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Susceptibility of porin- and lipopolysaccharide-deficient mutants of *Escherichia coli* to a homologous series of esters of *p*-hydroxybenzoic acid

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Summary

The methyl (Me), ethyl (Et), propyl (Pr) and butyl (Bu) esters of *p*-hydroxybenzoic acid have been tested against wild type and porin- and/or lipopolysaccharide (LPS)-deficient mutants strains of *Escherichia coli*. The Bu ester was the most active inhibitor, the Me the least. The most sensitive strains to an individual ester were those with LPS-defective outer membranes, whilst the most resistant were the wild type (PCO479) and rough mutant (D21) strains. The results are discussed in relation to some of the physical properties of the parabens, especially their hydrophobic nature.

Introduction

Recent studies from this laboratory have considered the effects of cationic bactericides on some envelope mutants of *Escherichia coli* and *Pseudomonas aeruginosa* (El-Falaha et al. 1985). These organisms have been useful in obtaining information about the role of the outer membrane in bacterial resistance to antiseptics and preservatives.

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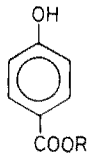

R = Me, Et, Pr
or Bu

Fig. 1. Chemical structures of esters of *p*-hydroxybenzoic acid. Me = methyl; Et = ethyl; Pr = propyl; Bu = butyl.

We next wished to examine the activity against some porin- and/or lipopolysaccharide (LPS) *E. coli* mutants of a homologous series of antibacterial agents. One such series, which has been studied extensively in the past (Hugo and Russell, 1982) and which contains as members useful pharmaceutical and cosmetic preservatives (Haag and Loncrini, 1984; Wallhausser, 1984) consists of the methyl (Me), ethyl (Et), propyl (Pr) and butyl (Bu) esters of *p*-hydroxybenzoic acid (4-hydroxybenzoic acid; Fig. 1). The physical and chemical properties of these agents are well known, and consequently their activity against these *E. coli* strains has been investigated in the light of some of these properties, notably their oil : water partitioning. Hansch et al. (1972) described an antimicrobial structure–activity relationship in these esters which correlated well with octanol/water partition coefficients. Shibasaski (1969) showed a linear relationship between antimicrobial activity and alkyl chain length of esters (Me to octyl). Dymicky and Huhtanen (1979) correlated antimicrobial activity against *Clostridium botulinum* with pK_a values and chain length of esters.

Materials and Methods

Nutrient media

These were Brain Heart Infusion (BHI) broth and agar, purchased from Oxoid, Basingstoke, Hants. The final pH was 7.4.

Chemicals

The Me, Et, Pr and Bu esters of *p*-hydroxybenzoic acid were purchased from Nipa Laboratories, Pontypridd. The compounds were dissolved in hot Water-for-Injections, B.P., sterilized and incorporated whilst still warm into BHI agar (final vol. 20 ml).

Organisms

These consisted of various *E. coli* strains, as listed in Tables 2 and 3. Organisms were grown overnight at 37°C in BHI broth, to a density of ca. 10^9 cfu/ml. For sensitivity testing to parabens, cultures were used diluted, as described below.

Test methods

Cultures were diluted 1 : 1000 in BHI broth. By means of a Denley multipoint inoculator (Denley Instruments, Billingshurst, Sussex), 1 μl vols. of diluted cultures were spotted on to a series of plates of overdried (37°C, 3 h) BHI agar containing various concentrations of Me (range 0–1500 μg/ml), Et (0–500 μg/ml), Pr (0–400 μg/ml) or Bu (0–240 μg/ml) paraben. Paraben solutions were prepared with the aid of heat and added when warm to BHI agar. Plates were incubated for 24 h at 37°C, and the concentration of a paraben that completely prevented growth was recorded as the minimum inhibitory concentration (MIC). Experiments were carried out in triplicate.

Results and Discussion

Table 1 summarizes the chemical and physical properties of the parabens, and also lists some published MIC values against *E. coli* and, for comparison, against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* As would be expected, aqueous solubility decreases as the homologous series is ascended. Partitioning between oil and water demonstrates that solubility in oil increases with an increasing number of carbon atoms in the ester group. The higher the ester, the more effective it is antimicrobially with concentration up to a solubility-limiting chain length (Ferguson, 1939).

The MIC values provided by Haag and Loncrini (1984) for the esters against *E. coli* in Table 1 tend to be higher than MICs against various *E. coli* strains used in the present study (Table 2). However, the inoculum level listed by them in Table 1 is ca. 10^6–10^7 cfu, whereas in the investigation it has been ca. 10^3. Inoculum size affects to some extent the inhibitory activity of these preservatives (El-Falaha et al., 1983; Table 1).

Table 2 lists the *E. coli* strains studied, whether there is any deletion in a porin or a deficiency in LPS, together with the MIC values (μg/ml) of the Me, Et, Pr and Bu esters. In addition, a comparison is presented between a mutant and the wild-type strain, PC0479, in the form of a ratio of MIC of an ester against PC0479: MIC of that ester against the test mutant. Thus, the higher the ratio, the more likely it is that that ester can pass through the outer membrane barrier. (This assumes that the inner membrane has the same composition in all of the test strains and that it responds equally to that particular ester.) With the LPS-deficient strains, the ratio increases from Me to Bu. This is also so, but to a lesser extent, with those strains which have no deficiency in their LPS, even though they may lack one or more porins; the rough mutant D21 presents the same order of response to a particular ester as PC 0479. These results, therefore, suggest that as the homologous series of esters is ascended, the LPS-deficient mutants are penetrated more readily, resulting in the highest PC0479/mutant MIC ratio with the Bu ester. Eklund (1980) showed that the activity of the parabens in inhibiting growth and in inhibiting alanine or other uptake into vesicles increased with increasing side-chain length. These findings implied that their mechanism of action was related to their membrane solubility, as had earlier been

TABLE 1

SOME CHEMICAL, PHYSICAL AND MICROBIOLOGICAL PROPERTIES OF ESTERS OF *p*-HYDROXYBENZOIC ACID

Property	Me ester	Et ester	Pr ester	Bu ester	Reference
Molecular weight	152	166	180	194	
Solubility in water (g/100 g) at					Haag and Loncrini (1984)
15°C	0.16	0.08	0.023	0.005	
25°C	0.25	0.11	0.04	0.015	
80°C	3.2	0.86	0.45	0.15	
K_w^0 (mineral oil)	0.02	–	0.5	3.0	Parker (1982)
K_w^0 (vegetable oil)	7.5	–	80.0	280.0	Parker (1982)
K_w^0 (arachis oil)	2.4	13.4	38.1	239.6	Furr and Russell (1972b)
Log P (octanol: H_2O)	1.96	2.47	3.04	3.57	Hansch et al. (1972)
MIC value *					Haag and Loncrini (1984)
E. coli	2000	1000	500	500	
S. aureus	2000	1000	500	125	
Ps. aeruginosa	4000	> 2000	> 1000	> 1000	
MIC value **					El-Falaha et al. (1983)
E. coli	800	560	350	160	
Ps. aeruginosa	1000	700	350	160	
Concn. (mM) giving 50% inhibition of growth and uptake process in					Eklund (1980)
E. coli ML308-225	5.5	2.2	1.1	0.4	
Ps. aeruginosa ATCC9027	3.6	2.8	> 1.0	> 1.0	
B. subtilis ATCC6633	4.3	1.3	0.9	0.46	

* MIC vs 10^6–10^7 cfu/ml.
** MIC vs 2×10 cfu.

postulated by Hansch et al. (1972). Hansch et al. (1972) described a general parabolic relationship between log 1/C and log P (where C is an inhibitory concentration and P the partition coefficient in an octanol–water system) using *B. subtilis* as test organism.

Our results have been re-presented in Table 3, in the sense that all the molar MIC values obtained have been related to the effect of Me paraben on a particular strain. Thus, the figures depicted in Table 3 are the ratios of MIC of Me paraben against a strain: MIC of test ester against the same strain. When the Me : Et MIC ratios are considered, it can be seen (Table 3) that slightly higher values are obtained with the LPS-deficient strains. The ratios are more pronounced with these strains when the Me : Pr MIC ratios are observed, and considerably more so when the Me : Bu MIC ratios are noted.

TABLE 2

INHIBITORY CONCENTRATIONS OF ESTERS OF *p*-HYDROXYBENZOIC ACID AGAINST *E. COLI* STRAINS

E. coli strain	Protein deficiency *	LPS deficiency *	Me ester			Et ester			Pr ester			Bu ester		
			MIC **		PC0479: test strain	MIC **		PC0479: test strain	MIC **		PC0479: test strain	MIC **		PC0479: test strain
			(a)	(b)		(a)	(b)		(a)	(b)		(a)	(b)	
PC0479	–	–	600	3.95×10^{-3}	1	450	2.7×10^{-3}	1	280	1.58×10^{-3}	1	200	1.03×10^{-3}	1
D21	–	Rough	800	5.26×10^{-3}	0.75	500	3.01 ± 10^{-3}	0.9	300	1.67×10^{-3}	0.93	250	1.29×10^{-3}	0.8
D21f2	–	Deep rough	600	3.95×10^{-3}	1	250	1.51×10^{-3}	1.8	100	5.56×10^{-4}	2.8	40	2.06×10^{-4}	5
PC2040	OmpF	Heptoseless	400	2.63×10^{-3}	1.5	200	1.20×10^{-3}	2.25	100	5.56×10^{-4}	2.8	50	2.58×10^{-4}	4
CE1054 †	OmpA OmpF	–	400	2.63×10^{-3}	1.5	250	1.51×10^{-3}	1.8	150	8.33×10^{-4}	1.87	80	4.12×10^{-4}	2.5
CE1055	OmpA OmpF	Heptoseless	400	2.63×10^{-3}	1.5	200	1.20×10^{-3}	2.25	100	5.56×10^{-4}	2.8	30	1.55×10^{-4}	6.7
CE1056	OmpC		400	2.63×10^{-3}	1.5	250	1.51×10^{-3}	1.8	175	9.72×10^{-4}	1.6	100	5.16×10^{-4}	2
CE1057	OmpC OmpF	Heptoseless	500	3.29×10^{-3}	1.2	250	1.51×10^{-3}	1.8	100	5.56×10^{-4}	2.8	40	2.06×10^{-4}	5
CE1058 †	OmpA OmpC OmpF	–	400	2.63×10^{-3}	1.5	250	1.51×10^{-3}	1.8	175	9.72×10^{-4}	1.6	100	5.16×10^{-4}	2
CE1059	OmpA OmpC OmpF	Heptoseless	400	2.63×10^{-3}	1.5	200	1.2×10^{-3}	2.25	50	2.78×10^{-4}	5.6	20	1.03×10^{-4}	10
CE1122	OmpF	–	300	1.97×10^{-3}	2	200	1.2×10^{-3}	2.25	125	6.94×10^{-4}	2.24	100	5.16×10^{-4}	2
CE1131	OmpA	–	400	2.63×10^{-3}	1.5	250	1.51×10^{-3}	1.8	175	9.72×10^{-4}	1.6	100	5.16×10^{-4}	2

* Information from Alphen et al. (1977) and from the Phabagen Collection, University of Utrecht.

** MICs as (a) μg/ml or (b) on a molar basis.

† In BHI broth: Alphen et al. (1977).

TABLE 3

RELATIVE EFFICACY OF ESTERS OF *p*-HYDROXYBENZOIC ACID AGAINST *E. COLI* STRAINS

E. coli strain	Protein deficiency *	LPS deficiency *	Ratio of MICs ** (Me: other esters for each strain)		
			Me/Et	Me/Pr	Me/Bu
PC0479	–	–	1.46	2.5	3.83
D21	–	Rough	1.75	3.15	4.08
D21f2	–	Deep rough	2.62	7.1	19.17
PC2040	OmpF	Heptoseless	2.2	4.73	10.19
CF1054	OmpA OmpF	–	1.74	3.16	6.38
CE1055	OmpA OmpF	Heptoseless	2.19	4.73	16.97
CE1056	OmpC	–	1.74	2.71	5.1
CE1057	OmpC OmpF	Heptoseless	2.18	5.92	15.97
CE1058	OmpA OmpC OmpF	–	1.74	2.71	5.1
CE1059	OmpA OmpC OmpF	Heptoseless	2.19	9.46	25.53
CE1122	OmpF	–	1.64	2.84	3.82
CE1131	OmpA	–	1.74	2.71	5.1

* Information from Alphen et al. (1977).

** Actual MIC values are listed in Table 2. Ratios have been determined on a molar basis.

Deficiency in one or more porin channels would be expected to have little direct role to play in sensitivity or resistance of a strain to any paraben. However, porin-deficient strains with intact LPS show an intermediate resistance to Pr and Bu esters, and it may well be that some phospholipid (see below) is exposed at the cell surface of these strains (Alphen et al., 1977). This point will be considered in more detail later. The LPS-depleted mutants were the most sensitive strains to the parabens, and especially to the Pr and Bu esters (Tables 2 and 3). If Nikaido's theory is correct (Nikaido and Vaara, 1985), then the presence of phospholipid in the outer leaflet of the outer membrane of these mutants would enable adequate penetration of hydrophobic agents, and in particular the higher members of this homologous series, through this part of the cell envelope, so that they may reach their presumed target site, the inner (cytoplasmic) membrane (Furr and Russell, 1972a, b and c). However, some doubt persists as to whether, in fact, there is an increase in phospholipid relative to LPS in LPS mutants (Shales and Chopra, 1982). Leive et al. (1984) investigated tetracycline antibiotics of various hydrophobicities against 'normal' and mutant *E. coli* strains. As hydrophobicity increased, the 'normal' cells (no defect in LPS or porin) became more resistant, whereas mutants altered in outer membrane permeability became more sensitive (decreasing MIC values).

Furr and Russell (1972b) found that the uptake of the Pr and Bu esters to isolated cell walls of *Serratia marcescens* was less than to whole cells. This suggested that these esters were taken up from aqueous solution and diffused through the outer cell layers to the inner membrane. The assay procedures used did not permit detection of any significant uptake of Me or Et ester to whole cells or isolated walls. More

sophisticated techniques did, however, demonstrate Me and Et uptake by *E. coli* cells (Lang and Rye, 1973). It was also found that the intracellular : extracellular concentration ratios for the Me, Et and Pr esters were, respectively, 3.5, 6.4 and 18.5, but that irrespective of size, ester molecules at their site of action were equally effective in inhibiting growth, so that differences in activity reflected differences in uptake (Lang and Rye, 1973). Both groups of workers agreed that uptake proceeded by a general dissolution of ester into the cell with no specific sites existing for uptake at the cell surface. This implies that the Me and Et esters have greater difficulty in reaching the inner membrane or that they cause less damage to this membrane than do the higher esters, a point previously suggested in studies with whole cells and spheroplasts of *Serratia marcescens* (Furr and Russell, 1972c). The situation is not, therefore the same as that noted above for hydrophobic tetracyclines (Leive et al., 1984).

Table 4 compares the partition coefficients (expressed as log P) of the esters in an octanol : water system (Hansch et al., 1972) with log 1/C values, where C is the MIC value, expressed in molar terms (see Table 2). Log 1/C has been plotted against log P for different 'groups' of strains, the results being presented in Fig. 2 for the wild-type strain PC0479 and the deep rough mutant D21f2. It is noticeable that the slope of the response is much greater with the mutant. Similar findings (not shown) to D21f2 were obtained with the other LPS mutants: strain PC2040 and CE1057 gave reasonably linear responses, but with CE1055 and CE1059 there was a deviation from linearity (enhanced effect) above the Pr and Et ester, respectively. The lines (not shown) for strains CE1054, CE1056, CE1058 and CE1131 were

TABLE 4

RELATIONSHIP BETWEEN LOG P AND LOG 1/C VALUES OF PARABENS

Physical property or *E. coli* strain	Ester *			
	Me	Et	Pr	Bu
Log P **	1.96	2.47	3.04	3.57
PC0479	2.403	2.567	2.808	2.987
D21	2.279	2.521	2.778	2.89
D21f2	2.403	2.822	3.255	3.686
PC2040	2.580	2.919	3.255	3.589
CE1054	2.580	2.822	3.079	3.385
CE1055	2.580	2.919	3.255	3.811
CE1056	2.580	2.822	3.012	3.288
CE1057	2.483	2.822	3.255	3.686
CE1058	2.580	2.822	3.012	3.288
CE1059	2.580	2.919	3.556	3.987
CE1122	2.706	2.919	3.158	3.288
CE1131	2.580	2.822	3.012	3.288

* Values against *E. coli* strains are log 1/C, where C is the minimum inhibitory concentration expressed in terms of molarity (see Table 2).

** In octanol : water (data from Hansch et al., 1972).

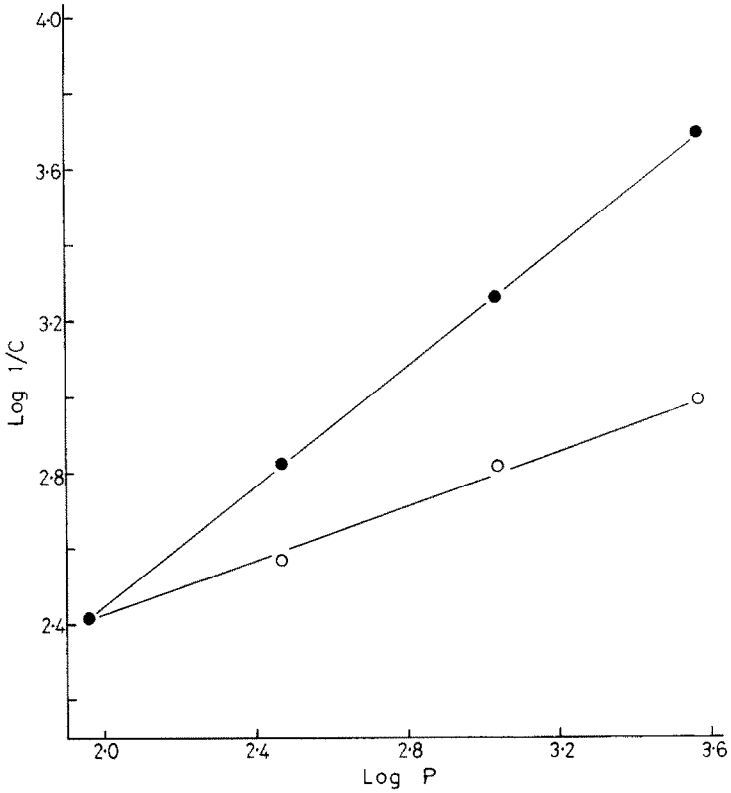

Fig. 2. Structure–activity relationships for hydroxybenzoates acting against some *E. coli* strains (log 1/C is the $\log_{10}$ of the reciprocal of the MIC molar value, and log P is the $\log_{10}$ of the partition coefficient). ○——○, PC0479 (wild-type); ●——●, D21f2 (deep rough).

reasonably linear, and only slightly steeper than the response with PO0479, although the values of log 1/C were consistently higher than for the parent strain.

Fig. 3 depicts the best straight lines by computer analysis of the data, when log S (where S is comparative sensitivity, i.e. MIC of an ester vs PC0479: MIC of that ester vs test organism), obtained from Table 2, is plotted against log P. The responses tend to fit into two patterns, the LPS-deficient mutants (Fig. 3a) and the outer mutants with no LPS deficiency (Fig. 3b). Gradients of the lines of the latter group are much lower. Only with strain CE1054 is the gradient statistically different from zero. Interestingly, this strain is considerably more sensitive to phospholipase when grown in BHI broth (as it was here) than in yeast extract broth, suggesting that in BHI more phospholipid is exposed at or near the cell surface. A further point of interest is to compare the slopes of the lines of the LPS-deficient mutants in relation to their protein deficiency; these gradients are 0.53 (CE1059, $A^-C^-F^-$), 0.38 (CE1057, C^-F^-), 0.38 (CE1055 A^-F^-), 0.37 (D21f2; any porin deficiency?) and 0.25 (PC2040, F^-). Tentatively, therefore, it appears that there may be some relationship between porin deficiency and paraben sensitivity. The situation with

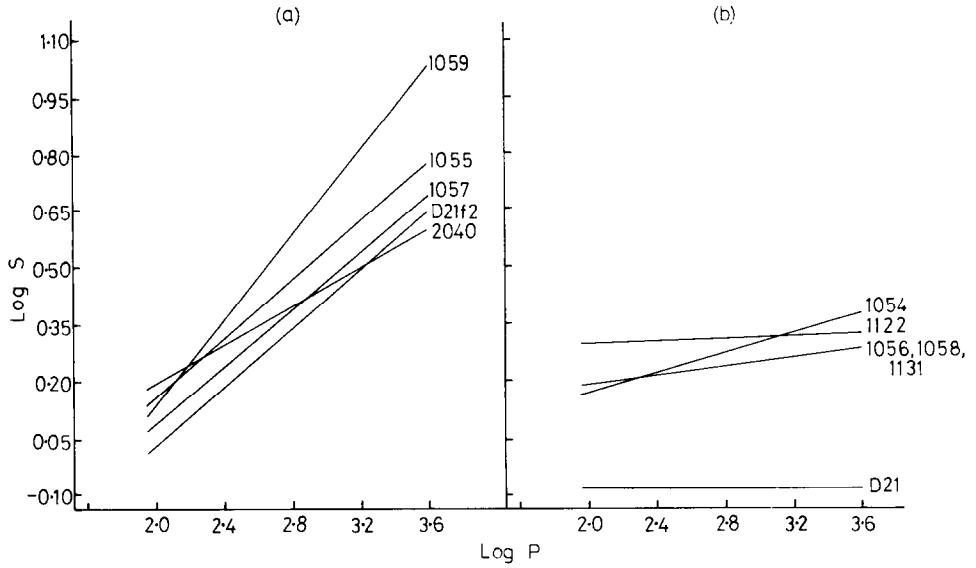

Fig. 3. Best straight lines when log S (MIC of ester vs PC0479:MIC vs test strain) is plotted against log P (partition coefficient) for (a) deep rough (heptoseless) mutants and (b) other strains. Numbers on the graphs refer to the strain numbers.

those strains showing no LPS loss but having a porin deficiency is less clear-cut, although these strains are more sensitive than PC0479 to the parabens, especially the higher esters.

Some preliminary studies have also been made with nutrient agar in place of BHI agar, for tests with the Pr and Bu esters. MIC values tended to be rather higher in nutrient agar, the reason for which might be changes in outer membrane composition. This will be considered in further detail in future experiments. It may be important to note that strains CE1054 and CE1058 suffer further porin loss in BHI as compared to yeast broth and become sensitive to phospholipases, implying that phospholipid is exposed to a greater extent in these strains, at least, in BHI (Alphen et al., 1977).

We have also examined the effects of the four esters on a strain (SC12, 155) of *E. coli* kindly provided by Dr. R.B. Sykes, Squibb Institute for Medical Research, New Brunswick, NJ, U.S.A. This strain was stated to be defective in penicillin binding protein (PBP) 1b, and as such uses PBP 1a as a compensatory protein. MIC values in BHI agar of the Me, Et, Pr and Bu esters in μg/ml (in brackets, in molar terms) were, respectively, 300 (1.97×10^{-3}), 200 (1.2×10^{-3}), 75 (4.17×10^{-4}) and 20 (1.03×10^{-4}), giving the following molar inhibition ratios: Me/Et 1.64; Me/Pr 4.72; Me/Bu 19.13. This strain is highly sensitive to β-lactams, and our findings imply that it might well have a deletion in outer membrane LPS, since it is one of the more sensitive strains to the parabens. It is also sensitive to the hydrophobic antibiotics erythromycin and rifampicin.

In the light of these earlier investigations and of the results obtained in the

present study, further investigation using various culture media with these *E. coli* mutants to show the uptake of parabens to whole cells and isolated cell components and the effects of the drugs on inner membrane damage would be instructive. In addition, the strains could prove useful in studying paraben combinations.

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References

Alphen, L. van, Lugtenberg, B., Boxtel, R. van and Verhoef, K., Architecture of the outer membrane of *Escherichia coli* K12. I. Action of phospholipases A_2 and C on wild type strains and outer membrane mutants. Biochim. Biophys. Acta, 466 (1977) 257–268.

Dymicky, M. and Huhtanen, C.M., Inhibition of *Clostridium botulinium* by *p*-hydroxybenzoic acid *n*-alkyl ester. Antimicrob. Ag. Chemother., 15 (1979) 798–801.

Eklund, T., Inhibition of growth and uptake processes in bacteria by some chemical food preservations. J. Appl. Bact., 48 (1980) 423–432.

El-Falaha, B.M.A., Russell, A.D. and Furr, J.R., Sensitivities of wild-type and envelope-defective strains of *Escherichia coli* and *Pseudomonas aeruginosa* to antibacterial agents. Microbios, 38 (1983) 99–105.

El-Falaha, B.M.A., Rogers, D.T., Furr, J.R. and Russell, A.D., Surface changes in *Pseudomonas aeruginosa* exposed to chlorhexidine diacetate and benzalkonium chloride. Int. J. Pharm., 23 (1985) 239–243.

Ferguson, J., The use of chemical potentials as indices of toxicity. Proc. Roy. Soc. Series B, 127 (1939) 387–404.

Furr, J.R. and Russell, A.D., Some factors influencing the activity of esters of *p*-hydroxybenzoic acid against *Serratia marcescens*. Microbios, 5 (1972a) 189–198.

Furr, J.R. and Russell, A.D. Uptake of esters of *p*-hydroxybenzoic acid by *Serratia marcescens* and by fattened and non-fattened cells of *Bacillus subtilis*. Microbios, 5 (1972b) 237–246.

Furr, J.R. and Russell, A.D., Effect of esters of *p*-hydroxybenzoic acid on spheroplasts of *Serratia marcescens* and protoplasts of *Bacillus megaterium*. Microbios, 6 (1972c) 47–54.

Haag, T.E. and Loncrini, D.F., Esters of *para*-hydroxybenzoic acid. In Kabara, J.J. (Ed.), Cosmetic and Drug Preservation—Principles and Practice, Marcel Dekker, New York and Basel, 1984, pp. 63–77.

Hansch, C., Courbeils, J.L. and Leo, A., The antimicrobial structure–activity relationship in esters of 4-hydroxybenzoic acid. Chim. Ther., 6 (1972) 427–433.

Hugo, W.B. and Russell, A.D., Types of antimicrobial agents. In Russell, A.D., Hugo, W.B. and Ayliffe, G.A.J. (Eds.), Principles and Practice of Disinfection, Preservation and Sterilisation, Blackwell Scientific, Oxford, 1982, pp. 8–106.

Lang, M. and Rye, R.M., A correlation between the antibacterial activity of some *p*-hydroxybenzoate esters and their cell uptake. Microbios, 7 (1973) 199–207.

Leive, L., Telesetsky, S., Coleman, W.G. and Carr, D., Tetracyclines of various hydrophobicities as a probe for permeability of *Escherichia coli* outer membranes. Antimicrob. Ag. Chemother., 25 (1984) 539–544.

Nikaido, H. and Vaara, M., Molecular basis of bacterial outer membrane permeability. Microbiol. Rev., 49 (1985) 1–32.

Parker, M.S., The preservation of pharmaceutical and cosmetic products. In Russell, A.D., Hugo, W.B. and Ayliffe, G.A.J. (Eds.), Principles and Practice of Disinfection, Preservation and Sterilisation, Blackwell Scientific, Oxford, 1982, pp. 287–307.

Shales, S. and Chopra, I., Outer membrane composition in *Escherichia coli* and the poor activity of hydrophobic antibiotics against enteric bacteria. J. Antimicrob. Chemother., 9 (1982) 329–331.

Shibasaki, I., Antimicrobial activity of alkyl esters of *p*-hydroxybenzoic acid. J. Ferment. Technol., 47 (1969) 167–177.

Wallhausser, K.H., Antimicrobial preservatives used by the cosmetic industry. In Kabara, J.J. (Ed.), Cosmetic and Drug Preservation–Principles and Practice, Marcel Dekker, New York and Basel, 1984, pp. 607–745.