DETERMINATION OF PARTITION COEFFICIENT OF BENZO[*b*]THIOPHENES BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract: The octanol-water partition coefficients (log P) for a series of benzo[*b*]thiophenes were estimated by high performance liquid chromatography on a C_{18} reversed-phase column, using methanol-water as mobile phase. Measured values for the benzo[*b*]thiophenes ranged from 2.08 to 4.18. The experimental log P values showed good correlation with those predicted by the Dixon's method.

Introduction

The octanol/water partition coefficient (log P), as a measure of the hydrophobicity of a compound, plays an important role in many biological processes. Based on the extensive work of Hansch's group (1,2), it is now generally accepted that log P values are particularly suitable for characterizing the interactions between chemical substances and biological systems. Thus, it finds numerous applications in quantitative structure-activity relationship (QSAR) studies (3-8).

Reversed-phase high-performance liquid chromatography has increasingly been used as an alternative method for a rapid measurement of the hydrophobicity of bioactive compounds (9-13). Chromatographic experiments have many practical advantages over the direct determination of partition coefficients, i.e. small amounts of materials are required, impurities can be separated during the measurements, there is no need for concentration determination, and the process can be easily automated (14).

Evaluation of the partition coefficients of organic compounds by HPLC correlation is best performed under conditions which maximize the similarities in the chromatographic process and the partitioning mechanism occurring in a biphasic octanol-water system (15-17). Although this ideally requires employing a totally aqueous mobile phase in the HPLC system, the excessively long retention times which would be obtained for lipophilic compounds under these conditions make such an approach impractical.

Several researchers have advocated the extrapolation of retention data obtained at many mobile phase compositions to obtain capacity factors at totally aqueous conditions for log P estimation (18,19). Although many successful examples associated with this log k_w approach have been reported (20-22), the multiple experiments required for this approximation drastically increases the time required for each log P determination, and thus makes this unattractive for the rapid estimation of the log P's of large numbers of compounds.

It should be noted that although the partition mechanism between the stationary phase and the mobile phase is complex, the extensive studies so far reported (23,24) show that, with methanol-water as eluent, the hydrogen-bonding behavior of the HPLC system is similar to that of the octanol-water system and the logarithm of the capacity factor (log k') values are correlated with log P values by a Collander-type equation 1 where a and b are empirical constant which characterize the solvent system (25,26).

$$\log P = a \log k' + b \qquad (1)$$

As part of our efforts to obtain benzo[*b*]thiophenequinones as potential antiprotozoal agents (27-29), benzo[*b*]thiophenes **1-14** were synthesized in our laboratory (30), *via* heterocyclization of o-nitrobenzaldehydes with methyl thioglycolate in basic media (31,32).

Since lipophilicity could play an important role in the biological activity of benzothiophene-containing biological active compounds and no precedents regarding this structural property have been reported, we decided to evaluate this parameter on benzo[*b*]thiophenes **1-14**. Here we wish to report results on the evaluation of the partition coefficients of benzo[*b*]thiophenes **1-14** by reversed-phase high performance liquid chromatography and their correlation with the lipophilicity predicted by the methods of Ghose-Crippen and Dixon.

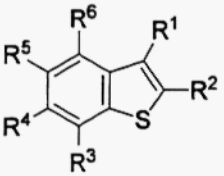
Experimental

Materials:

All the solvents and chemical substances used were analytical grade. Benzo[*b*]thiophenes **1-14** (Table 1) were synthesized by using the Beck's method (27,31,32) and standard organic group transformations. Analytical samples of the heterocycles were obtained by column chromatography on silica gel followed by recrystalization.

HPLC-grade methanol (Fisher Scientific, Fair Lawn, NJ,USA) and distilled-deionized water (Millipore) were used. All the samples were dissolved in methanol at 1mg/mL.

Table-1: *Benzo[b]thiophenes prepared for the lipophilicity evaluation*



Benzothiophene	R^1	R^2	R^3	R^4	R^5	R^6
1	H	CO_2Me	H	H	H	H
2	H	CH_2OH	H	H	H	H
3	H	CO_2Me	H	H	Cl	H
4	H	CH_2OH	H	H	Cl	H
5	H	CHO	H	H	Cl	H
6	H	$CH=N\text{-}NMe_2$	H	H	Cl	H
7	H	CO_2Me	H	NO_2	H	H
8	Br	CHO	H	H	Cl	H
9	Br	CH_2OH	H	H	Cl	H
10	Br	$CH=N\text{-}NMe_2$	H	H	Cl	H
11	H	CH_2OH	OMe	H	H	OMe
12	H	CHO	OMe	H	H	OMe
13	H	CO_2Me	OMe	H	H	OMe
14	NH_2	CO_2Me	OMe	H	H	OMe

Apparatus:

All retention measurements were made using a Merck Hitachi System equipped with an L-6250 intelligent pump and a Lachrom L-7420 model UV-VIS detector. Samples injection was performed using a Rheodyne model 7251 injector fitted with a 20 µl sample loop. Chromatographic data were recorded and processed on a Star Chromatography Workstation (Varian Associates,Inc. Version 4.51). The stationary phase was a C_{18} column (Supelco, 250mmx4.6 mm i.d,. 5µ particle size). The mobile phase was a mixture of methanol and water (70:30). The flow rate was set at 1mL/min and the pH was adjusted to 3.0 with acetic acid to avoid the effect of acid dissociation of the samples.

Procedures:

Sixteen compounds (Table 2) of widely varying functionality and structure types were used as standards in the determination of log P values. The capacity factors (k') for benzo[*b*]thiophenes and standards were calculated using equation 2 where t_R is the sample retention time and t_0 is the retention time of an unretained substance, determined by methanol

$$k' = (t_R - t_0)/t_0 \quad (2)$$

The correlation between the partition coefficient of the samples and their capacity factor was determined using equation 1. The partition coefficient values were calculated using the Ghose (33) and Dixon´s methods implemented in the Spartan package (34) (Table 3).

Table-2: *Reported octanol-water partition coefficients (log P)* and experimental capacity factors (log k').*

Compound	log P	log k'	Compound	log P	log k'
Benzyl alcohol	1.10	-0.535	*p*-Nitrochlorobenzene	2.41	0.141
Phenylacetic acid	1.41	-0.449	Toluene	2.69	0.332
Phenol	1.46	-0.541	Chlorobenzene	2.84	0.299
Benzoic acid	1.87	-0.409	p-Dichlorobenzene	3.39	0.536
Anisole	2.11	0.006	Azobenzene	3.82	0.464
Benzene	2.13	0.053	Biphenyl	4.04	0.688
Salicylic acid	2.26	-0.344	Phenyl ether	4.21	0.705
p-Nitrotoluene	2.37	0.026	Anthracene	4.45	0.989

(*) Literature log P values were taken from reference 2.

Results and Discussion:

The retention values (log k') of the standards eluted under our experimental conditions were plotted against the reported log P values to examine the accuracy of the correlation between both parameters. It can be described by equation 3:

$$\log P = 2.067 \log k' + 2.406 \quad (3)$$
$$n = 16 \quad r^2 = 0.956 \quad s = 0.03$$

In order to obtain a better correlation the application of Prodigy ODS column (Phenomenex, 250 mmx4.6 mm i.d,. 5µ particle size) was evaluated. These columns are fully end-capped to suppress residual silanol groups influences. However, the relatively low regression coefficient achieved ($r^2 = 0.91$, data no shown) suggest that different partitioning behavior between C_{18} and ODS column may be caused by differences other than those caused by analyte interactions with silanol groups of the stationary phase of C_{18}. We consider that equation 3 can be regarded as a good Collander type equation, therefore a C_{18} column appears suitable for the benzo[*b*]thiophenes log P prediction.

The experimental log P values were compared with those calculated using the Ghose and Dixon's method (Table 3.). The Dixon method ($r^2 = 0.72$) has a reasonably consistent variability throughout the log P range studied (2.08-4.18), it also shows that this is not the case for the Ghose-Crippen method. The fact that the later method underestimates all the values measured by HPLC indicates that it is not appropriated for the prediction of log P values of benzo[*b*]thiophene structural related with **1-14**.

Table-3: Experimental and calculated log P values.

Benzothiophene	log P_{exp}	log P_{Ghose}	log P_{Dixon}	Benzothiophene	log P_{exp}	log P_{Ghose}	log P_{Dixon}
1	3.21	1.74	3.13	**8**	3.89	1.91	4.05
2	2.08	1.35	2.68	**9**	3.60	2.84	4.16
3	3.81	2.30	3.68	**10**	4.54	2.56	4.09
4	2.71	1.91	3.19	**11**	2.11	1.10	2.54
5	2.94	1.77	3.13	**12**	2.75	0.96	2.76
6	4.18	2.43	3.29	**13**	-	1.49	3.64
7	3.06	1.78	3.47	**14**	2.97	-0.23	2.46

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