Retention in RP-HPLC: Lipophilicity Determination of Substituted Biphenyls by Reversed-Phase High Performance Liquid Chromatography

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RETENTION IN RP-HPLC: LIPOPHILICITY DETERMINATION OF SUBSTITUTED BIPHENYLS BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The RP-HPLC capacity factors (k') of a series of substituted biphenyls were determined on a C_{18} column with methanol/water as the mobile phase. A linear relationship was found between log k' and the volume fraction of methanol (φ) for each of the 25 tested compounds with the correlation coefficient > 0.99. High correlation was found between log k'_w and S, which are intercept and slope of plot of log k' vs. φ, respectively. The values of S and log k'_w were quantitatively correlated with the solvatochromic parameters of the solutes and two statistically significant equations were established which showed that solute volume and hydrogen bond basicity were of major importance in influencing the retention of these studied compounds. The obtained log k'_w was chosen as a measure of the solute hydrophobicity and will be used for the on-going Quantitative Structure-Activity Relationship (QSAR) studies of the substituted biphenyls.

INTRODUCTION

The lipophilicity character of a chemical has long been shown to play a basic role in determining distribution phenomena, such as the adsorption on soil or sediment and the bioconcentration by aquatic organisms.[1,2] In addition, it influences the ecotoxicity of a chemical.[3] Lipophilicity is generally defined as the tendency of a chemical to distribute between an immiscible nonpolar solvent and water. The logarithm of the partition coefficient of a chemical in the n-octanol/water system (Kow), which is usually measured by "shake flask" method, is widely used because of its simplicity and some similarity between n-octanol and biological membranes. However, the conventional "shake flask" method has limited application range up to log Kow = 4, and it is time-consuming and requires considerable amounts of pure stable compounds.[4] It has been proven that the retention capacity factor (k') of a compound in a reversed-phase high performance liquid chromatography (RP-HPLC) system, is a reliable indirect descriptor of the lipophilicity of a compound.[5-11] Moreover, some studies have shown that log k'_w, the retention capacity factor which is extrapolated from a binary phase to 100% water in a RP-HPLC system, is an even better descriptor of lipophilicity than the isocratic factor because it is independent of any organic modifier effects and it reflects polar-non-polar partitioning in a manner similar to shake-flask measurements.[6,12-13]

The 4-alkyl or alkoxy-4'-cyanobiphenyls is an important group of liquid crystal materials.[14] With their more and more extensive uses, these compounds, along with their precursors, become environmentally and toxicologically important. However, there have been few investigations on their environmental behavior.[15,16] Our interest in lipophilicity resulted from a need to develop quantitative structure-activity relationships (QSARs) for their toxicities to *Daphnia magna* and Alga. These compounds are generally very non-polar; for example, by applying the fragmental method of Hansch and Leo,[17] the calculated log Kow for 4-n-butyl-4'-cyanobiphenyl is higher than 5. Direct measurement of their n-octanol/water partition coefficients by the conventional 'shake flask' method is difficult because of their highly lipophilic characteristics.

In this paper, first we are reporting a systematic study of the lipophilicity of these compounds by using a RP-HPLC method, and the measured data will be used in the on-going research of quantitative structure-activity relationships (QSARs) for the compounds. Second, based on the linear solvation energy relationships (LSERs), we are using solvatochromic parameters to study the major solute factors governing the retention of these studied compounds in RP-HPLC.

MATERIALS AND METHODS

Instruments

The HPLC system (Shimadzu, Japan) consisted of a SCL-8A system monitor, a LC-8A pump, a C-R4A integrator, and a SPD-6AV ultraviolet spectrophotometer as the detector. A C_{18} reversed-phase Nucleosil 7 (Dalian Institute of Chemical Physics, Academic Sinica) (15 x 4.6 mm i.d.) column was used.

Chemicals

The structures of the 25 substituted biphenyls are shown in Table 1. These compounds were supplied by the Yantai Valiant Fine Chemicals Co. Ltd., Yantai, P. R. China. The purity for each of the chemicals is greater than 99%. The water used as a mobile phase component was double-distilled. Methanol was analytical grade and redistilled before use. Sodium nitrate was also analytical grade.

Measurement of Log k'

The log k' values were determined for each compound. The mobile phases were made by mixing methanol with water in the proportions 100:0, 90:10, 85:15, 80:20, and 75:25 (v/v). The flow rate was 1.0 mL/min. All measurements were made in, at least, duplicate. The average reproducibility of each determination was better than 1.0% relative. The capacity factors (k') were determined using $k' = (t_r-t_0)/t_0$, where t_r is the retention time of the compound, and t_0 is the void volume or the dead time. An aqueous solution of sodium nitrate was used for the measurement of t_0.

The regression analysis was performed using the "Statgraphics" program (STSC, Inc., 1987).
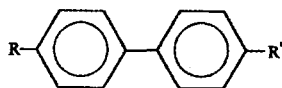
RESULTS AND DISCUSSIONS

The relationship between log k' and methanol concentration in mobile phase given by Snyder et al.[18] is :

$$\log k' = \log k'_w - S \varphi \tag{1}$$

Table 1

The Structure of the Substituted Biphenyls

R—⟨◯⟩—⟨◯⟩—R'

No.	R	R'
1	H	H
2	$CH_3C(O)$	H
3	CH_3CH_2	H
4	CH_3CH_2	CN
5	CH_3CH_2	I
6	$CH_3H_7C(O)$	H
7	C_4H_9	H
8	C_4H_9	CN
9	C_4H_9	I
10	$C_4H_9C(O)$	H
11	C_5H_{11}	H
12	C_5H_{11}	CN
13	C_5H_{11}	I
14	CH_3CH_2O	CN
15	$CH_3(CH_2)_2O$	CN
16	$CH_3(CH2)_3O$	CN
17	$CH_3(CH_2)_4O$	CN
18	CH_3CH_2O	I
19	$CH_3(CH_2)_2O$	I
20	$CH_3(CH_2)_3O$	I
21	$CH_3(CH_2)_4O$	I
22	OH	CN
23	OH	I
24	Br	H
25	Br	Br

where k'_w represents the k' value for a compound if pure water is used as eluent, S is the slope of the regression curve, and φ is the volume percentage of methanol in the mobile phase. For each studied compound, linear correlation was found between log k' and φ, and the correlation coefficients were all > 0.99. The values of S and the extrapolated log k'_w are given in Table 2.

Table 2

The Values of Solvatochromic Parameters for the Studied Compounds

No.	R	R′	S^a	Log $K'_w{}^a$	$V_x/100^b$	$\pi^*{}_2{}^b$	$\alpha_2{}^b$	$\beta_2{}^b$
1	H	H	3.897	3.663	0.920	1.20	0.00	0.28
2	$CH_3C(O)$	H	3.178	2.887	1.116	1.55	0.06	0.67
3	CH_3CH_2	H	5.032	4.899	1.116	1.14	0.00	0.30
4	CH_3CH_2	CN	4.351	4.036	1.215	1.34	0.22	0.67
5	CH_3CH_2	I	6.162	6.168	1.297	1.36	0.10	0.32
6	$C_3H_7C(O)$	H	4.270	4.044	1.312	1.51	0.06	0.67
7	C_4H_9	H	5.487	5.462	1.312	1.10	0.00	0.30
8	C_4H_9	CN	4.798	4.711	1.411	1.30	0.22	0.67
9	C_4H_9	I	6.554	6.707	1.493	1.32	0.10	0.32
10	$C_4H_9C(O)$	H	4.972	4.765	1.410	1.49	0.06	0.67
11	C_5H_{11}	H	6.312	6.332	1.410	1.08	0.009	0.30
12	C_5H_{11}	CN	6.221	6.028	1.509	1.28	0.22	0.67
13	C_5H_{11}	I	7.185	7.393	1.591	1.30	0.10	0.32
14	CH_3CH_2O	CN	3.859	3.514	1.260	1.44	0.28	0.89
15	$CH_3(CH_2)_2O$	CN	4.542	4.227	1.358	1.42	0.28	0.89
16	$CH_3(CH_2)_3O$	CN	5.239	4.951	1.456	1.40	0.28	0.89
17	$CH_3(CH_2)_4O$	CN	5.803	5.571	1.554	1.38	0.28	0.89
18	CH_3CH_2O	I	5.570	5.525	1.342	1.42	0.16	0.54
19	$CH_3(CH_2)_2O$	I	6.227	6.223	1.440	1.40	0.16	0.54
20	$CH_3(CH_2)_3O$	I	6.515	6.577	1.538	1.38	0.16	0.54
21	$CH_3(CH_2)_4O$	I	6.839	6.907	1.636	1.36	0.16	0.54
22	OH	CN	2.467	1.885	1.064	1.53	0.82	0.88
23	OH	I	4.158	3.784	1.146	1.55	0.70	0.53
24	Br	H	4.729	4.576	1.051	1.35	0.10	0.24
25	Br	Br	5.431	5.344	1.182	0.70	0.20	0.20

[a] S and log k'_w are the slope and intercept values of plot of log k' vs. φ (the volume percentage of methanol in the mobile phase), respectively.
[b] Solvatochromic parameters of the solutes calculated according to references 23, 24.

The relationship between the slope S and the intercept values (log k'w) was investigated for the tested compounds, and a good linear correlation was observed:

$$S = (0.770\pm0.062) + (0.876\pm0.012) \log k'_w \qquad (2)$$

$$n = 25, \ S.D. = 0.078, \ r = 0.9979$$

In the above equation, and elsewhere, n is the number of the data points, while S.D. and r indicate the average standard deviation and the correlation coefficient of the fit, respectively. It is clear that for the studied compounds, S and log k'_w are highly correlated and this may be a reflection of the suitability of the methanol/water system for estimating the lipophilicy of the compounds.[6]

In order to investigate the main factors controlling the retention of these compounds in RP-HPLC, the methodology of linear solvation energy relationships (LSERs) in conjunction with solvatochromic parameters, was used to describe the retention in RP-HPLC.[19-22] According to this approach, variation in capacity factors with solute structure can be related to their potential for intermolecular interactions as follow:

$$\log k' = SP_0 + mV_x/100 + s\pi^*_2 + a\,\alpha_2 + b\,\beta_2 \qquad (3)$$

where V_x, π^*_2, α_2, and β_2 are parameters that describe the solute, and m, s, a, and b are the corresponding regression coefficients. SP0 is the intercept of the relationship defined by eqn. 3. The V_x term measures the intrinsic molar volume; π^*_2 is a measure of solute dipolarity/polarizability, α_2 is the hydrogen bond donor ability (HBD) or HBD acidity, and β_2 is the hydrogen bond acceptor ability (HBA) or HBA basicity. The values of solvatochromic parameters for the studied compounds were calculated according to the references[23,24] and are given in Table 2.

Many studies have shown that S is dependent not only on the HPLC system but rather on solute structure.[25-28] Therefore both log k'w and S can be described by the same solute parameters used to describe log k' and the general equations are as follows:[26]

$$\log k'_w = SP_w + m_w V_x/100 + s_w\pi^*_2 + a_w\alpha_2 + b_w\beta_2 \qquad (4)$$

$$S = SP_s + m_s V_x/100 + s_s\pi^*_2 + a_s\alpha_2 + b_s\beta2 \qquad (5)$$

where the subscripts w and s designate fitting coefficients for log k'_w and S, respectively. By regression analysis, the LSER equations of S and log k'_w for the data listed in Table 2 are as shown in eqns. 6 and 7:

$$S = (-0.681 \pm 0.458) + (5.666 \pm 0.346)\,V_x/100 - (2.984 \pm 0.279)\,\beta_2 \qquad (6)$$

$$n = 25, \quad S.D. = 0.312, \quad R = 0.9648$$

$$\log k'_w = (-1.40\pm0.459) + (6.380\pm0.347)\ V_x/100 \qquad (7)$$

$$- (3.606\pm0.280)\ \beta_2$$

$$n = 25,\ \ S.D. = 0.313,\ \ R2 = 0.9728$$

In the above two equations, the $\pi*_2$ and α_2 terms are not included because they are not significant at the 95% confidence level, and V_x and β_2 are the most important parameters in the equations. So, it is clear that for the studied compounds, solute size and hydrogen bond basicity are the two major retention governing factors in RPLC, and this is in accordance with other studies.[19-22,29] Eqn. 6 shows that increasing hydrogen bond basicity results in a decrease in S, whereas increasing the size of the solute leads to an increase in S.

Therefore, as the solute becomes increasingly hydrophobic, S will become increasingly positive; in contrast, as the solute becomes more hydrophilic and more polar, S will decrease when other conditions remain the same. Eqn. 7 shows that increasing solute size and/or decreasing hydrogen bond basicity will increase solute lipophilicity.

CONCLUSION

The retention capacity factors (log k') for the 25 substituted biphenyls were measured at five different compositions of the eluent, and the log k'_w was extrapolated from the linear relationship between log k' and the volume fraction of methanol in the mobile phase (φ) for each studied compound. The log k'_w may be an advantageous lipophilic parameter used in QSAR studies.

LSER equations were established for S and log k'_w, which are slope and intercept of plot of log k' vs. φ, respectively. The results show that solute size and hydrogen bond basicity are the two major factors influencing the retention of these studied compounds in RP-HPLC.

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