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A New R_L Constant for the Calculation of $\log P$ and π Values in Congeneric Compounds

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Retention volumes of iodinated diethylstilbestrols and estradiols measured by high-pressure liquid chromatography on a silica column were found to be linearly related to their experimental partition coefficients ($\log P$). The $\log P$ values calculated from the new R_L parameter correlate well with experimental values and with those calculated by Hansch π -constants. The magnitudes of π -values for iodine in monosubstituted estradiols are discussed in terms of the contribution of steric and »proximity« effects to these constants. The reported technique may be a significant addition to the methodology of obtaining lipophylic-hydrophylic constants in structure-activity relationship studies.

The lipophylic nature of molecules seems to play a major role in determining the interaction of drugs with biological systems¹. Hansch and Fujita developed a rational quantitative approach to structure-activity correlations² by using multiple regression analysis and free-energy related parameters. The recent conclusion of Leo and Hansch³ that the »highest level of confidence can be placed in calculated $\log P$ values when (1) the $\log P$ of a parent solute is known, (2) π -constant of the required substituent(s) is available, and (3) the substituents either do not have any effect on groups already present in the parent or else this effect has been previously determined« is based upon research over last ten years and represents the keystone for further investigations. The common approach for the assesment of lipophylic character was so far either the classical method² of measuring partition coefficients or by using thin layer chromatography⁴. Recently, partition high-pressure liquid chromatography (HPLC) has also been used for the determination of $\log P$ values⁵. In this paper we present the determination of partition coefficients of diethylstilbestrol (1), estradiol (2) and their iodo derivatives, compounds of significant biological and clinical importance⁶. During investigations of iodinated estrogens⁷ we observed an apparent correlation between retention volumes of substrates

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examined on the absorption high pressure liquid chromatography column and their partition coefficients. Substrates partitioning on a silica column can be, to a certain extent, correlated with their distribution on a thin layer chromatogram. A known R_M parameter derived from tlc data is used widely in quantitative structure activity relationship⁸. A similar approach was applied in calculations of $\log P$ values using retention times and volumes from a reverse phase HPLC technique^{9a,b,c}. Relative polarities of congeneric compounds can be directly related to their R_f values in a given solvent; such a definite relationship is less obvious when based on retention volumes on a silica HPLC column. The reasonable approach to this problem, within a series of substituted phenolic compounds, is to assume that the largest retention volume of the parent solute has the physical meaning analogous with this of the starting point on a thin layer chromatogram. The magnitude of the retention volume of some highly nonpolar material e.g. pentane simulates the solvent front value on tlc. On these bases the new physical constant R_L (being somewhat similar to a R_M value⁸) can be defined as

$$R_L = \log \left[\frac{(V_R^{PC} - V_R^p) - \frac{V_R^{PC} - V_R^C}{V_R^{PC} - V_R^p}}{\frac{V_R^{PC} - V_R^C}{V_R^{PC} - V_R^p}} \right] \quad (1)$$

where v_R is the retention volume using chloroform as a solvent; superscripts PC, C and p denote the parent compound, substituted compound and pentane respectively. We found that the relationship between $\log P$ in the octanol water system ($\log P_{o/w}$) and the R_L constant can be expressed as

$$\log P_{o/w} + 10 R_L = K_L \quad (2)$$

According to Hansch statement³, partition coefficients of iodo derivatives of estradiol and diethylstilbestrol in an octanol-water system can be derived from $\log P$ values of parent solutes (Table I and II) using the known equation (3)²

$$\log P_x = \log P_H + \pi_x \quad (3)$$

where P_H is the partition coefficient for a parent solute and P_x is that for a derivative.

Partition coefficient for diethylstilbestrol is known¹⁰ ($\log P_{o/w} = 5.07$), so that $\log P_{o/w}$ values for the monoiodo diethylstilbestrol (3), diiodo diethylstilbestrol (4) and triiodo diethylstilbestrol (5) are calculated using equation 3. The application of equation (2) gives fairly consistent K_L values for compounds 3, 4, and 5. The magnitudes of $\log P_{o/w}$ parameters calculated for these structures

TABLE I

Calculation of $\log P_{o/w}$ Values for Iodinated Diethylstilbestrols and Iodinated Estradiols from Adsorption HPLC Data^a

Compound	v_R /ml	R_L ^b	K_L ^c	exper. $\log P_{o/w}$	calc. $\log P_{o/w}$	π_I
Diethylstilbestrol (1)	10.59			5.07	5.07	
I ₁ -diethylstilbestrol (3)	6.26	1.0850	17.11	6.26	6.33	1.26
I ₂ -diethylstilbestrol (4)	5.09	0.9715	17.17	7.45	7.46	1.13
I ₃ -diethylstilbestrol (5)	3.71	0.8620	17.26	8.64	8.56	1.10
Estradiol (2)	13.59			3.64	3.64	
4-I ₁ -estradiol (6)	7.64	1.2510	16.73	4.22	4.28	0.64
2-I ₁ -estradiol (7)	6.72	1.1830	16.80	4.97	4.96	1.32
2,4-I ₂ -estradiol (8)	2.90	1.1290	16.84	5.55	5.50	

^a experimental conditions: silica gel μ -Porasil column, solvent chloroform, actual pressure 1500 p.s.i. (1 p.s.i. = 6673.03 Pa) flow rate 1 ml/min; ^b calculated from eq 1 using v_R for pentane 3.04 ml; ^c K_L average for compounds 3-5 is 17.18 and for compounds 6-8 is 16.59.

TABLE II

Calculation of $\log P_{chl/w}$ Values for Iodinated Diethylstilbestrols and Iodinated Estradiols from Adsorption HPLC Data^a

Compound	v_R ' ^b	R_L ' ^c	exper. $\log P_{chl/w}$	calc. $\log P_{chl/w}$
Diethylstilbestrol (1)	5.295		4.25	4.24
I ₁ -diethylstilbestrol (3)	3.130	0.7467	5.51	5.55
I ₂ -diethylstilbestrol (4)	2.545	0.6214	6.83	6.85
I ₃ -diethylstilbestrol (5)	1.855	0.4973	8.15	8.16
Estradiol (2)	6.793		2.68	2.66
4-I ₁ -estradiol (6)	3.816	0.920	3.17	3.30
2-I ₁ -estradiol (7)	3.358	0.850	4.18	4.12
2,4-I ₂ -estradiol (8)	2.951	0.790	4.67	4.76

^a experimental conditions: silica gel μ -Porasil column, solvent chloroform, working pressure 1500 p.s.i. (1 p.s.i. = 6673.03 Pa) flow rate 1 ml/min; ^b v_R ' = 0.5 v_R in ml; ^c K_L average for compounds 3-5 is 0.8209.

using equation (2) are in a good agreement with those constants obtained applying equation (3) (Table I), thus demonstrating the validity of the proposed method.

Calculations and analysis of $\log P_{o/w}$ values for 4-iodoestradiol (6), 2-iodoestradiol (7), 2,4-diiodo estradiol (8) and estradiol itself (2) are more complicated. Retention volumes of monoiodinated estradiols 6 and 7, were experimentally found to be different⁷. This observation indicates either an inconsistency in the magnitude of π_I value in the examined set of congeneric structures or a non-validity of equations (1) and (2) for the family of iodinated estradiols. Consequently, the experimental determination of $\log P$ values for compounds 2, 6, and 7 was necessary to verify calculations of partition coefficients using the R_L parameter. Substrates concentration of these compounds in chloroform-water partitioning were quantitatively determined by means of highly sensitive and

reproducible HPLC technique. The detailed procedure used in these measurements is given in the Experimental section. From experimental $\log P_{\text{chl/w}}$ for estradiol (2), 4-iodo estradiol (6) and 2-iodo estradiol (7), their $\log P_{\text{o/w}}$ values are obtained by an application of the previously reported equation (4) which refers to acidic compounds^{12,13}:

$$\log P_{\text{chl/w}} = 1.1 \log P_{\text{o/w}} - 1.34 \quad (4)$$

The agreement between experimental partition coefficients and these derived by the application of the equation 2 is evident from data tabulated in Table I. Furthermore, these results indicate clearly on differences in π values for 4-iodo estradiol (6) and 2-iodo estradiol (7), making simple calculation of $\log P$ values for 6 and 7 by application of equation (3) highly inaccurate.

The $\log P$ values in the chloroform-water partitioning were found to be related to the logarithmic R_L' parameter. This constant (eq. 5) is defined to refer

$$R_L' = \log \left[\frac{V_R'^{\text{PC}} - V_R'^{\text{P}} - \frac{V_R'^{\text{PC}} - V_R'^{\text{C}}}{V_R'^{\text{PC}} - V_R'^{\text{P}}}}{\frac{V_R'^{\text{PC}} - V_R'^{\text{C}}}{V_R'^{\text{PC}} - V_R'^{\text{P}}}} \right] \quad (5)$$

to v_R' values (one half of the retention volume on silica HPLC column using chloroform as a solvent) measured on the parent solute (PC), substituted compound (C) and pentane (p), respectively. It was possible to show that using R_L' parameter the relationship between $\log P$ values in two different solvents (octanol and chloroform) are related in a following way (eq 6):

$$\log P_{\text{chl/w}} = \log P_{\text{o/w}} - R_L' \quad (6)$$

Partition coefficients for monoiodo diethylstilbestrol (3), diiodo diethylstilbestrol (4) and triiodo diethylstilbestrol (5), as well as these for 4-iodo estradiol (6), 2-iodo estradiol (7) and 2,4-diiodo estradiol (8) are calculated using equation 6. The numerical values of these constants are given in Table II. They correlate well with $\log P_{\text{chl/w}}$ for iodinated diethylstilbestrols (3–5) obtained by the application of equation 4 as well as with experimental $\log P_{\text{o/w}}$ for iodinated estradiols (6–8).

The $\log P$ value for diiodo estradiol (8) was not experimentally determined¹⁴ but was obtained by calculations (eq 6 and 5). On the basis of the additivity principle of π values in polysubstituted compounds^{2,15}, partition coefficient for estradiol was back calculated ($\log P_{\text{chl/w}}$ for estradiol = 2.664) from $\log P_{\text{chl/w}}$ for 4-iodo estradiol (6), 2-iodo estradiol (7) and 2,4-diiodo estradiol (8) (eq 3). It

was found to be in a good agreement with the experimental value ($\log P_{\text{chl/w}}$ for estradiol = 2.68). Moreover, the $\log P_{\text{o/w}}$ for estradiol was calculated by summing new Hansch fragment constants³ as shown in eq 7:

$$\log P = \sum_1^h a_n f_n \quad (7)$$

The resulted value ($\log P_{\text{o/w}}$ for estradiol = 3.69¹⁶) was very close to this derived from eq (5) using either experimental or calculated partition coefficients in chloroform-water system ($\log P_{\text{o/w}}$ for estradiol = 3.64). These calculations provide a strong evidence that the presented calculative approach for $\log P$ values holds firmly not only in the case of 2,4-diiodo estradiol but for the family of iodinated estradiols.

The most important practical implication of the method reported here is that for a congeneric structure for which a partition value has not been or perhaps cannot be determined, a given retention volume in chloroform may serve for a computation of the $\log P$ value. The equation 8 derived from equations (2) and (6)

$$\log P_{\text{chl/w}} = K_L - 10 R_L - R_L' \quad (8)$$

lends itself to the calculation of partition coefficients for compounds 1—8 identical to magnitudes of those parameters tabulated in Table II. It is clear that $\log P$ values of only two members of congeneric series are required to be known in order to calculate K_L values and partition parameters for the whole set of congeneric structures.

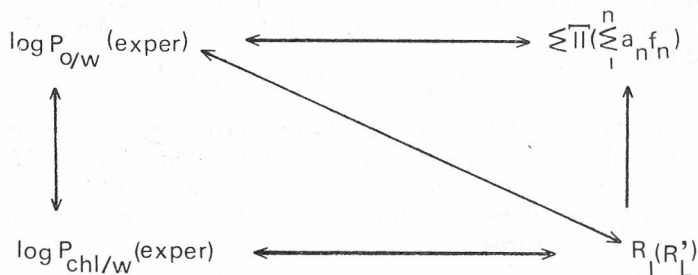
Despite slight discrepancies between the $\log P_{\text{chl/w}}$ determined experimentally and those calculated for 4-iodo estradiol (6) and 2-iodo estradiol (7), it is obvious that the π values for iodine atom in 4-iodo estradiol (6) and 2-iodo estradiol (7) differ markedly in regard to the position of substitution. The π value in 4-iodo estradiol (6) ($\pi = 0.65$) is smaller than the π value in 2-iodo estradiol (7) ($\pi = 1.45$) or in other *ortho* monoiodinated phenols ($\pi = 1.19$)^{2,15}, indicating necessarily the relative increase in hydrophobicity of 4-iodo estradiol (6) compared with 2-iodo estradiol (7). An examination of both Dreiding and space-filling molecular models of estradiol (2), 4-iodo estradiol (6) and 2-iodo-estradiol (7) shows not only more pronounced pseudoequatorial orientation of the C_5-C_6 bond in 4-iodo estradiol (6) but also definitely less eclipsing between the C_3-OH and C_4-I bonds in 4-iodo estradiol in comparison to the equivalent bonds in estradiol (2) and 2-iodo estradiol (7). Comparing uv spectral data⁷ from 4-iodo estradiol and 2-iodo estradiol (7), a smaller bathochromic shift is observed in spectrum of 4-iodo estradiol (6). This can be associated with the iodine substitution at the C-4 position causing a planarity distortion of the ring A and a decrease in the iodine-aromatic electrons conjugation. Therefore, the OH group in 4-iodo estradiol (6) is less shielded by bulky iodine atom than that in 2-iodo estradiol (7) and is more available for an external hydrogen bonding. As a consequence, the $\log P$ value of 4-iodo estradiol (6) is smaller and v_R on a silica column is greater in comparison with identical parameters determined for 2-iodo estradiol (7).

The higher π value in 2-iodo estradiol (7) ($\pi = 1.45$) compared to common π value for *ortho* iodine in phenols ($\pi = 1.19$) implies an increase in the lipophylic character of 2-iodo estradiol (7). This can be reasonably rationalized

either in terms of an intramolecular I—OH hydrogen bonding¹⁷ or in a possible contribution of Rekker's proximity effects to the magnitude of $\log P_{\text{chl/w}}$ ^{18a,b}. Two electronegative substituents (OH and I) in 2-iodo estradiol (7) are probably forced to be close enough for either mutual electronic interactions or sharing the same solvating water molecule(s).

Verloop's opinion that »there is considerable intercorrelation between the van der Waals radii and the constants of the ortho substituents«¹⁹ can be well correlated with our observations that steric effects have crucial influence on the magnitude of π values for *ortho* iodine atoms. It seems that partition coefficients are extremely sensitive towards such interactions.

In considering mutual relationships between Hansch π constants or fragment constants, partition coefficients in octanol and chloroform, and R_L (R'_L) parameters derived from HPLC data the following scheme illustrates the possibilities for interconversion of these parameters:



Being all equilibrium constants in free energy relationship, it seems that all above parameters are equally valid in the methodology of obtaining hydrophylic-lipophylic constants in structure-activity relationship studies.

The simplicity and accuracy of the reported technique indicates the determination of hydrophobic coefficients by HPLC to be an attractive alternative to the conventional methods. Further studies will hopefully establish the generality of this method.

EXPERIMENTAL

Analytical HPLC was performed using a μ -Porasil (silica gel), 1 ft* \times 0.125 in** column, on a Waters Associates Model ALC/GPC-200 liquid chromatograph. The analytical work in this study was identical to the previously published procedure⁷. The purity of reagent grade estradiol (Sigma Chem. Co.) was checked by ir and mass spectral data; it was found to be better than 98.9%. 4-Iodo estradiol, 2-iodo-estradiol and 2,4-diiodoestradiol were synthesised as described in the literature⁷. Diiodinated estradiol was removed from reaction products on a preparative silica gel HPLC column. Both monoiodinated estradiols were used together in the determination of partition data. Spectrograde chloroform (50 ml) was washed with water (10 \times 50 ml) prior to use. Both solvents (chloroform and water) were mutually saturated one with each other in a preequilibration process during 24 hours. In a typical partition experiment estradiol (2 mg) was allowed to dissolve in equal volumes (50 ml) of water and chloroform. The mixture was treated as described previously^{2c} to ensure true partitioning. The mixture was allowed to stand overnight. Five aliquots from the aqueous layer (5 \times 5 ml) were evaporated in vacuo until dryness

* 1 foot = 0.3048 m

** 1 inch = 0.0254 m

followed by analyses of chloroform solutions ($5 \times 50 \mu\text{l}$) on a silica gel HPLC column. Solid residues obtained from the aqueous layer were dissolved in chloroform ($5 \times 100 \mu\text{l}$) and were also examined analytically by means of HPLC technique. Areas corresponding to the solute signals from both layers were determined by a cut-and-weight method.

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SAŽETAK

Nova R_L konstanta za računanje log P i π vrijednosti u homolognim serijama spojeva

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Volumeni zadržavanja jodiranih dietilstilbestrola i estradiola mjereni su na koloni silika-gela upotrebom visokotlačne tekućinske kromatografije; nađeno je da su oni linearno pridruženi koeficijentima raspodjele odgovarajućih spojeva. Računanja vrijednosti log P provedena su uz upotrebu nove konstante R_L , odnosno primjenom Hanschove metode. Računate vrijednosti log P podudarale su se s eksperimentalnim podacima. Razmatrana je veličina π -vrijednosti za atom joda u monosupstituiranom estradiolu u skladu s utjecajem steričkih efekata i »efekata blizine« na tu vrijednost. Iznescena metoda može se smatrati doprinosom metodologiji proučavanja odnosa između kemijske strukture i biološke aktivnosti.

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