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A structure-activity correlation for the antibacterial action of a series of *m*-alkoxy phenols against *Escherichia coli*

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Recent publications (Kaufman & Koski 1975; Davis et al 1976; Beezer et al 1980, 1981; Anderson et al 1981) have emphasized the need to correlate partition coefficient data with biological activity data determined at the same, preferably biologically significant, temperature. To date most of the n-octanol water partition coefficients have been reported as being measured at 'room temperature' (Leo et al 1971) whereas the biological data used in correlations have been determined over a temperature range from 18 to 37 °C (Hansch & Fujita 1964; Lien et al 1968).

The antibacterial activities of the *m*-alkoxy phenols described in Table 1 have been determined (Klarmann et al 1931) at a temperature of 18 °C, against both *Salmonella typhosa* (Gram-negative) and *Mycobacterium pyogenes var. aureus* (Gram-positive). The activity was shown to depend upon the test organism used; the activity against Gram-negative bacteria increased to a maximum at the *m*-hexoxy derivative and decreased thereafter. The 'Hansch' correlation (Hansch & Fujita 1964) proposes that biological activity is determined by lipophilicity and by the solubility limits in aqueous compartments. Phenols are cytoplasmic poisons (Roberts & Rahn 1946; Judis 1962; Hedgecock 1966) and hence their lipid solubilities will determine, to a large extent, their ability to cross the cell membrane. It has also been demonstrated (Gale & Taylor 1947; Maurice 1952; Tomcsik 1955) that cell walls and cell membranes are damaged by phenols.

Microcalorimetric determination of phenol coefficients for these *m*-alkoxy phenols against *Escherichia coli* (NCTC 8196), shows that quantitative data may be obtained rapidly (30 min compared with 2–3 days for official procedures) and also provide a precise measure of the relative biological potencies of the compounds (Beezer et al 1981). These data, combined with published partition coefficient data determined at the same temperature (Beezer et al 1980), might demonstrate a new correlation between the two. This paper reports just such a correlation and discusses the limitations imposed by discrepancies in the temperatures at which the data were obtained.

Table 1 shows the partition coefficients (K_D) as reported previously, determined by Beezer et al (1980) and as calculated by Lien et al (1968). By incorporating the early data for biological activity (Klarmann et al 1931) with these calculated partition coefficients Lien et al (1968) derived the following equations:
for Gram-positive bacteria:

$$\log(\text{phenol coefficient}) = 0{\cdot}871 \log K_D - 1{\cdot}164 \quad (1)$$

where n (number of compounds) = 11, r (correlation coefficient) = 0·994 and s (standard deviation) = 0·115
for Gram-negative bacteria:

$$\log(\text{phenol coefficient}) = 0{\cdot}18 (\log K_D)^2 + 1{\cdot}628 \log K_D - 1{\cdot}777 \quad (2)$$

where n = 11, r = 0·975, s = 0·208
The *m*-octoxy and *m*-nonoxy derivatives were excluded from these equations without explanation; inclusion of these two phenols yields for Gram-positive bacteria:

$$\log(\text{phenol coefficient}) = 0{\cdot}817 \log K_D - 1{\cdot}057 \quad (3)$$

where n = 13, r = 0·98.

The phenol coefficients determined by microcalorimetry (Beezer et al 1981) and by the official procedure are shown in Table 2; no maximum in activity is encountered and the results resemble those reported for Gram-positive bacteria (Klarmann et al 1931). The microcalorimetric data were obtained with the use of acetone as co-solvent to enable bactericidal concentrations of the higher members to be achieved. The relationships between the calculated partition coefficients and the phenol coefficients shown in Table 2 are:

$$\text{A: } \log(\text{phenol coefficient}) = 0{\cdot}822 \log K_D - 1{\cdot}286 \quad (4)$$
$$n = 7,\ r = 0{\cdot}996;$$

$$\text{B: } \log(\text{phenol coefficient}) = 0{\cdot}884 \log K_D - 1{\cdot}176 \quad (5)$$
$$n = 9\ r = 0{\cdot}993;$$

$$\text{C: } \log(\text{phenol coefficient}) = 0{\cdot}823 \log K_D - 1{\cdot}212 \quad (6)$$
$$n = 9\ r = 0{\cdot}993$$

These relationships are all rather similar and are comparable with equation (3). It appears, initially, that the antibacterial activity for these compounds increases in parallel with the partition coefficients. However inspection of the data in Table 2 shows that the phenol coefficients for *m*-heptoxy and *m*-octoxy phenols are marginally lower than would be predicted from the

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Table 1. Experimental and calculated logarithms of partition coefficient values.

Phenol	Experimental[a]	Calculated[b]
Resorcinol	0·83	0·80
Phenol	1·53[c]	1·46
m-Methoxy	1·63	1·58
m-Ethoxy	2·04	2·08
m-Propoxy	2·66	2·58
m-Butoxy	3·10	3·08
m-Pentoxy	3·70	3·58
m-Hexoxy	[4·12]	4·08
m-Heptoxy	[4·73]	4·58
m-Octoxy	[5·15]	5·08
m-Nonoxy	[5·76]	5·58
m-Phenoxy		3·21
m-s-Pentoxy		3·38

[ ] predicted values.

[a] The experimental partition coefficients were determined at 303K (Beezer et al 1980).

[b] Values calculated (Lien et al 1968) from an experimental value of 1·58 for *m*-methoxy phenol by adding 0·50 log units for each CH_2 group. No mention is made of the temperature at which determinations were made.

[c] Taken from published data (Davis et 1976) on the effect of temperature on n-octanol water partition coefficient of phenol.

values for the lower homologues suggesting that even in the presence of a co-solvent a maximum in activity may eventually be observed, perhaps, again, because of limiting solubilities.

The experimentally determined partition coefficient values at 303K for phenol, resorcinol, *m*-methoxy to *m*-pentoxy phenols (Table 1) and the projected values for *m*-hexoxy to *m*-octoxy phenols give the following relationships with the three sets of phenol coefficients (Table 2):

A: log (phenol coefficient) =

$$0{\cdot}861 \log K_D - 1{\cdot}266 \quad (7)$$

$$n = 7 \quad r = 0{\cdot}993;$$

B: log (phenol coefficient) =

$$0{\cdot}799 \log K_D - 1{\cdot}134 \quad (8)$$

$$n = 9 \quad r = 0{\cdot}991;$$

C: log (phenol coefficient) =

$$0{\cdot}804 \log K_D - 1{\cdot}201 \quad (9)$$

$$n = 9 \quad r = 0{\cdot}992$$

There is no great difference between equations (4–6) and (7–9). Table 2 indicates, however, that there are differences between the calculated and experimental values of K_D, indeed there is a detectable oscillation in the values of log (experimental K_D) (Beezer et al 1980). This oscillation has subsequently been shown to be determined largely by oscillation in the heats of solution of the phenols in water (Beezer et al 1983). This oscillation in the heat of solution of odd and even members of the series of phenols means that there could

Table 2. Phenol coefficients for resorcinol monoethers determined by: (A) standard AOAC test, phosphate buffered glucose; (B) standard AOAC test with 10% v/v acetone as co-solvent; (C) microcalorimetry.

Phenol	A	B	C
Phenol	1	1	1
m-Methoxy	1·5	1·4	1·2
m-Ethoxy	3·1	2·9	2·4
m-Propoxy	8·0	7·4	7·4
m-Butoxy	21	29	25
m-Pentoxy	80	74	74
m-Hexoxy	200	180	170
m-Heptoxy	—	440	380
m-Octoxy	—	700	640

conceivably be two quite distinct correlations, one for each sub-series, the equations such as (1)–(9) representing averaged values. While the present set of data is not large enough to test this possibility the oscillations observed confirm the need to determine both partition coefficient and biological activity data under identical conditions before there is any attempt to correlate the parameters measured.

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