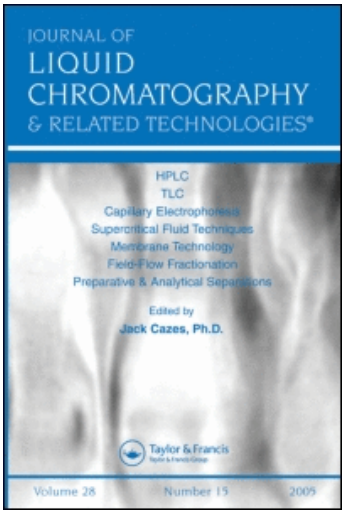

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DETERMINATION OF OCTANOL-WATER PARTITION COEFFICIENTS FOR A SERIES OF IMIDAZOLIDINEDIONES BY A NOVEL COMBINATION OF MICRO SHAKE-FLASK AND HPLC TECHNIQUES

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ABSTRACT

Octanol-water partition coefficient (Log P) values for a series of 19 imidazolidinedione analogues have been determined by high performance liquid chromatography. To obtain a regression equation between log P and chromatographic log k' values, a modified micro shake-flask method has been developed. In the shaking step, a simple syringe and test tube system was devised and has proved sufficient to ensure the proper distribution of analytes between the two phases. In addition, HPLC was used not only as a detector for the shake-flask method, but also as a technique for determining the capacity factors of the compounds. Further technique modifications include alternatives to the use of octanol and dimethyl sulfoxide (DMSO) in HPLC thereby eliminating the detrimental effect of these solvents on the stationary phase. Our micro shake-flask method is a modified version of the technique developed by Ford et al. which used a Mixxor-separator device for distribution of solute and an HPLC apparatus for the determination of the ratio of solute concentrations in the two phases [H. Ford, Jr., C. L. Merski, and J. A. Kelley, J. Liq. Chromatogr., 14, 3365, 1991]. Finally,

calculated log P values for this imidazolidinedione series have also been determined and compared to experimental data. The relationship between lipophilicity and anticonvulsant activity for the molecules has thus been examined.

INTRODUCTION

The lipophilicity of a chemical compound is the single most important correlate in quantitative structure-activity relationship (QSAR) studies [1]. The most widely used measure of lipophilicity is the octanol-water partition coefficient, expressed as log P [2]. Experimental log P values can be determined either by the time-consuming shake-flask method or by a reversed-phase HPLC technique [3,4]. Although the HPLC determination is the more efficient of the two methods, it requires a reference system which is based on log P values determined by the classical shake-flask technique. Ford and coworkers introduced a rapid and simple microscale method, in which the conventional shake-flask was replaced by a Mixxor apparatus [5]. This technique requires only a 10 μg sample, making the distribution faster and more efficient. Since Ford et al used HPLC instead of other conventional methods to determine the contents of the two phases, the largest source of error in the experiment was eliminated. In our study, their technique was adopted for the shake-flask method. However, HPLC was used not only as a detector for the shake-flask method, but also as a technique for determining the capacity factors of the compounds. To our knowledge, this useful combination of the two techniques has not been previously reported. Other modifications to the micro scale method of Ford et al have also been included either to simplify equipment requirements or to alleviate other problems inherent in the use of some HPLC solvents.

Imidazolidinediones (hydantoins) constitute an important class of anticonvulsant compounds. It is well known that a correlation exists between the log P values of anticonvulsants and their activities [6]. However, no similar relationship has been demonstrated for hydantoins, despite numerous SAR studies for these compounds [7-9]. To examine whether the same relationship holds true for the hydantoin subclass of anticonvulsants, we synthesized and determined the activity of 19 structurally related hydantoin derivatives. Their log P values were determined by both experimental and theoretical methods.

The purpose of this study was to develop a novel combination of micro shake-flask and HPLC techniques for the experimental determination of log P. Hydantoin anticonvulsant compounds were used to validate our novel method for log P determination. The relationship between hydantoin biological activities and log P values was also investigated.

MATERIALS

All hydantoin analogues were synthesized by standard methods and were characterized fully by ^{1}H, ^{13}C NMR, IR. The C5 monosubstituted hydantoins were prepared by the reaction of an appropriate α-amino acid with potassium cyanate [10]. Analogues having benzyl substitution at N3 were synthesized by benzylation using benzyl bromide [11]. The 3-ethyl and 3-phenyl analogues were obtained by the reaction of an appropriate alkyl or aryl isocyanate with an α-amino acid [12]. The C5 disubstituted compounds were produced by the Bucherer Bergs synthesis [13]. All chemicals and solvents were purchased from Aldrich or BDH as HPLC grade materials. Anticonvulant activities were measured using the picrotoxin method [14].

EXPERIMENTAL

A BECKMAN System Gold Module 126 liquid chromatograph equipped with a BECKMAN System Gold UV Module 166 detector operating at 215 nm was used for the analysis. The HPLC system was controlled by a NEC PC-8300 computer. The stationary phase was VYDAC ODS RP-C18 (4.6 mm x 250 mm, 5 μ, 300 Å) and the mobile phase was comprised of 30 (methanol):70 (aqueous at pH 2) v% mixture; the aqueous component is a 0.1 v% solution of trifluoroacetic acid (TFA). The flow rate was set to 1 mL/min. All experiments were performed at room temperature (23-25 C). Approximately 1 μg of each compound was dissolved in 0.5 mL pH 2 TFA solution. A 25 μL aliquot of this solution was injected onto the HPLC column by a 100 μL Hamilton 802 chromatographic syringe. The column dead time t_0 was measured as the first distortion of the baseline after injection of denaturated methanol (t_0= 2.01 min.).

TABLE 1. Experimental Parameters for Micro Shake-Flask Analysis.

Compounds	A (μL)	B (μL)
Hydantoin	300	300
5,5-diphenylhydantoin*	10	1000
3-methyl-5,5-diphenylhydantoin*	10	1000
5-methylhydantoin	300	300
5,5-dibenzylhydantoin*	10	1000
5-benzylhydantoin	300	300
3,5-dibenzylhydantoin*	10	1000

* These compounds were dissolved in MeOH instead of buffer (pH 7) at the beginning of the micro shake-flask method.

Experimental log P values were determined using a modified micro shake-flask method [5]. For each anticonvulsant, a 5 mg/mL solution was prepared in either a pH 7.0 potassium phosphate aqueous buffer or methanol (see Table 1). A 20 μL aliquot from this buffer was dissolved in 1.0 mL octanol-saturated pH 7.0 potassium phosphate buffer or methanol (Table 1). Then 1.0 mL buffer saturated n-octanol was added. To achieve solute distribution we departed from the previously described mixer apparatus of Ford at al. and employed a simple glass syringe and test tube system. This distribution was effected by applying 30 piston strokes of the glass syringe to the two phase mixture with repetitive ejection into and recovery from a glass test tube. After 15 mins, the two phases were separated, and each phase was transferred to a 1.5 mL Eppendorf microcentrifuge tube. They were centrifuged in a Hettich benchtop centrifuge at 2500xg for 5 mins. From the octanol phases, A mL (Table 1) was transferred into a centrifuge tube. This tube was placed in a LABLONCO Freeze Dry-50 lyophilizer connected to a SAVANT Speed Vac concentrator, and the octanol was removed. The dry compound was dissolved in B μL (Table 1) of a pH 7.0 buffer (henceforth this phase will be referred to as the "octanol" phase). 25 μL aliquots from both phases were injected into an HPLC chromatograph using a 25 μL loop injector. The relative concentration of the samples in each phase was then determined by an HPLC analysis. The chromatographic conditions were the same as described above. The P

value was obtained from the ratio of peak areas in the "octanol" and buffer phases, respectively. Three independent log P measurements were performed for each sample.

RESULTS AND DISCUSSION

Ford and coworkers used a mixer apparatus for partitioning the sample between octanol and water [5]. In our study, this apparatus was replaced by an ordinary 5 cm^3 glass syringe and a 10 cm^3 glass test tube. Log P results obtained using this simple equipment showed consistency and reproducibility as demonstrated by the standard deviations in log P's (see Table 1). This simple and inexpensive test tube and syringe system permits anyone with access to HPLC equipment to use this reliable micro shake-flask method for log P determination.

A major problem with the HPLC detection of analytes in the shake-flask technique arises if the UV absorption peak of the solute coincides with that of octanol. Unfortunately, this is the case for hydantoin compounds, whose λ_{max} values are close to 215 nm. This problem was solved by removing octanol from the 10 μL octanol phase aliquot containing the equilibrated solute by lyopilization. The resultant dry analyte was redissolved in a large amount (1000 μL) of pH 7 buffer to overcome precipitation of the analyte in the aqueous buffer. Henceforth this phase become the "octanol phase". The lyophilization of octanol also eliminated the need to wash the column regularly to remove detrimental octanol that would lead to peak distortions. If the UV absorption of the solute interferes with that of octanol, this procedure offers an excellent way of circumventing this problem.

We have also avoided using dimethyl sulfoxide (DMSO) as a preliminary solvent because its application would lead to more serious peak distortions in the chromatogram than those caused by octanol. Instead, we used a pH 7 buffer or methanol for less or more hydrophobic compounds, respectively.

In the HPLC technique, log k' is usually determined by either the isocratic or polycratic approach. In the isocratic approach, log k' is measured at a certain eluent composition, whereas in the polycratic approach log k'_{ϕ} values are determined at several ϕ, and the results are extrapolated to 100% water content. As opinions are divided as to the advantages and disadvantages of the two approaches, the isocratic method was used in this study. A 30% methanol solution

was employed as the mobile phase because this composition gave the most practical retention times. The advantages of using methanol as an organic modifier as opposed to other modifiers are well known [15]. The pH of the aqueous eluent was set to 2 to ensure that all compounds are fully protonated.

Table 2 shows the compounds with their measured and calculated log P values; bioactivities on a scale of 0 through 4 are also included. Five molecules which reflected the full spectrum of retention times were chosen as standards. For these five compounds, both the micro shake-flask log P and the HPLC log k' values were determined. For the remaining compounds, only their log k' values were determined; their log P's were obtained from the following regression equation:

$$\log P = a \log k' + b$$
$$(n=5,\ s=0.09,\ r^2=0.998)$$

As statistically shown, the correlation between log P and log k' is excellent for those five molecules. As a further check on the validity of our procedure, the obtained log P values for hydantoin and phenytoin (both were part of the standard set) were compared to earlier experimental data. In the literature, several log P's are available for phenytoin in the 2.23-2.47 range [16-18], and they compare well with our value of 2.27. Similarly, the hydantoin log P of -1.63 from this study is very close to the former value of -1.69 [18].

A regression analysis was also carried out between the measured and calculated log P values according to the following equation:

$$\log P = a\ \mathrm{ALOGP} + b$$
$$(n=19,\ s=0.06,\ r^2=0.95)$$

The two most conspicuous outliers are 3-phenylhydantoin and 1-methyl-3-hydantoin (Table 2). The structural similarity between these compounds suggests that some parameters associated with the N^3-C_{benzyl} link do not correctly take the electronic effects into consideration, thereby overestimating true log P values. (3-benzyl-5-phenylhydantoin possesses a somewhat high log P, although this is not out of line with the precision of the method [15]). Excluding those two outliners, one arrives at an improved r^2 value of 0.97.

TABLE 2.

Compounds	t_R (min)	log k'	log P	ALOGP	A‡
hy†	2.53	-0.69	-1.53, -1.63±0.01*	-1.18	0
5,5-diphenylhy	36.17	1.21	2.28, 2.29±0.01*	2.14	4
1-methyl-3-phenylhy	5.04	0.15	0.14	0.95	1
3,5-dibenzylhy	67.03	1.49	2.84	2.70	0
5-benzyl-3-phenylhy	35.86	1.21	2.27	2.71	0
3-ethyl-5-benzylhy	18.05	0.88	1.61	1.39	0
3-methyl-5,5-diphenylhy	70.64	1.51	2.88	2.38	3
5-isopropylhy	3.76	-0.10	-0.36	0.02	1
5-isobutylhy	5.84	0.25	0.35	0.44	1
5-methylhy	2.94	-0.40	-0.95, -0.86±0.02*	-0.86	0
3-benzylhy	8.53	0.49	0.82	0.79	2
5-phenyl-3-ethylhy	10.43	0.60	1.05	1.11	0
5,5-dimethylhy	3.19	-0.28	-0.72	-0.64	0
5-phenyl-3-methyl-5 ethylhy	19.45	0.92	1.69	1.73	2
3-phenylhy	4.21	0.00	-0.15	0.72	2
5-benzylhy	6.01	0.27	0.39, 0.44±0.02*	0.81	0
5-phenylhy	4.45	0.05	-0.05	0.53	3
5,5-diphenyl-2S-hy	47.81	1.34	2.53	2.69	3
5,5-dibenzylhy	44.22	1.30	2.46, 2.41±0.01*	2.78	0

* Log P's were measured by the micro shake-flask method.

† hy = hydantoin. ‡ Anticonvulant activities were measured using the picrotoxin method [14].

Regression analysis reveals no correlation between the anticonvulsant activities and the experimental log P values for the series (see eqn. (3)):

$$\log (\text{activity}) = a \log P + b$$

($n=19$, $r^2=0.13$)

This finding is in contrast to the strong dependence of general anticonvulsant activity on their log P values [6]. A possible explanation is that hydantoin binding to the receptor site is a specific geometric process. This is supported by the fact that whereas 5,5-diphenyl substitution seems necessary for high activity, the 5,5-dibenzyl compound is inactive.

CONCLUSION

A novel combination of HPLC measurement of log P values and the use of HPLC as a detection technique in a micro shake-flask method has been introduced and validated on a series of 19 hydantoin analogues. It is hoped that the technique described here will find widespread application due to its simplicity and reliability. No correlation between the activities and log P values for the series anticonvulsant compounds has been found.

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