Octanol–Physiological Buffer Distribution Coefficients of Lipophilic Amines by Reversed-Phase High-Performance Liquid Chromatography and Their Correlation with Biological Activity

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The use of an isotonic, pH 7.4, 1-octanol saturated phosphate buffer with added *N,N*-dimethyloctylamine (DMOA) at 1–4 mM on persilated RP-18 reversed-phase high-performance liquid chromatography can give excellent agreement ($r > 0.99$) with 1-octanol bulk phase shake–flask distribution coefficients for lipophilic amines, such as phenothiazines and tricyclic antidepressants. This system can be a superior model of biological partitioning compared to other reversed-phase high-performance liquid chromatography systems, e.g., 20%, v/v, CH_3CN in the same buffer, usually with or without added DMOA. Requirements for an adequate data set are discussed. Histamine-releasing activity in rat mast cells for a series of 14 phenothiazines and tricyclic antidepressants is best correlated by this optimized system ($r = 0.929$) compared to the organic system on C-18 ($r = 0.873$). Addition of 4 mM DMOA to the organic system improves the correlation ($r = 0.913$); this may indicate that the mode of activity is nonspecific binding of the lipophilic amine to the mast cell. On the other hand, binding of seven phenothiazines to BSA was found to involve a specific interaction of the aliphatic nitrogen to the protein. Correlation using the optimized system with a $+pK_a$ term ($r = 0.980$) was superior to the other systems. The best organic modifier correlation was $r = 0.939$, without a pK_a term. This could be interpreted as indicating that the latter system already contains a contribution from the basicity of the aliphatic nitrogen, which is supported by other evidence. Finally, the inhibition of (Na^+,K^+)ATPase by nine lipophilic amines was about equally well correlated by the optimized system as by the 20%, v/v, CH_3CN system with added DMOA ($r \approx 0.96$). Omitting the DMOA from the organic system gave a poorer correlation ($r = 0.911$). This is consistent with the putative mechanism of action of ATPase, a membrane-bound enzyme. Binding of drug occurs to the membrane lipids, inducing a conformational change indirectly in the enzyme, which is then deactivated. Since the drug does not directly interact with the enzyme, there is less discrimination between the different partitioning systems. The apparent discrimination observed for the histamine release therefore requires further clarification. The use of several high-performance liquid chromatography systems can help clarify the quantitative structure–activity relationships.

In previous papers of this series[1,2] we have shown that a 1-octanol coated reversed-phase column in a high-performance liquid chromatography (RP-HPLC) system can accurately and rapidly determine 1-octanol partition (P) coefficients for neutral compounds and P, distribution (D) and ionization (pK_a) coefficients for acidic compounds. The RP-HPLC system gives values in excellent agreement with bulk phase shake–flask (SF) values and accurately accounts for the effect of ion-pair partitioning in the case of lipophilic acids.[2] For the case of lipophilic amines, however, the existing methodology does *not* give sufficiently accurate results due, presumably, to the interaction of the aliphatic amine side chain (of compounds such as the phenothiazines, tricyclic antidepressants, etc.) with residual silanol sites. Compounds are retained too long—appear too lipophilic—and give unsymmetrical peak shapes. Compounds with *hindered* amines behave normally.

Baker et al.[3] have questioned the use of 1-octanol in correlating biological data and have proposed the use of standard organic modifiers (e.g., MeOH,[3] CH_3CN, etc.) with RP packing materials. It has been shown previously[4-9] that organic modifiers do not correlate with 1-octanol retention indexes, due to differences in the hydrogen-bonding properties of the two systems. Henry et al.[8b] have found moderate correlations for a number of HPLC systems for a series of sulfonamides and barbiturates. In a different approach to the preparation of stable 1-octanol columns, Miyake and Terada[10] directly absorbed 1-octanol to hot silica, claiming improvement over the method of Mirrlees et al.[11] However, no data on lipophilic amines were reported. Molnár and Horváth[12] used aqueous buffers on RP-18 Lichrosorb to study relatively hydrophilic amines of physiological interest. No comparisons were made to SF values, nor were any correlations of biological data attempted. Several authors[13,14] have obtained quantitative evidence that peak deformation in RP-HPLC is caused by interaction of the solute with residual silanol sites. Both Schaper[15] and Ezumi and Kubota[16] have published more extensive studies on the simultaneous[1,2] determination of P, D, and pK_a by SF methods.

In this report, we examine the correlation of $\log k'_{CH_3CN}$, $\log k'_{octanol}$, and $\log D_{HPLC}$ vs. $\log D_{SF}$ (i.e., $\log D_{shake\text{-}flask}$) for a diverse group of lipophilic amines. We have found conditions—modifications of those reported by Wahlund[17,18]—which give excellent agreement between $\log k'$ and $\log D_{SF}$ under physiological buffer conditions. Because of the critical role of ion-pair partitioning[9,19] with lipophilic

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Table I. Effect of Buffer Components on Log k' (C-18) and Log D_{SF}[a]

	compd	log k' A	log k' B	log D_{SF} A	log D_{SF} B
A	chlorpromazine	1.911	1.751	3.221	3.167
B	promazine	1.415	1.117	2.480	2.538
C	promethazine	1.512	1.271	2.849	2.790
D	amitriptyline	1.419	1.279	2.830	2.499
E	mepacrine	0.889	0.574	1.911	2.219
F	doxepin	1.025	0.778	2.217	2.199
G	imipramine	1.145	0.934	2.490	2.581
H	cyproheptadine	1.715	1.527	3.110	3.142
I	indoramin	0.536	0.413	2.313	2.278
J	tilorone	0.701	0.325	1.471	1.314

[a] Buffer A = 0.01 μ PO_4 + 0.14 μ NaCl, pH 7.4; buffer B = 0.15 μ PO_4, pH 7.4; both saturated with 1-octanol, column length 3 cm.

amines (the importance of the counterion has long been recognized),[20] we have chosen "physiological" (isotonic) buffer systems as our standard buffer conditions. Of course, many "physiological" buffers are available and we have limited this initial study to just one, the phosphate buffer saline (PBS) buffer, because it is one of the simplest isotonic buffers. Hoefnagel, Hoefnagel, and Wepster[32] have shown that in the area of physical organic chemical correlations of rates and equilibria the nature of the solvent can have a dramatic effect on substituent "constants" of charged substituents. The most accurate comparisons are when both system and model are determined under identical conditions. We also examine the correlation of these retention indexes with biological data. Although the diversity and amount of these data do not allow for firm conclusions, some interesting patterns emerge.

Results and Discussion

Optimization of High-Performance Liquid Chromatography Conditions for Lipophilic Amines. Our procedure for determining the lipophilicity of neutral and acidic compounds involves the use of C-18 Corasil with 1-octanol saturated 0.15 μ PO_4 (pH 7.4; μ = ionic strength), called buffer B, as the mobile phase.[1,2] As shown in Table I and Figure 1a, these conditions are totally unsatisfactory in the case of lipophilic amines, with $r \approx 0.89$ compared to $r \approx 0.99$ for neutral and acidic compounds. Therefore, we have undertaken an examination of the RP-HPLC conditions in order to improve the correlation between SF and RP-HPLC methods. In comparison, Figure 1c shows the same compounds chromatographed under optimized conditions, to be described below. The agreement is now seen to be excellent, of the same order of magnitude as for neutral and acidic compounds, and well within the general level of accuracy for such measurements (<<5%).

Buffer. Most of the lipophilic amines with which we are concerned have a $pK_a > 7$, which means that they will be partially or fully protonated at physiological pH, 7.4. A lipophilic cation will tend to partition as an ion pair,[18,20] and this partitioning will be affected by the nature of the available anions. A phosphate buffer with added NaCl can attempt to mimic isotonic physiological conditions, although it will certainly not have the vast array of available ions present in, for example, plasma. (Note: the addition of low concentrations of NaCl will not harm the Teflon-

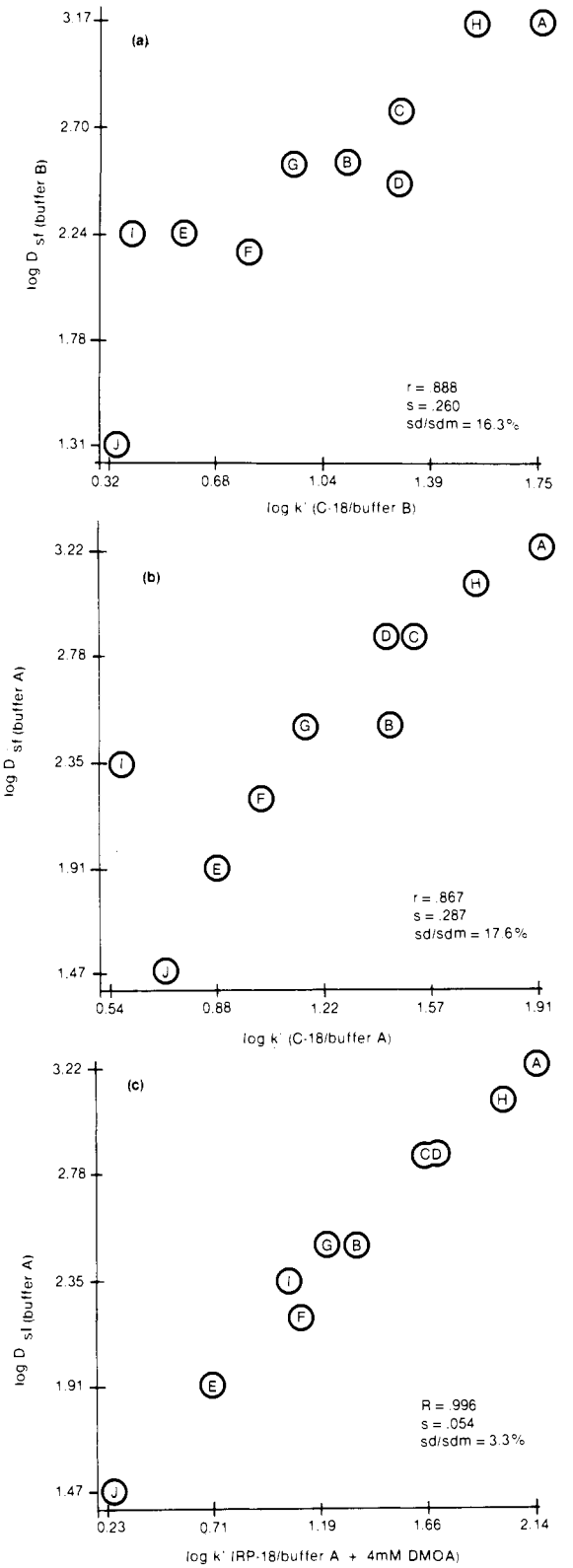

Figure 1. Comparison of log k' determined on different columns and with different mobile phases with shake-flask distribution coefficients determined with the same buffer. See Tables I and IV for data. (a) Conditions used for neutral and acidic compounds[1,2] using buffer B; note poor linearity for these lipophilic amines. (b) Linearity increased somewhat by use of PBS isotonic buffer A; note promazine and indoramin still deviate. (c) Excellent linearity is obtained by changing column packing to RP-18 and adding the lipophilic *N,N*-dimethyloctylamine to buffer A. DMOA competes for the free silanol sites on the column packing material, converting log k' to a purer measure of partitioning. Compounds: A = chlorpromazine; B = promazine; C = promethazine; D = amitriptyline; E = mepacrine; F = doxepin; G = imipramine; H = cyproheptadine; I = indoramin; J = tilorone.

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coated stainless-steel tubing and valves if it is washed out with octanol-saturated distilled water after each session.) Comparison of two buffers, A [0.01 μ PO_4 + 0.14 μ NaCl] (phosphate buffer saline, PBS; isotonic) with B [0.15 μ PO_4], both pH 7.4 and saturated with 1-octanol, is shown in Table I and eq 1a. Numbers in parentheses are 95% confidence limits; n = number of compounds; s = standard deviation of the regression; r = correlation coefficient; F = overall F test for significance of correlation; SD/SDM = s/standard deviation of data (to compare, roughly, with the experimental error). The physiological buffer, A, differs by a constant amount (0.31) from the other buffer, B based on eq 1a.

$$\log k'_A = \underset{(0.14)}{0.31} + \underset{(0.13)}{0.92} \log k'_B \quad (1a)$$

$n = 10$; $s = 0.08$; $r = 0.987$; $F_{1,8} = 293.7$; SD/SDM = 5.7%

We have also determined SF D values with this same pair of buffers (Table I). In this case, we find eq 1b. This correlation is of lower precision and there is no longer a constant difference between the two buffers. The lower correlation may be due to the larger error inherent in SF determinations, but eq 1a,b confirm, with a data set obtained under consistent conditions, fundamental differences in the two procedures, as noted above.

$$\log D_{SF(A)} = \underset{(0.63)}{0.10} + \underset{(0.25)}{0.97} \log D_{SF(B)} \quad (1b)$$

$n = 10$; $s = 0.17$; $r = 0.953$; $F_{1,8} = 78.6$; SD/SDM = 10.8%

Comparing Figures 1a and 1b we find that the statistical correlation has not improved; however, Figure 1b appears more regular, if not completely linear. The greatest deviant is indoramin 1, which does not contain an alicyclic aliphatic amine, as do all other compounds (except cyproheptadine, which has a relatively unhindered cyclic amine). Indoramin is not expected to ion pair to the same extent as the other compounds; therefore, we might expect log k' to be "too low" compared to the other compounds. As we will demonstrate below (cf. Figure 1c), this explanation does not appear to be correct.

Addition of Aliphatic Amines. Wahlund[13,17,18] has demonstrated that it is necessary to use hydrophobic amines or quaternary ammonium compounds to prevent peak deformation and improve resolution when separating lipophilic amines on HPLC. We have investigated tetraethylammonium chloride ($Et_4N^+Cl^-$) and dimethyloctylamine (DMOA) as agents to swamp out binding to residual silanol sites. Under the conditions examined, $Et_4N^+Cl^-$ did not eliminate peak tailing. Retention times were slightly decreased. Therefore, other quaternary amines were not examined in this study. On the other hand, DMOA not only eliminated peak tailing in most instances, but also improved the correlation between log k' and log D_{SF}. This can be seen in Table II and eq 2 and 3. The addition of 1 mM DMOA to the physiological buffer A has considerably improved the agreement between HPLC and bulk-phase SF D values. For most compounds studied, 1 mM DMOA sufficed; however, for a series of guanidines, 4 mM DMOA was required to eliminate peak deformation, and this concentration has been used in most subsequent work.

$$\log D_{SF} = \underset{(0.26)}{1.57} + \underset{(0.25)}{0.85} \log k'_{C\text{-}18/0} \quad (2)$$

$n = 25$; $s = 0.344$; $r = 0.832$; $F_{1,23} = 51.72$; SD/SDM = 11.6%

$$\log D_{SF} = \underset{(0.10)}{1.71} + \underset{(0.12)}{1.00} \log k'_{C\text{-}18/1} \quad (3)$$

$n = 25$; $s = 0.164$; $r = 0.964$; $F_{1,23} = 305.3$; SD/SDM = 5.5%

Table II. Effect of Column Packing Materials and DMOA on Log k' Compared to Shake-Flask[a]

compd	log k' RP-18/1	C-18/1	C-18/0	RP-8/1	SF
nitrobenzene	0.602	0.028	0.028	0.564	1.880
anisole	0.928	0.332	0.353	0.872	2.086
benzene	0.946	0.352	0.349	0.874	2.13
chlorobenzene	1.631	1.027	1.050	1.563	2.873
benzophenone	1.916	1.307	1.332	1.785	3.101
nortriptyline	0.883	0.447	0.995	1.162	1.980
lidocaine	0.401	−0.208	−0.114	0.229	1.647
imipramine	1.269	0.818	1.145	1.194	2.490
mesoridazine	0.588	0.252	0.923	1.045	1.805
doxepin	1.115	0.632	1.025	1.044	2.217
propoxyphene	1.263	0.656	0.795	1.124	2.359
tilorone	0.234	0.007	0.701	0.183	1.471
quinidine	0.840	0.220	0.495	0.623	2.068
mepivacaine	0.051	−0.564	−0.429	−0.098	1.268
mepacrine	0.761	0.329	0.888	0.807	1.911
fluanisone	1.669	0.996	1.175	1.448	2.930
dibucaine	1.651	1.064	1.215	1.588	2.906
promazine	1.351	0.927	1.465	1.378	2.480
promethazine	1.670	1.172	1.512	1.595	2.849
indoramin	1.049	0.297	0.536	0.858	2.313
clozapine	1.734	1.136	1.396	1.663	2.993
chlorpheniramine	0.273	0.007	0.466	0.466	1.380
cyproheptadine	1.999	1.516	1.715	1.853	3.110
acridine	2.146	1.501	1.556	1.993	3.291
chlorpromazine	2.144	1.656	1.911	2.061	3.221

[a] Buffer A (Table I) saturated with 1-octanol. Number to right of solidus indicates the concentration of DMOA added (mM). See Experimental Section for further details. SF = shake-flask log D; buffer A without added DMOA. RP-18 and RP-8 on 2-cm columns; C-18 on 3-cm columns.

Packing Materials. C-18 Corasil, RP-8 Lichrosorb, and RP-18 Lichrosorb were studied. In general, 18-C is better than 8-C, and the porous RP-18 (10 μM) is better than pellicular C-18 Corasil (25 μM) in mimicking SF values, as shown in Table II and eq 3–5. Equation 5 shows

$$\log D_{SF} = \underset{(0.17)}{1.24} + \underset{(0.13)}{0.99} \log k'_{RP\text{-}8/1} \quad (4)$$

$n = 25$; $s = 0.186$; $r = 0.954$; $F_{1,23} = 232.2$; SD/SDM = 6.3%

$$\log D_{SF} = \underset{(0.06)}{1.21} + \underset{(0.04)}{0.98} \log k'_{RP\text{-}18/1} \quad (5)$$

$n = 25$; $s = 0.064$; $r = 0.995$; $F_{1,23} = 2131.0$; SD/SDM = 2.2%

unit slope, 0.98 ± 0.04, and essentially perfect correlation between the two processes; SD/SDM = 2.2%, the limit of accuracy of our measurements. Identical unit slopes indicate that, on the average, a unit change in lipophilicity, as measured by HPLC, is identical with those measured by SF. The goodness-of-fit parameters differ for the three systems, however.

RP-18 packing material saturated with 1-octanol before packing gave much better results than in situ coating with 1-octanol saturated buffer;[1,2] no differences were observed with C-18 Corasil. Slurry packed RP-18 suffers gradual depletion of the 1-octanol from the packing and must be recoated with direct injections of 1-octanol as performance

Table III. Agreement between Log D Obtained on 2- and 10-cm Columns (RP-18/4)

compd	log D 10 cm[a]	log D 2 cm[b]	Δ
mepivacine	1.221	1.264	−0.043
tilorone	1.435	1.482	−0.047
chlorpheniramine	1.528	1.514	0.014
lidocaine	1.573	1.587	−0.014
mesoridazine	1.837	1.784	0.053
mepacrine	1.986	1.966	0.020
quinidine	2.030	2.031	−0.001
nortriptyline	2.045	2.010	0.035
indoramin	2.274	2.267	0.007
doxepin	2.357	2.324	0.033

[a] Standards for 10-cm column: aniline, benzaldehyde, acetophenone, nitrobenzene, anisole, benzene. Calibration line: $\log D = 0.5655 + 1.017 \log k'$ ($s = 0.056$; $r = 0.997$; $F_{1,5} = 859$; SD/SDM = 3.4%). [b] Standards for 2-cm column: aniline, benzaldehyde, nitrobenzene, anisole, benzene, chlorobenzene, benzophenone. Calibration line: $\log D = 1.257 + 0.9823 \log k'$ ($s = 0.034$; $r = 0.999$; $F_{1,5} = 2898$; SD/SDM = 1.9%).

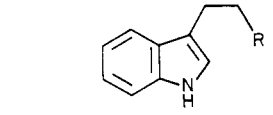
warrants:[1] this is not a problem with the C-18 Corasil, which is not stripped. For determination of log D, column length is not a variable, since overlapping series on 20 and 100 mm columns packed with RP-18 gives the values shown in Table III and eq 6. Note the zero intercept, unit

$$\log D_{20\text{mm}} = \underset{(0.10)}{0.10} + \underset{(0.05)}{0.94} \log D_{100\text{mm}} \quad (6)$$

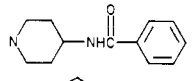
$n = 10$; $s = 0.026$; $r = 0.998$; $F_{1,8} = 1726$; SD/SDM = 2.4%

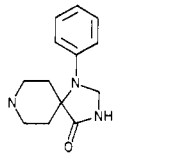
slope, and very high correlation. Columns should be selected such that $1 < k'$ for as many compounds as possible in order to have reliable results. We have not established an upper limit for k'; inaccuracy in measuring the maximum of a broad peak is offset by the larger k'. However, in order to have measurable peaks, larger samples are required. In this study we have obtained good results with compounds having $k' \approx 1000$.

Returning to Figures 1b and 1c, we can now assign the "errant" behavior of indoramin to the interaction of the *other* members of the class with free silanol sites of the packing material. Having only a hindered cyclic amine (or indole) N precludes such interaction in **1**. Indoramin is



1, R =





2, R =

normal; when the silanol interaction is blocked for the other compounds by addition of DMOA, all compounds fall in line. Indoramin *can* probably ion pair. We have studied an analogue of indoramin, **2**,[23] at 11 pH values (using C-18 with 1-octanol saturated 0.10 M PO_4) and were able to detect ion pairing with log $P = 3.20 \pm 0.05$, log $P' = 1.90 \pm 0.02$, and $pK_a = 7.57 \pm 0.08$ by curve fitting.[9] The pK_a value agrees well with the literature[25] value reported for indoramin (7.7).

Summary. Table IV gives a consistent set of data for RP-18, C-18, and CH_3CN on C-18, all with 4 mM DMOA, and also CH_3CN on C-18 without DMOA, together with experimentally determined SF D values using the same buffer, without DMOA. DMOA has no effect on SF values, when present, for the compounds tested. The effect of DMOA on acidic groups in the solute molecule has not been specifically examined at this time. Addition of DMOA is only required if interaction of basic groups with residual silanol sites is anticipated. As shown for indoramin, steric hindrance eliminates this interaction; this was also observed for substituted pyridines in our earlier work.[1]

Table V gives a squared correlation matrix (uncorrected for degrees of freedom) for the first 29 compounds, where all data are available. Values in parentheses include the six most lipophilic compounds for which SF values were not obtained. Addition of these compounds tends to increase the cross-correlations, because of increased range. The effect of increased sample size is insignificant in this case. The most significant observation is the large discrepancy for the $CH_3CN/0$ system with the number of points. This suggests that the $CH_3CN/0$ system does not model the most lipophilic compounds consistently (because of the good agreement among the other systems). Correlations vs. SF values are given in Table VI for the first 29 compounds.

It can be seen that RP-18/4 (eq 7) is as good as RP-18/1 (eq 5). This can be contrasted with eq 10 using 20%, v/v, CH_3CN for which there is very poor agreement, as noted previously.[4-9] The addition of 4 mM DMOA to 20%, v/v, CH_3CN considerably improves the agreement (eq 9). The data are not sufficiently extensive and the structures are not sufficiently diverse to allow a complete factor analytical investigation of Table IV; there is only one major factor, accounting for 97.9% of the total variance within this data set by principal components analysis.

All of the above work has been performed at physiological pH, 7.4, and for this reason we have not concerned ourselves with the simultaneous determination of ionization constants[1,2,9] by fitting theoretical curves through log D vs. pH curves. We present limited data in Table VII at several pH values. The agreement at different pH values is well within the experimental limits of this method, but there is a slight trend to divergence at lower pH. DMOA has been optimized at pH 7.4. Higher DMOA concentrations at pH 6.0 have been shown to lower log k' in preliminary studies.

Correlation of High-Performance Liquid Chromatography Distribution Coefficients with Biological Data. We have demonstrated that it is possible to make a RP-HPLC system behave essentially *exactly* as a bulk phase 1-octanol partitioning system, a system which has been the reference standard for the past 2 decades.[9] The question naturally arises, is 1-octanol the best system for biological correlations? One deficiency of previous studies[3] has been a choice of compounds with very limited structural variation. Since CH_3CN and 1-octanol values can be highly correlated for series with constant hydrogen-bonding properties,[4,9] one must choose compounds with a wide range of structural types in order to choose between these two systems. Otherwise the choice between systems can depend upon minor statistical quirks in the data and not upon underlying mechanisms. Henry et al.[3b] obtained

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Table IV. Consistent Data Set[a]

compd	log k' RP-18/4	C-18/4	CH_3CN/4	CH_3CN/0	SF	log D_{calcd}[b]
nitrobenzene	0.57	0.021	0.118	0.160	1.88	
anisole	0.885	0.381	0.264	0.279	2.09	
benzene	0.901	0.353	0.273	0.29	2.13	
chlorobenzene	1.61	1.022	0.773	0.807	2.84	
benzophenone	1.87	1.301	1.005	1.059	3.1	
nortriptyline	0.767	0.363	0.349	0.899	1.98	2.008
lidocaine	0.336	−0.087	0.005	0.036	1.65	1.58
imipramine	1.227	0.806	0.498	1.058	2.49	2.465
mesidorazine	0.537	0.135	−0.196	0.394	1.80	1.78
doxepin	1.086	0.642	0.330	0.886	2.22	2.325
propoxephene	1.259	0.769	0.483	0.673	2.36	2.497
tilorone	0.229	0.113	−0.412	0.051	1.47	1.475
quinidine	0.788	0.214	−0.312	−0.084	2.07	2.029
mepivacaine	0.008	−0.492	−0.462	−0.472	1.27	1.254
mepacrine	0.721	0.344	−0.064	0.480	1.91	1.963
fluanisone	1.64	1.063	0.805	0.896	2.98	2.875
dibucaine	1.642	1.11	0.501	0.915	2.91	2.877
promazine	1.345	0.907	0.505	1.252	2.48	2.582
promethazine	1.669	1.176	0.757	1.252	2.85	2.904
indoramin	1.028	0.348	−0.055	0.136	2.31	2.267
clozapine	1.725	0.95	0.817	1.151	2.99	2.959
chlorpheniramine	0.262	0.074	−0.329	0.334	1.38	1.507
cyproheptadine	1.984	1.529	1.037	1.43	3.11	3.217
acridine	2.103	1.447	0.685	0.775	3.29	3.335
chlorpromazine	2.144	1.667	1.086	1.632	3.22	3.375
amitriptyline	1.641	1.214	0.753	1.264	2.83	2.853
acetophenazine	1.301	0.808	0.366	0.728	2.62	2.527
desipramine	0.432	0.108	0.182	0.807	1.45	1.695
clomipramine	2.072	1.593	1.041	1.479	3.24	3.265
perphenazine	2.287	1.749	1.1	1.473	nd[c]	
trifluopromazine	2.459	1.996	1.315	1.736	nd	
thioradizine	2.411	1.942	1.284	1.717	nd	
chlorprothixene	2.614	2.142	1.389	1.658	nd	
prochlorperazine	2.767	2.292	1.571	1.907	nd	
trifluoperazine	3.076	2.58	1.775	1.96	nd	

[a] See Table II for explanation of symbols. CH_3CN is 20%, v/v, CH_3CN in buffer A. RP-18/4 is considered the "optimized" set of conditions. Minor discrepancies between SF values given in this table and Table II are due to redeterminations with this batch of buffer A. Column lengths: RP-18/4, 2 cm; C-18/4, 3 cm; CH_3CN, both on 10 cm C-18 columns. [b] From eq 7. [c] Not determined.

Table V. Squared Correlation Matrix for Table IV[a]

system[b]	A	B	C	D	E
A RP-18/4	100	97 (98)	86 (93)	68 (81)	98
B C-18/4		100	86 (93)	77 (86)	93
C CH_3CN/4			100	79 (87)	83
D CH_3CN/0				100	61
E SF					100

[a] Numbers represent the percent variance explained; numbers in parentheses are for all 35 compounds, others are for 29 compounds. [b] See Tables IV and II for explanation of symbols.

moderate correlations of various HPLC systems vs. biological activity for sulfonamides and barbiturates, both sufficiently diverse in structure. However, the moderate correlations suggest the possibility that other factors (electronic, steric?) might play a role in the biological activity. In order to focus on the choice of partitioning systems, the biological activity should ideally be determined *solely* by partitioning properties. Otherwise statistical problems can distort the choice.

Furthermore, as we have shown in Table I, buffer components can have a considerable[20] effect on D; for many compounds these distribution coefficients are *not* related to P by the often used eq 11a but by the more complicated

$$\log D = \log P - \log [1 + ([H]/K_a)] \quad (11a)$$

$$\log D = \log [P + P'([H]/K_a)] - \log [1 + ([H]/K_a)] \quad (11b)$$

eq 11b, where P' is the partition coefficient for the ion pair.[9] K_a is the dissociation constant for the protonated base. Therefore, in the case of lipophilic amines, one must be very cautious in "correcting" tabular data obtained under different experimental conditions,[24] since eq 11a is not applicable and P' is seldom known. One should also be cautious of using calculated contributions such as π or f for very polar or charged groups due to ion pairing. Ideally, data must be collected under uniform ionic strength and with uniform buffer components and be compared to identical systems for maximum accuracy. Otherwise, the interpretation of correlation equations is confounded by several different sources of error.

Table VI. Log $D_{SF} = a$ Log $k' + b$ for Table IV

system[a]	a	b	s^b	r	$F_{1,27}$	SD/SDM	eq
RP-18/4	0.98 (0.05)	1.24 (0.06)	0.079	0.992	1681.0	2.4%	7
C-18/4	1.04 (0.11)	1.67 (0.10)	0.169	0.964	351.1	5.1%	8
CH_3CN/4	1.22 (0.12)	1.92 (0.13)	0.263	0.909	128.8	8.0%	9
CH_3CN/0	0.92 (0.29)	1.72 (0.26)	0.365	0.780	41.9	12.1%	10

[a] See Table IV and II for explanation of symbols. [b] $n = 29$.

Table VII. pH Effects on Log D_{HPLC}[a] and Log D_{SF}[b]

compd		pH 6.0	6.43	6.95	7.40	8.00
mepacrine	SF:	−0.05	0.58	1.24	1.91	2.75
	HPLC:	0.34	0.70	1.35	1.96	2.76
chlorpromazine	SF:	2.25	2.58	2.91	3.22	
	HPLC:	2.50	2.68	3.02	3.38	

[a] RP-18/4; see Table II for explanation of symbol. [b] Same buffer without 4 mM DMOA.

In the case of our study on *neutral* tuberins,[4,9] we found a better correlation using 1-octanol HPLC log P values over 25%, w/w, MeOH HPLC values for the correlation of activity vs. the lipophilic *Mycobacterium phlei*. However, in order to obtain a good correlation it was necessary to add both field and resonance electronic effects to both equations and also a dummy parameter to the 1-octanol equation in order to handle hydrogen-bonding substituents. We concluded that the origin of the dummy parameter was to "correct" 1-octanol back toward a purer alkane phase of the waxy *Mycobacteria*. The 1-octanol equation had $r = 0.952$ ($s = 0.14$), while the 25%, w/w, MeOH equation had $r = 0.867$ ($s = 0.257$); thus, even for this well-designed set of data, it was not possible to make a clear and decisive choice between the two methods.

While there are innumerable studies on lipophilic amines, unfortunately most contain only a very small number of compounds of one or the other subclass and, therefore, insufficient variation in hydrogen-bonding properties to significantly differentiate between alkane (CH_3CN/C-18) and alcohol (1-octanol/RP-18) HPLC systems. In addition, in order to clearly differentiate between partitioning model systems, the biological activity should be nonspecific or have at least a minimum of "special" factors in order to focus on the partitioning process. The cationic portion of the molecule may be involved directly in the activity, in which case the pK_a may represent the basicity of the N or acidity of the NH^+. However, the pK_a may also represent a "correction" because the partitioning model may not be correct. As discussed in excellent reviews by Kubinyi[26] and Martin,[27] simple linear QSAR may not be correct for ionized species. Once again, the available literature that we have been able to uncover is probably *not* adequate in terms of either quantity (to support the additional regression parameters in the more complicated models[26,27]) or diversity (to differentiate between partitioning models) to answer the above questions. We discuss below three examples which contain enough compounds of sufficient diversity to enable one to make a plausible, *initial* argument toward the latter point. *However, we must await a better data set to answer all of the above questions with certainty.*

Frisk-Holmberg and van der Kleijn[28] studied the histamine-releasing activity in rat mast cells at pH 7.0 for 14 phenothiazines and tricyclic antidepressants for which we have measured log D values. Distribution coefficients ("apparent partition coefficients") were also determined by the authors but were extrapolated, using the dubious eq 11a, considerably outside of the observed pH range in order to provide "true" partition coefficients.

The data are given in Table VIII; log k' values have been taken from Table IV and the results are given in Table IX. Correcting log D_{calcd}, which was obtained by regression between log D_{SF} and log k' for RP-18/4 (i.e., from eq 7), to pH 7.0 of the experiment can be accomplished by use of eq 11a because of the small change in pH. Thus, log D_1 − log D_2 = log $[(K_a + [H_2])/(K_a + [H_1])]$. The nearly constant p$K_a$ for this series of compounds gives rise to nearly constant corrections, 0.285 − 0.400. The resulting correlation is given by eq 20. As can be seen in Table IX,

$$\log (1/ED_{50} \times 10^{-3}) = \underset{(0.13)}{0.53} \log D^{7.0}_{calcd} + \underset{(0.36)}{0.82} \quad (20)$$

$n = 14$; $s = 0.167$; $r = 0.932$; $F_{1,12} = 78.3$; SD/SDM = 10.5%

RP-18/4 is about equal to C-18/4; both are superior to CH_3CN/4. Omitting the 4 mM DMOA destroys the correlation for CH_3CN/0. Because of limited data, the SF comparisons, eq 16 and 17, have only nine compounds, but RP-18/4 is again slightly superior. There are an insufficient number of compounds to clarify the reason for the lower correlation with SF. Using Frisk-Holmberg and van der Kleijn's log P_{app} (apparent partition coefficient) values, we obtain eq 18, which can be compared to the superior equation (eq 19) for the same compounds. The authors report $r = 0.78$ for 14 compounds. The correlation between log P_{app} and log $D^{7.0}_{calcd}$ is low, $r = 0.836$, suggesting experimental problems in the determination of P_{app}. For a smaller group of six phenothiazines, Hulshoff and Perrin[29] found a high correlation with R_M values for the free base determined on oleyl alcohol coated Kieselguhr G plates with MeOH–H_2O containing ammonia and KCl at pH^x = 10.5. However, the activity values were corrected to those expected for the free base by a model. Further improvement was obtained by adding activity of about 1% due to the protonated species, $r = 0.907$–0.986. There do not appear to be enough compounds to fully justify these conclusions, especially since the pK_a values used in the corrections were compiled from different sources. Ideally, these constants should be determined under the same conditions as the other parameters.[1,2,21]

Addition of pK_a to log k' was insignificant for all equations, except eq 15 and 18; see eq 21 and 22. Equation 21 is as good as eq 12, except for the lower overall F (r will be higher because of the additional term). Since addition of 4 mM DMOA, eq 14, both improves the correlation and eliminates the significance of an added pK_a term, it would appear that this term is a correction of log $k'_{CH_3CN/0}$ (toward pure partitioning) and does not involve the biological activity. We therefore conclude that the RP-18/4 system is the superior model for biological activity compared to organic modifiers and that the release of histamine from rat mast cells at pH 7.0 is a nonspecific property of lipophilic amines, determined by the extent of binding. Since the best equation (eq 12 or 20) only explains 86% of the variance and the biological error is probably somewhat better than 14%, it is still possible that the binding occurs in a more complicated manner than indicated by these simple linear equations. For example, both the neutral compound and the ion pair may bind, but with different affinities. More complicated models cannot be fit with the small amount of data available at this time.[26,27]

Hulshoff and Perrin[29] have obtained the BSA binding constants by circular dichroism for a series of phenothiazines at pH 7.44. Because there are only seven compounds in common between these two studies (Table VIII) and because the TLC R_M values for the free base used by the authors were partly calculated, we have limited our

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Table VIII. Biological and Partition Data

			histamine release[a]				BSA binding[g]			ATPase[i]		
no.	compd	pK_a	P_{app}[k]	obsd	calcd[b]	Δ	obsd	calcd[h]	Δ	obsd	calcd[j]	Δ
1	promazine	9.42[e]	2.15	2.05	1.97	0.08	3.21	3.25	−0.04	−1.17	−1.03	−0.14
2	chlorpromazine	9.36[e]	2.92	2.52	2.40	0.12	3.66	3.66	0.00	−0.21	0.09	−0.31
3	promethazine	9.4[c]		1.82	2.15	−0.33				−0.37	−0.57	0.21
4	trifluoperazine	8.1[c]	3.90	2.92	2.89	0.03	3.91	3.98	−0.07	1.69	1.40	0.29
5	thioradazine	9.5[c]		2.72	2.54	0.18	3.81	3.82	−0.01	0.79	0.47	0.32
6	prochlorperazine	8.1[c]	2.40	2.75	2.73	0.02	3.83	3.82	0.01	0.87	0.97	−0.10
7	perphenazine	7.8[c]	2.95	2.63	2.47	0.16	3.57	3.52	0.04	0.09	0.29	−0.21
8	acetophenazine	7.8[d]		2.12	1.95	0.17						
9	chlorprothixene	7.6[c]	2.67	2.57	2.65	−0.08						
10	amitriptyline	9.4[c]	2.3	1.85	2.13	−0.28						
11	nortriptyline	9.73[f]	1.8	1.76	1.67	0.09						
12	imipramine	9.5[c]	2.3	1.79	1.91	−0.12				−0.99	−1.19	0.21
13	clomipramine	9.5[c]	2.8	2.22	2.36	−0.14						
14	desipramine	10.2[c]	1.7	1.58	1.49	0.09						
15	trifluopromazine	9.21[e]					3.88	3.81	0.07	0.27	0.54	−0.27

[a] Log $(1/ED_{50} \times 10^{-3})$.[28] [b] Equation 12. [c] Cited in ref 28. [d] Estimated from analogues. [e] Cited in ref 29. [f] Cited in ref 25. [g] Log K, ref 29. [h] Equation 23b. [i] Log [%/(100 − %)], percent inhibition at 1×10^{-4} M.[30] [j] Equation 27. [k] Apparent partition coefficient.[28]

Table IX. QSAR for Histamine Release[a]

system	X	a	b	n	s	r	$F_{1,n-2}$	SD/SDM	eq
		A. Log $(1/ED_{50} \times 10^{-3}) = aX + b$ (All Compounds)[b]							
RP-18/4	log k'	0.53 (0.13)	1.26 (0.26)	14	0.171	0.929	75.2	10.7%	12
C-18/4	log k'	0.56 (0.14)	1.47 (0.22)	14	0.173	0.927	73.1	10.8%	13
CH_3CN/4	log k'	0.82 (0.23)	1.50 (0.24)	14	0.188	0.913	59.9	11.8%	14
CH_3CN/0	log k'	0.98 (0.34)	0.90 (0.49)	14	0.225	0.873	38.4	14.1%	15
		B. Log $(1/ED_{50} \times 10^{-3}) = aX + b$ (Omitting Compounds without Log D_{SF})							
buffer A	log D_{SF}	0.39 (0.28)	0.97 (0.74)	9	0.192	0.779	10.8	23.7%	16
RP-18/4	log k'	0.41 (0.27)	1.40 (0.41)	9	0.184	0.800	12.4	22.7%	17
		C. Log $(1/ED_{50} \times 10^{-3}) = aX + b$ (Omitting Compounds without Log P_{app})							
e	log P_{app}	0.62 (0.32)	0.67 (0.85)	11	0.273	0.827	19.5	18.7%	18
RP-18/4	log k'	0.52 (0.12)	1.27 (0.24)	11	0.142	0.956	96.0	9.8%	19

system	X	a	b	c	n	s	r	$F_{2,n-3}$	SD/SDM	eq
		D. Log $(1/ED_{50} \times 10^{-3}) = aX + bpK_a + c$								
CH_3CN/0	log k'	0.81 (0.28)	−0.19 (0.13)	2.84 (1.34)	14	0.167	0.938	40.2	10.5%	21
e	log P_{app}	0.36 (0.28)	−0.29 (0.19)	3.91 (2.45)	11	0.184	0.934	27.3	12.6%	22

[a] Reference 28. See Tables IV and II for explanation of symbols. [b] See Table VIII. [c] Table VIII, compounds 4-7 and 9; log D_{SF} from Table IV. [d] Table VIII, compounds 3, 5, and 8. [e] pH 7.0, μ = 0.15 Sörensen's buffer with 1-octanol (see ref 28).

Table X. Log $K = a \log k' + bpK_a + c$ for BSA Binding

system[b]	a	b	c	s	r	F^e	SD/SDM	eq
RP-18/4	0.42 (0.20)		2.71 (0.48)	0.103	0.924	29.1	17.1%	23a
RP-18/4	0.52 (0.15)	0.13 (0.11)[c]	1.31 (1.21)	0.059	0.980	49.4	9.9%	23b
C-18/4	0.42 (0.19)		2.88 (0.37)	0.098	0.932	33.3	16.2%	24a
C-18/4	0.52 (0.16)	0.11 (0.12)[d]	1.72 (1.22)	0.064	0.977	41.8	10.7%	24b
CH_3CN/4	0.57 (0.25)		3.00 (0.33)	0.098	0.931	32.7	16.3%	25
CH_3CN/0	0.94 (0.20)		2.13 (0.67)	0.093	0.939	37.1	15.4%	26

[a] Reference 29. Data from Table VIII. [b] See Tables IV and II for explanation of symbols. [c] $p < 0.05$. [d] $p < 0.10$. [e] $F_{1,5}$ for eq 23a, 23b, 24, and 25 and $F_{2,4}$ for eq 23b and 24b; $n = 7$.

studies to intercomparisons between our four HPLC systems. The range in log K for these seven compounds is quite small, and more detailed comparisons are not warranted. We have included correlations with pK_a because the authors found evidence for parallelism of log K vs. R_M based on the pK_a of the subclass. The results are given in Table X. There is a *slight* trend toward higher correlation with CH_3CN/0 when pK_a is omitted. This may be due to the interaction of the aliphatic nitrogen (or NH^+) with the free silanol sites; the interaction may mimic interaction with the phenothiazine binding site on BSA. Addition of pK_a to the correlation, however, shows the reverse trend, with RP-18/4 being the best two-parameter model. The positive coefficient with pK_a indicates that more basic amines bind more strongly, but the microscopic interpretation is not yet clear. The pK_a term is less significant with C-18/4 and insignificant with CH_3CN/4 and CH_3CN/0. This trend tends to confirm that the CH_3CN values are sensitive to hydrogen-bonding interactions with free silanol sites. The interaction is blocked by addition of DMOA *and* 1-octanol. RP-18/4 is closer to a pure partitioning mechanism, while CH_3CN/0 represents a blend of partitioning and absorption. The large change upon addition of 4 mM DMOA to CH_3CN in the previous case, eq 14 and 15, for similar compounds shows that the two biological processes are not the same. Histamine release is apparently nonspecifically inhibited by lipophilic amines, while BSA binding appears to contain some spe-

Table XI. Logit % = a Log k' + b for Inhibition of (Na^+,K^+)ATPase[a]

system[b]	a	b	s	r	$F_{1,7}$[c]	SD/SDM	eq
RP-18/4	1.40 (0.36)	−2.92 (0.80)	0.269	0.962	85.8	10.4%	27
C-18/4	1.45 (0.36)	−2.32 (0.65)	0.266	0.963	88.0	10.3%	28
CH_3CN/4	1.99 (0.45)	−2.08 (0.53)	0.241	0.969	108.0	9.3%	29
CH_3CN/0	2.66 (1.08)	−4.02 (1.70)	0.404	0.911	34.1	15.6%	30

[a] Reference 30. Data from Table VIII. [b] See Tables IV and II for explanation of symbols. [c] n = 9.

cificity with regard to basicity of the aliphatic nitrogen. Furthermore, the pK_a term enters eq 21 with a negative term and was thought to be a correction of CH_3CN/0 toward a purer partitioning system (see above). For BSA binding, a positive pK_a term enters eq 23b and 24b, and the effect is thought to be due to a specific interaction of the aliphatic amine with a binding site on the BSA.

The authors corrected log K (pH 7.44) to that expected by their model for the free base and obtained two sets of lines with R_M (pH^x 10.5) for the free base. They then corrected a second time to introduce about 4% binding of the protonated species and noted an improvement in correlation. Propagation of errors in pK values was not considered. We interpret the results of Table X to indicate that binding is governed both by increasing distribution of the free base and ion pair and by the basicity of the aliphatic nitrogen. However, the small number of compounds makes these conclusions tentative. One cannot examine the role of steric hindrance about the aliphatic NH^+, nor can one test more complicated binding models.[26,27] Hulshoff and Perrin[29] also discuss the conflicting direct experimental evidence in the literature.

Davis and Brody[30] have studied the inhibition of (Na^+,K^+)ATPase in rat brain at pH 7.5 by nine of the compounds in Table VIII. We correlate the logit of the percent inhibition at 1×10^{-4} M (logit % = log [%/(100 − %)]; this is a preferred transformation compared to log % because it is linear over a wider range of percent and has better distribution properties. Table XI shows the results of correlation vs. the four HPLC systems. Addition of pK_a was insignificant for all systems.

In this case, all three DMOA systems give similar high correlations with CH_3CN/4 superior in terms of overall F. The addition of DMOA to CH_3CN significantly improves the ability to correlate the biological data compared to CH_3CN/0. This might indicate that the basicity of the aliphatic N is not critical. Indeed, it is thought that the mode of action for these types of compounds is nonspecific binding of the drug to the membrane. This induces a change in membrane fluidity, which in turn induces a change in the conformation—hence, activity—of the membrane-bound enzyme.[31] Since there is no direct interaction between drug and enzyme, there is lower discrimination found between the three partitioning systems as models for the binding process. This observation may be compared to the previous example, in which pK_a was found to be critical because the drug (presumably) bound at a (somewhat) specific site on the BSA protein, and to the binding to mast cells, in which the optimized 1-octanol system was superior.

Different model systems may be required for different biological circumstances. In fact, the *pattern* of correlations that we have obtained has been helpful in interpretation of the results on these three less than ideal data sets. While the 1-octanol (lipophilic alcohol) system does perform well for the three diverse biological systems examined herein, we do not feel that this study provides an unequivocal answer. Comparisons of small differences in statistical parameters are probably justified in this case because the range in independent variable (log k') is similar and residuals are evenly distributed about zero and show no trends for the key equations. The main difficulty, as stated above, is that the hypothesis that 1-octanol performs better than alkane partitioning or distribution coefficients can only be adequately tested by a much larger, much more diverse set of compounds with differing hydrogen-bonding capabilities. Unfortunately, we have not been able to uncover (or produce at this time) sufficient data on the nonspecific actions of lipophilic amines to fully answer this question or to explore the more accurate models for handling activity of both free base and ionized species.[26,27] Finally, we would recommend the use of the above conditions, without DMOA, for neutral, acidic, and hindered basic compounds.

Experimental Section

The general procedure of Unger and Feuerman[2] was used. All HPLC runs were conducted at room temperature in an air-conditioned room. All packing materials were first persilated.[21] C-18 Corasil (Waters, 37–50 μM) columns were dry-packed; RP-8 and RP-18 Lichrosorb (Merck, mean 10 μM) columns were packed by a modification of the slurry packing method of Bristow.[22] The persilated and dried packing material was first slurried in ca. 3 mL of 1-octanol and then poured into a precolumn (0.25 × 4 in.) which was connected to an empty column. The free end of the precolumn was connected to the HPLC pump. The HPLC reservoir was filled with 1-octanol saturated H_2O and a flow rate of 0.5 cm^3/min was used initially, eventually increasing to 4 cm^3/min or until the inner pressure exceeded 2500 psi. The column was held vertically and continuously flushed with 1-octanol saturated H_2O until no 1-octanol drops could be found in the eluent (ca. 4 h). The packed column was then disconnected from the precolumn and the performance of the column tested with standard compounds.

Stainless-steel tubing of ca. 2-mm i.d. with an interior coating of Teflon (ca. 0.001 in. thick) was purchased from Alltech Associates. Different column lengths (2, 3, 10, and 50 cm) were packed to accommodate different lipophilicities. Flow rate was constant at 2–3 mL/min. Generally, best results were obtained when $1 < k' = (t_x - t_0)/t_0$ where t_0 is the retention time of the unretained internal standard (e.g., MeOH). Column length was selected such that most (preferably all) compounds would satisfy this criteria, but ease of analysis was also a factor. For the compounds studied herein, the 2- and 10-cm columns were usually adequate.

The mobile phase consisted of 0.01 μ PO_4 [KH_2PO_4 (1.47 mM)/K_2HPO_4 (8.06 mM)] with 0.14 μ NaCl (called buffer A); μ = ionic strength. The 0.15 μ PO_4 buffer (called buffer B) was 45.5 mM K_2HPO_4 and 13.5 mM KH_2PO_4. Buffer A, the isotonic "phosphate buffer saline" or PBS buffer, was first saturated with 1-octanol (Fisher certified) and then was supplemented with appropriate amounts of neutralized *N*,*N*-dimethyloctylamine, DMOA (ICN Pharmaceuticals). Slight amounts of HCl and/or NaOH were then added to adjust the final pH^x reading to 7.40 (because of the 1-octanol, these are not true pH, but the adjustment serves to standardize different batches as to acidity). Mobile phases were passed through Millipore filters immediately before use to degas and purify. Samples were dissolved in MeOH or preferably MeOH/buffer, with MeOH serving as internal standard. Calculation of k' for multiple injections during the

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course of a session showed that k' was constant to a very high degree. Day-to-day variation was larger, about 3%, and linear-regression equations could be used to relate all log k' back to a given day by appropriate choice of standards. For situations in which a very high correlation between log D_{SF} and log k' were obtained, $r > 0.99$, use of standard compounds allowed log D to be calculated each day. Standards were all measured by shake-flask methods (see below) in the identical buffers (without DMOA). For buffer A, log D values are as follows: 2-butanone, 0.28; aniline, 0.91; benzaldehyde, 1.45; acetophenone, 1.65; nitrobenzene, 1.88; anisole, 2.09; benzene, 2.13; benzophenone, 3.10; chlorobenzene, 2.84. Reproducibility in log D was also about 3%. The 20%, v/v, CH_3CN (MCB, Chromatoquality) was prepared by diluting 20 volumes of organic with buffer A to 100 volumes.

Shake-flask partitionings were carried out in 16 × 100 mm culture tubes with an aluminum-lined screw cap. A typical partitioning was conducted in 10 mL of the same isotonic buffer A as used in the HPLC work and varying amounts of buffer-saturated 1-octanol, depending upon the lipophilicity of the sample. Samples were shaken on an automatic shaker for at least 2 h, and the tubes were then centrifuged at 2000 rpm to clarify the two phases. Spectrophotometric determination (Bausch & Lomb Spectronic 200 UV) of concentrations was used for pure and stable compounds; for impure or unstable samples, concentrations in both phases were determined by standard analytical HPLC methods using computer integration or cut-out tracings to determine relative amounts of compound. Final sample concentrations of 10^{-4} to 10^{-5} M in buffer and 10^{-3} to 10^{-4} M in 1-octanol were obtained. Each sample was determined at 3-4 dilutions and extrapolated to infinite dilution if a concentration trend was observed; otherwise, means were taken. Typical precision was 1-2%.

All computer correlations were performed on a commercial APL language system (Proprietary Computer Systems, Van Nuys, CA), using our published regression programs.[9]

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Mechanism of Action of (2-Haloethyl)nitrosoureas on DNA. Isolation and Reactions of Postulated 2-(Alkylimino)-3-nitrosooxazolidine Intermediates in the Decomposition of 1,3-Bis(2-chloroethyl)-, 1-(2-Chloroethyl)-3-cyclohexyl-, and 1-(2-Chloroethyl)-3-(4′-*trans*-methylcyclohexyl)-1-nitrosourea

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Three examples of the postulated but hitherto unisolated 2-(alkylimino)-3-nitrosooxazolidines (**2**) have been prepared containing cyclohexyl, *trans*-4-methylcyclohexyl, and 2-chloroethyl groups at the 2 position, respectively. These compounds correspond to intermediates previously postulated to be formed in the aqueous decomposition of the antitumor agents 1-(2-chloroethyl)-3-cyclohexyl- (CCNU), 1-(2-chloroethyl)-3-(4′-*trans*-methylcyclohexyl)- (MeCCNU), and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), respectively. Compounds **2** decompose under physiological conditions to give a range of products similar to those formed from the corresponding (2-chloroethyl)nitrosoureas, including the hitherto unrecognized 2-hydroxyethyl *N*-alkylcarbamates (**9**). Compounds **2a** and **2b** are converted with hydrochloric acid into CCNU and MeCCNU, respectively, suggesting that **2a** and **2b** may be reaction intermediates of decomposition. The corresponding 3-alkyl-1-nitroso-1-(2-hydroxyethyl)ureas (**4**) were characterized and, since they also decompose to give the same products as **2**, may arise from the ring opening of **2**. The intermediacy of compounds **4** can explain the formation of hydroxyethylated nucleosides isolated by other workers from the reaction of (2-chloroethyl)nitrosoureas on polynucleotides.

1-3-Bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3-[4′-*trans*-methylcyclohexyl]-1-nitrosourea (MeCCNU), and other (2-haloethyl)nitrosoureas are of clinical value in the treatment of Hodgkin's disease,[1,3] brain tumors[2], lymphomas,[3] and other malignant diseases. BCNU, CCNU, and related (2-chloroethyl)nitrosoureas have been found to give rise to electrophiles upon aqueous decomposition, which may alkylate DNA[4-7] and other macromolecules in the cell. For example, chloroethyl cations, or their equivalent, may alkylate a base in DNA and, following labilization of the C–Cl bond, this can result in an interstrand cross-link.[5-7] Another major decomposition product is the alkyl isocyanate which can result in carbamoylation reactions of amino groups in macromolecules.[8-12] Such reactions may, in part, underlie the cytotoxic action of these agents. Extensive studies on the chemistry of nitrosoureas and their hydroxylated metabolites[13-16] and the method of formation of products from

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