walters (2) who showed that the bactericidal effectiveness of the monofluorophenols against *E. coli* was in the order: $m > p = o$.

The bactericidal effects of the trifluoromethylphenols correlate well with their partition coefficients (*P*) between aqueous buffer and *polar* nonaqueous phases (Fig. 1). No such correlation is observed if cyclohexane is used as the nonaqueous phase, because *o*-trifluoromethylphenol has a partition coefficient three times higher than would be expected from Collander's (5) rule relating, for a set of congeners, partition coefficients determined using various nonaqueous phases. A similar observation was made by Pinney and Walters (2) for the monofluorophenols and by Burton *et al.* (1) for a number of substituted phenols. The latter workers attributed the anomalous partition coefficients of the *o*-isomers to their intramolecular hydrogen bonding, and they suggested that this gave rise to low partition coefficients in hydrogen-bonding solvents because of low solute–solvent interaction.

However, it must be remembered that a partition coefficient is a measure of relative, not absolute, solubility. Water is more polar and is a better hydrogen-bonding agent than is oleyl alcohol. Since the

[1] Metrohm E436.
[2] Using a Dipolmeter, type DM01, and a Bellingham and Stanley Abbe "60" refractometer.
[3] Grubb-Parsons Spectromaster.
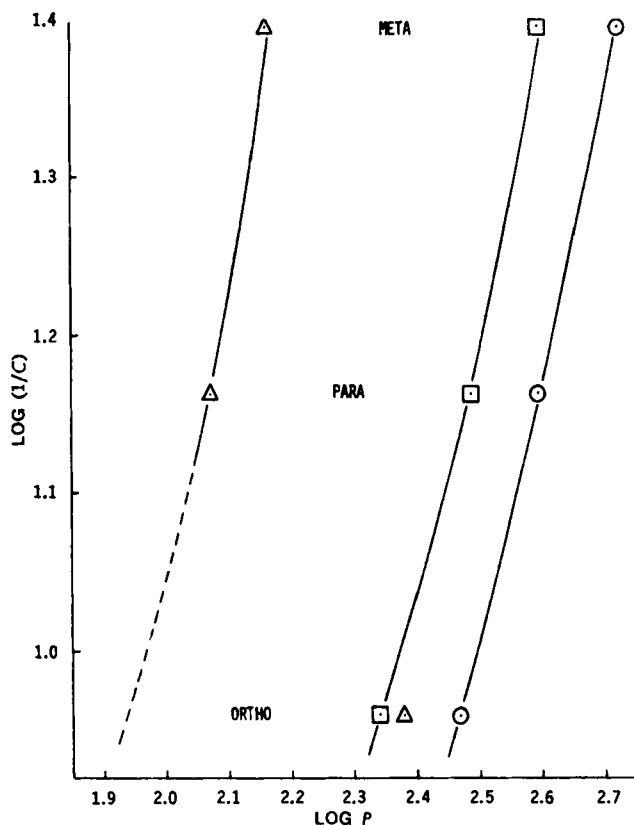[4] Research and Industrial Instruments Co., London, England.

Figure 1—*Correlation between bactericidal effects and partition coefficients of the isomeric α,α,α-trifluoromethylphenols. Partition coefficients are between aqueous buffer, pH 5, and oleyl alcohol (○), methyl oleate (□), and cyclohexane (△). Partition coefficients in cyclohexane–buffer are shown X 100.*

Table II—Partition Coefficients of the Cresols between Certain Organic Phases and McIlvaine's Buffer, pH 5

Organic Phase	Meta-	Para-	Ortho-
Oleyl alcohol	65.1	66.5	74.4
Methyl oleate	42.2	38.4	57.8
Cyclohexane	0.74	0.77	1.55

ciently strong to resist rupture in polar solvent, then the o-isomer may have the higher biological activity. This is the case for the isomeric nitrophenols. Although partition coefficients are not available, aqueous solubility data (o-isomer, 2.1 g. l.$^{-1}$ at 20°; p-isomer, 15 g. l.$^{-1}$ at 20°) show that the intramolecular hydrogen bond is not ruptured in water; Fujimoto (6) showed that the o-isomer is a more effective bactericide against *Salmonella typhosa*.

If the o-isomer cannot form an intramolecular hydrogen bond, then the rank order of partition coefficients should not be affected by choice of nonaqueous phase; Table II shows this to be the case with the isomeric cresols. In all solvent systems, o-cresol has the highest partition coefficient; and it is significant that Burton *et al.* (1) reported that this isomer also has the highest bactericidal effectiveness of the three cresols against *P. aeruginosa*.

The pKa values of the isomeric trifluoromethylphenols parallel their partition coefficients, *i.e.*, $m > p > o$. This is probably fortuitous, since the partition coefficients were determined using buffer at pH 5, when all three isomers would be virtually completely undissociated. Furthermore, since the bactericidal activities were also determined at pH 5, one would not expect the observed small difference in pKa to be significant. However, the bactericides act on or in the cell, where the pH is about 7.4. Here the extent of ionization will be significant; and since primarily it is undissociated phenol which acts as bactericide, effectiveness will be a function of pKa. Thus, the effect of pKa in this series of compounds is to reinforce, at the site of action, the effect of partition in the random walk to the site of action.

In conclusion, solvent systems for the determination of *in vitro* parameters of biological activity must be chosen carefully. Rytting *et al.* (7) recently argued, from a thermodynamic standpoint, for the use of hydrocarbon solvents. However, this could lead to erroneous predictions of biological activity, as would have been the case for the present results. From a practical viewpoint, we prefer the use of polar liquids such as oleyl alcohol or octanol.

effect of intramolecular hydrogen bonding is to reduce polarity and intermolecular hydrogen bonding, an intramolecularly hydrogen-bonded compound should have a higher partition coefficient than its p-isomer in oleyl alcohol–buffer.

Nonetheless, the present results and those of Burton *et al.* (1) show that if an o-substituted phenol can form an intramolecular hydrogen bond, then it generally has a lower partition coefficient than has its p-isomer in oleyl alcohol–buffer. It is suggested that this is largely because the intramolecular hydrogen bond is ruptured in polar solvents, and the compound then distributes itself according to its polarity and/or intermolecular hydrogen-bonding ability, which must be greater than those of the corresponding p-isomer. Table I shows that, for the compounds examined here, both the dipole moment[5] and hydrogen-bonding ability (as measured by the shift of the hydroxyl stretching frequency produced by a hydrogen-accepting solvent) are greater for the o-isomer than for the p-isomer. It thus becomes clear, as emphasized in Fig. 1, that the partition coefficient of an o-isomer, capable of intramolecular hydrogen bonding, is anomalous *in inert solvent* and not, as Burton *et al.* (1) suggested, in polar solvent.

A further confirmation of this comes from the isomeric chlorophenols. o-Chlorophenol is known to participate in intramolecular hydrogen bonding; yet the solubility of this isomer is greater in water (28.5 g. l.$^{-1}$ at 20°) than is that of the p-isomer (27.1 g. l.$^{-1}$ at 20°). It is, therefore, not surprising that Burton *et al.* (1) found the partition coefficient (oleyl alcohol–buffer) and effectiveness against *Pseudomonas aeruginosa* of o-chlorophenol to be lower than those of the p-isomer.

On the other hand, if an intramolecular hydrogen bond is suffi-

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[5] Strictly speaking, dipole moments should be determined in a hydrogen-bonding solvent in which weak intramolecular hydrogen bonds may be assumed to be ruptured.

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