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Determination of octanol/water partition coefficients for long-chain homologs of orcinol from cereal grains*

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Polycratic reversed-phase high-performance liquid chromatography (RPHPLC) was used for estimation of the octanol/water partition coefficient for three highly hydrophobic long chain orcinol homologs.

The homologs studied (C 15:0, C 17:0 and C 19:0) showed high preference for hydrophobic phase as evidenced by their high octanol/water partition coefficient (log $P_{o/w}$) values of 7.02–7.74; 8.71–9.47 and 10.49–11.32 for the 95% prediction interval, respectively. Experimentally estimated values were compared with log P values calculated with the use of several fragmental systems. The experimental values of log $P_{o/w}$ are in best agreement to those calculated with the use of the Klopman system (Klopman, G., Namboodiri, K. & Schochet, M., 1985, *J. Comput. Chem.* 6, 28–38). The lack of appropriate standard compounds with known log $P_{o/w}$ in the range over 6 markedly affected the accuracy of experimental determinations.

An important problem in studying biological activities of compounds is the often-found relationship between biological properties of molecules and their hydrophobic properties characterised by relative solubilities in aqueous and nonaqueous phases (the partition coefficient, P) [1, 2]. Most of biologically active compounds that act on cells have to pass the barrier of their plasma membrane and/or cellular membrane systems. The importance of log $P_{o/w}$ (partition coefficient in octanol/water) and its close relationship to the transport properties of drugs and their interaction with receptors was demonstrated by Hansch & Fujita [3]. Thus there is great interest in estimation of partition coefficients of molecules, usually expressed as $\log_{10} P$.

Resorcinolic lipids, long chain homologs of orcinol, exhibit a marked biological effect which is strongly dependent on the preferential interaction of their molecules with cellular membranes, but also with proteins and nucleic acids (e.g [4–18]). Unlike other widely known sources of resorcinolic lipids (e.g. cashew, ginkgo, mango) cereal grains contain a broad spectrum of homologs that vary both in side chain unsaturation and length. The presence of C13–C27 chains in grain has been demonstrated [8, 19, 20]. There is only few data concerning partition coefficients of compounds similar in nature [4]. Therefore estimation of log P for various homologs of cereal grain resorcinolic lipids became important in our studies concerning their biological activity.

In the past the traditional shake-flask method for determining log $P_{o/w}$ was most frequently used. However, this method is tedious and has very limited application to compounds of very

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[1] Abbreviations: AR, alkylresorcinol(s); RPHPLC, reversed-phase high-performance liquid chromatography; RPLC, reversed-phase liquid chromatography.

high hydrophobicity. Most of the available data on partition coefficients concern compounds of moderate hydrophobicity [2, 21]. For determination of log P, reversed-phase liquid chromatography (RPLC) has been widely applied in recent years as a rapid and precise method (e.g. [22–24]). Many of early RPLC methods used capacity ratios measured by monocratic methods to estimate log $P_{o/w}$. According to these methods the capacity ratios (denoted k_f, where f = volume fraction of water in the eluent) are determined at a single eluent composition for all the compounds in a series. Log k'_f values are then correlated with log $P_{o/w}$ [23]:

$$\log P_{o/w} = a \log k'_f + b \quad (1)$$

However, an increasing number of papers report the use of retention data measured by polycratic methods to derive parameters that are correlated with log $P_{o/w}$. In these methods, capacity ratios are measured for each compound at a number of different eluent compositions and regression analysis of the log k'_f (f) data is used to obtain the intercept (log k'_w, i.e., the extrapolated values of log k_f for 100% water as the eluent). This variable is then correlated with log $P_{o/w}$ by using the equation (1) [23, 25, 26].

In this paper we present values of log $P_{o/w}$ for three resorcinolic lipid homologs estimated by polycratic RPLC method, and their comparison with values calculated with the use of several computing approaches.

MATERIALS AND METHOD

Resorcinolic lipid homologs, 5-n-pentadecylresorcinol (C 15 : 0 AR), 5-n-heptadecylresorcinol (C 17:0 AR), 5-n-nonadecylresorcinol (C 19:0 AR), 5-n-heneicosylresorcinol (C 21:0 AR) and 5-n-tricosylresorcinol (C 23:0 AR) were isolated from rye grains by chromatography according to the previously published procedure [8].

Chromatography. A LDC/Milton Roy chromatographic system equipped with ConstaMetric III pumps, Rheodyne 7125 injection system with 20 µl sample loop and differential refractometer (KNAUER) was used. The output of detector was connected through A/D converter (digital multimeter Metex M-3850) to personal computer (386DX) running home-written software to acquire and store data. Separations were performed at room temperature (22°C) on octadecylsilane silica (7 µm) column 4.6 mm × 150 mm (LMIM, Hungary) using methanol-water mixtures as mobile phase at 1.5 ml/min. All mobile phases were filtered through 0.45 µm filters before use and deaerated with the use of Sartorius filtration-degassing set-up. The column inlet was protected with in-line 0.45 µm filter. The column dead time used to calculate the capacity ratios was determined for each mobile phase using $NaNO_3$. Changes in volume fraction of water in mobile phase were accomplished by controlling the pumps with LDC Gradient Master set on isocratic mode. All runs were repeated at least 7 times. Regression analyses were performed by using statistical analysis.

Compounds with know values of log $P_{o/w}$ [2, 21]: thymol, naphtalene, anthracene and dodecanol were of the highest purity available from Sigma-Aldrich (U.S.A.).

RESULTS AND DISCUSSION

Separation of resorcinolic lipids by RPLC encounters serious problems due to their high hydrophobicity. At water content in mobile phase in the range of several percent the increase of retention time is logarithmically proportional to the chain length. Although initially it was assumed that all five homologs would be analysed, the results obtained for mobile phases containing as much as 10% water showed that the retention times of C 21:0 and C 23:0 homologs were excessively long (over 80 min) and they were beyond practical detection due to peak diffusion. Other homologs also showed very high values of retention time with a further, even small, increase of volume fraction of water in the mobile phase. These properties of the compounds studied had two significant implications in further experiments. First, they limited the water volume fraction to only 0.2 and second, the number of the homologs studied had to be limited to only three, those with the shortest chains. For predicting the log k'_w (capacity factor in pure water as mobile phase) linear regression analysis of log k_f (f) to obtain values of the slope and intercept describing each log k_f (f) curve, was applied. The linearity of this relationship for resorcinolic

Table 1

Parameters describing the relationship between log k′ and volume fraction of water in mobile phase for standard compounds and resorcinolic lipids homologs obtained by linear regression analysis of experimental data

	Regression parameters		r^2*	n**	Standard error of estimate
	a	b			
Thymol	−0.632	0.034	0.973	9	0.071
Naphtalene	−0.390	0.033	0.982	9	0.063
Anthracene	−0.288	0.046	0.996	8	0.040
Dodecanol	−0.159	0.047	0.989	7	0.057
C 15:0 AR	0.043	0.069	0.994	11	0.071
C 17:0 AR	0.124	0.085	0.993	9	0.060
C 19:0 AR	0.217	0.102	0.992	8	0.060

* Correlation coefficient; ** number of experiments

lipids over the whole range of the water volume fraction had to be assumed. However, this assumption might not be right in the case of all compounds [23, 25]. The values describing the relationship between log k' and the volume fraction of water for the homologs studied and standard compounds are presented in Table 1. The obtained values of log k'_W (Table 2) were then correlated with log $P_{o/w}$ data for standard compounds (Fig. 1). The differences in the values of $P_{o/w}$ for standard compounds found in literature [2, 21] considerably affected the values determined for the resorcinolic lipids

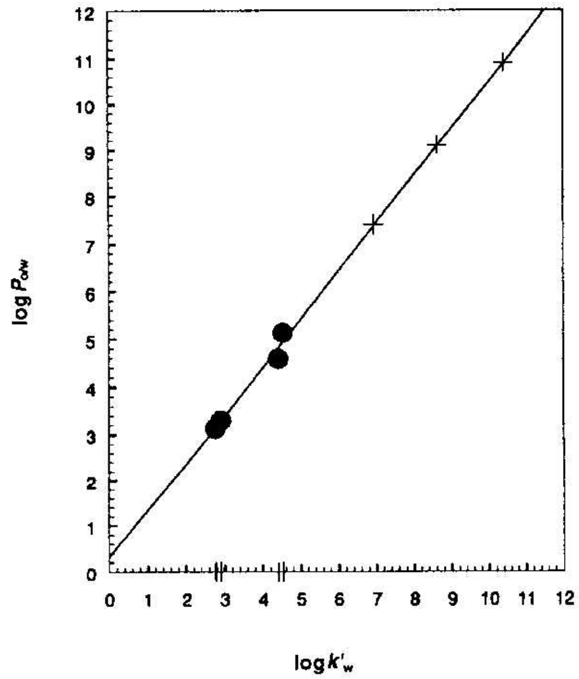

Fig. 1. Correlation of log k'_W for the studied homologs of resorcinolic lipids and literature log $P_{o/w}$ data [2, 21] for standard compounds.

log $P_{o/w}$ = 1.01875 log k'_w (±0.0181) + 0.31355 (±0.1161); r^2 = 0.9984, standard error of fit = 0.1301; ●, standards, +, resorcinolic lipids.

Table 2

Values of log k'_W (capacity factor in mobile phase containing only water) obtained by regression analysis of data from Table 1, and their correlation with log $P_{o/w}$

	log k'_W	log $P_{o/w}$*
Thymol	2.768 (0.071)	2.8–3.3
Naphtalene	2.910 (0.063)	3.31–3.54
Anthracene	4.312 (0.040)	4.45–4.59
Dodecanol	4.541 (0.057)	5.13
C 15:0 AR	6.943 (0.071)	
C 17:0 AR	8.620 (0.060)	
C 19:0 AR	10.401 (0.060)	

* Values taken from literature [2, 21]

Table 3

Experimentally estimated log $P_{o/w}$ values for resorcinolic lipids at 95% prediction interval

Homolog	log $P_{o/w\,(exp)}$ (95% prediction interval)
C 15:0 AR	7.02–7.74
C 17:0 AR	8.71–9.47
C 19:0 AR	10.49–11.32

Table 4

Comparison of log $P_{o/w}$ for resorcinolic lipid homologs calculated by various methods and obtained experimentally by the RPHPLC method

Homolog	Hansch & Leo [2]	Rekker & de Kort [27]	Klopman *et al.* [28]	Rekker & de Kort modif.*	Experimental**
C 15:0	9.0	8.8	7.6	8.6	7.4
C 17:0	10.3	9.8	8.6	9.6	9.2
C 19:0	11.6	10.9	9.5	10.6	10.9

*Calculation based on literature data for log $P_{o/w}$ of resorcinolic fragment; ** mean values taken from Table 3

studied. The values of log $P_{o/w}$ estimated for C 15:0, C 17:0 and C 19:0 alkylresorcinols within 95% confidence interval are shown in Table 3. One can easily find that for hydrophobic compounds, especially for C 19:0 homolog, the accuracy of the true value of log $P_{o/w}$ is within 1 log unit. Taking into account the differences in log $P_{o/w}$ between successive homologs (average 1.762 per C_2H_4 unit) it can be also expected that higher homologs may show the values of log $P_{o/w}$ as high as 16 (for C 25:0 AR).

The above mentioned values per one CH_2 unit are by over 30% higher than calculated by Hansch & Leo [2] according to their fragmental system for computational prediction of log $P_{o/w}$, over 65% higher than reported by Rekker & de Kort [27] but approximately in the same range (about 13%–11% higher) than stated in the work of Klopman *et al.* [28]. For comparison, the values of log $P_{o/w}$ for the resorcinolic lipids studied, calculated according to these systems, are shown in Table 4. The values obtained by the method of Klopman *et al.* [28] fall well into the 95% prediction interval of the experimentally estimated values (cf. Table 3). The procedure of Rekker & de Kort, when applied only partially (log $P_{o/w}$ for resorcinol was taken from literature data) for calculating the effect of aliphatic chain, showed even better agreement with experimental values (see also Table 5).

Table 5

Comparison of the reliabilities of four methods

Method	Standard error*	Mean $\|\Delta\|$**	Max $\|\Delta\|$
Hansch-Leo [2]	1.24	1.19	1.60
Rekker [27]	0.92	0.73	1.41
Klopman [28]	0.86	0.71	1.37
Rekker modif.	0.75	0.64	1.16

* $\sqrt{\sum \Delta^2/n}$; ** $\Delta = \log P_{(obs)} - \log P_{(calc)}$

The results obtained point to difficulties in application of both experimental and computational methods for estimation of partition coefficients of highly hydrophobic compounds. Itokawa *et al.* [4] estimated experimentally log $P_{o/w}$ for various mono- and dihydroxy pentadecylbenzenes, showing a marked effect of position of –OH substituents upon the hydrophobicity of these molecules. Our data for 1,3-dihydroxy-5-n-pentadecylbenzene (not studied previously) suggests that all-*meta* substitution creates the least hydrophobic molecules. However, further studies are necessary to evaluate the biological relevance of this fact.

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