J. Pharm. Pharmacol. 1995, 47: 345–347
Received April 18, 1994
Accepted December 6, 1994

Determination of Octanol-water Partition Coefficients by an HPLC Method for Anticonvulsant Structure-activity Studies

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Abstract

Octanol-water partition coefficients have been measured and calculated for eight clinically relevant anticonvulsants.

For some compounds, these are the first experimentally available log P values. For the remainder of the molecules, new values have been suggested. The utility of the micro shake-flask experimental method and the ALOGP calculational method in determining anticonvulsant log P values has been demonstrated.

The discovery of new anticonvulsant drugs is a continuing neuropharmacological priority. To aid in the design of new active compounds, quantitative structure-activity relationship (QSAR) studies are routinely employed (Fauchere 1989). As the lipophilicity of a drug is a crucial factor in determining its bioavailability to the receptor microenvironment, the octanol-water partition coefficient, expressed as log P, plays a central role in anticonvulsant QSAR (Lien et al 1975; Hopfinger 1985).

Up to 1960, anticonvulsant drug design was dominated by cyclic ureides (barbiturates, phenytoin), acyclic ureides (phenacemide) and cyclic amides (phenylethylmalonamide). By the 1980s, two new clinically important anticonvulsants had been introduced (carbamazepine, valproate), and in the 1990s additional novel anticonvulsant molecules (clobazam, vigabatrin) are emerging. Despite the fundamental role of log P in anticonvulsant QSAR studies, no consistent experimental technique has been applied to this diversity of molecular structures.

The goals of this study were to apply the micro shake-flask log P determination method to a series of eight structurally diverse anticonvulsant molecules and to compare these results with other experimental determinations of log P.

Materials and Methods

For experimental log P measurements, a micro shake-flask method was employed. The method, introduced by Ford et al (1991), is rapid, reliable, and requires only small quantities of the analyte. Coupled with an HPLC detection technique, it has provided reliable results in earlier studies (Henczi et al 1993, 1994). For calculation of log P, the atomic contribution method was used (Ghose et al 1988).

Materials

Valproic acid (Abbott, Chicago), clobazam (Hoechst, Germany) and Vigabatrin (Merrell-Dow, Kansas City) were acquired from pharmaceutical suppliers. All other compounds were purchased from Aldrich Chemical Co., Milwaukee. All solvents were HPLC grade and purchased from BDH, Toronto.

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Procedures

Experimental log P values were determined using a modified micro shake-flask method (Ford et al 1991; Henczi et al 1993, 1994). For each anticonvulsant, a 5-mg mL^{-1} solution was prepared in a pH 7·0 potassium phosphate buffer. A 20-μL aliquot from this buffer was dissolved in 1·0 mL octanol-saturated pH 7·0 potassium phosphate buffer. Buffer (1 mL) saturated with *n*-octanol was added and 30 piston strokes were applied to the solution in a 10-mL glass syringe. After 15 min, the two phases were separated, and each phase was transferred to a 1·5 mL Eppendorf microcentrifuge tube. Centrifugation was in a Hettich benchtop centrifuge at 2500 *g* for 5 min. From the octanol phases, A μL (Table 1) was transferred into a centrifuge tube. This tube was placed in a Lablanco Freeze Dry-50 lyophilizer connected to a Savant Speed Vac concentrator, and the octanol was removed. The compound remaining after octanol lyophilization was dissolved in B μL (Table 1) of a pH 7·0 aqueous buffer (henceforth this phase will be referred to as the octanol phase). Twenty-five microlitres of both phases was injected onto an HPLC chromatograph using a 25-μL loop injector. The relative concentration of the samples in each phase was then determined by HPLC analysis. The P value was obtained from the ratio of peak areas in the octanol and buffer phases, respectively.

HPLC conditions. HPLC analyses employed a Beckman System Gold Module 126 liquid chromatograph equipped with a Beckman System Gold UV Module 166 detector operating at 215 nm; a Hewlett Packard 3394A integrator was used for peak analysis. The HPLC system was controlled by an NEC PC-8300 computer. A Vydac ODS RP-18 (4·6 × 250 mm, 5 μ, 300 Å) column was employed as the stationary phase, whereas the mobile phase consisted of a methanol/0·1% aqueous trifluoroacetic acid (pH 2·0) mixture. The ratio of the two solvents in the mixture was varied

Table 1. Experimental parameters for HPLC analysis.

Compounds	Methanol : 1% trifluoroacetic acid	Retention (min)	A (μL)	B (μL)
Phenytoin	30 : 70	36·17	10	1000
Carbamazepine	45 : 55	9·62	10	1000
Phenacemide	45 : 55	4·22	300	600
Barbitone	45 : 55	3·72	300	600
Phenylethylmalonamide	30 : 70	4·46	10	1000
Valproic acid	60 : 40	8·02	10	1000
Clobazam	55 : 45	6·24	10	1000
Vigabatrin	20 : 80	3·04	300	300

depending on the solute (Table 1). The flow rate was fixed at 1·0 mL min^{-1}. All experiments were performed at room temperature (21°C).

Calculations

Log P values were calculated from the formula ALOGP = $\sum n_i a_i$, where n_i is the number of atoms of type i, and a_i is the corresponding atomic contribution to log P (Ghose et al 1988).

Results and Discussion

The measured and calculated log P values, together with literature data, are shown in Table 2. For phenytoin and barbitone, the agreement of the micro shake-flask method with literature data is excellent. There is only a slight difference for phenacemide. Moreover, our calculated value (0·66) for phenacemide is in excellent agreement with the measured log P. However, calculated values can only be used as rough guides to predict log P as they can differ from experimental values by as much as 0·5–1·0 log P units even for relatively simple compounds (Ghose et al 1988). With regard to clobazam, both our calculated ALOGP and the CLOGP (Hansch & Bjorkroth 1987) data for this compound suggest that the previously reported value of 0·95 is too low. Our measurement confirms this, even though the obtained value of 2·12 is 0·66 log P units below the ALOGP value. With regard to valproic acid, our results confirm the value of 2·75 as opposed to 0·204. For the remaining three compounds (carbamazepine, phenylethylmalonamide, vigabatrin), for which no literature data were available, the measured and calculated partition coefficients are in excellent agreement. For the vigabatrin ALOGP value, a −2·30 correction factor was included because of the zwitterionic state of that compound (Leo 1990). However, no correction factor was used for the log P values of phenylethylmalonamide and valproic acid, even though these compounds are partially dissociated at pH 7·0 in aqueous solution. Since the difference between the pH of the buffer and the pK_a of these two organic acids is well below 4 pH units (2·0 and 2·5 units for valproic acid and phenylethylmalonamide, respectively), the octanol phase consists of undissociated compounds in both cases (Leo et al 1971). This assumption is borne out by the agreement between log P and ALOGP values in both cases.

The pharmacodynamic interaction of an anticonvulsant drug with its neuronal receptor necessitates the ability of the drug to traverse the blood-brain barrier to ensure bioavailability to the receptor microenvironment. As demonstrated by Hansch & Bjorkroth (1987), log P is the single most important factor in determining the solubility properties of a drug and hence reaching brain receptor sites.

In this study, the applicability of the micro shake-flask method for the experimental log P determination of structurally diverse anticonvulsants has been demonstrated. Furthermore the capacity of ALOGP to provide reasonable log P values has likewise been demonstrated. Accordingly, these techniques may be employed in anticonvulsant QSAR studies.

Table 2. Experimental and calculated log P data for the eight anticonvulsant compounds.

Compounds	Literature		Present study	
	Experimental	Calculated[a]	Experimental	Calculated (ALOGP)[b]
Phenytoin	1·96[c], 2·23[d], 2·40[e], 2·47[e]	2·08[c]	2·29	2·14
Barbitone	0·61[c], 0·65[c]	0·64[c]	0·65	0·75
Valproic acid	0·204[f], 2·75[g]	2·72[j]	2·60	2·66
Phenacemide	0·87[e]	0·87[j]	0·66	0·66
Clobazam	0·95[c]	2·44[c]	2·12	2·78
Carbamazepine	—	2·18[h]	2·19	2·67
Phenylethylmalonamide	—	0·009[j]	1·81	1·65
Vigabatrin	—	−2·98[j]	−2·16	−2·21[i]

[a]CLOGP values calculated by the Hansch method (Leo et al 1971), [b]Henczi et al (1994), [c]Ghose et al (1988), [d]Leo (1993), [e]Tamura et al (1987), [f]Hansch & Leo (1979), [g]Fowler et al (1989), [h]Abbott & Achermpong (1988), [i]a factor of −2·30 was added to account for the zwitterionic structure (Hansch & Bjorkroth 1987), [j]values calculated by the MEDCHEM software.

Acknowledgements

The authors thank Drs Riopelle, Dostaler and Ross for technical advice and for access to HPLC equipment. We also thank Mr T. Hunter for providing access to the Hewlett Packard Integrator. We acknowledge the assistance of the referee in providing calculations with MEDCHEM software. D. F. Weaver acknowledges an Ontario Ministry of Health Care Scientist Award and an MRC operating grant.

References

Abbott, F. S., Achermpong, A. A. (1988) Quantitative structure-anticonvulsant activity relationships of valproic acid, related carboxylic acids and tetrazoles. Neuropharmacology 22: 875–879

Fauchere, J. L. (ed.) (1989) QSAR: Quantitative Structure-activity Relationships in Drug Design. Alan R. Liss Inc., New York

Ford, H., Merski, C. L., Kelley, J. A. (1991) A rapid microscale method for the determination of partition coefficients by HPLC. J. Liquid Chromatogr. 14: 3365–3386

Fowler, D. W., Eadie, M. J., Dickinson, R. G. J. (1989) Transplacental transfer and biotransformation studies of valproic acid and its glucuronides in the perfused human placenta. J. Pharmacol. Exp. Ther. 249: 318–323

Ghose, A. K., Pritchett, A., Crippen, G. M. (1988) Atomic physicochemical parameters for three dimensional structure directed quantitative structure-activity relationships III. J. Comp. Chem. 9: 80–90

Hansch, C., Bjorkroth, J. P. (1987) Hydrophobicity and central nervous system agents: on the principle of minimal hydrophobicity in drug design. J. Pharm. Sci. 76: 663–687

Hansch, C., Leo, A. J. (1979) Substituent Constants for Correlation Analysis in Chemistry and Biology. Wiley, New York

Henczi, M., Weaver, D. F., Torkos, K. (1993) Experimental measurement on partition coefficients by RP-HPLC for a glycine related peptide series. Danube Symposium, Budapest

Henczi, M., Nagy, J., Weaver, D. F. (1994) Determination of octanol-water partition coefficients for a series of imidazolidinediones by a novel combination of the micro shake-flask and HPLC techniques. J. Liquid Chromatogr. 17: 2605–2613

Hopfinger, A. J. J. (1985) Computer-assisted drug design. Med. Chem. 28: 1133–1139

Leo, A., Hansch, C., Elkins, D. (1971) Partition coefficients and their uses. Chem. Rev. 71: 525–554

Leo, A. J. (1990) Methods of calculating partition coefficients. In: Hansch, C., Sammes, P. G., Taylor, J. B., Ramsen, C. A. (eds) Comprehensive Medicinal Chemistry. Vol. 4, Pergamon Press, New York, pp 295–319

Leo, A. J. (1993) Calculating log P_{oct} from structures. Chem. Rev. 93: 1281–1306

Lien, G. J., Liao, R. C. H., Shinoda, H. G. (1979) Quantitative structure-activity relationships with dipole moments of anticonvulsants and CNS depressants. J. Pharm. Sci. 68: 463–465

Tamura, A., Kawase, F., Sato, T., Fujii, T. (1987) Binding of chlorpromazine, phenytoin and aspirin to the erythrocytes and lipoproteins in whole human blood. J. Pharm. Pharmacol. 39: 740–742