Journal of Chromatography, 384 (1987) 349–356
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 999

MEASUREMENT OF LIPOPHILICITY BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

COMPARISON WITH CALCULATED LIPOPHILICITY VALUES

JULIE J. SABATKA*, DOUGLAS J. MINICK, THOMAS K. SHUMAKER, GORDON L. HODGSON Jr. and DAVID A. BRENT
Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, NC 27709 (U.S.A.)

SUMMARY

Reversed-phase high-performance liquid chromatography (HPLC) was used to measure the lipophilicities of a series of biphenyl acids and their non-acid precursors. Values obtained by HPLC for the precursors were as predicted from the calculated lipophilicity values. For the biphenyl acids, a systematic deviation in the correlation of calculated log P and log k' was observed (hydrogen-bond acceptors and non-hydrogen-bonding substituents described two separate lines in the correlaton). An explanation of these results is presented, based on steric effects which control the way the biphenyl acids interact with the stationary phase.

INTRODUCTION

Octanol–water partition coefficients (log P), as measured by the standard shake-flask method, are widely used in structure–activity studies. This method is time-consuming and limited by problems such as difficulty in measuring very high or low partitions, and variance in the results due to impurities, dissociation, decomposition, poor detectability, and emulsion formation. Despite these limitations, a large data base of measured values exists. In an effort to avoid the problems associated with the shake-flask method, calculation methods and chromatography have been used to measure lipophilicity. Chromatography avoids many of the problems and has the added advantage of being easily amenable to automation.

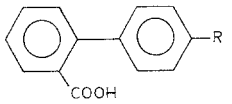
In this study, reversed-phase high-performance liquid chromatography (RP-HPLC) was used to measure the lipophilicities of a series of biphenyl acids (Table I) and their precursors (*p*-bromobenzene analogues containing substituents similar to those varied in the acid series, Table II). The goals of this study were: (i) to use the lipophilicity constants from HPLC and the calculated log P values independently to develop quantitative structure–activity relationships for the biphenyl acids and their precursors; (ii) to investigate the correlation between the HPLC constants and calculated log P values.

The first of these goals was unsuccessful. Neither HPLC nor calculated log P

0021-9673/87/$03.50

TABLE I

BIPHENYL ACIDS: SUBSTITUENTS AND LIPOPHILICITY DATA



Compound number	*Substituent*	*log k'*	*Calculated log P*
1	H	0.397	3.773
2	Cl	1.04	4.506
3	Br	1.19	4.656
4	OCH_3	0.693	3.736
5	S(*n*-hexyl)	3.56	6.996
6	SO(*n*-butyl)	1.44	3.815
7	SO(*n*-hexyl)	2.31	4.873
8	$SO_2N(C_2H_5)_2$	1.5	4.071
9	$SO_2N(C_2H_5)(C_3H_7)$	2.083	4.6
10	$SO_2N(C_3H_7)_2$	2.34	5.129
11	SCF_3	1.88	5.46
12	CH_3	0.922	4.422
13	C_2H_5	1.35	4.951
14	Isopropyl	1.85	5.35
15	Butyl	2.46	6.009
16	*tert.*-Butyl	2.21	5.749
17	Phenyl	2.18	5.661
18	CF_3	1.42	4.69
19	CHO	0.522	3.195
20	COOH	−0.109	3.561
21	$CO_2C_3H_7$	2.132	4.853

values was useful for predicting the activity of this series (the structure–activity study will be published later). However, a correlation between HPLC lipophilicity constants, and log *P* values was found for both acids and precursors, and some interesting results emerged from these correlations.

EXPERIMENTAL

The distinguishing features of our HPLC method are the highly deactivated stationary phase (made by further deactivation of Corasil C_{18}) and the mobile phase of pH and ionic strength similar to blood (taking into account the organic solvent effect on the mobile phase pH)[1].

Apparatus

The HPLC equipment consisted of an autoinjector (Micromeretics, Norcross, GA, U.S.A.), two high-pressure pumps (Waters Model M-45) and a variable-wavelength UV detector (Water Model 450, Waters Chromatography Division of Millipore, Milford, MA, U.S.A.). The columns were made of 316 SS (5 × 0.46 cm I.D.).

TABLE II

BROMOBENZENE PRECURSORS: SUBSTITUENTS AND LIPOPHILICITY DATA

Compound number	*Substituent*	*log k'*	*Calculated log P*
1	H	0.881	3.005
2	F	0.96	3.148
3	Cl	1.32	3.718
4	Br	1.44	3.868
5	OCH_3	1.08	3.064
6	SCH_3	1.43	3.648
7	$SOCH_3$	−0.02	1.1414
8	$SO_2N(C_2H_5)_2$	0.825	3.258
9	$SO_2(C_2H_5)(C_3H_7)$	1.55	3.787
10	$SO_2N(C_3H_7)_2$	1.94	4.316
11	C_2H_5	1.78	4.183
12	C_6H_5	2.35	4.893
13	CF_3	1.51	3.888
14	$CO(CH_2)_3CH_3$	1.868	4.107
15	$CO(C_6H_5)$	1.75	4.159
16	$CO_2C_2H_5$	1.46	3.579

Materials

In all experiments, HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA, U.S.A.), HPLC purified water (Hydro Services, Durham, NC, U.S.A.), and reagent-grade buffer salts (Mallinckrodt, Paris, KY, U.S.A.) were used.

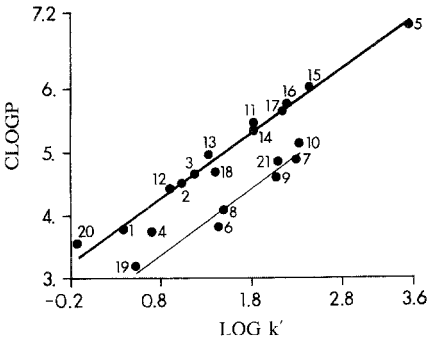
Procedures

Columns were packed by a tap-fill procedure with highly deactivated stationary phase [made by further deactivation of Corasil C_{18} (Waters)][1]. Mobile phases were of pH and ionic strength similar to blood (taking into account the organic solvent effect on the mobile phase pH)[1].

For all experiments the flow-rate was 2 ml/min, detection was at 254 nm, and injection volumes were 10 μl. All mobile phases were filtered through 0.45 μm filters (Millipore, Bedford, MA, U.S.A.).

Capacity factors, k', were the average of three measurements. The retention of an injection of methanol was used to determine the void volume.

The mobile phase for the biphenyl acid series [methanol–buffer (1:4)] was prepared from 1.6123 g $NaH_2PO_4 \cdot H_2O$ and 11.7620 g $Na_2HPO_4 \cdot 7H_2O$. The phosphates were dissolved in 800 ml water, and 200 ml of methanol was added. The apparent pH of the resulting solution was 7.79. The calculated ionic strength was 0.15 molal. The mobile phase for the *p*-bromobenzene series [methanol–buffer (2:3)] was prepared from 1.6075 g $NaH_2PO_4 \cdot H_2O$ and 11.1945 g $Na_2HPO_4 \cdot 7H_2O$. The phosphates were dissolved in 600 ml water, and 400 ml methanol was added. The apparent pH of the mobile phase was 8.14, the calculated pH 7.40. The calculated ionic strength was 0.15 molal.



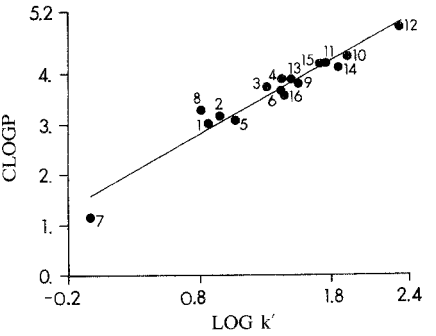


Fig. 1. Biphenyl acids: correlation of calculated log P (CLOGP) with log k' (LOG k'). Curve 1 (thin line) is the correlation of hydrogen-bond acceptors. Curve 2 (heavy line) is the correlation for non-hydrogen bonders. Regression data are in Table III.

Fig. 2. p-Bromobenzene precursors: correlation of calculated log P (CLOGP) with log k' (LOG k'). Regression data are in Table III.

Calculated log P values were determined using the CLOG P program, Version 3.3. Structures were entered with the SMILES program (both Ponoma Medicinal Chemistry Project, Ponoma College, Claremont, CA, U.S.A.). Conformational analysis and energy minimization of the biphenyl acid structure model were carried out using Macro Model (Clark Still, Columbia University, New York, NY, U.S.A.).

RESULTS AND DISCUSSION

A comparison of the data plots for the biphenyl acids (Fig. 1) and their p-bromobenzene precursors (Fig. 2) shows a striking difference. While a single line described the correlation of log k' with log P for the p-bromobenzene series, two lines best described the same correlation for the acid series. For the biphenyl acids, there is a separation between hydrogen-bond acceptors (curve 1) and non-hydrogen-

TABLE III

LINEAR REGRESSION DATA FOR THE CORRELATIONS OF CALCULATED LOG P AND LOG k'

r = correlation coefficient; s = standard deviation; F = overall F test for the correlation, n = number of compounds.

Series	*Calculated for log P = a log k' + b*					
	a	*b*	*r*	*s*	*F*	*n*
Biphenyl acids						
Curve 1	1.0386	2.5350	0.9800	0.1514	121.27	7
Curve 2	1.0194	3.434	0.9873	0.1606	464.45	14
Overall	0.9511	3.2538	0.8787	0.4499	64.38	21
p-Bromobenzene precursors						
Overall	1.4449	1.616	0.9695	0.2102	218.96	16

bonding substituents (curve 2). No hydrogen-bond donors were studied. Note that a dicarboxylic acid (Table I, compound 20) was included in the correlation. Under our chromatographic conditions, this compound is a dianion and should act as a hydrogen-bond acceptor. However, its log P was calculated for the neutral molecule. Lowering the calculated log P by four log units to correct for the anion substituent would place this point below the hydrogen-bond acceptor line. This result would be expected for a strong hydrogen-bond acceptor, such as an anion.

Table III gives the linear regression data for the correlations. In comparing the overall regression with the regression for fitting two lines to the data, the null hypothesis of equality of variances is tested by $F_0 = s_1^2/s_2^2$, where $s_1 \geqslant s_2$. F_0 has an F distribution with $n_1 - 1$ and $n_2 - 1$ degrees of freedom. The null hypothesis is rejected if $F_0 > F_{0.99}\ (V_1, V_2)$, where $F_{0.99}\ (V_1, V_2)$ is the appropriate critical value; *i.e.* prob$[F > F_{0.99}\ (V_1, V_2)] \leqslant 0.01$. Using this criterion, the fit of the individual curves is significantly better (to the 99% confidence level) than the fit of the overall correlation. When substituents similar to those used for the biphenyl acid series are para substituted on bromobenzene a significantly better fit results when a linear regression is used to fit all of the data to a single line, rather than two lines.

Several authors have reported systematic deviations in the correlation of HPLC data with log P. Unger *et al.*[2] have reported observing two lines in correlations comparing retention on an octanol-coated C_{18} column with an octanol-saturated aqueous mobile phase with the retention on a C_{18} column with a methanol–water mobile phase. The compounds studied were tuberin [N-(β-styryl)formamide] analogues. The substituents could be divided into non-hydrogen-bonding and hydrogen-bonding [*i.e.* SO_2PH, OCH_3, $N(CH_3)_2$] groups. As with our data, the hydrogen-bonding compounds (all hydrogen-bond acceptors) appeared more lipophilic by HPLC than by log P. However, Unger *et al.* found that a single line described the correlation of measured log P with retention data obtained on the octanol-coated C_{18} system. Therefore, one would expect two lines in correlations of log P with HPLC data from the methanol–water system.

Haky and Young[3] reported a second line for a series of phenolic calibration standards when they were compared to non-phenolic compounds. These compounds (which are strong hydrogen-bond donors) were predicted to be less lipophilic by HPLC than by log P. Haky and Young[3] and Davis[4] explain this deviation by hydrogen bonding with octanol. Yamagami *et al.*[5] showed the same sort of deviations for phenolic compounds and related this to the relative basicity of the methanol compared to octanol. Addition of tetrahydrofuran (THF) (a stronger base than methanol) to the mobile phase eliminates the deviation of the phenols by increasing their retention. Yamagami *et al.*[5] attribute this effect to the stronger hydrogen-bond acceptor properties of THF and the higher concentration of THF in the stationary phase[6]. While the first effect alone would tend to increase the solvation of the solute in the mobile phase and thus decrease retention, the increased possibility of such interactions with the THF in the stationary phase leads to longer retention.

Leo *et al.*[7] demonstrated that correlations between partition coefficients measured in apolar solvents *versus* water and those measured in octanol *versus* water were best described by dividing solutes into classes according to hydrogen-bonding characteristics. They found that strong hydrogen-bond donors were "minus deviants" and hydrogen-bond acceptors were "plus deviants". Examination of our data

and that of Unger *et al.*[2] suggests that hydrogen-bond acceptors may be further separated into groups (one for substituents like OCH_3, O-*tert.*-butyl etc. and another for SO_2CH_3, SO-*n*-butyl etc.), according to the strength of the interaction. This is supported by published data on hydrogen-bond acceptor strength[9].

While systematic deviations are reported for hydrogen-bond donors and acceptors as compared to non-hydrogen-bonding substituents, our data show this sort of selectivity for the biphenyl acids but not for the *p*-bromobenzene compounds. Differences in selectivities between these solute classes can be explained by differences in the effects of induction on the hydrogen-bond accepting capabilities of the substituents. The two functionalities [4-bromo compared to 4-(2-carboxyphenyl)], create different electronic environments for the substituents. In the *p*-bromobenzenes, induction would decrease the electron density of the substituents, and therefore decrease the hydrogen-bond accepting capabilities, making one line for all compounds more likely. This electronic effect could be the cause of the lack of selectivity for the *p*-bromobenzenes. By contrast, the narrow range of pK_a values and the lack of correlation of pK_a with sigma values (Table IV) indicate that there are no major inductive effects for the biphenyl acids; therefore, induction cannot be the cause of the two lines observed in the correlation.

The size and shape of molecules also contribute to the type of interaction which occurs with the stationary-phase surface, and could explain the differences in selectivity observed between the biphenyl acids and the *p*-bromobenzene compounds. Lochmuller *et al.*[8] have reported that a bonded phase of chain length greater than C_{12} is required to solvate a molecule the size of benzene in the stationary phase. The larger size of the biphenyl acids may restrict complete solvation of these molecules by the stationary phase, allowing the hydrogen-bond acceptor substituents to participate in the retention in a way different from that of the apolar substituents. The

TABLE IV

COMPARISON OF pK_a VALUES AND SIGMA *PARA* VALUES FOR THE BIPHENYL ACIDS

Substituent	*Sigma* para*	*pK_a***
OCH_3	−0.27	3.50
CH_3	−0.17	3.78
C_2H_5	−0.15	3.94
Cl	0.23	3.29
Br	0.23	3.65
CO-*n*-butyl	0.48***	3.45
COC_6H_5	0.43	3.30
COOH	0.45	2.30, 4.68
CF_3	0.54	3.83
$SO_2N(C_3H_5)_2$	0.57§	3.69
$SO_2N(C_3H_7)_2$	0.57§	4.26

* From ref. 10.
** Measured in our laboratory.
*** Value for COC_2H_5.
§ Values for SO_2NH_2.

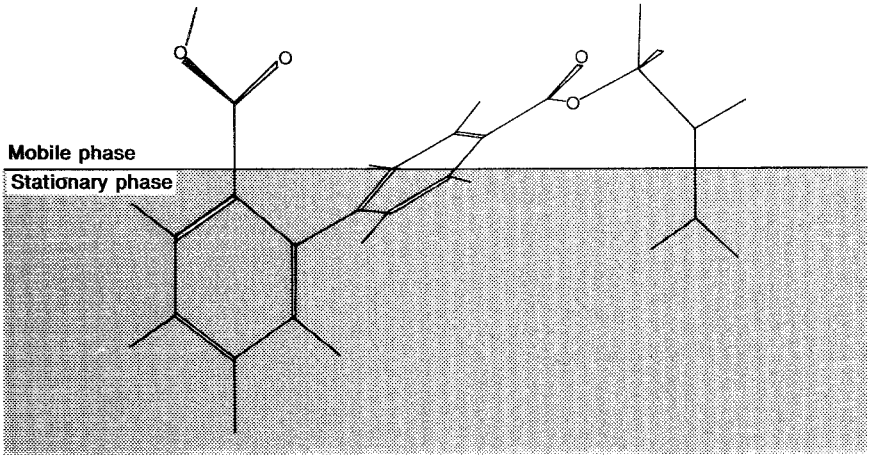


Fig. 3. An energy-minimized conformation of a biphenyl acid interacting with the stationary phase (substituent, CO_2-*n*-propyl).

smaller *p*-bromobenzene compounds would be completely solvated by the stationary phase, allowing no differentiation between hydrogen-bonding and non-hydrogen-bonding substituents. The ability of the stationary phase to solvate one class of solutes completely and not another, due to size restrictions, may account for the selectivity differences observed for this HPLC system.

Perhaps more significantly, the shape of the biphenyl acids (Fig. 3 shows an energy-minimized conformation of the molecule) driven by the position of the carboxylate, better explains why hydrogen-bond acceptors were differentiated in the correlation for the biphenyl acids and not for the *p*-bromobenzenes. The biphenyl acids contain a carboxylic acid group that is anionic under these HPLC conditions. Solvation of this group by methanol and water molecules would force this portion of the molecule to remain in contact with the mobile phase. This in turn, places the hydrogen-bond acceptors substituted on the other ring in a position to accept hydrogen-bonding interaction with methanol molecules coating the surface of the stationary phase (see Fig. 3 for a representation of this orientation in the stationary phase). This interaction would be absent from biphenyl acids with apolar substituents, and would explain the longer retention for the hydrogen-bond acceptors.

CONCLUSION

These results show that correlating lipophilicities measured by HPLC with octanol–water partition coefficients may not be straightforward, for some structural types, because other effects (*e.g.* hydrogen bonding) also influence the data. Experiments are currently being performed to test the proposals advanced in this paper to explain the deviations of the correlation.

REFERENCES

1 D. A. Brent, J. J. Sabatka, D. J. Minick and D. W. Henry, *J. Med. Chem.*, 26 (1983) 1014.

2 S. H. Unger, J. R. Cook and J. S. Hollenberg, *J. Pharm. Sci.*, 67 (1978) 1364.
3 J. E. Haky and A. M. Young, *J. Liq. Chromatogr.*, 7 (1984) 675.
4 S. S. Davis, *J. Pharm. Pharmacol.*, 25 (1973) 982.
5 C. Yamagami, H. Takami, K. Yamamoto, K. Miyoshi and N. Takao, *Chem. Pharm. Bull.*, 32 (1984) 4994.
6 R. M. McCormick and B. L. Karger, *Anal. Chem.*, 52 (1980) 2249.
7 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
8 C. H. Lochmuller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
9 R. W. Taft, D. Gurka, L. Joris, P. von R. Schleyer and J. W. Rakshys, *J. Am. Chem. Soc.*, 91 (1969) 4801.
10 C. Hansch and A. J. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, John Wiley, New York, 1979.