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High-performance liquid chromatographic study of the correlation between the physico-chemical parameters of furan and benzene aldehydes and the rate of their metabolism by yeast

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ABSTRACT

A kinetic study of the metabolism of furan and benzene aldehydes *in vivo* by *Candida scottii* by high-performance liquid chromatography showed that the biotransformation proceeded via the stages aldehyde → alcohol → carboxylic acid. It was shown by regression analysis that a good correlation exists between the rate constant and chromatographic retention, Hammett's σ constant and molar volume for both the furan and benzene derivatives.

INTRODUCTION

The correlation between structure and biological activity is an important problem in biochemistry, biotechnology and pharmacochemistry. Hansch's approach made it possible to correlate the activity with hydrophobic, electronic, steric and other properties of molecules [1,2]. Recently we used reversed-phase high-performance liquid chromatography (RP-HPLC) to investigate the behaviour of some inhibitors of yeast growth in the presence of *Candida* cells [3–5]. An example of the kinetic curves for the metabolism of furfural is presented below (Fig. 1 [4]). Analysis of the kinetic data showed that the transformation of aldehydes can be described by a general scheme for both furan and benzene derivatives: RCHO → RCH_2OH → RCOOH, where R is substituted furyl or aryl. It is probable that the oxidation of the primary alcohol to the acid proceeds through an intermediate form but we were not able to detect it by kinetic or analytical methods.

Only a few researchers have considered the correlation between metabolism rate and structure [6]. In this work, we investigated the kinetics of aldehyde metabolism by *Candida scottii* cells and attempted to establish its dependence on the structure and physico-chemical properties.

EXPERIMENTAL

Kinetic measurements

The biomass of *C. scottii* strain [7] was previously grown in buffered medium containing glucose. The fermentation runs were performed in the absence of carbohydrate nutrition in aerobic batch conditions at 30°C. The cell concentration was 20 and 10 g/l for the benzene and furan aldehydes, respectively. In each run 1 mmol/l of the aldehyde was added. Aliquots of 2 ml were filtered, diluted tenfold and analysed by the RP-HPLC method described below. First-order rate constants, k, were calculated as average from 6–8 measurements.

Chromatography

The HPLC system consisted of an LKB (Brom-

TABLE I

METABOLISM RATE CONSTANT, RETENTION INDICES, HAMMETT σ CONSTANTS AND VAN DER WAALS VOLUMES OF FURAN AND BENZENE DERIVATIVES

No.[a]	Substance	k (min^{-1})	k'	σ^b	$V_w{}^c$
1	Furylacrylic acid	0.00136	1.50	−0.22	79.9
2	5-Hydroxymethylfurfural	0.00642	1.02	0	55.6
3	Furfuryl alcohol	0.00832	1.00	0	53.2
4	5-Methylfurfural	0.0236	3.15	−0.17	56.2
5	Furfural	0.0372	1.60	0	45.1
6	Furylacrolein	0.0728	6.8	−0.10	61.2
7	5-Nitrofurfural	0.22	1.50	0.78	59.3
8	5-Nitrofuryl acrolein	0.517	5.5	0.68	75.5
9	Syringaldehyde	0.00104	7.08	−0.21	90.2
10	2,4-Dihydroxybenzaldehyde	0.00575	3.63	0.85	66.3
11	Vanillin	0.0127	4.68	−0.29	75.8
12	Veratraldehyde	0.0157	13.49	−0.19	84.7
13	4-Hydroxybenzaldehyde	0.0266	3.31	−0.37	61.5
14	3-Hydroxy-*p*-anisaldehyde	0.0552	5.25	−0.17	75.8
15	Protocatechuic aldehyde	0.126	3.02	−0.27	66.3
16	3-Hydroxybenzaldehyde	0.138	3.47	0.10	61.5

[a] Numbers correspond to curve numbers in Figs. 2 and 3.
[b] Taking as zero unsubstituted furfural or benzaldehyde [8].
[c] Taken from ref. 9.

ma, Sweden) Model 2150 pump, a Varian (Vienna, Austria) Model 2080 column oven, a Kratos (Ramsey, NJ, USA) Model 783 UV detector, a Spherisorb C_6 (10 μm) column (25 cm × 4.6 mm I.D.) and a Shimadzu (Kyoto, Japan) Chromatopac C-R3A data system. Previously found absorption maxima for each aldehyde were used as detection wavelengths. The mobile phase was acetonitrile–water (6:94, v/v). The elution rate was 1.5 ml/min and the column oven was thermostated at 60°C.

For kinetic measurements the same conditions were used but for the analysis of aldehydes having long retention times (as furylacrolein, veratraldehyde and syringaldehyde) the acetonitrile concentration in the eluent was 8.5% (v/v).

TABLE II

REGRESSION ANALYSIS OF EXPERIMENTAL DATA FOR FURAN ALDEHYDES (MODELS 1–4) AND BENZALDEHYDE (MODELS 5–8) ACCORDING TO GENERAL EQUATION LOG $k = b_0 + b_1$ LOG $k' + b_2 \sum\sigma + b_3 V_w$

Model No.	Parameters taken into account	Regression coefficients (b), 95% confidence intervals (S) and F factors		
		b_0		
		b_0	S_0	F_0
1	Hydrophobicity	−2.05	0.86	2.25
2	Hydrophobicity and molar volume	−0.95	5.16	0.1
3	Hydrophobicity and electronic effect	−2.16	0.42	4.11
4	Hydrophobicity, electronic effect and molar volume	−0.59	0.08	102
5	Hydrophobicity	−0.71	1.46	0.94
6	Hydrophobicity and electronic effect	−0.62	1.64	0.57
7	Electronic effect and molar volume	1.97	3.53	1.24
8	Hydrophobicity, electronic effect and molar volume	2.88	0.68	4.68

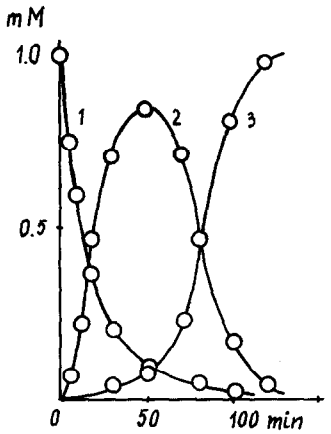

Fig. 1. Kinetics of furfural metabolism by *Candida scottii*. Curves: 1 = Furfural; 2 = furfuryl alcohol; 3 = furan-2-carboxylic acid.

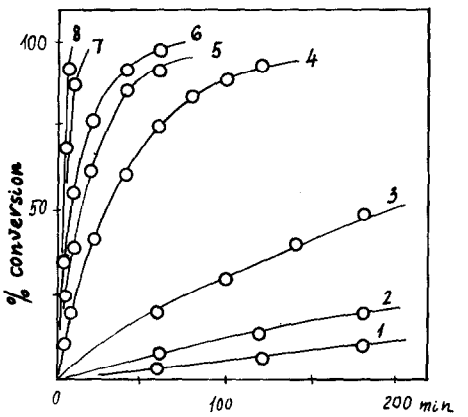

Fig. 2. Dependence of conversion of furan aldehydes on time. For curve numbers, see Table I.

RESULTS

Kinetic curves for the metabolic transformation of furfural by *C. scottii* are given in Fig. 1. The appearance of these curves is typical of consecutive reactions and shows that metabolism proceeds through two stages: furfural → furfuryl alcohol → furan-2-carboxylic acid. This conclusion was confirmed by experiments in which furfuryl alcohol and furan-2-carboxylic acid were preparatively isolated from fermentation media and their chemical structures were proved [4,5]. The same was established for each furan and benzene derivative.

Kinetic curves describing the first stage of metabolism of furan derivatives are presented in Fig. 2 as conversion–time dependences and in Fig. 3 as logarithm of concentration–time dependences. Data for furfuryl alcohol and furylacrylic acid are given for comparison. It is seen (Fig. 3) that at a constant cell concentration the metabolism is described by a first-order reaction. This is also true for the benzaldehyde derivatives.

We investigated the correlation of the kinetic data with hydrophobic, electronic and steric characteristics of substrate molecules. The chemical and structural parameters were the chromatographic retention index, k', which is proportional to hydrophobicity [8], the sum of the substituent Hammett constants, $\sum\sigma$, calculated as described by Barlin and Perrin [9], and the Van der Waals volume, V_w [10]. These data, which are summarized in Table I, were treated by regression analysis as described by

Regression coefficients (b), 95% confidence intervals (S) and F factors									F factor (overall)		Correlation coefficient
b_1			b_2			b_3			Theory	Calc.	
b_1	S_1	F_1	b_2	S_2	F_2	b_3	S_3	F_3			
1.58	0.72	3.36	–	–	–	–	–	–	5.99	3.37	0.60
1.82	1.88	4.18	–	–	–	−0.019	0.052	0.88	5.79	1.83	0.65
1.37	0.35	9.72	1.58	0.012	20.1	–	–	–	5.79	16.8	0.93
1.65	0.16	462	1.69	0.13	699	−0.028	0.04	173	6.59	110	0.99
−1.43	1.42	0.66	–	–	–	–	–	–	5.99	1.43	0.44
−1.62	1.24	1.69	−0.51	0.65	0.55	–	–	–	5.79	0.92	0.52
–	–	–	−0.70	1.09	1.81	−0.051	0.041	6.98	5.79	4.45	0.76
1.56	0.87	3.12	−0.72	0.48	2.26	−0.078	0.018	19.4	6.59	4.49	0.80

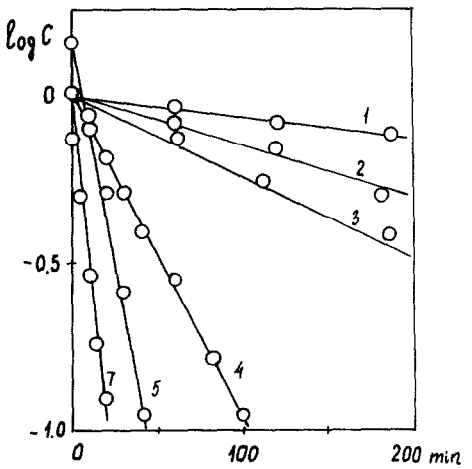

Fig. 3. Conversion of furan aldehydes plotted as logarithm of concentration *versus* time. For curve numbers, see Table I.

Afifi and Azen [11]. Four combinations of independent variables (log k', $\sum\sigma$ and V_w) for both furan (models 1–4) and aromatic aldehydes (models 5–8) were considered. For each model the regression coefficients, 95% confidence intervals of the regression coefficients, F factors and correlation coefficients were calculated, and are presented in Table II. Moreover, for each two subsets the squared correlation matrix was calculated (Table III), showing the extent of collinearity of the independent variables.

DISCUSSION

The results in Tables II and III show that the correlation is high in the furan series but not so high in the benzene series. However, in many instances reported for biological systems poorer correlations were considered to be acceptable [6]. It has been noted in many papers that the correlation is high when the structure of substances is similar within the series. Another factor is, of course, the identity of the set of ferments responsible for the transformation. If we assume that our case is analogous to those reported elsewhere [12], then the alcohol aldehyde dehydrogenase (ADH) is responsible for aldehyde reduction and our results may be explained in terms of the ADH model obtained by Hansch and Bjorkroth [12] from X-ray data treated by a computer graphic method. According to this model, the ferment active site consists of a hydrophobic cavity, one end of which has a polar group, so that complexing of the substrate depends on both hydrophobic and electronic properties of corresponding parts of the molecule.

Our results for benzaldehydes appear to be in agreement with this scheme. Introduction of hydrophobic groups increases the rate of metabolism and we have a positive coefficient for log k' (b_1 in model 8). Introduction of electron-attracting groups decreases the electron density on the carbonyl oxygen atom, thus decreasing the rate of metabolism and the coefficient for $\sum\sigma$ (b_2) is negative. The influence of hydrophobic properties of the substituents on the rate of metabolism is the same in the series of furan derivatives. The coefficient b_1 is also positive in model 4. However, the influence of electronic properties is different here, as the coefficient for the σ term in positive. One may assume that another mechanism exists in this instance.

TABLE III

CORRELATION MATRIX

Model 4			Model 8		
1.000	0.134	0.347	1.000	−0.208	0.849
0.134	1.000	0.176	−0.208	1.000	−0.271
0.347	0.176	1.000	0.849	−0.271	1.000

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