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DETERMINATION OF PARTITION COEFFICIENTS OF PYRIDO[1,2-a]-PYRIMIDIN-4-ONE DERIVATIVES BY TRADITIONAL SHAKE, THIN-LAYER CHROMATOGRAPHIC AND GAS–LIQUID CHROMATOGRAPHIC METHODS

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SUMMARY

In the investigation of quantitative structure–activity relationships, one of the most important linear free-energy related parameters is the logarithmic value of water–1-octanol partition coefficients (log P) and π values derived from that.

Partition coefficients of eighteen pyrido[1,2-a]pyrimidin-4-one compounds were measured for the water–1-octanol solvent system by spectrophotometric methods. R_M values and corrected ΔI values of the compounds were also determined by thin-layer chromatographic and gas–liquid chromatographic methods, respectively. The excellent correlations found between these log P, R_M and corrected ΔI values prove that liquid–liquid partition data can be obtained not only by thin-layer chromatography or high-performance liquid chromatography but also by gas–liquid chromatography.

INTRODUCTION

In the study of quantitative structure–activity relationships log P values for the water–1-octanol system are used for the characterization of hydrophobicity[1,2]. This liquid–liquid system is accepted as the best for characterizing the distribution of drugs in living systems. 1-Octanol plays a specific role because it shows similarity to the

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lipoid phase of living beings. Many attempts have been made to find a substitute for the traditional shake method for the determination of water–1-octanol partition coefficients. As stated by Collander[3], a linear relationship exists between the logarithmic values of the partition coefficients measured in two different solvent systems, as shown by eqn. 1:

$$\log P_2 = a \log P_1 + b \tag{1}$$

where a and b are constants, and P_1 and P_2 relate to the partition coefficients in the two solvent systems.

Leo[4] showed the limitations of eqn. 1. Linearity between $\log P_1$ and $\log P_2$ values will exist if one of the following requirements is met: the primary solvation forces in the two solvent systems, or the solutes under consideration, are sufficiently similar. This latter similarity can be assumed in the case of a homologous series of solutes and possibly also in case of congeneric solutes.

Relationships were investigated among results obtained by thin-layer chromatography (TLC), gas–liquid chromatography (GLC) and shake methods. The close linear relationship gives information on the common character of the predominant solute–solvent interactions in the applied chromatographic system. The advantages of chromatographic methods are as follows: small amounts of substances are needed, impurities do not disturb the measurements, and there is no need to find proper analytical methods for measuring the concentrations in the two solvents. If the solubility is low in water or 1-octanol, chromatographic methods give more accurate results. Dearden and Tomlinson[5,6] found good correlation between pharmacological activity and R_M values measured on several stationary phases of congeneric compounds. In their opinion the dynamic partition coefficients obtained by chromatographic methods are more similar to *in vivo* distribution processes than a static equilibrium.

Pyrido[1,2-*a*]pyrimidin-4-one compounds synthesized by Mészáros and co-workers[7–10], which show biological activity in the central nervous system, were chosen as model compounds.

THEORETICAL AND EXPERIMENTAL

Determination of partition coefficients by shake method

The compounds were dissolved in water with Sörensen buffer (pH 5), and the appropriate amount of 1-octanol was added to the solutions so that the concentration in the water phase could be measured spectrophotometrically. After the mixture had been shaken for 30 min in a shaking machine, the two phases were separated in a centrifuge at 750 g for 5 min. Samples were taken by a syringe from the lower water phase and the concentrations were measured by using a Spektromom 195 spectrophotometer in the UV range. It seemed to be enough to determine only the decrease of concentration in the water phase. The partition coefficients were calculated from eqn. 2:

$$P = \frac{E_0 - E_1}{E_1} \cdot \frac{v_{\text{water}}}{v_{1-\text{octanol}}} \tag{2}$$

where E_0 is the measured absorbance in water before partitioning, E_1 is the measured absorbance in water after partitioning, and $v_{water}/v_{1-octanol}$ is the ratio of the water and octanol volumes.

The measurements were carried out at room temperature ($20 \pm 1°C$). Note that the P values obtained are only distribution coefficients, because these slightly basic compounds may accept protons at the given pH and the true partition coefficient relates only to the same molecular state.

Determination of partition data by TLC

The basic principle[6] of the determination of partition data by TLC is shown in eqn. 3:

$$R_M = \log \left(\frac{1}{R_F} - 1 \right) = \log P + \log V_s/V_m \tag{3}$$

where R_F is the measured retention factor, P is the partition coefficient, and V_s/V_m is the layer constant.

According to eqn. 3, R_M values are directly proportional to $\log P$ values. Boyce and Millborrow[11], Biagi et al.[12], Tomlinson[6] and others[13–22] have already applied reversed-phase TLC for the determination of R_M values. Paraffin oil or silicon oil as lyophilic stationary phases and acetone with buffer, dioxan or ethanol as hydrophilic mobile phases were used. Biagi et al.[12] used a mixture of several percent of acetone in water as a mobile phase to get R_F values between 0.2–0.8. A linear relationship has been found between the acetone concentration and R_M values. The R_{M_0} values at zero concentration of acetone can be calculated from the intercept of the given straight line. So results could be extrapolated to pure water. Waisser et al.[17] have criticized Biagi's method and they pointed out that non-extrapolated R_M values showed better correlation to the partition coefficients. The difference between R_M values of a substituted and a parent compound (ΔR_M) is equivalent to the Hansch hydrophobic substituent constant. Biagi et al.[12,19] and others[20,21] pointed out the relationship between not only $\log P$ and R_M but also π and ΔR_M values. In the case of acidic or basic compounds R_M values were corrected[22].

R_F and R_M values of pyrido[1,2-a]pyrimidine-4-one derivatives were measured as follows: Kieselgel 60 F_{254} (Merck, Darmstadt, G.F.R.) plates were used after keeping them at 160°C for 1 h. Compounds were dissolved in 0.5% concentration in methanol. Developments were carried out in a chromatography tank lined with filter paper containing 100 ml of mobile phase (methanol–water, 30:70) for a preliminary saturation of 1 h. The distance of development was 15 cm. Detection was at 254 nm.

Determination of liquid–liquid partition data by GLC

According to Kaliszan's recent review[23], GLC retention data are not related to the hydrophobic properties of the solute. During GLC separation, van der Waals and polar rather than hydrophobic interactions take place. The lack of correlation between GLC retention data and hydrophobic parameters has been shown by several authors[24,25].

On the contrary Sheehan and Langer[26], Conder et al.[27,28], and Boček[29] showed the theoretical possibility of using GLC to obtain liquid–liquid partition

data. In this paper a more general approach is shown and proved by measurements.

Gas chromatographic retention of a certain compound is in direct proportion to its partition coefficient between the carrier gas and the stationary phase. In order to obtain liquid–liquid partition coefficients, retention properties should be measured on two columns that differ only in the polarity of the stationary phase.

Special care has to be taken avoid adsorption. The Kováts index was used as a retention property. The gas–liquid partition coefficient, K, of a solute is related to the Kováts index as follows:

$$\log K = \frac{b\,I}{100} - zb + \log j\, t'_{R_z}\, v_0 - \log V_L \tag{4}$$

where I is the Kováts index, b is the slope of the line obtained by plotting the number of carbon atoms and $\log t'_{R_z}$ values, t'_{R_z} is the adjusted retention time of the n-paraffin having z carbon atoms, j is the gas compressibility correction factor, v_0 is the carrier gas flow-rate, and V_L is the stationary phase volume.

From eqn. 4 it can be seen that under given gas chromatographic conditions z, b, j, t'_{R_z}, v_0 and V_L can be regarded as constants, therefore eqn. 4 can be rewritten as follows:

$$\log K = \frac{b\,I}{100} + \text{constant} \tag{5}$$

The quotient of gas–liquid partition coefficients related to two stationary phases with different polarities can be considered as a liquid–liquid partition, and it may be expressed by a retention index according to eqn. 6:

$$\log P = \log \frac{K_1}{K_2} = \frac{b_1 I_1}{100} - \frac{b_2 I_2}{100} + \text{constant}_1 - \text{constant}_2 \tag{6}$$

Subscripts 1 and 2 relate to the two stationary phases. From eqn. 6 a corrected ΔI value is defined in the following way:
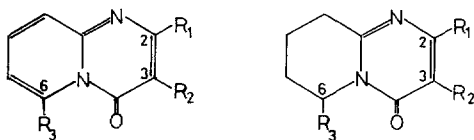
$$\text{corrected } \Delta I = \frac{b_1 I_1}{100} - \frac{b_2 I_2}{100} \tag{7}$$

These corrected ΔI values of the pyrido[1,2-a]pyrimidine-4-one derivatives were correlated to $\log P$ values.

Retention indices of the model compounds (Table I) were measured on four stationary phases (Carbowax 20M, OV-25, OV-17, OV-1) under the conditions shown in Table II. Index values have already been published[30]. Several corrected ΔI values can be obtained from the retention indices on the four stationary phases. The highest correlation between $\log P$ and corrected ΔI occurred when Carbowax 20M and OV-25 were considered; presumably these two supports have the right difference between their polarities.

TABLE I

MODEL COMPOUNDS INVESTIGATED

Compound No.	R_1	R_2	R_3
1a	H	H	H
1b	CH_3	H	H
1c	H	H	CH_3
1d	H	CH_3	H
1e	CH_3	H	CH_3
1f	H	CH_3	CH_3
1g	CH_3	H	C_2H_5
1h	C_2H_5	H	CH_3
1i	H	C_2H_5	CH_3
1j	CH_3	C_2H_5	CH_3
1k	CH_3	C_3H_7	H
1l	CH_3	C_3H_7	CH_3
1m	C_3H_7	C_2H_5	CH_3
2a	H	H	H
2c	H	H	CH_3
2e	CH_3	H	CH_3
2f	H	CH_3	CH_3
2j	CH_3	C_2H_5	CH_3

RESULTS AND DISCUSSION

Partition data obtained by the three methods are summarized in Table III. Relationships between chemical structure and log P or π values have been detailed elsewhere[30,31]. In this paper we would like to underline the relationship of the results

TABLE II

CONDITIONS OF GAS CHROMATOGRAPHIC PROCEDURE

Chromatograph: Hewlett-Packard 5710 A
Integrator: Chinoin Digint 21
Column: 6 ft. × 1/4 in. glass
Column packing: Chromosorb W DMCS
Stationary phases: 5% OV-17, 5% OV-1, 5% OV-25, 5% Carbowax 20M
Carrier gas: nitrogen
Carrier flow-rate 30 ml/min
Column temperature: 220°C
Detector temperature: 250°C
Injector temperature: 250°C
Applications of sample with Hamilton Microliter Syringes
Sample size: 1 μl
Solvent: chloroform
Attenuator: 128 × 10

TABLE III

PARTITION DATA BY THE SHAKE METHOD TLC AND GLC

	Shake method*		TLC*			GLC**				
	log P	S.D.	R_F	S.D.	R_M	$I_{CW\ 20M}$	S.D.	I_{OV-25}	S.D.	Corr. $\Delta I_{CW20M-OV-25}$
1a	0.204	±0.007	0.43	0.03	0.129	2645	3	2124	5	0.256
1b	0.572	0.009	0.34	0.03	0.281	2689	2	2189	2	0.219
1c	0.736	0.009	0.28	0.03	0.412	2622	2	2150	2	0.192
1d	0.771	0.012	0.31	0.02	0.346	2638	4	2161	2	0.177
1e	1.053	0.006	0.22	0.03	0.561	2663	1	2214	4	0.152
1f	1.265	0.029	0.18	0.03	0.666	2622	2	2198	10	0.125
1g	1.625	0.010	0.12	0.02	0.861	2656	2	2245	1	0.101
1h	1.592	0.006	0.13	0.03	0.837	2718	1	2292	4	0.110
1i	1.793	0.009	0.11	0.02	0.918	2659	1	2255	4	0.090
1j	1.856	0.012	0.09	0.02	1.026	2673	1	2297	4	0.040
1k	1.752	0.009	0.08	0.02	1.054	2769	2	2358	1	0.080
1l	2.221	0.012	0.04	0.01	1.443	2736	4	2374	5	0.017
1m	2.375	0.017	0.02	0.01	1.633	2732	1	2405	5	−0.031
2a	−0.144	0.015	0.47	0.03	0.050	2580	1	2128	2	0.171
2c	0.299	0.007	0.38	0.03	0.222	2488	2	2081	3	0.125
2e	0.668	0.001	0.32	0.03	0.336	2545	2	2159	1	0.085
2f	0.823	0.010	0.26	0.02	0.465	2483	3	2121	1	0.063
2j	1.428	0.008	0.14	0.02	0.793	2540	1	2241	2	−0.036

* $n = 20$.
** $n = 6$.

TABLE IV

COMPARISON OF MEASURED AND PREDICTED log P VALUES

|	log $P_{oct/water}$	Predicted log P				
		By TLC		By GLC		
	Dependent value	Predicted value	Residual	Predicted value	Residual	
1a	0.204	0.306	−0.102	0.290	−0.086	
1b	0.572	0.547	0.025	0.585	−0.013	
1c	0.736	0.754	−0.018	0.799	−0.063	
1d	0.771	0.650	0.121	0.759	0.012	
1e	1.053	0.990	0.063	1.113	−0.060	
1f	1.265	1.157	0.108	1.129	−0.065	
1g	1.625	1.465	0.160	1.518	0.107	
1h	1.592	1.427	0.164	1.449	0.144	
1i	1.793	1.556	0.237	1.607	0.186	
1j	1.856	1.727	0.130	1.933	−0.077	
1k	1.752	1.771	−0.019	1.683	0.069	
1l	2.221	2.386	−0.166	2.182	0.037	
1m	2.378	2.688	−0.313	2.567	−0.192	
2a	−0.144	0.181	−0.325	−0.056	−0.088	
2c	0.299	0.453	−0.155	0.290	0.009	
2e	0.668	0.634	0.035	0.588	0.081	
2f	0.823	0.838	−0.016	0.753	0.069	
2j	1.428	1.358	0.071	1.500	−0.072	
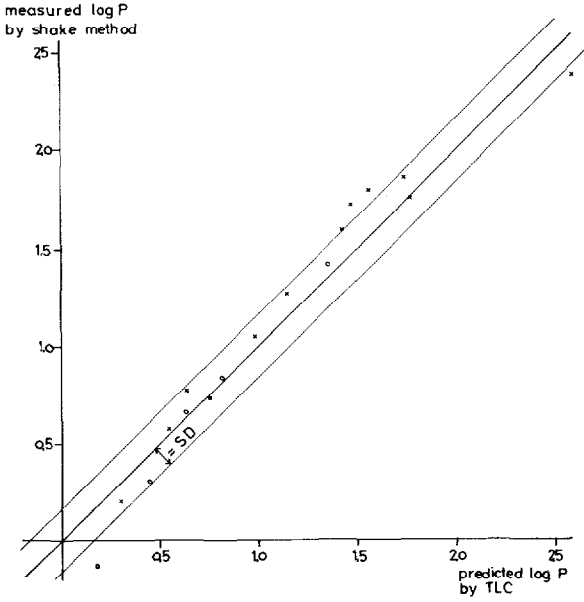
Fig. 1. The relationship of log *P* values measured by the shake method and predicted by TLC. O, Saturated compounds; ×, unsaturated compounds.
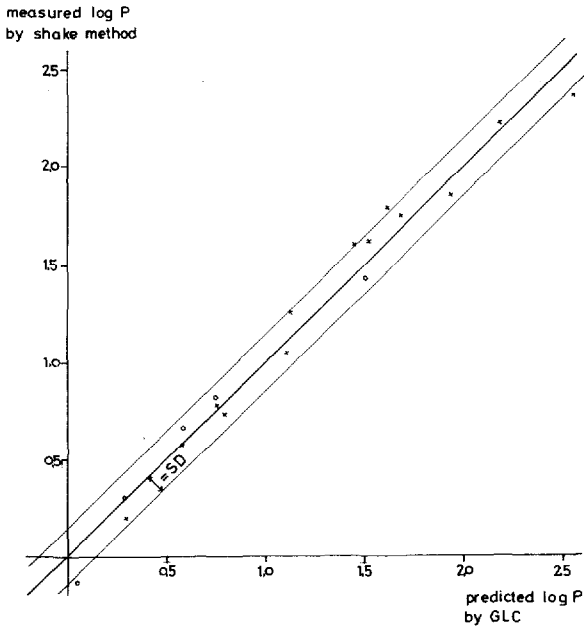

Fig. 2. The relationship of log *P* values measured by the shake method and predicted by GLC. O, Saturated compounds; ×, unsaturated compounds.

TABLE V

DATA OF CORRELATION AND THEIR STATISTICS

$y = ax + b$

Compound	y	x	a	b	r
	log P	R_M	1.452	0.235	0.977
1a–1m	log P	corr. ΔI	–7.917	2.321	0.987
	R_M	corr. ΔI	–5.334	1.422	0.988
	log P	R_M	2.079	0.161	0.990
2a–2j	log P	corr. ΔI	–7.501	1.230	0.991
	R_M	corr. ΔI	–3.595	0.668	0.998
	log P	R_M	1.584	0.102	0.975
1a–2j	log P	corr. ΔI	–6.523	1.874	0.737
	R_M	corr. ΔI	–4.202	1.128	0.771

from TLC and GLC. The values of log P measured by the shake method and those predicted by TLC and GLC are shown in Table IV and Figs. 1 and 2. Correlation data calculated by a least squares method are shown in Table V. In the first part of the table the three r values are not so good where corrected ΔI values were correlated with R_M or log P values; however, R_M and log P show good correlation. Because the limitations of eqn. 1, saturated and unsaturated compounds were considered separately and in such cases the r values became much greater and more significant for all of the compounds. At the same time, the correlation of log P with R_M did not change significantly. This is understandable because the solvent system represented by the two gas chromatographic stationary phases (Carbowax 20M and OV-25) essentially differs from the water–octanol system.

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