LIPOPHILIC–HYDROPHILIC PROPERTIES AND RETENTION OF PHENYLHYDRAZONOPROPANEDINITRILES BY BIOLOGICAL SYSTEMS

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Apparent partition coefficients in n-octanol–buffer system, solubility in the buffers, and retention by mitochondria from rat liver, by *Mycobacterium phlei* and by *Saccharomyces cerevisiae* (after 10 min inucbation) have been characterized for 13 arylsubstituted phenylhydrazonopropanedinitrile derivatives. Regression analysis has shown linear dependence of logarithms of the apparent partition coefficients on the published π parameters characterizing lipophilicity of the substituents. The apparent partition coefficients are inversely proportional to the solubility of the phenylhydrazonopropanedinitriles. The retention by the biosystems studied increases linearly with increasing lipophilicity, being independent of reactivity of the phenylhydrazonopropanedinitriles. The non-linear dependence of concentration of the phenylhydrazonopropanedinitriles remaining in the medium on the lipophilicity indicates that a lipophilic-hydrophilic equilibrium is established in the given time. The retained amount of the derivatives tested decreases with increasing pH values. The dependences are Z-shaped and have been described by the equations derived from the model presented by application of non-linear regression analysis.

Uncouplers of oxidative and photosynthetic phosphorylation are compounds able to interfere with synthesis of adenosine triphosphate in a cell by specific uncoupling of the oxidation from phosphorylation[1–3]. So far acid–base properties of the uncouplers have been mainly stressed with respect to explanation of mechanism of the uncoupling effect. According to the Mitchell theory[4,5], the presence of a ionisable group in their molecule is mainly responsible for perturbation of the proton gradient and the consequent inhibition of synthesis of adenosine triphosphate in the case of interaction of the uncoupler with the membrane of mitochondria, chloroplasts, or procaryotes. According to this idea, the uncouplers are dissolved in lipidic part of the membranes and cause the above-mentioned perturbation of pH gradient by the proton transfer. Another idea considers the uncouplers to be compounds which can chemically modify the proteins involved in the oxidative and/or photosynthetic phosphorylation[6–8]. One possible experimental verification able to decide

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which of the said ideas is more correct consists in confrontation of the presumed efficiency-limiting properties of the uncouplers, *i.e.* lipophilicity, acid–base properties, and chemical reactivity, with their uncoupling effect.

The present communication deals with characterization of lipophilicity of phenylhydrazonopropanedinitriles (called also[9] carbonyl cyanide phenylhydrazones) which are considered to be representative uncouplers and are applied to most varied experiments concerning studies of bioenergetical processes[10–16].

EXPERIMENTAL

Materials and Methods

The 4-OCF_3- and 3-Cl-phenylhydrazonopropanedinitriles were supplied by the firms Calbiochem (San Diego, USA), Boehringer (Mannheim. FRG), and Sigma (St. Louis, USA). The other phenylhydrazonopropanedinitriles were obtained by diazotization of the corresponding anilines and subsequent azo coupling reaction with malononitriles[9,17]. The spectrophotometry was carried out with an SP 30 UV VIS apparatus (Pye Unicam, Cambridge, GB) and a Specord UV VIS apparatus (Zeiss, Jena, GDR).

Solubility of the phenylhydrazonopropanedinitriles was determined by spectrophotometric determination of their concentrations in McIlvaine[18] citrate–phosphate buffers with pH 3·0, 5·0, and 7·2 and in the respective organic solvents. The saturated solutions of phenylhydrazonopropanedinitriles were prepared by 5 h shaking at 25°C and subsequent centrifugation at 10 000 *g*. The solubility values of the individual derivatives were determined as average values from three measurements each, the maximum error being ±5%.

The apparent partition coefficients were determined for the system n-octanol–buffer (Mc Ilvaine pH 3·0, 5·0, 7·2) by calculation from the relation (*1*)

$$P = (V_A/V_0)\,((A_0/A_A) - 1)\,, \qquad (1)$$

where V_A and V_0 mean the volumes of the aqueous and the n-octanol phases, respectively, and A_0 and A_A mean the initial and the equilibrium absorbances of the aqueous solution of the substance, respectively. n-Octanol (0·1 to 0·5 ml) was added to 10 ml solution of the respective phenylhydrazonopropanedinitrile in the buffer saturated with n-octanol. After 5 h shaking at 25°C, the solutions were separated by centrifugation at 600 *g* for 10 min, and the absorbance of aqueous phase was measured after temperation at 25°C. The buffer solution saturated with n-octanol at 25°C was used as the reference solution. The used concentrations of phenylhydrazonopropanedinitriles were $1 \cdot 10^{-5}$ mol l^{-1}. The found values of the partition coefficients are mean values from three measurements each, the maximum deviation being ±5%. The mixing of buffers with n-octanol did not change the pH values of aqueous phase.

The retention by the biosystems tested (yeasts, bacteria, mitochondria) was carried out in the following way: Fresh solutions of $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1} phenylhydrazonopropanedinitriles containing 0·25% dimethyl sulphoxide were treated with a suspension of the microorganisms or mitochondria. After 10 min incubation at 25°C, the suspension was centrifugated, and the supernatant liquid was submitted to spectrophotometry to find the absorbance of the non-retained portions A_x of the phenylhydrazonopropanedinitriles. Values of the non-retained and retained portions were determined from the relations (*2*) and (*3*)

$$c/c_0 = A_x/A_0\,, \qquad (2)$$

$$\text{Retention } (\%) = (1 - A_x/A_0) \cdot 100 , \qquad (3)$$

where A_0 stands for the initial absorbance of phenylhydrazonopropanedinitrile without the biological suspension corrected with respect to volume change, and c and c_0 mean the actual and the original concentrations of phenylhydrazonopropanedinitriles in the medium, respectively.

The stock suspension of the yeasts *Saccharomyces cerevisiae* Hansen was prepared in the physiological solution and contained $7{\cdot}5 \cdot 10^6$ cells per ml. The suspensions for the retention experiments were composed of 3·0 ml buffer (Mc Ilvaine, pH 3·0) and 20 µl stock yeast suspension, or of 2·8 ml buffer (Mc Ilvaine, pH 7·2) and 200 µl basic suspension. The pH dependence of the retention was determined with the use of citrate–phosphate Mc Ilvain buffers of pH 3·0, 4·5, 6·0, 6·8, 7·5, and borate Clark-Lubs buffer[18] of pH 9·0. In this case the suspension for use in the retention determination contained 3 ml buffer solutions and 50 µl stock yeast suspension. The yeasts were separated by centrifugation at 2 000 *g* (10 min).

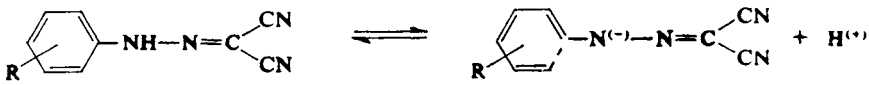
The bacteria *Mycobacterium phlei* (supplied by Dr V. Majtán, Research Institute of Preventive Medicine, Bratislava) were cultivated on the Šula medium at 28°C for 48 h, using a reciprocal shaker machine. The basic suspension of the cells in physiological solution contained 15 mg proteins per ml. The suspensions for the retention experiments were composed of 3·9 ml Mc Ilvaine buffer solution of pH 3·0 and 100 µl basic suspension of cells, or of 3·5 ml Mc Ilvaine buffer solution of pH 7·2 and 500 µl basic suspension. The bacteria were separated by centrifugation at 4 000 *g* (10 min).

The mitochondria used were isolated from rat liver by the method of Chance and Hagihara[19]. The suspension for the retention experiments contained 3·5 ml Tris buffer of pH 7·2 (with 0·25 mol l^{-1} saccharose and 0·2 mmol l^{-1} EDTA) and 500 µl basic suspension of the mitochondria (17·2 mg proteins per ml). The mitochondria were separated by centrifugation at 10 000 *g* (20 min).

The second order rate constants (k, 1 mol^{-1} s^{-1}) characterizing the reactivity of phenylhydrazonopropanedinitriles towards cysteine and the pK constants of these substances were determined spectrophotometrically by the method described in ref.[20]. The lipophilicity parameters π were taken from the work by Hansch and coworkers[21], and the non-linear regression analysis was carried out by the method described by Fletcher and Powell[22].

RESULTS AND DISCUSSION

Distribution of xenobiotics in a biosystem is determined by their hydrophobicity[23] which is expressed most often by means of the partition coefficient in the n-octanol–water system[21]. The partition coefficient of such dissociable compounds as phenylhydrazonopropanedinitriles (see Eq. (*4*)) depends on the composition (pH, salt concentration) of the phases[24].



CH C (4)

In this case, the hydrophobicity is characterized by the apparent partition coefficient determined at defined conditions. Table I gives its values in the system n-octanol–

–buffer solution (pH 3·0, 5·0, 7·2) found for 13 phenylhydrazonopropanedinitriles along with the corresponding π constants[21] of the substituents.

The apparent partition coefficient is defined as follows:

$$P_{app} = (\sum_{i=1}^{N} [CX_i]_o + [CH]_o + [C]_o)/(\sum_{i=1}^{N} [CX_i]_A + [CH]_A + [C]_A), \quad (5)$$

where the square brackets denote the concentration (provided that the concentration is sufficiently low) in water and in n-octanol (the subscripts A and o, respectively), CX_i means the ion pair with the i-th univalent cation X_i, and N means the overall number of the univalent cations able to form the ion pairs with C (see Eq. (*4*)). Eq. (*5*) can be transformed into Eq. (*11*) by application of the dissociation constant K_a (Eq. (*6*)), the association constants K_i of formation of the ion pairs (Eq. (*7*)), and the partition coefficients of the i-th ion pair P_i (Eq. (*8*)) and of the non-ionized and ionized phenylhydrazonopropanedinitrile (P_{CH}, Eq. (*9*), and P_C, Eq. (*10*), respectively).

$$K_a = [C]_A[H]_A/[CH]_A, \quad (6)$$

$$K_i = [CX_i]_A/[C]_A[X_i]_A, \quad (7)$$

$$P_i = [CX_i]_o/[CX_i]_A, \quad (8)$$

$$P_{CH} = [CH]_o/[CH]_A, \quad (9)$$

$$P_C = [C]_o/[C]_A, \quad (10)$$

$$\log P_{app} = \log P_{CH} - \log (A\, 10^{pH} + 1) + \log (B\, 10^{pH} + 1), \quad (11)$$

where

$$A = K_a(\sum_{i=1}^{N} K_i[X_i]_A + 1),$$

$$B = K_a(\sum_{i=1}^{N} P_iK_i[X_i]_A + P_C)/P_{CH}.$$

Provided that concentrations of the ions capable of formation of ion pairs do not change substantially within the whole pH range, the relative magnitudes of the constants A and B determine graphical shape of the pH dependence of log P_{app} (Fig. 1). The curves *1* and *2* apply to the cases of $A < B$ and $A > B$, resp. The log P_{app} values of phenylhydrazonopropanedinitriles (Table I) decrease with increasing pH, hence $A > B$ at the conditions used by us (Eq. (*11*)). Table I also gives

(for comparison) the π constants[21] characterizing the hydrophobicity of the substituents present in the benzene ring. The results of linear regression analysis characterizing the linear dependence of log P_{app} on π are summarized in TableII (dependence *A*). From Table II it follows that the apparent partition coefficients determined for

TABLE I

Apparent partition coefficients (log P_{app}) of R-phenylhydrazonopropanedinitriles and the π values characterizing the lipophilicity of the R substituents. The partition coefficients determined for the n-octanol–buffer system (pH 3·0, 5·0, 7·2) at 25°C

No	R	pH 3·0	pH 5·0	pH 7·2	π
I	H	2·64	2·48	1·85	0·00
II	4-CH_3	3·20	2·91	2·10	0·56
III	4-Cl	3·39	2·98	2·23	0·71
IV	3-Cl	3·38	2·97	2·19	0·71
V	4-$COCH_3$	2·10	1·01	1·15	−0·55
VI	4-NO_2	2·37	2·27	1·17	−0·28
VII	2-NO_2	2·90	2·29	1·27	—
VIII	3-OH	2·28	2·32	1·48	−0·67
IX	4-CH_2CH_2Cl	3·58	3·17	2·44	0·82
X	4-N=N—C_6H_5	4·25	3·60	3·06	1·69
XI	4-OCF_3	3·68	3·14	2·42	1·04
XII	4-$NHCOCH_3$	1·83	1·73	1·07	−0·97
XIII	4-$COCH_2Cl$	2·15	2·20	1·29	—

TABLE II

Dependences of the apparent partition coefficients on the π constants of the substituents (A) and on solubility *S* (B) in the form $\log P = a\pi + b$ and $\log P = a \log S + b$, respectively, determined by linear regression analysis. *n* number of compounds, *r* correlation coefficient, *s* standard deviation, F the F-test

Dependence	pH	*a*	*b*	*n*	*r*	*s*	F
A	3·0	0·923	2·716	11	0·994	0·090	330·3
A	5·0	0·671	2·502	11	0·981	0·117	102·3
A	7·2	0·748	1·715	11	0·968	0·170	59·5
B[a]	3·0	−1·066	−1·507	11	0·892	0·281	15·6
B[a]	5·0	−0·774	−0·508	11	0·912	0·183	19·8
B[a]	7·2	−0·902	−1·239	12	0·947	0·208	38·1

[a] The derivative *XII* (R = 4-$NHCOCH_3$) was excluded from the correlation.

pH 3·0 express the partition of the neutral form of the phenylhydrazonopropanedinitriles (the slope $a \sim 1$, and the value b approaches the log P_{app} value of the parent derivative).

Hydrophilicity of a compound can be expressed by its solubility in water. Table III gives the solubility values of the phenylhydrazonopropanedinitriles studied (determined in the Mc Ilvain buffer solutions of pH 3·0, 5·0, 7·2 at 25°C). The solubility increases with increasing pH value, which is due to better solvation of the ionized form of the phenylhydrazonopropanedinitriles. On the basis of thermodynamical considerations[25,26] a linear dependence with a slope close to -1 can be expected between log P and log S. The corresponding equations summarized along with the statistical parameters in Table II (dependences B) illustrate a good accordance of our data with theory. The derivative *XII* with the 4-$NHCOCH_3$ substituent deviates markedly from the regression straight line log P *vs* log S (it is not involved in the regression analysis), whereas the same derivative fulfils well the log P *vs* π correlation.

Table IV gives solubilities of the parent derivative in various organic solvents and complements thus the picture of lipophilic–hydrophilic properties of the substances investigated. The solubilities in the given series of solvents increase with increasing solvent polarity.

Table V summarizes the results characterizing the distribution of the phenylhydrazonopropanedinitriles between the incubation medium and the biological systems studied (the mitochondria isolated from rat liver, the bacteria *M. phlei*, and the yeasts *S. cerevisiae*) after 10 min.

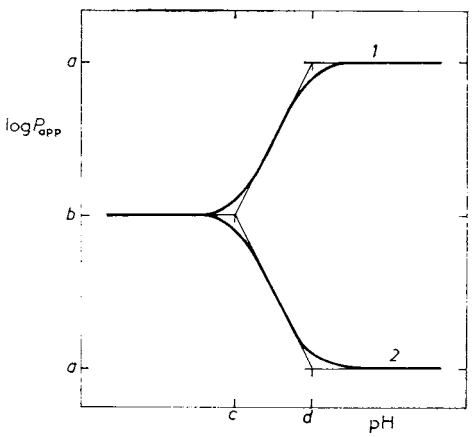


FIG. 1

Schematic representation of pH dependence of the apparent partition coefficient (Eq. (*11*)) for $A < B$ (1) and for $A > B$ (2). $a = \log . (P_{CH}B/A)$, $b = \log P_{CH}$, $c = -\log X$, $d = -\log Y$; X is the smaller and Y is the greater of the constants A, B

Fig. 2 presents the dependence of logarithms of the retained portions (pH 7·2) of the individual derivatives on their reactivities characterized by the logarithm of the rate constant of reaction of the compounds studied with cysteine. This thiol imitates nucleophilic groups of biological systems with which phenylhydrazonopropanedinitriles can react[8]. From Fig. 2 it is obvious that the retention is independent of the chemical reactivity of the compounds investigated, hence no covalent bond is involved between the compounds and components of the biological systems.

TABLE III

Solubilities (log S) in mol l^{-1} of the R-phenylhydrazonopropanedinitriles in buffer solutions of different pH values at 25°C

R	pH 3·0	pH 5·0	pH 7·2
H	−3·59	−3·55	−3·07
4-CH_3	−4·28	−4·38	−3·58
4-Cl	−4·36	−4·49	−3·65
3-Cl	−4·35	−4·42	−3·56
4-$COCH_3$	−3·60	−3·55	−2·71
4-NO_2	−3·96	−3·88	−2·74
2-NO_2	−3·93	−3·84	−3·11
3-OH	−3·47	−3·28	−2·95
4-CH_2CH_2Cl	−4·88	−4·70	−4·08
4-N=N—C_6H_5	—	—	−4·92
4-OCF_3	−4·95	−4·61	−4·05
4-$NHCOCH_3$	−4·35	−4·24	−3·58
4-$COCH_2Cl$	−3·88	−3·65	−3·19

TABLE IV

Solubility (mol l^{-1}) of phenylhydrazonopropanedinitrile in organic solvents at 25°C

Solvent	Solubility	Solvent	Solubility
Heptane	$1·51 . 10^{-3}$	Methanol	$5·02 . 10^{-1}$
Hexane	$1·56 . 10^{-3}$	Ethanol	$3·22 . 10^{-1}$
Tetrachloromethane	$1·90 . 10^{-2}$	Acetone	$2·45 . 10^{0}$
Benzene	$4·89 . 10^{-1}$	Dimethylformamide	$2·78 . 10^{0}$
Diethyl ether	$3·30 . 10^{-1}$	Dimethyl sulphoxide	$1·01 . 10^{0}$

TABLE V

Distribution of R-phenylhydrazonopropanedinitriles between the medium and biosystem after 10 min at 25°C. $A = -\log(c/c_0)$, where c_0 and c mean the original concentration and that remaining in the medium, respectively; B = (log of retention in %)

R	Mitochondria		*M. phlei*				*S. cerevisiae*			
	pH 7·2		pH 3·0		pH 7·2		pH 3·0		pH 7·2	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
H	0·26	1·66	0·07	1·20	0·10	1·32	0·13	1·40	0·21	1·59
4-CH_3	0·35	1·74	0·13	1·40	0·20	1·56	0·20	1·57	0·40	1·78
4-Cl	0·45	1·81	0·15	1·46	0·27	1·67	0·24	1·63	0·49	1·83
3-Cl	0·56	1·86	0·15	1·46	0·28	1·68	0·21	1·58	0·45	1·81
4-$COCH_3$	0·17	1·51	0·05	1·00	0·07	1·20	0·09	1·28	0·11	1·34
4-NO_2	—	—	0·07	1·15	0·14	1·43	0·10	1·32	0·14	1·44
2-NO_2	0·23	1·62	0·11	1·36	0·13	1·40	0·26	1·66	0·19	1·55
3-OH	0·23	1·62	0·10	1·32	0·13	1·40	0·05	1·02	0·06	1·11
4-CH_2CH_2Cl	0·53	1·85	0·24	1·63	0·27	1·67	0·25	1·64	0·56	1·86
4-N=N—C_6H_5	1·35	1·98	0·83	1·93	0·62	1·88	0·69	1·90	0·83	1·93
4-OCF_3	0·65	1·89	0·23	1·61	0·23	1·68	0·28	1·68	0·53	1·85
4-$NHCOCH_3$	0·22	1·60	0·06	1·11	0·05	1·01	0·03	0·79	0·04	0·91
4-$COCH_2Cl$	—	—	—	—	—	—	—	—	0·18	1·54

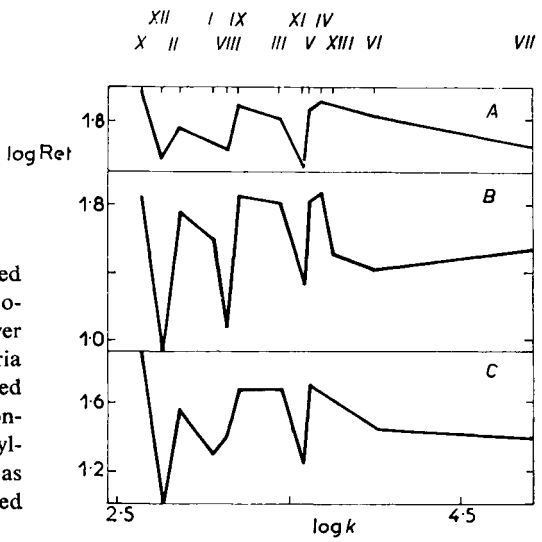


FIG. 2

Dependence of logarithm of the retained portion (log Ret) of phenylhydrazonopropanedinitriles by mitochondria from rat liver (*A*), by *S. cerevisiae* (*B*), and by bacteria *M. phlei* (*C*) on the reactivity expressed by logarihtms of the second order rate constants (log *k*) of the reactions of phenylhydrazonopropanedinitriles with cysteine as a model thiol. The derivatives are denoted as in Table I

The shape of the dependences indicates that there exists a similarity between the retentions of the phenylhydrazonopropanedinitriles by the individual biological objects. Therefrom it follows that the retention of the phenylhydrazonopropanedinitriles represents a biophysical process of distribution between lipidic and aqueous phases.

From the simulations of the transport kinetics in the model systems composed of alternating aqueous and lipidic phases[27,28] it follows that the dependence of the ratio c/c_0 of the actual to the original concentration in the extracellular phase on the partition coefficient P after a constant time of distribution can be described by Eq. (*12*)

$$\log (c/c_0) = \sum_{i=1}^{N} B_i \log (C_i' P_M + 1) + D , \qquad (12)$$

where C_i' and D are constants, B_i and N are integers, in a non-equilibrium process being always $B_i = \pm 1$ and $N = 2$; $N = 3$ to 5, if a part of the compounds is in lipophilic-hydrophilic equilibrium whereas the rest is not; and $N = 1$ in the case when all the compounds studied attained the equilibrium state within the time given in the experiment. If Eq. (*12*) is to be applied to real biological systems, then the partition coefficient P_M of the membrane–water system must be expressed by means of the partition coefficient P found for the model n-octanol–water system with application of the Collander equation[29] (*13*)

$$P_M = dP^e , \qquad (13)$$

where d and e are constants dependent on the solvent type. After introduction into Eq. (*12*) it is obtained

$$\log (c/c_0) = \sum_{i=1}^{N} B_i \log (C_i P^e + 1) + D . \qquad (14)$$

TABLE VI

Dependence of concentration (c) of phenylhydrazonopropanedinitriles remaining in the medium after 10 min incubation with biosystems according to Eq. (*14*), determined by non-linear regression analysis[22], c_0 $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}; the statistical parameters as in Table II

Biosystem	pH	D	C_i	*e*	*n*	*r*	*s*	*F*
Mitochondria	7·2	−0·179	$1{\cdot}063 \cdot 10^{-3}$	1·329	11	0·986	0·073	33·5
M. phlei	3·0	−0·032	$7{\cdot}395 \cdot 10^{-5}$	1·112	12	0·973	0·062	18·2
	7·2	0·043	$6{\cdot}448 \cdot 10^{-2}$	0·543	12	0·952	0·059	11·9
S. cerevisiae	3·0	−0·010	$4{\cdot}407 \cdot 10^{-3}$	0·660	12	0·942	0·073	8·8
	7·2	0·160	$1{\cdot}852 \cdot 10^{-1}$	0·548	13	0·973	0·067	23·9

Table VI presents a summary of the best descriptions of the experimental data by Eq. (*14*). It is seen that $N = 1$ in all the cases investigated. Introduction of further members into Eq. (*14*) did not improve the statistical parameters sufficiently significantly (the correlation coefficient and the F value increased by 1·1% at the most, and the standard deviation decreased by 2·4% in the best case, with regard to the values for $N = 1$ in both the cases). Therefrom it can be concluded that at the given conditions all the phenylhydrazonopropanedinitriles investigated attained the lipophilic-hydrophilic equilibrium in the biological systems studied. The e constant characterizes the membranes of the biological system with respect to n-octanol (Eq. (*13*)). Fig. 3 gives a comparison of the measured and calculated retention values of the compounds studied by the mitochondria from rat liver.

The retained portion of the compound (Ret) represents the ratio of the amounts of the compound in lipidic and aqueous phases of the biological system (n_L and n_A, respectively) and of the total amount (n) of the compound. For lipophilic compounds:

$$\text{Ret} = (n_L + n_A)/n \sim n_L/n \,. \qquad (15)$$

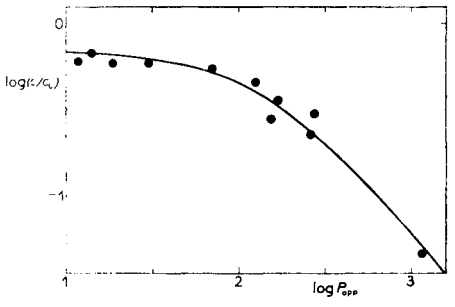


FIG. 3

Dependence of concentration (c) of the phenylhydrazonopropanedinitriles remaining in the medium after 10 min incubation with mitochondria of rat liver on the lipophilicity of the compounds studied (log P_{app}). c_0 the initial concentration of phenylhydrazonopropanedinitriles ($2{\cdot}5 \,.\, 10^{-5}$ mol l^{-1}). The line corresponds to the respective equation in Table VII

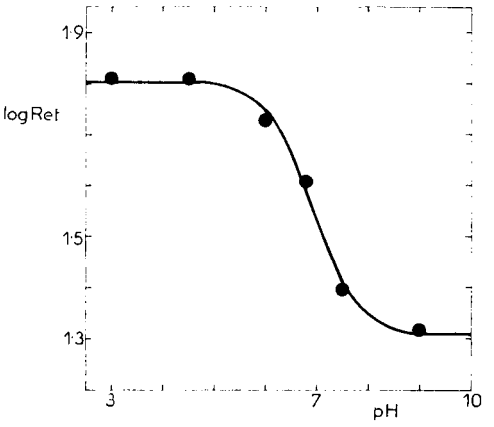


FIG. 4

The pH dependence of retention of 3-chlorophenylhydrazonopropanedinitrile by *S. cerevisiae* after 10 min incubation. The line corresponds to the respective equation in Table IX

The partition coefficient is the ratio of concentrations of the compound in the lipidic and aqueous phases (c_L and c_A, respectively).

$$P = c_L/c_A = n_L(V_A + V_E)/(n - n_L)\,V_L \sim n_L(V_A + V_E)/nV_L\,, \qquad (16)$$

where V_A and V_E are the volumes of aqueous phases of the biological system and of the extracellular aqueous phase, respectively, and V_L stands for the volume of the lipidic phases. The approximation given in Eq. (*16*) applies to the compounds of low lipo-

TABLE VII

The dependence of the retained portions (Ret) of phenylhydrazonopropanedinitriles after 30 min on the apparent partition coefficient P_{app} according to Eq. (*17*), determined by linear regression analysis. For the symbols of the statistical parameters see Table II

Biosystem	pH	*a*	*b*	*n*	*r*	*s*	F
Mitochondria	7·2	0·226	1·304	11	0·958	0·122	44·6
M. phlei	3·0	0·333	0·398	12	0·943	0·090	36·1
	7·2	0·343	0·850	12	0·897	0·113	18·5
S. cerevisiae	3·0	0·387	0·308	12	0·910	0·176	21·7
	7·2	0·409	0·833	13	0·826	0·184	10·7

TABLE VIII

Values of the R-phenylhydrazonopropanedinitrile portions retained by *S. cerevisiae* in media of different pH at 25°C after 10 min

R	log (retention in %)					
	pH 3·0	pH 4·5	pH 6·0	pH 6·8	pH 7·5	pH 9·0
H	1·60	1·54	1·40	1·30	1·26	1·15
4-CH_3	1·74	1·73	1·64	1·49	1·03	0·98
4-Cl	1·76	1·81	1·71	1·60	1·44	1·37
3-Cl	1·81	1·81	1·73	1·61	1·39	1·32
4-$COCH_3$	1·70	1·67	1·50	1·36	0·98	0·85
2-NO_2	1·72	1·72	1·56	1·37	1·11	1·01
4-CH_2CH_2Cl	1·71	1·73	1·64	1·54	1·33	1·30
4-N=N—C_6H_5	1·84	1·84	1·82	1·62	1·49	1·38

philicity and for diluted suspensions. Combination of Eqs (*13*), (*15*), and (*16*) gives Eq. (*17*)

$$\log \text{Ret} = a \log P + b \,, \qquad (17)$$

whose validity is limited to the compounds having $\log P$ within a certain range and to dilute suspensions of microorganisms. Table VII presents the results of linear regression analysis of our data. It is seen that the said approximations are valid well in three out of five cases ($r > 0{\cdot}9$).

It can be stated that the retention of phenylhydrazonopropanedinitriles by biosystems of different structure depends on lipophilic-hydrophilic properties of the respective derivatives. The retention degree is decreasing with increasing pH value of medium. (Table VIII). Combination of Eqs (*11*) and (*17*) gives Eq. (*18*) whose validity has the same limitations as Eq. (*17*).

$$\log \text{Ret} = a \log (B\, 10^{\text{pH}} + 1) - \log (A\, 10^{\text{pH}} + 1) + b \qquad (18)$$

The constants a, b, A, B determined by non-linear regression analysis from the data of Table VIII are given in Table IX. Graphical comparison of the measured and calculated values is given in Fig. 4 for the 3-Cl derivative. It can be presumed that the dependence of the retention of phenylhydrazonopropanedinitriles by the other biosystems tested on the pH values of medium will be analogous to that of the yeasts.

TABLE IX

Dependence of the retained portions (Ret) of R-phenylhydrazonopropanedinitriles by a *S. cerevisiae* suspension (10 min) on the pH value of medium according to Eq. (*18*), determined by non-linear regression analysis[22]. For the symbols of the statistical parameters see Table II, $n = 6$

R	a	b	A	B	r	s	F
H	0·101	1·608	$1{\cdot}234 \cdot 10^{-4}$	$2{\cdot}832 \cdot 10^{-9}$	0·998	0·021	56·9
4-CH_3	0·377	1·755	$1{\cdot}870 \cdot 10^{-6}$	$1{\cdot}351 \cdot 10^{-8}$	0·971	0·186	3·0
4-Cl	0·391	1·782	$4{\cdot}698 \cdot 10^{-7}$	$3{\cdot}902 \cdot 10^{-8}$	0·995	0·039	16·8
3-Cl	1·620	1·805	$1{\cdot}737 \cdot 10^{-7}$	$8{\cdot}539 \cdot 10^{-8}$	0·997	0·036	29·1
4-$COCH_3$	1·050	1·668	$2{\cdot}939 \cdot 10^{-7}$	$4{\cdot}686 \cdot 10^{-8}$	0·992	0·099	10·5
2-NO_2	0·497	1·719	$9{\cdot}572 \cdot 10^{-7}$	$3{\cdot}330 \cdot 10^{-8}$	0·998	0·045	38·8
4-CH_2CH_2Cl	1·594	1·715	$1{\cdot}908 \cdot 10^{-7}$	$1{\cdot}021 \cdot 10^{-7}$	0·992	0·053	10·2
4-N=N—C_6H_5	0·910	1·851	$2{\cdot}302 \cdot 10^{-7}$	$7{\cdot}049 \cdot 10^{-8}$	0·996	0·041	19·5

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