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Biologically based QSARs: study of cardanol derivatives on interaction with *Saccharomyces cerevisiae*

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Summary

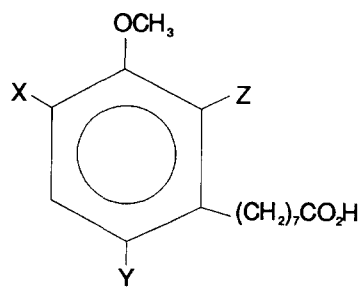
Derivatives of the cashew nut component shell liquid have been examined for antifungal activity by microcalorimetry. The dose/response data are shown to be useful in establishing a Linear Free Energy Relationship. The data are the first to show that such a biologically based quantitative structure-activity relationship (QSAR) can be applied to antifungals – and to derivatives of natural products.

Introduction

Cardanol, a mixture of related 3-alkyl phenols differing in their degree of unsaturation in the C_{15} side-chain, can be obtained from the oil of the cashew nut. The oils extractable from the cashew nut have been the subject of study for potential industrial applications (Aggarwal, 1975; Sundararamaiah, 1976). Extracts of the oil have also been shown to have antibacterial and antifungal properties (Gulati et al., 1964; Adawadkar and El Sohly, 1981). In an attempt to examine the chemical and biological properties and the potential applications of extracts and derivatives of cardanol a study of these materials as a source of new aromatic compounds has been initiated (De Morais, 1990).

This includes the first report of some derivatives of 8-phenyloctanoic acid obtained by oxidation of cardanol (Scheme 1).

Work from this laboratory has demonstrated (Beezer et al., 1986, 1987), for a limited series of compounds, the existence of a biological group additivity parameter. It has, furthermore, been ex-



Scheme 1. (a) X = Y = Z = H; (b) X = Z = H; Y = Cl; (c) X = Y = Z = Cl; (d) X = Z = H; Y = Br; (e) Z = Y = H; X = NO_2; (f) X = Z = H; Y = NO_2 (De Morais and Bolton, 1990).

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tended to demonstrate (Beezer et al., 1988) the existence of a Collander relationship for biological response data. The experimental technique used to evaluate biological response was microcalorimetry (Beezer, 1980). The demonstration of linear free energy relationships (LFERs) for biological response was based upon studies of homologous series of compounds.

Thus, (i) the availability of some ring-substituted derivatives of 8-phenyloctanoic acid not based upon homologous series and (ii) the objective of exploring the potential applications of these compounds have led us to the present microcalorimetric investigation.

To expand further the QSAR nature of the work from this laboratory, we have studied, in detail, the antifungal activities of compounds listed above as a, b, d, f. Compounds c and e were subjected only to a qualitative examination.

Materials and Methods

Compounds

The preparation, identification and purity of the 8-aryloctanoic acids have been described elsewhere (De Morais and Bolton, 1990).

Saccharomyces cerevisiae (NCYC 239)

The growth, preservation in liquid nitrogen, medium and handling of cultures and inocula were as reported previously (Perry et al., 1989).

Microcalorimetry

The conduct of microcalorimetric experiments has been extensively described in earlier papers on this topic (Beezer et al., 1986, 1987, 1988). The compounds were added in methanol (2 cm^3) to 50 cm^3 of medium plus cells for presentation to the microcalorimeter.

Results and Discussion

The quantitative results for the bioassay of compounds a, b, d and f are reported in Table 1. Also listed in Table 1 are the calculated (Hansch and Leo, 1979) values of the octanol/water partition coefficients (P) for the four compounds subjected to quantitative investigation. The regression coefficients slopes and intercepts for the linear log dose/response lines are also displayed in Table 1. $Log(dose)_{max}$ is defined, as previously (Beezer et al., 1986, 1987, 1988), as the maximum dose of the drug which can be administered without eliciting a response. Qualitative data on all compounds are reported in Table 2. Fig. 1 displays the appearance of a typical microcalorimetric output. It will be noted that Fig. 1 and the qualitative information displayed in Table 2 indicate that there are two differing modes of action amongst this group of related compounds.

A plot of log P vs $log(dose)_{max}$ for compounds a, b, d and f is linear (regression coefficients: intercept = 5.9820; slope = −1.2460; r^2 = 0.9954). There appears, therefore, to be an LFER for this group of compounds which is based upon biological response as determined microcalorimetrically. The compounds themselves are phenyl derivatives and possess a charged moiety (the carboxylic acid group) and a hydrophobic moiety (the hydrocarbon chain and the substituted methoxyphenyl group). The hydrophobic character of the compounds is apparent from their (calculated) log P values and from the need to add the drugs to a medium which, in the microcalorimetric experiment, contains 4% methanol. Blank experiments showed that methanol in this concentration had

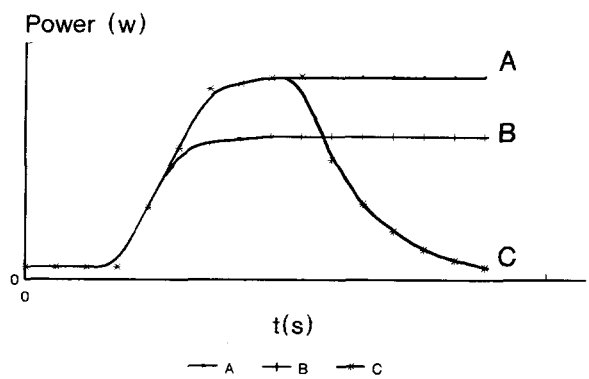

Fig. 1. Representative power-time curves. (A) Control; (B) a drug treated incubation showing reduction of power to another plateau value; (C) a drug treated incubation showing a signal decreasing to the base-line, i.e. cell death. See text and Table 2.

TABLE 1

Concentration/response data and regression parameters of the derived log dose-response line

Compound	Concentration (μmol dm^{-3})	Response (%)[a]	Regression parameters
a	160	90	Intercept = −159.53
	120	80	slope = 114.02
	80	56	r^2 = 0.9968
	40	23	$\log(\text{dose})_{max}$ = 1.3991
			log P = 4.28
b	70	95	Intercept = −67.36
	35	66	slope = 89.71
	17.5	40	r^2 = 0.9992
	8.75	17.6	$\log(\text{dose})_{max}$ = 0.7509
			log P = 5.06
d	120	81	Intercept = −34.95
	60	69	slope = 57.33
			r^2 = 0.9525
	30	56	$\log(\text{dose})_{max}$ = 0.6096
	15	28	log P = 5.21
f	135.2	96	Intercept = −188.38
	67.6	52	slope = 132.67
	33.8	14	r^2 = 0.9986
	16.9	−24	$\log(\text{dose})_{max}$ = 1.4199
			log P = 4.17

[a]All results are the mean of at least triplicates. Response is reported as a percentage, i.e. in arbitrary units.

only a small and non-lethal effect upon the *S. cerevisiae* culture. It is encouraging to note that the existence of LFERs in directly determined biological response has now been extended to antifungal compounds. We might, therefore, anticipate that a Collander relationship should hold for, for example, $\log(\text{dose})_{max}$ data determined for *S. cerevisiae* and *Candida albicans*.

TABLE 2

Observations on the form of the calorimetric power-time curve showing differing modes of action of the added compounds

Compound[a]	Observation
a	decrease
b	plateau
c	decrease
d	plateau
e	plateau
f	decrease

[a]All compounds were added at a concentration of 2 mg per 50 cm^3 of incubation medium (glucose buffer: at pH 4.5).

The differing modes of action of the compounds is interesting — some apparently leading to cell death and some simply reducing the metabolic activity of the *S. cerevisiae*. The known mode of action of phenols is, at low concentration, that of disrupting the cell membrane and thus permitting leakage of cytoplasmic constituents (Perry et al., 1980). It is, presumably, a similar mechanism which is responsible for the effects of these methoxy arenes. However, as pointed out above, the fact that these compounds also possess a long hydrocarbon chain with a charged head group (i.e. a phospholipid-like molecule) will modify their behaviour. The surprising feature of the quantitative results is that these, apparently, differing modes of action do not present any discontinuity in the LFER.

The most widely used LFER applied to aromatic systems is the Hammett equation.

$\log_{10} k_x/k_H = \sigma\rho$

Although it may be applied successfully to heterolytic and homolytic processes, and to both kinetic and equilibrium measurements, it fails to reflect substituent effects in these pharmacological studies, since the order of σ values is H < Cl, Br < NO_2 for both *meta* and *para* substituents (cf. Table 1).

Overall, therefore, the conclusions of this work are (i) that microcalorimetry can provide a rapid (each experiment requires approx. 30 min) and precise method of screening natural products and their derivatives for biological, in this case antifungal, activity and (ii) that the data reveal the existence, for another series of compounds, of an LFER. Importantly, the observations reported here relate to antifungal activity and this communication is the first to report such a relationship for directly determined biological activity.

Further studies on natural products and their derivatives are in progress in this department to interpret further and to elaborate such directly determined QSARs.

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