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Note

Octanol–aqueous partition, distribution and ionization coefficients of lipophilic acids and their anions by reversed-phase high-performance liquid chromatography

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We have described[1] a method for the simultaneous determination of 1-octanol–aqueous partition, distribution and ionization coefficients (P, D and K_a, respectively; $P = D[(K_a/H) + 1]$ where H is the hydrogen ion concentration) by reversed-phase high-performance liquid chromatography (HPLC). 1-Octanol–aqueous partition coefficients for neutral species are of considerable interest in the area of quantitative structure–activity relationships (QSAR)[2], but the cumbersomeness of the classical shake-flask method is a deterrant to the experimental measurement of large numbers of these parameters. Reversed-phase HPLC allows rapid determination of P, D and K_a on small amounts of (possibly) impure material and is therefore of considerable practical interest to the pharmaceutical, pesticide and environmental industries.

In this note we wish to present further evidence that our method is applicable, in particular, to lipophilic acids and ion-pair partitioning, since Wahlund and Beijersten[3] have reported that lipophilic acids can partition to the hydrophobic support under certain conditions used in his studies (1-pentanol on C_2, C_8 and C_{18} bonded supports). Such partitioning to the hydrophobic support would diminish the parallelism between bulk phase and reversed-phase HPLC partitioning if it were to occur. However, this does not appear to be the case under conditions used in our procedure[1]. For example, $\log P$ for the lipophilic acid naproxen ((+)-6-methoxy-α-methyl-2-naphthaleneacetic acid) was reported[1] to agree excellently with literature shake-flask values ($\log P = 3.20$ compared to 3.18 for literature). These results were obtained at $pH < pK_a$ so that it was not possible to estimate partitioning of the anion (P'). We report here $\log P$, $\log P'$ and pK_a for two other lipophilic acids, xanthone-2-carboxylic acid[4] and tiopinac (6,11-dihydro-11-oxodibenzo[*b*,*e*]thiepin-2-acetic acid), a new non-hormonal anti-inflammatory agent[5]. These results confirm that our procedure correctly models bulk phase partitioning for lipophilic acids, including ion-pair partitioning.

EXPERIMENTAL

The procedure of Unger *et al.*[1] was used except that a Waters Model 204 constant-flow high-performance liquid chromatograph was attached to a Scientific Products Model 4000 data system in order to improve accuracy in determining log

k' ($k' = (t_x - t_0)/t_0$, where t_0 is the dead time (volume) and t_x is the sample time). Electronics were slightly modified such that the data system was activated as the injection lever was thrown. Ambient temperature was 22 ± 1°. Stainless-steel columns of 2 mm I.D. and 2.9, 10 and 50 cm length were packed with persilated Corasil C_{18}. Buffers were of 0.15 ionic strength (μ) (with added sodium chloride as necessary; columns were stripped after each workday by purging with water and then methanol; recoating took about 75–100 ml at 5 ml/min) and were saturated by shaking with excess 1-octanol (99.97% certified) and allowing to clarify for about two days. Mobile phases were then filtered through Millipore filters to degas and purify immediately before use. Analytical runs were performed at 2 ml/min as soon as the baseline had stabilized for 5 min. Dimethylformamide was used to dissolve samples (with buffer) and to determine t_0. Triplicate injections of each of the standards was made (2.9-cm column: anisole, chlorobenzene, benzophenone; 10-cm column: benzaldehyde, acetophenone, anisole; 50-cm column: catechol, acetanilide and benzaldehyde). The correlation coefficient for log k' *vs.* log D for standards was always $r \approx 0.999$ with a slope of essentially 1.0. An appropriate amount of dissolved sample was placed into the Waters injector with a 25-μl Hamilton chromatography syringe such that peak areas remained approximately constant.

Shake-flask values were determined[2] at 3–4 dilutions and log D obtained by averaging (xanthone-2-carboxylic acid) or by linear extrapolation (tiopinac). Solutions of tiopinac were protected from light.

Computer work was on an APL language system (Proprietary Computer Computer Systems, Van Nuys, Calif., U.S.A.) or on the Syntex IBM 370/158 for non-linear least squares[1].

RESULTS AND DISCUSSION

Horváth *et al.*[6] have derived equations for the case of both ionized and neutral species partitioning into a lipoid phase (also for multiple ionizations or zwitterionic partitioning) during reversed-phase HPLC. If log D is determined from the standard regression line, then the appropriate equations can be written as eqn. 1a for acid and eqn. 1b for acid and anion partitioning:

$$\log D = \log P - \log (1 + (K_a/H)) \quad (1a)$$

$$\log D = \log (P + P'K_a/H) - \log (1 + (K_a/H)) \quad (1b)$$

An example of ion-pair partitioning is provided by the lipophilic xanthone-2-carboxylic acid (I), which is the parent of a series of 7-substituted xanthone-2-carboxylic acids studied as mast cell inhibitors[4]. The partitioning behavior of I at 7 pH values was well fit by the standard model eqn. 1a with $r = 0.99$ and, in fact, gave insignificant P' when fitted to eqn. 1b. However, points at the most basic pH values deviated systematically, being much more "lipophilic" than expected. Additional points were then taken at still more basic pH values (pH 7.32 and 9.43) by classical shake-flask methods, above the operating range of the column. The combined data (collected in Table I) then fit eqn. 1b[7]. The derived constants from the non-linear fit of eqn. 1b are: $\log P = 3.12 \pm 0.07$; $\log P' = -0.21 \pm 0.07$; and $pK_a = 3.73 \pm 0.10$; with $n = 9$, standard deviation of fit = 0.086 and correlation coefficient between observed and calculated =

0.998. As can be seen in Table I, at pH 2.42–2.47 and pH 5.78–5.82, the agreement between the two methods is excellent. Log D determined at either about 6 or 99% ionization agrees excellently between shake-flask and reversed-phase HPLC methods. The data is internally consistent, being fit by eqn. 1b to a very high precision, irrespective of source of the data. The fourth column of Table I gives calculated log D (eqn. 1b) at all pH values, including those not used in the statistical analysis. Complete log P, log P' and pK_a were obtained for an additional 17 analogs of I in less than one month, demonstrating the productivity of this method[8]. This procedure for log P is more direct than that suggested by Wahlund and Beijersten[3].

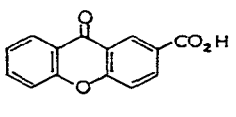
TABLE I

PARTITION DATA ON XANTHONE-2-CARBOXYLIC ACID

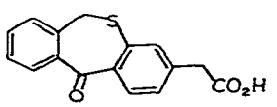
pH	*log D*			*Difference (observed – calculated, eqn. 1b)*
	Observed (±S.D.)	*Eqn. 1a***	*Eqn. 1b*	
2.42*,**	3.10 ± 0.14	3.099	3.099	0.001
2.47	3.053 ± 0.007	3.097	3.097	−0.044
3.35	2.977 ± 0.003	2.969	2.969	0.008
3.84	2.865 ± 0.005	2.760	2.761	0.104
4.06	2.617 ± 0.002	2.623	2.624	−0.007
4.35	2.348 ± 0.002	2.407	2.407	−0.059
4.83	1.855 ± 0.003	1.987	1.989	−0.134
5.78	1.190 ± 0.002	1.066	1.088	0.102
5.82*,**	1.10 ± 0.06	1.026	1.051	0.049
7.32*	−0.035 ± 0.03	−0.470	−0.020	−0.015
9.42*	−0.21 ± 0.03	−2.570	−0.208	−0.002

* Shake-flask, all other by reversed-phase HPLC.
** Not used in fitting eqn. 1b.
*** Calculated using log P and pK_a fit to eqn. 1b.

A second example is illustrated by the new non-hormonal antiinflammatory drug, tiopinac (II):



I



II

Partition data are presented in Table II. These data may be fitted to eqn. 1b, giving log $P = 2.97 \pm 0.06$; log $P' = 0.82 \pm 0.06$; and p$K_a = 3.71 \pm 0.10$; with $n = 7$, standard deviation = 0.069 and $r = 0.998$. These values compare quite favorably with log $P = 3.13 \pm 0.01$ by shake-flask and p$K_a = 3.82 \pm 0.28$ by solubility determination, both at 25° and $\mu = 0.10$. Log k' was found to decrease by 0.02 units at pH = 2.41 on going from $\mu = 0.10$ to $\mu = 0.15$; therefore, log P corrected = 3.11 ± 0.01; there is an insignificant effect on pK_a. Furthermore, pK_a as determined by reversed-phase HPLC is an apparent pK_a since it is determined in the presence of 1-octanol.

TABLE II

PARTITION DATA ON TIOPINAC

pH	*log D*			*Difference (observed — calculated, eqn. 1b)*
	Observed (±S.D.)	*Eqn. 1a**	*Eqn. 1b*	
2.40	2.932 ± 0.001	2.949	2.949	−0.017
3.34	2.813 ± 0.004	2.816	2.817	−0.004
3.92	2.654 ± 0.002	2.551	2.556	0.098
4.00	2.484 ± 0.006	2.500	2.506	−0.022
4.88	1.747 ± 0.001	1.772	1.815	−0.068
6.03	1.100 ± 0.000	0.648	1.042	0.058
7.36	0.802 ± 0.005	−0.680	0.833	−0.031

* Calculated using log P and pK_a fit to eqn. 1b.

This communication helps to establish that our procedure is applicable to lipophilic acids and ion-pair partitioning by confirming that, under these conditions, reversed-phase HPLC partitioning compares excellently with bulk phase distribution coefficients. Furthermore, anions of lipophilic acids can be considerably more lipophilic than previously thought[2]. This observation may have significant impact on various schemes to calculate partition coefficients used in QSAR studies[2,9].

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