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Quantitative structure–activity relationships: a group additivity scheme for biological response of *E. coli* to the action of *o*-, *m*- and *p*-alkoxyphenol

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Summary

Microcalorimetric assay of the antibacterial activity of 14 phenols shows that the activities can be expressed as two additive factors due to the aromatic portion and to the number of methylene groups.

The addition of phenols to respiring bacterial cells in a flow calorimeter produces a decrease in the power which can be used to compare the antibacterial potency of bactericides (Beezer et al., 1983a). In an extension of this work we have also shown (Beezer et al., 1986) that the interaction of respiring *E. coli* cells with a series of phenols could be factorised into two contributions, from the aromatic portion and from the methylene groups, the contribution from the CH_2 groups being linear with respect to carbon number in the alkoxy chain. We now report the extension of these studies to the *o*- and *p*-alkoxy phenols which shows that such group additivity parameters allow the calculation of biological response. Furthermore, the parameters used have an actual physical significance, in contrast to the de novo methods exemplified by Free and Wilson (1964).

The microcalorimetric methods, organisms, storage and use of inocula have been described (Beezer et al., 1983a). The catechol ethers from ethoxy to heptoxy were prepared by the method of Mauthner (1937) and were analysed by GLC before use. Hydroquinone alkyl ethers, methyl to heptyl, were purchased from Aldrich Chemical Co., crystallised from petroleum ether and analysed by GLC before use.

The microcalorimetric data (Table 1) were used to establish the relationships shown in Fig. 1 between dosage, the concentration of the phenol in the medium, and response, the decrease produced in the power output of the cells by exposure to the phenols in solution. This response we express as:

$$100 \times \left(\frac{\text{power}_{(\text{treated cells})} - \text{power}_{(\text{untreated cells})}}{\text{power}_{(\text{treated cells})}} \right)$$

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Table 1

Values of dose and response for the o- and p-alkoxyphenols

Compound	Dose (mM)	Response (%)	Compound	Dose (mM)	Response (%)
o-Me	47.02	−43	*p*-Me	31.16	−50
	57.41	−58		34.39	−58
	67.69	−67		37.39	−69
	86.12	−87		41.12	−87
o-Et	27.68	−61	*p*-Et	16.79	−55
	29.88	−64		18.00	−65
	32.48	−67		19.43	−79
	36.35	−80		20.28	−88
	41.83	−88			
o-Pr	10.34	−61	*p*-Pr	4.68	−55
	11.39	−71		5.01	−66
	12.14	−78		5.43	−82
	13.61	−90		5.89	−85
	15.19	−98			
o-Bu	2.43	−40	*p*-Bu	1.32	−55
	2.63	−69		1.51	−61
	2.92	−81		1.67	−65
	3.12	−89		2.24	−78
				2.82	−86
o-Pent	1.23	−70	*p*-Pent	0.50	−42
	1.40	−81		0.60	−61
				0.71	−70
	1.49	−86		0.84	−87
	1.55	−92		0.97	−97
o-Hex	0.27	−58	*p*-Hex	0.21	−52
	0.29	−65		0.22	−64
	0.32	−73		0.24	−62
	0.35	−87		0.26	−77
				0.27	−92
o-Hept	0.11	−53	*p*-Hept	0.07	−52
	0.12	−61		0.08	−61
	0.13	−77		0.09	−76
	0.14	−84		0.10	−91

In our earlier publication (Beezer et al., 1986), we defined "response" as simply the % of control values of power shown by treated cells, but inspection of the data on phenols and on polyene antibiotics (Beezer et al., 1983b) shows that low concentrations of these agents cause a small deflection in excess of the control values from untreated cells. The explanation offered for this "excess over control" was that very low concentrations of the agents damaged the membrane of the cells, allowing readier access to glucose and so temporarily raising metabolic activity; the cells nevertheless succumbed eventually to the effects of the drug. We have therefore modified our definition of response to allow some way of normalising this measurement between one batch of cells and another, though we have found that our procedure for storage in liquid nitrogen allows inocula to be stored apparently indefinitely. Response is now defined as a negative quantity, the depression of power caused by the presence of a phenol; this new definition should allow continuity of com-

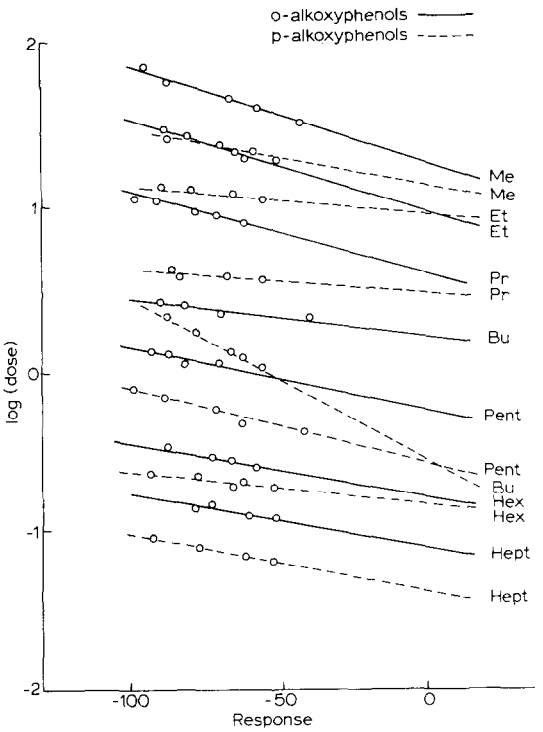

Fig. 1. Relationship between the dose (mM) and response for alkyl ethers.

parison between different batches of cells and between different cell lines. The data reported below for the *m*-compounds have been recalculated on this basis.

Fig. 1, constructed in this way, shows the expected linear relationship between log dose and response for the 14 phenols; it allows the calculation of the maximum dose or concentration of a phenol that will produce no response, [$\log(dose_{max})$]. It is apparent from Fig. 1 that in both the *o*- and *p*-series the behaviour of the butyl ether is atypical and we have previously drawn attention to the change in partitioning behaviour that occurs at the C_4 member of the series of *m*-alkoxyphenols. Yalkowsky et al. (1972) have also reported the anomalous behaviour of butyl *p*-aminobenzoate which represented a dividing line between two regular trends in the relationship between chain length and physical properties such as solubility in oil, water or hexane, melting behaviour, etc.

When values of $\log(dose_{max})$ are plotted against carbon number in the side-chain, straight lines are obtained:

o-alkoxyphenols:
slope −0.42, intercept 1.66, $r = -0.95$
m-alkoxyphenols:
slope −0.43, intercept 1.58, $r = -0.99$
p-alkoxyphenols:
slope −0.46, intercept 1.82, $r = -0.98$

The slopes of the three lines thus represent the log increment or contribution to response produced by addition of each methylene group whilst the intercepts are the log contributions of the three hypothetical "parent groups". These intercept values for the *o*-, *m*- and *p*-substituted parent groups differ considerably but the methylene contributions are remarkably constant the slight difference between *o*- and *p*- possibly reflecting the interaction between the hydroxyl and ether groups. The extent to which H-bonding or conformation of the alkyl chain may affect the values is of course unknown but it is encouraging to see that all three series yield linear plots which can be used to establish, or indeed to calculate, response for QSAR studies. Moreover, the small but significant differences observed in both slope and intercept values suggest the possibility of a very sensitive discrimination between closely related structures. The use of microcalorimetric bioactivity data in this way has been proposed previously (Perry et al., 1986) but on a less quantitative basis.

We conclude that the accuracy and reproducibility of microcalorimetric measurements of bioactivity would permit the establishment of a group additivity scheme that could genuinely distinguish between closely related structures.

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