pH-Metric log *P*. 4. Comparison of Partition Coefficients Determined by HPLC and Potentiometric Methods to Literature Values

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Received September 20, 1993, from the *Rhône-Poulenc Rorer, Dagenham Research Centre, Rainham Road South, Dagenham, Essex RM10 7XS, U.K., and §Sirius Analytical Instruments Ltd., Manor House, Lewes Road, Forest Row, East Sussex, RH18 5AF, U.K. Accepted for publication April 20, 1994®. ‡Present address: Department of Pharmacology, University of Leeds, Leeds, U.K.

Abstract □ The pK_a and log *P* of 20 compounds, including six substituted phenols, two substituted quinolines, *N*-methylaniline, five barbiturate derivatives, two phenothiazines, and several other molecules of pharmaceutical interest, were determined by the potentiometric technique at 25 °C and ionic strength 0.1 M (KNO_3). The log *P* values were determined also by partition HPLC. Three of the substances were of very low aqueous solubility, and for these the aqueous pK_as were determined by extrapolation from methanol–water solutions using the Yasuda–Shedlovsky technique. Values of log *P* obtained both by potentiometry and by partition HPLC, which ranged from 0.3 to 5.4, were in very good acordance with literature values. The general applicability of the potentiometric technique to ionizable compounds of diversely varied structures was demonstrated by the study.

The log of the octanol–water partition coefficient, log $P_{o/w}$ (or simply log *P*), of a substance is a key parameter in quantitative structure–activity relationships (QSAR),[1] used in the pharmaceutical industry (drug design and regulatory compliance), in the new-chemical manufacturing industry (environmental impact compliance), and in the environmental field (environmental fate of toxic substances).

We have been active in further developing the Dyrssen[2] dual-phase potentiometric titration technique for determining log *P*.[3-6] In part 1 of our series of papers, a method for estimating log *P* from "difference" plots was described;[4] in part 2, refinement of log *P* values by general nonlinear least squares was substantiated;[5] in part 3, a method for working with water-insoluble substances, using a methanol–water mixed-solvent approach, was elaborated.[6] The present paper is a validation study, where 20 compounds of considerably varied structures were selected for characterization by the pH-metric technique. Also included in the comparison were seven additional compounds (benzoic acid, alkoxyphenols, and propranolol) whose log *P* values were potentiometrically determined elsewhere.[3,5,6] The log *P* values were also measured in this study by partition HPLC. The log *P* values of the selected compounds are known from shake-flask measurements.

Experimental Section

Reagents—The preparation and standardization of HCl (Fisons) and KOH (Volucon, Rhône Poulenc) are described elsewhere.[3-5] Water-saturated partition-coefficient-grade octan-1-ol (Fisons) was used. Methanol (Fisons, analytical reagent, <0.05% water) was used without further purification. Phenol, 4-chlorophenol, 3-chlorophenol, 3,4-dichlorophenol, 4-iodophenol, quinoline, 3-bromoquinoline, and *N*-methylaniline were all obtained from Aldrich at a specified purity of >98%. 3,5-Dichlorophenol was obtained from the same source at a specified purity of >97%. Phenobarbitone (sodium salt), quinalbarbitone, amylbarbitone, pentobarbitone, chlorpromazine (hydrochloride salt), pericyazine, ketoprofen, celiprolol (hydrochloride salt), acebutolol (hydrochloride salt), and sulfamethazine were all materials from the Rhône-Poulenc Rorer research reference collection and were typical production material of pharmaceutical grade. All of the above compounds showed a single peak by partition chromatography.

Apparatus—The computerized titration instrument[3] we used to perform the pK_a and log *P* assays was equipped with a custom-designed pH sensing circuit (10^{15} Ω impedance) and a semimicro Ross-type double-junction combination pH electrode (Orion 8103SC). A six-way and a two-way valve (inert inner materials) were automatically driven to select any of six reagents (water, 0.5 M HCl, 0.5 M KOH, methanol, water-saturated octanol, and a surfactant solution to clean out the tubing after octanol dispensings). The water and methanol contained 0.1 M KNO_3 as an ionic strength adjuster. (Chlorpromazine was studied also in a medium with no added ionic strength adjuster.) Reagents were dispensed with a 5-mL stepper-motor-driven precision syringe, capable of a 0.00042-mL minimum-volume delivery. A circulating bath maintained the temperature of the sample solution at 25.0 ± 0.1 °C.

Titrations in the presence of octanol generally took longer to complete (20–60 min, depending on the sample and the requested pH range), than those in its absence (10–30 min). This was especially so when the octanol–water volume ratio exceeded 0.2. The instrument was programmed to accept a pH measurement as "stable" when the magnitude of the change in pH after a titrant addition was less than 0.01 min^{-1} (based on repetitive sequence of 15 pH readings taken over a 15-s interval). Titrant additions coincided with vigorous stirring (overhead stirrer) of the assay solution. After titrant addition the solution was stirred for an additional 15–30 s in the presence of octanol, before the pH reading sequence commenced. The actual pH readings were taken with stirring turned off. Such a scheme appeared to allow adequate time for sample equilibration to take place between the octanol–water phases.

Although the octanol was water-saturated, the water in the reagent bottle was not octanol-saturated. During calculations, a solubility of 0.0017 M for octanol in water[7] was used to correct the octanol–water volume ratio. Such corrections are only important for very small octanol volumes (<0.1 mL) relative to water volumes (5–20 mL).

Electrode Standardization—To establish the operational pH scale, the measuring circuit was first calibrated with a single aqueous pH 7 phosphate buffer and the Nernst slope was assumed. The operational scale was converted to the concentration scale p_cH (=−log [H^+]) using a four-parameter equation:[4,6]

$$pH = \alpha + S p_cH + j_H[H^+] + j_{OH}K_w/[H^+] \quad (1)$$

where K_w is the ionization constant of water. The parameters were empirically determined by a weighted nonlinear least squares procedure using data from the titration of a standard substance, ethylenediamine dihydrochloride,[4] or HCl[6] (in the case of mixed solvent pK_a determinations). Typical aqueous values of the adjustable parameters at 25 °C and 0.1 M ionic strength are $\alpha = 0.08 \pm 0.01$, $S = 1.001 \pm 0.001$, $j_H = 1.0 \pm 0.2$, and $j_{OH} = -0.6 \pm 0.2$.

pK_a Determination—For substances which are sparingly soluble in water it was necessary to work with $<7 \times 10^{-4}$ M solutions. Solutions with the water-soluble compounds were otherwise 0.002–0.025 M. The three water-insoluble substances, chlorpromazine, pericyazine and sulfamethazine, could only be dissolved in solutions with >34, >13, and >6 wt % methanol, respectively; sample concentrations were $<5 \times 10^{-4}$ M. The mixed solvent procedure is described elsewhere.[6] In most cases, standardized HCl was added to lower the pH to 2.5, and the solutions were titrated with KOH to about pH

® Abstract published in *Advance ACS Abstracts*, June 1, 1994.

0022-3549/94/1200-1280$04.50/0

Table 1—pK_a and log *P* Constants

compound	log *P*			pK_a	
	pH-metric	lit.[a]	HPLC	pH-metric	lit.[b]
Phenol	1.48 ± 0.01	1.46	1.46	10.01 ± 0.01	9.99[c]
4-Chlorophenol	2.45 ± 0.01	2.39	2.41	9.46 ± 0.01	9.42[d]
3-Chlorophenol	2.57 ± 0.01	2.50	2.50[e]	9.11 ± 0.01	9.08[d]
3,4-Dichlorophenol	3.39 ± 0.02	3.33	3.27	8.65 ± 0.01	8.63[f]
3,5-Dichlorophenol	3.63 ± 0.02	3.62	3.62[e]	8.22 ± 0.01	8.18[f]
4-Iodophenol	2.90 ± 0.02	2.91	2.92[e]	9.45 ± 0.05	9.20[c]
Quinoline	2.15 ± 0.08	2.03	2.10	4.97 ± 0.06	4.90[g]
3-Bromoquinoline	2.91 ± 0.04	3.03[h]	3.04	2.74 ± 0.03	2.69[g]
N-Methylaniline	1.65 ± 0.01	1.66	1.62	4.86 ± 0.01	4.85[g]
Phenobarbitone	1.53 ± 0.03	1.47	1.39	7.49 ± 0.02	7.45[d]
Butobarbitone	1.58 ± 0.07	1.65	1.64	8.00 ± 0.04	7.79[d]
Amylobarbitone	2.01 ± 0.03	2.07	2.10	8.07 ± 0.02	7.96[h]
Pentobarbitone	2.08 ± 0.06	2.07	2.10	8.18 ± 0.03	7.87[i]
Quinalbarbitone	2.39 ± 0.02	1.97	2.33	8.09 ± 0.01	7.92[j]
Chlorpromazine	5.39 ± 0.01	5.35	5.48[k]	9.24 ± 0.01	9.20[l]
Cation	0.35 ± 0.03				
Pericyazine	3.65 ± 0.08	3.52	3.89	8.76 ± 0.08	8.30[m]
Ketoprofen	3.14 ± 0.02	3.12	3.12[n]	4.29 ± 0.02	4.60[n]
Celiprolol	1.92 ± 0.04		2.22[o]	9.66 ± 0.03	
Acebutolol	1.75 ± 0.19	1.77	2.10[p]	9.41 ± 0.01	9.20[q]
Sulfamethazine	0.89 ± 0.03			7.80 ± 0.02	7.38[j]
Anion	0.24 ± 0.12	0.28		2.45 ± 0.03	2.36[j]
Benzoic acid	1.96 ± 0.02	1.97	1.83	4.21 ± 0.02	4.20[c]
4-Methoxyphenol	1.41 ± 0.03	1.34	1.30	10.27 ± 0.03	10.21[s]
4-Ethoxyphenol	1.81 ± 0.02	1.81	1.74	10.25 ± 0.01	
4-Propoxyphenol	2.31 ± 0.03	2.33	2.34	10.27 ± 0.01	
4-Butoxyphenol	2.87 ± 0.08	2.90	2.92	10.26 ± 0.08	
4-Pentoxyphenol	3.26 ± 0.11	3.50	3.49	10.13 ± 0.20	
(±)-Propranolol	3.35 ± 0.03	3.37		9.53 ± 0.06	9.72[r]
Cation	0.62 ± 0.03	0.48[r]			

[a] The lit. log *P* values quoted are those given by the "Daylight" Daymodels (version 3.53, supplied by Daylight Chemical Information Systems Inc., Irvine, CA) database as being the best available experimental determinations, except where no value is available, and other specified values are quoted. [b] The lit. pK_a values quoted are drawn from a variety of sources and are judged to be of high quality. [c] Reference 14. [d] Reference 14; ionic strength not stated. [e] Reference compound in the partition HPLC determination. [f] Reference 15. [g] Reference 16. [h] Reference 17. [i] Reference 18. [j] Reference 19. [k] Calculated from a value of log $D = 3.18$ at pH 7.00 using pK_a 9.30. [l] Reference 20. [m] Azzaro, University of Nice; private communication to the Pomona database. [n] Reference 21. [o] Determined at pH 10.95, using a modified set of calibrants. [p] Determined at pH 10.95; this gives a value of 2.11 after correction for ionization. [q] Reference 22. [r] Reference 10. [s] Reference 23.

11.5. After each titrant addition,the pH was measured. The initial estimates of the pK_as were obtained from the "difference" (Bjerrum) plots.[4] These approximate values were then refined by a weighted nonlinear least squares procedure.[5]

Potentiometric log *P* Determination—The octanol–water partition parameter, log *P*, where $P = [H_jA]_{octanol}/[H_jA]_{water}$, for a speices H_jA may be determined from the difference between the aqueous pK_a of the species and the *apparent* pK_a, p$_o$K$_a$, determined from a titration of the substance in the presence of octanol (or any other useful organic partition solvent that is immiscible with water). For water-insoluble substances, the aqueous pK_a, derived from Yasuda–Shedlovsky extrapolation,[6,8,9] is necessarily provided as a fixed contribution during the log *P* least squares refinement stage using the octanol data.

HPLC log *P* Determination—The method used is essentially that of Taylor,[10] and involved liquid–liquid partition chromatography between aqueous buffer and octanol supported on a porous silica medium. For convenience and speed, commercially available[11] 1 cm × 4.6 mm i.d. cartridges packed with 5-μm C1 coated silica were used; this silica has an average pore diameter of 120 Å. Octanol coating is carried out dynamically by Rheodyne injection of pure octanol onto the cartridge at a flow rate of 1 mL min^{-1} with the detector disconnected; typically 3 × 20 μL of the octanol is used. Calibration of the system is carried out by injecting dilute solutions of a mixture of seven or eight accurately known log *P* "standards" of mixed hydrogen-bond-donor and -acceptor strengths. The compounds used, together with their corresponding log *P* values in parentheses, are acetanilide (1.16), acetophenone (1.58), anisole (2.11), 3-chlorophenol (2.50), 4-iodophenol (2.92), benzophenone (3.18), diphenylamine (3.50), and diphenyl ether (4.00). A plot of log *k'*, the chromatographic capacity factor, against log *P* is linear with the coefficients of *a* and *b* in eq 2 typically being 1.00 and 0.5, respectively. Determination of

$$\log P = a \log k' + b \qquad (2)$$

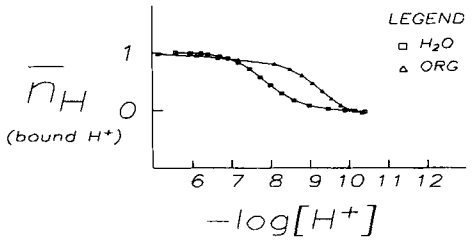

Figure 1—A typical difference plot, shown for quinalbarbitone. It is a plot of the average number of bound protons per molecule of drug substance ($\bar{n}_H$) versus p$_c$H (–log [H$^+$]).

log *P* or log *D* (the distribution coefficient) for the test solute was carried out by running a chromatogram for that solute and inserting the log *k'* value obtained into eq 2. The uncertainty in the result is expressed in the regression line for the calibration graphs obtained immediately preceding and following the unknown determination. The precision in replicate determinations is typically 0.02.

Theoretical Section

Function Minimized in Least Squares Refinement—The graphically determined approximate equilibrium constants form the "seed" values for the iterative least squares procedure for both pK_a and log *P* refinement. The refined values are those which produce a minimum in the sum of the weighted squares of residuals:

$$s = \sum_{i}^{N_o} \frac{(\mathrm{pH}_i^{obs} - \mathrm{pH}_i^{calc})^2}{\sigma_i^2(\mathrm{pH})} \quad (3)$$

N_o is the number of pH measurements; σ_i^2 is the estimated variance in the measured pH_i^{obs}. The model equation, pH_i^{calc}, is a function of the pK_a and log P parameters, as well as the independent variables.[5]

Weighting Scheme and "Goodness-of-Fit"—The weighting scheme used in eq 3

$$\sigma^2(\mathrm{pH}) = \sigma_c^2 + (\sigma_v\ \mathrm{dpH/d}V)^2 \quad (4)$$

is constructed from the variances where $\sigma_c = 0.005$ (units of pH), the fixed contribution to the variance in the measured pH, and $\sigma_v = 0.001$ mL, the estimated standard deviation in the volume of titrant. The weighting scheme properly recognizes that measurements of pH near the endpoint of a titration (where dpH/dV is large) are not as reliable as those from the buffered portion. After each iterative cycle a test of the progress of refinement is indicated by the "goodness-of-fit", GOF, which is defined by

$$\mathrm{GOF} = \left(\frac{S}{N_o - N_r}\right)^{1/2} \quad (5)$$

where N_r is the number of refined parameters (that is, the number of pK_as and log Ps). A GOF value of 1 is ideal. It would mean that on the average the calculated titration curve and the observed curve are about one standard deviation apart in pH (about 0.005 pH units in the buffer regions).

Results and Discussion

Dissociation Constants and Partition Coefficients—Table 1 lists the constants obtained from 37 separate titrations for the 20 studied compounds. Also included in Table 1 are values for seven additional compounds, determined elsewhere.[3,5,6] The GOF of the refinements were typically 0.5, ranging 0.1–1.1 and consistently being lower for the more water-soluble compounds. Although the pK_as were all determined at 0.1 M ionic strength (KNO_3), the values reported here have been "corrected" to zero ionic strength, I, using the relations $pK_a(0) = pK_a(I) - 2 \log f$ for monoprotic *weak acids* and $pK_a(0) = pK_a(I)$ for monoprotic *bases*, where f is the activity coefficient of the ions, derived from the Davies equation[12]

$$\log f = -Az^2\left(\frac{I^{1/2}}{1 + I^{1/2}} - 0.30I\right) \quad (6)$$

Chlorpromazine was assayed in the absence of ionic strength adjuster. Hence its pK_a and log P (neutral and ion-pair) are uncorrected in Table 1. Since this molecule is at the extreme high-log P end of the scale of comparison, we wished to determine its constants under conditions closest to those reported in the literature (namely, at near-zero ionic strength).

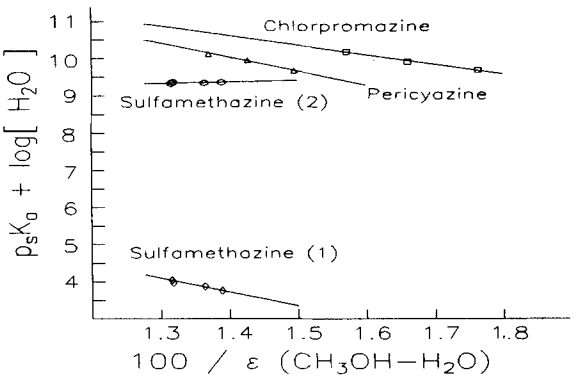

Figure 2—Methanol–water Yasuda–Shedlovsky plots for the three substances with very low water solubility. The dielectric constant, ϵ, of methanol-free water at 25 °C is 78.3, at which point $100/\epsilon = 1.28$; also at that point, log $[H_2O] = 1.74$.

Figure 1 shows a difference plot of quinalbarbitone. The aqueous difference curve, represented by squares, indicates an approximate pK_a of 7.9 (pH at $\bar{n}_H = 0.5$). The dual-phase difference curve, representing a titration in the presence of octanol (10% by vol) shows an *apparent* pK_a of 9.0. The higher value indicates that quinalbarbitone titrates as a weak acid. The difference between the true and the apparent pK_a was used to calculate an approximate log P of 2.1. This "seed" value refined by least squares to 2.39 ± 0.02.

Yasuda–Shedlovsky Plots—Figure 2 shows the Yasuda–Shedlovsky plots of $p_sK_a + \log [H_2O]$ vs $1/\epsilon$ for chlorpromazine, pericyazine, and sulfamethazine. It is characteristic of acids to have positive slopes and bases to have negative slopes. The diprotic molecule sulfamethazine has both a weak acid and a weak base ionization sites, as indicated by slopes of different signs. Table 2 summarizes the values extrapolated to zero methanol (corrected to zero ionic strength for pericyazine and sulfamethazine).

Correlation between Different Techniques—Figure 3 shows two log P correlation plots. The solid lines are based on *weighted* linear fits, where the literature values were assumed to be the independent variables ("error-free") and the pH-metric values were the dependent variables, with variances taken from the individual log P refinements as the basis of the weighting. A plot of log P (pH-metric) vs log P (lit.) exhibits a slope of 0.9928 and an intercept of 0.06 (r = 0.9941); a plot of log P (HPLC) vs log P (lit.) exhibits a slope of 1.0310 and an intercept of −0.03 (r = 0.9906).

Conclusion

Our study clearly indicates that log P values determined both by potentiometry and by partition HPLC are in good

Table 2 —Equilibrium Constants in Methanol–Water[a]

Chlorpromazine[b]		Pericyazine		Sulfamethazine		
R (wt %)	p_sK_a	R (wt %)	p_sK_a	R (wt %)		p_sK_a
49.6	8.16 ± 0.01	27.1	8.09 ± 0.04	15.6	7.94 ± 0.01	2.11 ± 0.02
42.2	8.41 ± 0.01	19.9	8.33 ± 0.02	12.2	7.90 ± 0.01	2.21 ± 0.02
34.5	8.61 ± 0.01	13.1	8.46 ± 0.05	6.3	7.88 ± 0.02	2.64 ± 0.03
				6.1	7.84 ± 0.01	2.34 ± 0.01
0.0[c]	9.24 ± 0.01	0.0[c]	8.76 ± 0.08	0.0[c]	7.80 ± 0.02	2.45 ± 0.03

[a] 25 °C, corrected to zero ionic strength (from 0.1 M KNO_3) in the cases of pericyazine and sulfamethazine. [b] No ionic strength adjuster used; average I = 0.001 M. [c] The value extrapolated to zero cosolvent using the Yasuda–Shedlovsky procedure (see the text).

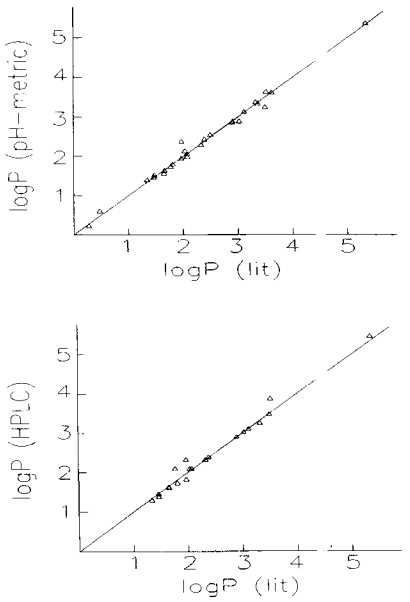

Figure 3—Correlation plots: log *P* (pH-metric) vs log *P* (lit) and log *P* (HPLC) vs log *P* (lit). The solid lines shown have a slope of one and a zero intercept.

agreement with the best available literature measurements of log *P* by the standard shake-flask method. This supports findings of previous potentiometric studies which examined fewer compounds.[2,13,20] Since the pH-metric technique can be applied to structurally diverse compounds without bias, it is a generally useful technique, when considering ionizable drug substances.

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Acknowledgments

Part of this work was supported by the U.K. Department of Trade and Industry, through SMART stages I and II grants.