0.98, respectively). It appears that this also holds for the more recent methods of calculating surface area.[4b,c] It should be emphasized that, regardless of whether it develops that it is a volume or a surface area calculation which can be refined to give the best relation with log $P$, we will still be far from a computerized system of log $P$ calculation by merely summing the sizes of assorted fragments. The surface area of fragments can differ appreciably, depending on how they are connected and an even greater log $P$ variation can arise by differences in character of the solute surface, depending on the order in which the fragments appear in isomeric solutes or whether or not they are in position to screen a dipole or H-bonding group.

If we are to untangle the effects on hydrophobic bonding caused by dipoles—localized, diffuse, or partly shielded—and by various types of hydrogen bonds, it is evident that we must first have a clear understanding of the very basic role that is played by solute size.

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(7) While most solutes with polar functional groups show very little temperature dependence of log $P$, argon and some alkanes show a marked decrease at 0 °C. This is being investigated further.
(8) It should be noted that it is the *localized* bond dipole that is important in determining log $P$, not the measured *overall* dipole; otherwise, *o*-dichlorobenzene would have a much lower log $P$ than *p*-dichlorobenzene (ortho, $\mu = 1.58$, log $P = 3.38$; para, $\mu = 0.0$, log $P = 3.39$).
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# Direct Measurement of Octanol–Water Partition Coefficients by High-Pressure Liquid Chromatography

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A technique is presented for the direct measurement of octanol–water partition coefficients by HPLC. The method involves running solutes in octanol-saturated water as the mobile phase against water-saturated octanol entrained on an inert support. Log $P$ correlates linearly with log $t_c$ for a number of standards. The measurable range in log $P$ (so far) is −0.3 to +3.7. A critical review of chromatographic methods in Hansch analysis is given.

Since the pioneering work of Meyer and Overton,[1] it has gradually become evident that nonspecific distribution plays an important part in determining biological activity. There are two parallel but essentially dissimilar ways in which this understanding may be exploited. The first is to attempt a correlation between biological potency and some thermodynamic quantity that may reasonably be imagined to reflect the distribution process, most commonly partition coefficient. For this purpose, any type of partition coefficient will do; success is the only criterion. The weakness of this approach is its inability to relate one such correlation to another. It was partly to overcome this weakness that Hansch explored the possibility of deriving additive group contributions to the partition coefficient that would allow this to be calculated for unsynthesized compounds (his other main contribution, the introduction of a multiparameter approach to the rationalization of biological activity,[2-4] does not concern us here). Octanol–water was chosen as the standard reference system for reasons connected with the easy availability of pure octanol and its intermediate degree of polarity, arguably much closer in its properties than a hydrocarbon to the biophase.[5] These group contributions or "$\pi$ values" are

$$\log P_R - \log P_H = \pi_R \quad (1)$$

defined by eq 1, in which $P_H$ and $P_R$ are, respectively, the octanol–water partition coefficient for the putative parent compound of a series and for that containing the substituent R. Despite evidence[6] that $\pi$ values are not always additive, usually for steric reasons but sometimes through electronic and hydrogen bonding effects, there are now many series of compounds in which $\pi$ appears to provide most of the reason for variation in biological potency.[2-5]

Partition coefficients are generally determined by some variant on the traditional shake-flask method.[7] This method is slow, tedious, often wasteful, and demanding in the standard of purity it requires. Consequently, there have been many attempts to develop chromatographic methods, to which in principle none of the above objections need apply. This is possible because some (not all) forms of chromatography are dominated by partitioning processes. It was shown by Martin and Synge[8] that $R_f$ relates

$$P = \text{constant } (1/R_f - 1) \quad (2)$$

$$R_M = \log (1/R_f - 1) \quad (3)$$

to $P$ according to eq 2; it follows that $R_M$, defined by Bate-Smith and Westall[9] according to eq 3, is linearly related to log $P$, and that $\Delta R_M$ is therefore analogous to $\pi$. (Green and Marcinkiewicz[10] had already shown, before Hansch's introduction[2] of $\pi$, that $\Delta R_M$ is additive for a number of benzenoid compounds.) The first exploitation of this relationship in a biological context is due to Boyce and Milbarrow,[11] who showed a relation between the

molluscicidal activity of some *N*-alkyltritylamines and their $R_M$ values on TLC plates; many such investigations, mostly by TLC but some involving paper chromatography, have followed.

Very recently, several authors[12-15] have demonstrated that HPLC can also be employed for this purpose. The advantages of HPLC over TLC lie in accuracy (i.e., definition of the peak), reproducibility, sometimes in ease of detection, and above all in dynamic range; as Tute[4] has pointed out, accuracy by TLC demands $0.2 < R_f < 0.8$, a range in $R_M$ of less than 1.5 decades where HPLC is capable of 3 to 4 decades (see below). Another advantage, which McCall emphasizes,[13] is that the support medium can be made almost totally inert. The importance of this factor has not always been appreciated. For example, Dearden and Tomlinson[16] in a TLC study using paraffin or octanol coated on a silica support show an excellent correlation for a series of acetanilides between $\pi$ (octanol–water) and the $\Delta R_M$ values obtained against both stationary phases. Since the substituents used were highly discriminating and there is no general correlation between octanol–water and hydrocarbon–water partition coefficients,[7] this is a very surprising result. Here the influence of the SiOH groups has almost certainly had the effect of imparting a spurious polarity to the paraffinic phase; it is noteworthy that Draber and Buchel[17] found *no* correlation ($r = 0.078$) between $\pi$ and $\Delta R_M$ for a series of hydrazones on paraffin-coated TLC plates and attributed this to hydrogen bonding with the silica support. It has also been noted[18] that TLC on silica is dominated by adsorption mechanisms and that a cellulosic support is in this respect better. Such a support has been used by Bird and Marshall[19] in a TLC study of some penicillins using octanol as the stationary phase. At pH 3 they found the almost unit slope relation of eq 4; this contrasts with the

$$R_M = 1.035 \log P - 1.892 \ (r = 0.997) \quad (4)$$

$$\Delta R_M = 0.832 \pi + 0.022 \quad (5)$$

octanol–water eq 5 of Dearden and Tomlinson[16] with its anomalously low slope. Boyce and Milbarrow,[11] using aqueous acetone as the mobile phase, noted that the incremental $R_M$ in a homologous series fell as the proportion of acetone rose, the result presumably of a convergence in solvent properties between the two phases. Since Dearden and Tomlinson[16] used the same medium, this factor also will help to explain the slope of eq 5. The slightly excessive slope of eq 4 was explained by Bird and Marshall[19] as due to the rather high concentration (0.5 M) of buffer employed; high ionic strengths are therefore also to be avoided, though this effect seems less important than the other (but see later).

The retention $k'$ of a compound by HPLC is related to elution time by

$$k' = (t_R - t_0)/t_0$$

where $t_R$ and $t_0$ are the retention times of a retained and unretained compound, respectively.[13] If $t_c = (t_R - t_0)$ is the corrected elution time

$$\log k' = \log t_c - \log t_0$$

$$\log P = \log t_c + \text{constant} \quad (6)$$

It is seen from the equivalence[12,13] of $\log k'$ and $R_M$ that $\log t_c$ is related to $\log P$ by an equation of type 6. The question of importance in the present context is whether the determination of $\log P$ by the HPLC method gives results close enough to those obtained by shake-flask partitioning to permit the accurate derivation of $\pi$ values (it is admitted, as noted at the start, that this may be irrelevant if nothing beyond a straight correlation with biological potency is required). The answer appears to depend on the class of compound studied. McCall[13] has measured $k'$ for a number of benzenoid compounds and, by normalizing their values to that for benzene itself, has shown agreement with shake-flask $\log P$ values to be generally good for simple hydrocarbons but to deviate progressively as the polarity of the solute increases; an error of up to 0.3 in $\log P$ is found for amines. The mobile phase was water and particular care had been taken to silanate residual SiOH groups, without which precaution correlation is much worse,[13] so the major source of error must be sought elsewhere. Similarly, Carlson et al.[14] publish correlations between $\log k'$ and $\log P$ for a number of substituted phenols and anilines whose apparent goodness of fit ($r = 0.96, 0.97$) conceals individual deviations that are unacceptably large (up to 0.2) if this is to be used as a method for deriving $\pi$ values. Haggerty and Rehagen[12b] and Henry et al.[15] note similar anomalies with respect to their own work. Since all these authors use as their stationary phase a bonded octadecylsilane support which must have essentially the partitioning characteristics of a hydrocarbon, and since there is no general correlation between octanol and hydrocarbon partitioning,[7] we believe this fact to represent the main source of the deviations noted above. It cannot be too strongly emphasized that, on all the available evidence, the only true model for octanol is octanol itself.

We therefore set out to determine whether HPLC could be adapted to measuring octanol–water partition coefficients directly. The attempt has succeeded, and the purpose of this paper is to present our method and results. A commercial Kieselguhr support is thoroughly silanized and slurry packed[20] at high pressure into a column of 4.6-mm bore; column lengths of 10–30 cm have so far been used. The packing is coated with water-saturated octanol, which is then eluted with octanol–saturated water until no more droplets of octanol can be removed. The column is now ready for use. Solutes are injected in octanol-saturated water at a concentration appropriate for uv detection; the same medium is used as eluent. This and the solutions are buffered to ensure the presence of the solute in the un-ionized form (the concentration of buffer is typically 0.01 M; see later). Pyridine *N*-oxide ($\log P$ −1.69[21]) or tyrosine ($\log P$ −2.26[22]) is added with each standard or unknown to define $t_0$. Two or three standards appropriate to the $\log P$ range under investigation are run daily; at constant flow rates this has proved sufficient, and indeed a surprising degree of reproducibility is found between days. Minor peaks due to impurities in unknowns can be ignored. Elution times of up to 2 h can be measured accurately. By varying the column length and flow rate, a range in $\log P$ of −0.3 to +3.7 has so far been attained. At a flow rate of 2 ml/min, recoating of the column is not normally required in less than 50 h of actual use.

The results for one such experiment employing a series of standards are displayed graphically on Figure 1; eq 7 defines the relation. Its slope is indistinguishable from

$$\log t_c = 1.0056 \log P \ (\pm 0.0181) - 0.6223 \ (\pm 0.0279) \quad (7)$$
$$n = 7; \ r = 0.999$$

unity, as it should be. Similar graphs have been constructed for other $\log P$ ranges by varying the column length and flow rate. Table I compares the literature

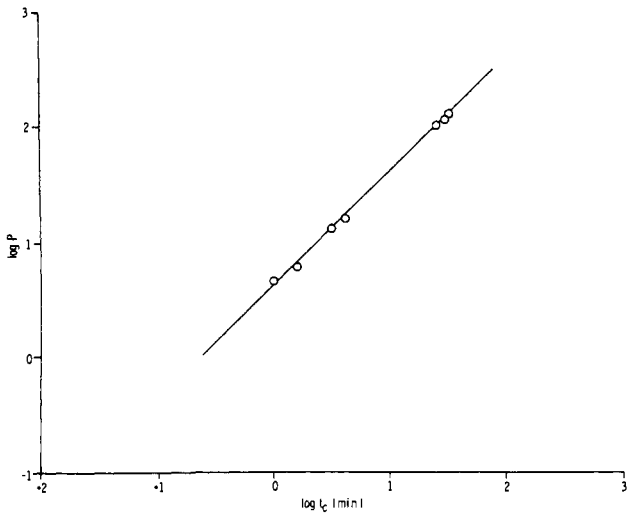


Figure 1. The relation between log $P$ and log $t_0$ for pyridine, practolol, acetanilide, β-picoline, quinoline, isoquinoline, and anisole (25-cm column, flow rate 2 ml/min).

Table I. Log $P$ Values of Standards by Conventional and HPLC Techniques

| Compound | Log $P$ | |
|---|---|---|
| | Lit. | HPLC[a] |
| Caffeine | −0.07[b] | −0.07 |
| 4-Aminopyridine | 0.28[c] | 0.26 |
| Pyridine | 0.65[d] | 0.66 |
| Practolol[e] | 0.79 | 0.78 |
| Aniline | 0.90[f] | 0.90 |
| Acetanilide | 1.16[f] | 1.14 |
| β-Picoline | 1.20[c] | 1.20 |
| Phenol | 1.46[f] | 1.45 |
| Quinoline | 2.03[d] | 2.04 |
| Isoquinoline | 2.08[g] | 2.09 |
| Anisole | 2.11[f] | 2.10 |
| $p$-Chlorophenol | 2.39[f] | 2.40 |
| 4-Methylquinoline | | 2.52 |
| Phenazine | 2.84[b] | 2.81 |
| Chlorobenzene | 2.84[f] | 2.84 |
| 3-Bromoquinoline | | 3.03 |
| Acridine | 3.40[h] | 3.39 |

[a] Means of two to eight runs. [b] S. M. Anderson, unpublished results. [c] M. S. Tute, unpublished results. [d] J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, 8, 150 (1965). [e] Reference 29. [f] Reference 2. [g] Reference 6a. [h] C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, 86, 1616 (1964).

values of log $P$ for the standards currently in use with the mean values we have obtained by HPLC through cross-comparison in a number of experiments. Two of these (4-methyl- and 3-bromoquinoline) are secondary standards for which there is no published log $P$ value but whose consistency of behavior is very high. All the rest agree to within our estimate of their probable error (±0.04). Since these compounds belong chemically to several disparate series, it is extremely improbable that this correlation is fortuitous. Similarly one might have expected the effect of unsilanized OH groups, if important, to have shown up as systematic deviations, e.g., for the proton-acceptor heterocyclic compounds. In fact, we do not know to what extent total silanation of the support medium matters. Where the octadecylsilane phase is effectively a monomolecular layer, ours is merely entrained, and it is quite possible that this massive coating of octanol makes the chemical nature of the support medium irrelevant. Nevertheless, we have preferred to play safe in this respect.

Log $P$ values for unknowns may therefore be estimated by comparing their log $t_c$ values with those for one or more standards. In practice, two well-spaced standards are used as a check on linearity. Any departure from unit slope renders the results doubtful; it may indicate that recoating is necessary (typically, this failing leads to retention times that are too short at the upper end of the scale). The standards are chosen from the list of Table I according to the pH range in use; for example, anisole and quinoline are useful alternatives for acidic and basic conditions, respectively. We have found this technique to be swift, easy, and reproducible in the accessible log $P$ range. This range so far is more limited than that attainable in direct partitioning, e.g., by multiple extraction or solvent ratio techniques, which correspondingly though are very vulnerable to errors brought about by solvent entrainment or impurities. It has proved, however, of particular value for the accurate measurement of heterocyclic log $P$ values, from which a series of appropriate $\pi$ scales are at present under construction.[23]

Two limitations to the method must be stressed. Firstly, compounds of very limited solubility tend to give anomalously low log $P$ values; sometimes their peaks even accompany the solvent front. In such cases we suspect that the partitioning process is too slow to reach equilibrium in the time it takes for elution to be completed. Decreasing the flow rate will sometimes effect a cure and, even when it does not, the change in apparent log $P$ value will help to pinpoint this source of trouble. It is notable that heterocyclic compounds, which are generally more soluble than their carbocyclic analogs, tend also to be better behaved. The other limitation concerns the use of ion correction. For a compound that can partition as cation or free base, eq 8 states the expected relation between experimental partition coefficient ($P_{exp}$) and pH

$$P_{exp} = P_+/(1 + K_a/[H^+]) + P_0/(1 + [H^+]/K_a) \quad (8)$$

where $P_+$ and $P_0$ refer to cation and neutral species, respectively (for an acid, these quantities are replaced by $P_0$ and $P_-$). This relation is sigmoidal, and while it is customary to use the ion-correction method by working on its linear section,[4,7] any portion of the curve may be used to calculate log $P_0$ provided that p$K_a$ is known. Line A on Figure 2 shows the result of using the conventional ion-correction method for propranolol,[24] a compound inaccessible to direct shake-flask estimation. All $P_{exp}$ values lie in the range $^1/_{20}$–20, which we regard as the outside limits for accurate estimation; there results[25] log $P_0$ 3.56 ± 0.06 (Tute[4] gives log $P_0$ 3.33 but bases this on a $P_{exp}$ value of 74; n.b. p$K_a$ 9.45[4] is correct[26]). Line B shows three determinations using the HPLC technique. It is clear that cation as well as neutral species is extracting; line B is theoretical for log $P_+$ 0.80 and log $P_0$ 3.39. The same (phosphate) buffer was used, but in the latter case at much higher concentration (0.1 M), and a salt effect is therefore suspected. A similar experiment with quinoline yielded log $P_0$ 2.29 (cf. Table I). When an exact pH has to be maintained, as distinct from that required merely to prevent solute ionization, consideration has to be given to the fact that the solute goes in as a "slug dose", so that relatively high buffer concentrations are inevitable. The problem is analogous to that met by Bird and Marshall.[19] At present, therefore, we do not recommend the use of ion-correction techniques.

The ability of octanol to stay on the column is presumably the result of its low volatility and low solubility in water. It seems probable that solvents which lack one

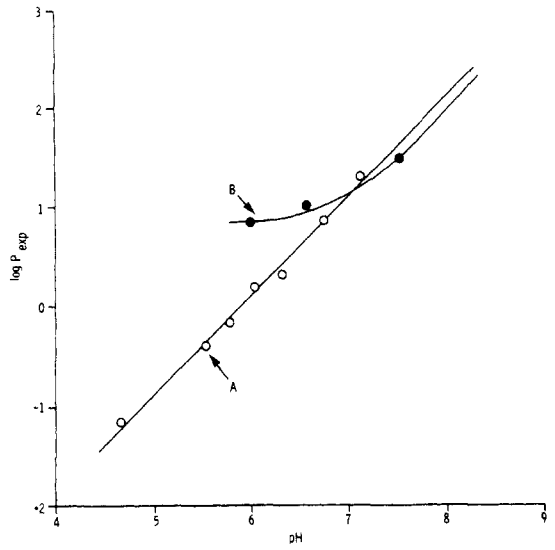


Figure 2. The experimental log $P$ value of propranolol[24] as a function of pH: (A) by the conventional partitioning technique at $I = 0.01$ (see Experimental Section); (B) by HPLC at $I = 0.1$ (see text).

or other of the foregoing attributes, e.g., ether, $CHCl_3$, $CCl_4$, or cyclohexane, are unlikely to behave so well.

## Experimental Section

**Preparation of Column.** Hyflo supercel[27] was passed through a 44-$\mu$ sieve, slurried with distilled water, and pumped through a series of water-filled Dreschel bottles of increasing size (2 × 125, 1 × 250, 1 × 500 ml) at a flow rate of 10 ml/min using a peristaltic pump. Distilled water was pumped through the apparatus until the liquid in the Dreschel bottles became clear. The fraction collected in the 500-ml Dreschel bottle appeared lighter in color and more homogeneous than the other fractions; it possessed under microscopic examination a highly inhomogeneous spread of particle shapes and sizes (5–50 $\mu$) but its handling properties proved satisfactory. After washing with acetone and drying at 120 °C, this material (5 g) was silanized by stirring overnight with 50 ml of a 2% solution of $Me_3SiCl$ in dry toluene. After degassing the material was filtered off, washed with toluene and methanol, and dried (4 h, 120 °C). A 4.6-mm bore column was then slurry packed with this material at 5000 psi according to the method of Majors.[20] The packing was coated by injecting successive 500-$\mu$l lots of water-saturated 1-octanol onto the column, which was then eluted with octanol-saturated water until no more droplets of octanol emerged. It is advisable to continue elution for about 0.5 h after the last droplet is seen, since while partitioning is unaffected, the uv microcell can be fouled this way. The column is now ready for use. It can be left in situ or, if removed for storage full of liquid will, if capped, remain stable indefinitely.

It is advisable to continue the original coating and elution procedure for 6–24 h; too short a time leads to split peaks, probably because of uneven or incomplete wetting out. For recoating, when this becomes necessary, 2 h is normally sufficient. The need for recoating is signalled by a departure of the standard graph from unit slope (see above), generally in the direction that compounds with expected long retention times come off too quickly.

**HPLC Technique.** Solutions for injection are prepared at about 1 mg/ml in water, according to the expected uv absorbance, though this is not critical if a detector–recorder combination with a high signal-to-noise ratio is used. Compounds not readily soluble at the required concentration may be predissolved in a few drops of some suitable solvent, e.g., methanol; tests have demonstrated that $t_c$ is unaffected, at least provided that the amount of cosolvent does not exceed 10%. Each solution, standard or unknown, contains pyridine $N$-oxide or tyrosine. All solutions and the eluent are prepared with octanol-saturated water containing a suitable buffer, most commonly 0.01 M HCl (for acids) or 0.01 M $Na_2CO_3$ (for bases), but also neutral phosphate buffer at similar concentrations where appropriate. The pump is started a few minutes before any solution is injected so as to allow the column and flow rate to stabilize; a flow rate in the range of 1–3 ml/min is normal, but a range of 0.2–8 ml/min has been employed. A typical injection volume is 10 $\mu$l. Standards are normally run first, followed by unknowns. If considered advisable, standards may be rerun before the end of the day, but in practice this has generally proved to be unnecessary unless the flow rate is changed. For each injection $t_0$ is signalled by the appearance of the tyrosine or pyridine $N$-oxide peak, and the constancy of $t_0$ is a useful check on the flow rate. The different log $P$ ranges are measured using columns of differing length; as a rough guide, a 10-cm column covers 1.8–3.7, a 20-cm column 0.6–2.2, and a 30-cm column −0.3 to 1.2 in log $P$. It is possible that the solvent programming technique pioneered by Haggerty[12] would allow this whole range and more to be covered by a single column, but so far we have not tried this.

The laboratory temperature is normally in the range 22 ± 2 °C, and no more precise temperature control has been attempted. While elution times could conceivably be affected by temperature variation, there is evidence[28] that log $P$ values are very insensitive in this respect, and the use of internal standards obviates the need for a correction.

Measurements were carried out using a Cecil 210 coil pump liquid chromatograph equipped with a Cecil 212 variable wavelength uv monitor capable of absorbance accuracy down to 210 nm which was connected to a Chessell BD8 single channel recorder.

**Other Techniques.** The log $P$ value of practolol[29] was obtained using conventional[7] shake-flask techniques. Those for propranolol[24] (line A of Figure 2) were obtained in collaboration with Dr. S. S. Davis of Aston University[25] on an Akufve 110 liquid–liquid extractor, an instrument capable of rapid equilibration and on which it is possible to observe directly when equilibration is complete[30] (here, almost within seconds).

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# Use of High-Pressure Liquid Chromatography for Quantitative Structure-Activity Relationship Studies of Sulfonamides and Barbiturates[1]

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Retention volumes for a group of sulfonamides obtained on three different HPLC columns were correlated with log $P$, p$K_a$, and biological activity. These data were compared with previously published $R_M$ values obtained from five different TLC systems. In a similar manner, previously published chromatographic data of barbiturates from five different HPLC systems were correlated with log $P$, p$K_a$, and biological activity. Depending on the chromatographic system, good correlations can be obtained with log $P$ or with biological activity, but not necessarily both, using the same chromatographic data.

The use of partition coefficients (log $P$) in an octanol–water system (the Hansch method) has become a standard method to carry out quantitative SAR studies.[2] The method requires either experimentally determining the partition coefficient or calculating log $P$ using tables of $\pi$ values for substituents.[3,4] The latter has its limitations, and there are innumerable compounds where log $P$ between octanol–water must be determined.[5] This can be a tedious process complicated by instability in aqueous media, analytical procedures, and the tendency for the compound to dissociate.[2a]

Recently HPLC has been used to determine log $P$ values.[6-8] This was a logical step as chromatography has been used to determine various physical constants. Paper impregnated with olive oil was used to determine $R_M$ values for a series of phenols to measure hyperconjugation.[9,10] Other correlations of chromatographic behavior with chemical structure have been done using ethyl oleate coated cellulose.[11,12] Structure and biological data have been correlated in thin-layer chromatographic systems using silica gels impregnated with a nonpolar phase.[13-16] Finally, fairly linear correlations can be obtained when comparing the ion-pair partition chromatography retention volumes of a series of biogenic amines with their $\pi$ values.[17] For our study, we selected sulfonamides due to the availability of biological data which have a reasonably well-defined end point and for comparison with previously published chromatographic studies in a variety of TLC systems.[14,18-21] There is also available an earlier traditional octanol–water system study.[22] For purposes of this study, the data from ref 20 were used as it had been utilized by Hansch in a previous study, and it appeared to be a well-controlled study. It was also decided to compare results obtained from three different HPLC columns: a bonded reverse phase pellicular packing, a pellicular packing coated with squalene, and a pellicular packing coated with octanol.

## Experimental Section

Statistical correlations were performed [Oregon State University Statistical Interactive Programming System (SIPS)] using the log of the retention volume (log $V_R$). This is defined as

$$\log V_R = \log [(t_R - t_0)(\text{flow rate})]$$

where $t_R$ = retention time of the compound and $t_0$ = retention time of the solvent front. It is not necessary to use the capacity factor ($k'$) which is defined as

$$\log k' = \log [(t_R - t_0)/t_0] = \log (t_R - t_0) - \log t_0$$

The log $t_0$ term becomes a constant and drops out. Both $V_R$ and $k'$ can be compared with some other variable (log $P$, log $1/C$, or $R_M$) independent of the flow rates. Normally the log of the retention time (log $R$) requires a constant flow rate [log $R$ = log $(t_R - t_0)$]. However, log $R$ can be compared with other physical constants at programmed flow rates provided the change in flow rate is linear.[6]

Solvents were of analytical reagent quality and were used without further purification. Sulfonamides and barbiturates were obtained from commercial sources.

HPLC was performed on a Waters Model ALC/GPC 201 liquid chromatograph, equipped with two M-6000 pumps, a Model U-6 K injection valve, and a Model 660 programmer. A Varian Model 635 uv–visible spectrophotometer equipped with low dead volume flow cells was used as a detector. Sulfonamides were analyzed at 255 nm and barbiturates at 245 nm. Peaks were recorded on a Soltec dual pen recorder, and retention times were determined using a stopwatch.

The bonded reverse-phase packing material Bondapak C-18 Corasil, the pellicular silica Corasil II, and the porous silica Porasil A were all obtained from a commercial source (Waters Associates, Inc., Milford, Mass.; particle size 37–70 $\mu$ in each case). Approximately 1% loadings of octanol and squalene were prepared on Corasil II using published solvent–evaporation methods, with ether as a solvent.[26] Likewise, 15% loadings of squalene and octanol on Porasil A were prepared for packing precolumns.

Stainless steel columns, 60 cm × 2 mm i.d., were packed using published "Tap-fill" procedures.[26] Acetate buffers of pH 4.0 and 5.0 were prepared.[27] Sorensen phosphate buffer, pH 6.5, was also used.[28] The pH of the column eluent was monitored at periodic intervals.

An appropriate precolumn was placed between the pump and the injector when using the 1% squalene and 1% octanol on Corasil II columns. Also, the buffers were presaturated with the