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The lipophilicity of deuterium atoms. A comparison of shake-flask and HPLC methods

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Summary

Isotopic effects are demonstrated in the lipophilicity, measured by shake-flask and HPLC methods, of a series of deuterated aromatic compounds. The results indicate that deuterated compounds are less lipophilic than their protium isomers by about -0.006 per deuterium atom on the log P_{oct} scale. This isotopic effect is satisfactorily accounted for by differences in molar volumes of isotopomers. The partition coefficient of benzene and toluene is critically evaluated in view of the volatility of these compounds.

Introduction

Binding of a substrate by the active site of an enzyme is a process comparable in some respects to the extraction of a solute from water into an organic solvent. Thus the rate and extent of binding of a substrate are related to its lipophilicity. Several physicochemical parameters have been used as an index of lipophilicity, e.g. TLC R_m and R_f values, HPLC capacity factors (log k), and partition coefficients measured in water/organic solvent systems (log P). Log P values have been measured and compiled during the last two decades for many different solvent systems, *n*-octanol/water being the system of choice in many cases (Hansch and Leo, 1979,

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1983). However, little is known about the partitioning behavior of labeled compounds in general and deuterated compounds in particular, as compared to their unlabeled isotopomers.

From HPLC measurements of various organic compounds, Tanaka and Thornton (1976) found that deuterated compounds have 0.2–0.5% smaller capacity factors (log k) than their protium isomers. The observation that deuterated molecules are eluted before the corresponding non-deuterated analogs in reversed-phase HPLC has been confirmed by others (Cartoni and Ferretti, 1976). Recently, Kovach and Quinn (1983) reported β-deuterium isotope effects in the partition coefficient for 3 types of carbonyl compounds and found protium compounds favored in the organic phase by 1–2%. These results indicate that deuteration of a compound slightly diminishes its lipophilic character. However, at present no exact rules are available.

Literature data show that deuterated compounds have smaller molar volumes than their corresponding isotopomers (Bartell and Roskos, 1966; Kiss et al., 1979). Deuterocarbons also exhibit lower polarizabilities and hence, presumably weaker London dispersion forces (Bartell and Roskos, 1966; Kiss et al., 1979; Kovach and Quinn, 1983).

This may be related to the fact that C–D bonds are a little shorter than C–H bonds (Kiss et al., 1979; Sutton, 1965). In a recent publication Testa and Seiler (1981) proposed for non-polar fragments a relationship between Rekker's hydrophobic fragmental constants, f_i (Rekker and de Kort, 1979), and fragmental van der Waals volumes, V_i, i.e. between lipophilicity and volume:

$$f_i = 0.0534\ V_i \tag{1}$$

This equation predicts that for a decreasing molar volume, as is the case for the deuterated analogs of a substance, the lipophilic character will also decrease, in agreement with the observations cited above. A second relation between lipophilicity and fragmental volume is seen in the following equation (Mayer et al., 1982a; van de Waterbeemd and Testa, 1983):

$$\frac{f_H}{f'_H} = \frac{0.182}{0.23} \cong \frac{4.62\ cm^3/mol}{5.81\ cm^3/mol} \tag{2}$$

In this equation f_H is Rekker's fragmental constant for H, as obtained from a statistical approach, i.e. a mean value from hydrogen linked to aliphatic and aromatic carbons. Hansch and Leo (1979) measured the partition coefficient of H_2 and calculated the fragmental value for H as $f'_H = (\log P_{H_2})/2$. The ratio f_H/f'_H appears to be identical to the ratio of the exposed volumes.

Similar to H, hydrophobic fragmental values are known for many other fragments (Hansch and Leo, 1979, 1983; Rekker and de Kort, 1979). However, a fragmental value for D is not known, making it impossible at present to estimate or calculate the isotopic effect in partition due to the introduction of one or more deuterium atoms in a molecule.

In the present study we compare partition coefficients (log P) obtained by the classical shake-flask method with capacity factors (log k) from reversed-phase high-performance liquid chromatography for a limited series of deuterated aromatic compounds and their isotopomers, namely benzene, toluene, *p*-xylene, pyridine and aniline. For the same compounds apparent molar volumes were measured, and the isotopic effects observed are compared.

Materials and Methods

Deuterated compounds (isotopic purity > 99%) were purchased from Aldrich (Belgium) and Merck (F.R.G.). *n*-Octanol was of quality 'purissimum', obtained from Fluka (Switzerland) and used without further purification. Buffers and salts were of analytical grade. Distilled water was used throughout. Aqueous and octanolic phases were mutually saturated.

Partition coefficients from shake-flask method (Leo et al., 1971; Mayer et al., 1982b)

For all compounds, aqueous solutions having an ionic strength $\mu = 0.12$ (NaCl or phosphate buffer) were prepared. For aniline and pyridine, buffer solutions of pH = 7.4 were used. The volume ratio of the two phases was chosen to leave 30–60% of the solute in the aqueous phase. Both phases were transferred to Sovirel tubes and submitted to gentle mechanical shaking for one hour, a time sufficient to ensure equilibrium. After centrifugation for 20 min at 8000 rpm the aqueous phase was analyzed using a Perkin Elmer model 557 UV spectrophotometer.

Special attention was paid to the determination of partition coefficients of volatile compounds, in particular benzene and toluene. Stock aqueous solutions were made in two types of flasks: a normal Erlenmeyer flask, and a volumetric flask with a long narrow collar, which reduces the liquid surface and hence evaporation to a minimum. The partition coefficient of *p*-xylene was not determined by this method, since the shake-flask method becomes very inaccurate for highly lipophilic compounds (log P > 3).

Reversed-phase high-performance liquid chromatography (RP-HPLC)

A Siemens S 101 chromatograph equipped with an Orlita pump type DMP-AE 10.4 was used. The detector was a Zeiss PM2DLC spectrophotometer with a measuring cell volume of 8 µl, operating at 254 nm. As stationary phase LiChrosorb RP-18, particle size 10 µm (Merck, F.R.G.) was employed. The 30 cm length and 4 mm i.d. column was prepared in our laboratory using a high-pressure packing technique (Hennion et al., 1978). Salts were of analytical grade; methanol (analyzed reagent grade) was from Merck (F.R.G.). Distilled water/methanol mixtures were used as mobile phase and all solutions were purified by filtration through a Millipore-Q system. Column dead-time (t_0) was determined using an aqueous solution of sodium bichromate as the non-retained compound. The capacity factor k was calculated as

$$k = \frac{t_R - t_0}{t_0} \tag{3}$$

where t_R is the retention time of the compound under investigation. The logarithm of the capacity factor (log k) is taken as lipophilic index by analogy to log P.

Apparent molar volumes

Apparent molar volumes, Φ_V (Barlow, 1980, 1983; Edward et al., 1977; Millero, 1971; Perron and Desnoyers, 1979), were calculated from high-precision density measurements, using Eqn. 4. By making measurements over a range of concentrations it is possible to extrapolate to the apparent molar volume at infinite dilution Φ_V^0, using Eqn. 5. This equation can be seen as the Redlich-Meyer equation (Millero, 1971) for non-electrolytes.

$$\Phi_V = \frac{MW}{\rho_{solv}} + \frac{1000(\rho_{solv} - \rho_{sol})}{\rho_{solv} \cdot C} \tag{4}$$

$$\Phi_V = \Phi_V^0 + j \cdot C \tag{5}$$

In these equations MW is the molecular weight of the solute, C the molar concentration, ρ_{solv} and ρ_{sol} the density of pure solvent, i.e. 2-propanol, and of the solution, respectively, and j a coefficient from regression analysis.

The densities were measured using an Anton Paar Precision Density Meter DMA 60, including a measuring cell DMA 601. Temperature was maintained at 20 ± 0.001°C by a Lauda RC-20 thermostat placed in a thermostated room. The precision in the density is $\pm 1.5 \times 10^{-6}$ g/cm^3. In the present measurements 2-propanol was used as solvent, since water shows solubility restrictions for the investigated compounds. The apparent molar volumes obtained can be converted to those in water using Eqn. 6 (Gryllaki, van de Waterbeemd and Testa, to be published):

$$(\Phi_V^0)_{H_2O} = 0.994(\pm 0.011)(\Phi_V^0)_{2\text{-propanol}} \tag{6}$$

$(n = 13; r^2 = 0.911; s = 4.480)$

Results and Discussion

Partition coefficients (log P_{oct})

In Table 1 the experimental partition coefficients measured in the *n*-octanol/water system are tabulated. Except for benzene (see below), all compounds show a significant difference between the deuterated compound and its protiated isomer, the latter being more lipophilic. The mean isotopic effect per D atom can be calculated as -0.0054 ± 0.0037 ($n = 4$).

Benzene is a special case, since it is rather volatile. Fujita and coworkers (1964) determined the partition coefficient of benzene by considering the partitioning between air in the volumetric flask and water. Using P(H_2O/air) as determined by Milligan (1924), they obtained with this method a value of 2.13 for benzene and 2.69

TABLE 1

n-OCTANOL/WATER PARTITION COEFFICIENTS OBTAINED BY THE SHAKE-FLASK METHOD

Compound	log P^{exp}	Isotopic effect	Isotopic effect per D atom	log $P_{lit.}$ [a]
C_6H_6	2.036 ± 0.010 [b]			2.13, 2.15, 2.15, 2.03, 2.04, 2.34
C_6D_6	2.032 ± 0.008 (N.S.) [c]	−0.004	−0.0007	
$C_6H_5CH_3$	2.612 ± 0.026			2.69, 2.73, 2.80, 2.94, 2.11
$C_6D_5CD_3$	2.572 ± 0.030 ($P < 0.05$)	−0.040	−0.0050	
$C_6H_5NH_2$	0.890 ± 0.004			0.90, 0.98, 0.89, 0.85, 0.91
$C_6D_5NH_2$	0.842 ± 0.013 ($P < 0.01$)	−0.048	−0.0096	
C_5H_5N	0.624 ± 0.005			0.65, 0.62, 0.64, 0.66, 0.78
C_5D_5N	0.592 ± 0.006 ($P < 0.001$)	−0.032	−0.0064	

mean isotopic effect per D atom: −0.0054 ± 0.0037

[a] Taken from Hansch and Leo, 1979, 1983.
[b] Standard deviation ($n \geq 4$).
[c] Significance of difference between isotopomers from Student's *t*-test.

for toluene. Ever since reference has been frequently made to these values without further verification.

In the present study we re-examined the partition coefficient of benzene and investigated the influence of volatility. Two types of flask were used to prepare aqueous stock solutions, namely an Erlenmeyer flask and one with a long narrow collar (volumetric flask). The Erlenmeyer flask has a relatively large liquid surface area. As a consequence each time the flask is opened for sampling, a portion of the benzene is lost. As clearly seen in Table 2 and Fig. 1, log P changes as a function of tube number (tube 1 contains the first sample, tube 4 the last). This volatility effect was overcome using the second type of flask, i.e. one with a narrow long collar. Now the volatility effect is reduced to a minimum and constant log P values are obtained. The log P value of benzene thus determined, 2.04, is 0.09 less than the original value (Fujita et al., 1964). Table 2 shows that the volatility effect for toluene is also overcome using a narrow-collar flask.

It should be remembered that the benzene log P value of 2.13 is a cornerstone in some hydrophobic fragmental systems suggesting possible improvements.

Lipophilicity measurements by HPLC

Numerous workers have demonstrated a close relationship between retention on

TABLE 2
VOLATILITY EFFECT ON THE PARTITION COEFFICIENT OF BENZENE AND TOLUENE

Tube no.	Stock solution in:			
	Erlenmeyer flask		Narrow-collar flask	
	C_6H_6	C_6D_6	C_6H_6	C_6D_6
1	2.135	2.103	2.023	2.040
2	2.153	2.133	2.039	2.022
3	2.204	2.164	2.037	2.030
4	2.249	2.185	2.045	2.037
mean	$\overline{2.185 \pm 0.052}$	$\overline{2.146 \pm 0.036}$	$\overline{2.036 \pm 0.009}$	$\overline{2.032 \pm 0.008}$
	$C_6H_5CH_3$		$C_6H_5CH_3$	$C_6D_5CD_3$
1	2.603		2.596	2.611
2	2.638		2.596	2.577
3	2.667		2.618	2.553
4	2.696		2.640	2.553
5			2.640	2.532
6			2.568	2.570
7			2.624	2.612
mean	$\overline{2.651 \pm 0.040}$		$\overline{2.612 \pm 0.026}$	$\overline{2.572 \pm 0.030}$

reversed-phase HPLC columns and *n*-octanol/water partition coefficients (Butte et al., 1981; Hammers et al., 1982; Miyake and Terada, 1982; Unger, 1980; Unger and Chiang, 1981). Several techniques have been employed, e.g. columns coated with a *n*-octanol layer as the stationary phase (Unger, 1980). It appears that even non-treated C_{18} packings mimic the octanolic phase quite well. The logarithm of the capacity factor (log k) as defined in the Experimental Section appears to be related linearily to log P. Depending on the polarity, log k can be determined at various mobile

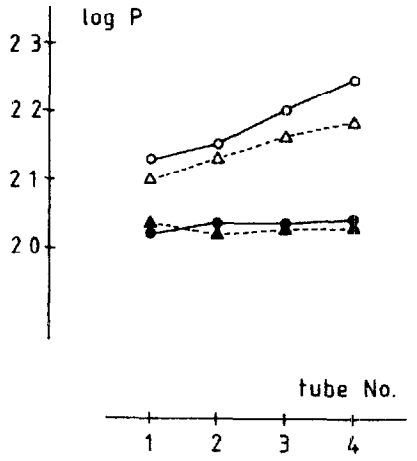

Fig. 1. *n*-Octanol/water partition coefficient of benzene (●———●, ○———○) and benzene-d_6 (▲- - - - -▲, △- - - - -△) determined in 4 tubes after sampling from stock aqueous solutions in Erlenmeyer flasks (○, △) or narrow-collar flasks (●, ▲).

TABLE 3

CAPACITY FACTORS FROM RP-HPLC

Compound	Formula	$\log k_{70}$ [a]	$\log k_{60}$	$\log k_{50}$	$\log k_0$ [b]	r
Benzene	C_6H_6	0.141 ± 0.005 [c]	0.408 ± 0.003	0.697 ± 0.003	2.083 ± 0.009	0.9997
	C_6D_6	0.126 ± 0.003	0.392 ± 0.003	0.676 ± 0.006	2.048 ± 0.007	0.9998
Toluene	$C_6H_5CH_3$	0.365 ± 0.005	0.685 ± 0.003	1.028 ± 0.003	2.681 ± 0.009	0.9998
	$C_6D_5CD_3$	0.346 ± 0.002	0.664 ± 0.002	1.002 ± 0.008	2.639 ± 0.008	0.9998
p-Xylene	$CH_3C_6H_4CH_3$	0.599 ± 0.007	0.970 ± 0.003	1.370 ± 0.005	3.293 ± 0.012	0.9998
	$CD_3C_6H_4CD_3$	0.589 ± 0.005	0.959 ± 0.002	1.349 ± 0.006	3.246 ± 0.010	0.9999
	$CD_3C_6D_4CD_3$	0.577 ± 0.006	0.946 ± 0.003	1.337 ± 0.004	3.233 ± 0.009	0.9999
		$\log k_{40}$	$\log k_{30}$	$\log k_{20}$		
Aniline	$C_6H_5NH_2$	0.208 ± 0.002	0.431 ± 0.002	0.657 ± 0.003	1.106 ± 0.001	0.9999
	$C_6D_5NH_2$	0.193 ± 0.005	0.413 ± 0.003	0.635 ± 0.004	1.077 ± 0.001	0.9999

[a] $\log k_{70}$ means methanol/water 70/30 (v/v).

[b] Extrapolated to 0% methanol.

[c] Standard deviation ($n \geq 3$).

phase compositions. Methanol/water is a frequently used mixture. Methanol can act both as proton donor and proton acceptor, therefore providing a genuine dilution medium for the aqueous phase, up to high proportions of methanol. For this reason it is often found that log k can be extrapolated linearily to 100% water giving log k_0 values which are close to log P_{oct} values obtained by the shake-flask method (Butte et al., 1981). We have confirmed this relationship for a series of monosubstituted benzenes (Eqn. 7) (El Tayar, van de Waterbeemd and Testa, to be published):

$$\log k_0 = 0.966(\pm 0.007) \log P_{oct}^{exp} \quad (7)$$

$$(n = 18;\ r^2 = 0.993;\ s = 0.077)$$

This equation is forced through the origin since the constant term was non-significant; the standard deviation appears in parentheses.

In Table 3 the experimental and extrapolated log k values are presented, while in Table 4 a comparison is made between log k_0 values of the various isotopomers. Pyridine is excluded from this comparison since small basic molecules interact strongly with the free acidic silanol groups, causing tailing and asymmetric peak shape, thereby leading to a deviant and non-comparable partitioning behavior (Nahum and Horvàth, 1981; Unger, 1980). Comparing Tables 1 and 4 indicates that as for the monosubstituted benzenes (Eqn. 7), log k_0 and log P are closely related. This permits to compare directly the isotopic effect per D atom as obtained by

TABLE 4

COMPARISON OF log k_0 VALUES BETWEEN ISOTOPOMERS, AND ISOTOPIC EFFECT PER D ATOM FROM HPLC METHOD

	log k_0	Isotopic effect	Isotopic effect per D atom
C_6H_6	2.083 ± 0.009		
C_6D_6	2.048 ± 0.007 ($P < 0.01$)	−0.035	−0.0058
$C_6H_5CH_3$	2.681 ± 0.009		
$C_6D_5CD_3$	2.639 ± 0.008 ($P < 0.01$)	−0.042	−0.0053
$H_3C_6H_4CH_3$	3.293 ± 0.012		
$D_3C_6H_4CD_3$	3.246 ± 0.010 ($P < 0.01$)	−0.047	−0.0078
$CD_3C_6D_4CD_3$	3.233 ± 0.009 ($P < 0.01$)	−0.060	−0.0060
$C_6H_5NH_2$	1.106 ± 0.002		
$C_6D_5NH_2$	1.077 ± 0.004 ($P < 0.01$)	−0.029	−0.0058

mean isotopic effect per D atom: −0.0061 ± 0.0010

TABLE 5

ISOTOPIC EFFECTS IN APPARENT MOLAR VOLUMES AT 20 °C

Compound	Φ_V^0 (apparent molar volume in cm^3/mol)	Isotopic effect		$\frac{(\Phi_V^0)_H}{(\Phi_V^0)_D}$	$\frac{(\log k_0)_H}{(\log k_0)_D}$ [d]
		Total	Per D		
C_6H_6 [a]	88.76 ± 0.33 [c]				
C_6D_6 [b]	87.92 ± 0.23	−0.84	−0.140	1.010	1.017
$C_6H_5CH_3$	105.46 ± 0.27				
$C_6D_5CD_3$	104.75 ± 0.51	−0.71	−0.089	1.007	1.016
$CH_3C_6H_4CH_3$	123.73 ± 0.53				
$CD_3C_6D_4CD_3$	122.65 ± 0.23	−1.08	−0.108	1.009	1.019
$C_6H_5NH_2$	86.28 ± 0.19				
$C_6D_5NH_2$	85.16 ± 0.07	−1.12	−0.224	1.013	1.027

mean isotopic effect per D atom: −0.140 ± 0.060

[a] Molar volume values 88.867 and 88. 864 (Bartell and Roskos, 1966; Kiss et al., 1979).
[b] Molar volume values 88.626 and 88.632 (Bartell and Roskos, 1966; Kiss et al., 1979).
[c] Standard deviation ($n \geq 4$).
[d] From Table 4.

HPLC (−0.0061 ± 0.0010) and that from the shake-flask method (−0.0054 ± 0.0037). It is observed that the S.D. for the HPLC method is smaller, as expected from its greater precision. Combining these two values leads to the conclusion that the isotopic effect per D is −0.006, H being more lipophilic than D.

Isotopic effects in apparent molar volumes

In Table 5 the apparent molar volumes of benzene, toluene, *p*-xylene and aniline are given for the isotopomers of each compound. We confirm the literature observations cited in the introduction and conclude that deuterated substances have smaller volumes, the isotopic effect being −0.140 ± 0.060 cm^3/mol per deuterium atom. Using Eqn. 1, this value predicts an isotopic effect in log P of ca. −0.007 per D atom, i.e. in agreement with the experimental value of −0.006.

It should be stated that apparent molar volume measurements are less accurate than the determination of HPLC log k values. However, a parallel in behavior is seen between log k_0 and Φ_V^0 when the ratios D/H for these properties are compared, as shown in Table 5. This suggests that the isotopic effect in lipophilicity is of steric origin and is accounted for by differences in molar volume between isotopomers.

Conclusions

The present study demonstrates that an isotopic effect can be ascertained when comparing the lipophilicity of deuterated compounds and their protium analogs.

This effect amounts to −0.006 per deuterium atom on the log P_{oct} scale, H being more lipophilic than D. Depending on which fragmental system is used one can derive fragmental constants for the deuterium fragment, either (Rekker and de Kort, 1979) as $f_D = f_H - 0.006 = 0.176$, or (Hansch and Leo, 1979) as $f'_D = f'_H - 0.006 = 0.22$. It is noteworthy that even the classical shake-flask method reveals isotopic effects. The HPLC technique confirmed these observations with a greater precision. With the small series of compounds investigated so far it is not possible to conclude whether isotopic effects are different for deuterium linked to aliphatic or aromatic carbons. This would require a much larger and well-selected series of deuterated compounds, and would be quite time-consuming. Although the changes in lipophilicity and molar volume due to the introduction of deuterium atoms might seem small, at the receptor level the changes might be more important. As has been shown for neuroleptics, the lipophilic optimum can be quite critical and small changes can give a considerable variation in activity (Jenner et al., 1983; van de Waterbeemd et al., 1983).

A second area in which lipophilicity isotope effects might be of importance is in the determination of the mechanisms of enzyme-mediated reactions. Isotope effects are often used to shed light on reaction mechanisms. The tacit assumption in such studies is that equal concentrations of unlabeled vs deuterated substrate form equal concentrations of enzyme–substrate complex. In fact, any difference in rate of product formation is a reflection and summation of all the steps (binding and catalytic) leading up to the formation of product. It now appears that inherent differences in lipophilicity will have to be taken into account, particularly in multideuterated substrates of membrane-bound enzymes such as cytochrome P-450.

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