(6) E. Kutter and C. Hansch, *J. Med. Chem.*, 12, 647 (1969).

(7) T. Fujita, *J. Med. Chem.*, 16, 923 (1973).

(8) C. L. Johnson, *Proc. Natl. Comput. Conf. Exposition*, 43, 473 (1974).

(9) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, 44, 3289 (1966).

(10) P. A. Dobosh, Quantum Chemistry Program Exchange, Program 141, Indiana University.

(11) M. S. Gordon, Quantum Chemistry Program Exchange, Program 135, Indiana University.

(12) Kennard et al., Ed., "Molecular Structures and Dimensions", Vol. A1, International Union of Crystallography, Polycrystal Book Service, Pittsburgh, Pa., 1972.

(13) The PROPHET system is a specialized computer resource developed by the Chemical/Biological Information-Handling Program of the National Institutes of Health and oriented toward the needs of pharmacologists and medicinal chemists studying the relationship between chemical structure and biological activity. A detailed description of the system features appears in *Proc. Natl. Comput. Conf. Exposition*, 43, 457 (1974).

(14) D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, 23, 420 (1958).

(15) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, 16, 1207 (1973).

(16) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).

(17) P. Zeller, A. Pletscher, K. F. Gey, H. Gutmann, B. Hegedus, and O. Straub, *Ann. N.Y. Acad. Sci.*, 80, 555 (1959).

(18) P. C. Chieh and G. J. Palenik, *J. Chem. Soc. A*, 576 (1971).

(19) J.-L. Coubeils, P. Courriere, and B. Pullman, *C. R. Hebd. Seances Acad. Sci., Ser. D*, 273, 1164 (1971).

(20) J. G. Topliss and R. J. Costello, *J. Med. Chem.*, 15, 1066 (1972).

(21) H. Weinstein, S. Maayani, S. Srebrenik, S. Cohen, and M. Sokolovsky, *Mol. Pharmacol.*, 9, 820 (1973); S. Srebrenik, H. Weinstein, and R. Pauncz, *Chem. Phys. Lett.*, 20, 419 (1973).

(22) Y. C. Martin, W. B. Martin, and J. D. Taylor, *J. Med. Chem.*, 18, 883 (1975).

(23) C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. F. Tedeschi, and A. J. Berger, *J. Med. Chem.*, 5, 1265 (1963).

Inhibition of Dihydrofolate Reductase. Structure–Activity Correlations of Quinazolines[1]

James Y. Fukunaga, Corwin Hansch,* and Edward E. Steller

Department of Chemistry, Pomona College, Claremont, California 91711. Received October 10, 1975

A quantitative structure–activity relationship (QSAR) has been formulated for quinazolines causing 50% inhibition of liver dihydrofolate reductase. The QSAR for the quinazolines is compared with QSAR for triazine and pyrimidine inhibitors. The three QSAR suggest new possibilities for the design of inhibitors of mammalian dihydrofolate reductase.

Dihydrofolate reductase has become a focal point for research since the discovery that it is a key enzyme in a variety of biochemical processes. The finding that methotrexate, a potent inhibitor of dihydrofolate reductase, is effective in cancer chemotherapy has stimulated great interest in finding differential inhibitors for tumor and normal human enzyme. The pioneering work of Baker and co-workers has resulted in the development of a triazine (I) which now shows promise in clinical trials in cancer chemotherapy.
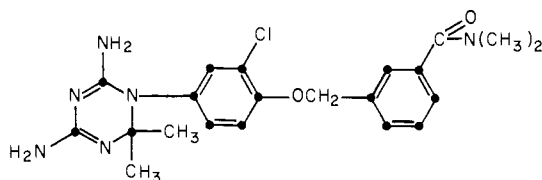
I

We have been interested in formulating QSAR from ligand interactions with dihydrofolate reductase in order to map the region around the active site. From this knowledge we would hope to be able to design more effective inhibitors.

Baker made a series of about 260 derivatives of I in which he varied substituents in the 2, 3, and 4 positions of the N-phenyl ring. The concentration of the triazine necessary to produce 50% inhibition of dihydrofolate reductase from tumor tissue was determined. Equation 1 was developed from this study.[2]

In eq 1, n represents the number of data points used in deriving the equation, r is the correlation coefficient, and s the standard deviation from the regression. Of the variables, π-3 refers to the hydrophobic interactions of substituents in the 3 position of the N-phenyl moiety,
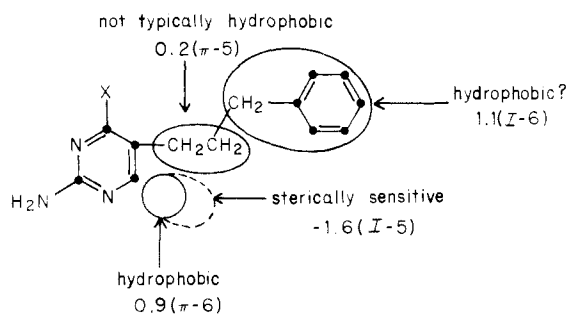
Chart I
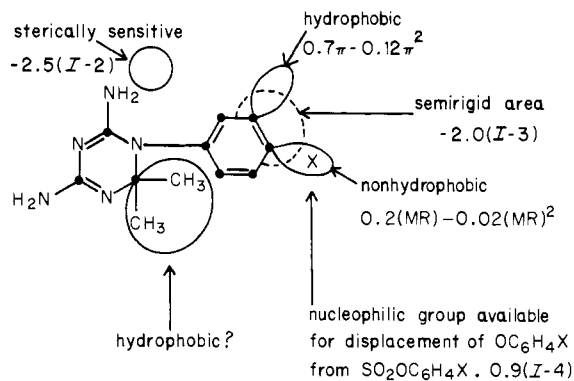
$$\log 1/C = 0.68(\pi\text{-}3) - 0.12(\pi\text{-}3)^2 + 0.23(\text{MR-4}) -$$
$$0.024(\text{MR-4})^2 + 0.24(I\text{-}1) - 2.53(I\text{-}2) -$$
$$1.99(I\text{-}3) + 0.88(I\text{-}4) + 0.69(I\text{-}5) + 0.70(I\text{-}6) +$$
$$6.49 \tag{1}$$

$$\begin{array}{ccc} n & r & s \\ 244 & 0.923 & 0.377 \end{array}$$

MR-4 refers to molar refractivity of substituents in the 4 position, and I-1–I-6 are indicator variables. I-1 takes the value of 1 for data points based on enzyme from Walker tumor and zero for those from L1210 leukemia enzyme. I-2 assumes the value of 1 for substituents in the 2 position, I-3 is for rigid structures (C_6H_5 or $CONHC_6H_5$ attached in the 3 or 4 position), I-4 is given the value of 1 for congeners having the active leaving group $-SO_2OAr$, I-5 receives the value of 1 for derivatives with a flexible bridge [$-O(CH_2)_x-$] between the N-phenyl group and a second phenyl ring, and I-6 assumes the value of 1 for certain amide functions between rings.
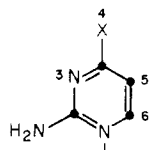
Chart II

The map of Chart I has been constructed from eq 1 to show the main features of substituent interaction with the enzyme.

From a second of Baker's studies using dihydrofolate reductase from pigeon liver, eq 2 has been formulated[3] for

$$\log [S]/[I]\ 0.5 = 1.12(I\text{-}6) + 2.17(I\text{-}2) +$$
$$0.90(\pi\text{-}6) - 1.23(I\text{-}4) + 1.18(I\text{-}1) - 1.61(I\text{-}5) +$$
$$1.63(I\text{-}3) + 0.26(\pi\text{-}5) - 3.12 \qquad (2)$$

$$\begin{array}{ccc} n & r & s \\ 108 & 0.932 & 0.520 \end{array}$$

pyrimidines of the type

when X = OH, SH, and NH$_2$. In eq 2, [S] refers to substrate concentration and [I] to inhibitor concentration causing 50% inhibition. I-6 takes the value of 1 for 5-(CH$_2$)$_n$C$_6$H$_5$ or 5-CH$_2$R (n = 0–4; R = four or five carbon atoms). π-5 with its small coefficient takes care of the interaction of small groups or the first part of long chains. The large coefficient of I-6 suggests that the bigger groups extend into the hydrophobic area which is better characterized by eq 1 as shown in Chart I. A value of 1 is assumed by I-1 when X = SH, I-2 when X = NH$_2$, I-3 for COO$^-$, I-4 when no substituent is present in the 5 position, and I-5 for 6-(CH$_2$)$_n$C$_6$H$_5$ (n = 0–2).

The map of Chart II can be constructed from eq 2. Chart II helps piece out the map of Figure 1 (assuming gross similarity between mammalian and avian enzymes). There is a definite hydrophobic site near the 6 position of the heterocyclic ring; however, very large groups will not fit into this pocket. Equation 2 (π-5) suggests that substituent space immediately off the 5 position is not typically hydrophobic. Because of the flexibility of the groups attached at the 5 position, evidence for the sterically sensitive site shown in Chart I cannot be adduced.

With the above equations and maps of the active site area of dihydrofolate reductase in hand, it was of interest to analyze and compare the extensive study of Hynes and Ashton[4] on quinazolines (II) inhibiting rat liver dihydrofolate reductase. Equations 3–11 have been derived from the data in Table I.

II
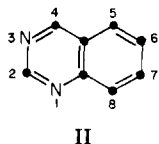
Method. Hydrophobic constants (π) were calculated in the usual manner.[2,3] For example, π for the naphthylvinyl group was estimated as

For the sulfur analogues (–SAr, SOAr, SO$_2$Ar), the following experimental values[5] were used as starting points: πS-C$_6$H$_5$ = 2.32; πSOC$_6$H$_5$ = –0.07; πSO$_2$C$_6$H$_5$ = 0.27. Hence π-SO-C$_6$H$_3$-3,4-Cl$_2$ = πSOC$_6$H$_5$ + 2πCl = –0.07 + 2(0.7) = 1.35. MR values were calculated from our recent compilation.[5] Sigma constants ($\mathcal{F}$ and $\mathcal{R}$) from ref 5 were also studied.

The following parameters were examined in the first approximation: π-5, π-6, MR-5, MR-6, $\mathcal{F}$-5, $\mathcal{R}$-5, $\mathcal{F}$-6, $\mathcal{R}$-6, σm-5, σm-6, σp-5, σp-6, I-1, I-2, I-3, I-4, I-5, and I-6. The position of attachment of the substituent to II is indicated by 5 or 6 in the above continuous variables. MR represents molar refractivity of the substituent scaled by 0.1. $\mathcal{F}$ and $\mathcal{R}$ refer to Swain and Lupton's inductive and resonance parameters,[5] σ is the Hammett constant,[5] and I represents indicator variable. These variables assume the value of 1 for the following structural features: I-1 for 2-OH or 2-SH; I-2 for 2-H; I-3 for 4-OH or 4-SH and the single case of unsubstituted II. I-4 is given the value of 1 for the following bridges from the 5 position to an aryl group: –S–, –SO–, SO$_2$, CH$_2$S, –CH=CH–. I-5 is a combination of I-1 and I-2 and I-6 assumes the value of 1 for 6-SO$_2$Ar.

Omitting I-5, there are 17 variables which means $2^{17} - 1 = 131071$ possible regression equations. Generating such a large number of equations is impractical; however, Furnival and Wilson[6] have recently published an algorithm which enables one to very rapidly calculate the sums of squares for each regression equation. We have programmed their algorithm to give the variance for each regression equation. With this algorithm, only a few minutes of CPU time is needed to calculate the necessary 131071 variances. It was found from a few such studies that none of the electronic parameters were significant; this is in line with previous findings on similar inhibitors of dihydrofolate reductase.[2,3] Also, only one squared term was found to be significant. The most significant terms were found to be MR-6, (MR-6)^2, π-5, I-1, I-2, I-3, I-4, and I-6. These eight terms could lead to $8(7)/2 = 28$ pairwise cross-product terms. Many of these yield singular matrices or meaningless results; hence, some study of the cross products must be undertaken before serious regression studies can be conducted. The following cross-product terms were found to yield singular matrices.

π-6·I-6	
π-5·I-1	I-1·I-2
π-5·I-2	I-1·I-4
π-5·I-6	I-2·I-4
π-6·I-4	I-4·I-6
MR-6·I-4	MR-6·I-6

The following were not studied because they lead to one or two nonzero values.

I-1·I-6	I-3·I-4
I-2·I-6	I-2·I-3

Two other cross-product terms have only two sets of values and variation is too small for meaningful results: π-6·I-6

= 1.59 or 1.69; MR-6·*I*-6 = 4.32 or 4.86.

A study of the remaining pairwise cross product-terms uncovered the importance of MR-6·*I*-1. At this point, the nine significant terms were studied via normal regression analysis, deleting various points until eq 3, based on 101 congeners, was judged to be the "best" equation.

$$\log 1/C = 0.810 \ (\pm 0.12) \ (\text{MR-6}) - 0.0635 \ (\pm 0.017)$$
$$(\text{MR-6})^2 + 0.775 \ (\pm 0.12) \ (\pi\text{-5}) - 0.734 \ (\pm 0.49)$$
$$(I\text{-1}) - 2.145 \ (\pm 0.38) \ (I\text{-2}) - 0.544 \ (\pm 0.21)$$
$$(I\text{-3}) - 1.395 \ (\pm 0.41) \ (I\text{-4}) + 0.776 \ (\pm 0.37)$$
$$(I\text{-6}) - 0.197 \ (\pm 0.12) \ (\text{MR-6}\cdot I\text{-1}) + 4.924$$
$$(\pm 0.23) \tag{3}$$

$$
\begin{array}{ccc}
n & r & s \\
101 & 0.961 & 0.441
\end{array}
$$
$$\text{ideal MR-6} = 6.4 \ (5.7\text{--}7.4)$$

Results

From a study of the variables outlined in the Method section, eq 3 emerged as the "best" equation. *C* in eq 3 is the molar concentration of inhibitor causing 50% inhibition of liver dihydrofolate reductase. The figures in parentheses are the 95% confidence limits, *n* represents the number of data points used to derive eq 3–11 (three points of Table I were omitted), *r* is the correlation coefficient, and *s* the standard deviation from the regression. The ideal value of MR is obtained from the partial derivative of eq 3. Congeners with substituents in the 6 position having MR values greater or less than 6.4 have lower activities (other factors being equal). The development of eq 3 is shown in Table II.

The most important variable is MR-6 followed by π-5. The substituents in the 5 position are, on the average, much smaller than those in the 6 position, which is one of the reasons MR-6 appears more important. Each of the equations in Table II has the lowest standard deviation in its class. For example, eq 7 has the lowest standard deviation of the 126 four-variable equations. With the exception of discontinuities of eq 6 and 7, there is a regular progression from eq 4 to 3. The variable *I*-1 comes in at eq 6. This variable takes the value of 1 for congeners having an SH or OH group in the 2 position. These congeners are, on the average, 65 times less active than those having a 2-NH₂ (other factors being equal). *I*-2 is given a value of 1 for 2-H. These congeners are about 100 times less active than the corresponding 2-NH₂ analogues. *I*-1 in eq 7 is displaced from eq 6 by the cross-product term MR-6·*I*-1. Using *I*-1 in eq 7 in place of MR-6·*I*-1 gives a considerably poorer correlation, indicating that even though this term is based on only seven points, it is quite significant. *I*-1 alone has an adverse affect on inhibitory power and, when MR-6 is large, the affect appears to increase. This result must be taken with reservation since MR-6·*I*-1 has essentially only two values, 0 or 4.9. Since the distribution of values is so poor, not much reliance can be placed on this term until a better set of congeners is studied.

The only exponential term is MR-6 which suggests that more active congeners could be made by means of larger hydrophobic groups in the 5 position.

The degree of collinearity among the variables is shown in the squared correlation matrix of Table III. One fortunate result is that the collinearity between MR-6 and π-6 is low, indicating that 6-space (space in or on the enzyme off the 6 position of II) is not typically hydrophobic. Unfortunately, collinearity between π-5 and MR-5 is high which tends to compromise the conclusion that

Chart III
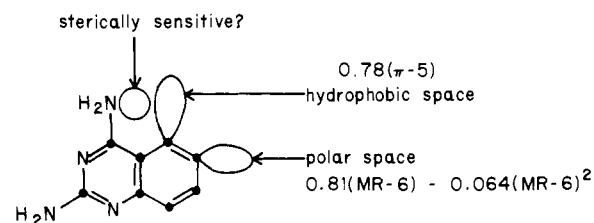
5-space is hydrophobic. However, the π-3 and MR-3 vectors on which eq 1 rests are much more orthogonal (r^2 = 0.5) so that the two data sets point to a rather large hydrophobic pocket. There is moderately high correlation between *I*-4 and MR-5 and π-5 which tends to obscure the role of *I*-4.

Discussion

From eq 3, a map of the area around the binding site can be constructed (Chart III). Chart III is similar to Chart I in that both have hydrophobic sites and polar sites in comparable positions. Chart II suggests additional hydrophobic space near the 8 position of II. However, this space is limited. Possibly a substituent such as 8-Br could be used to increase inhibitory power. The initial slope of π-5 in eq 1 is similar to that of eq 3; however, eq 3 has no exponential term in π-5 so that greater activity can be expected from placing more hydrophobic groups in this position. Optimum hydrophobicity for 5-substituents is about 2.8 (π₀) for eq 1. Equation 3 does not require a (π-5)² term and has several well-fit 5-substituents with π >3.0 which suggests either a better fit of 5-substituents of II to the hydrophobic site or a difference in the two enzymes. The data on which Chart I is based were obtained using enzymes from L1210 leukemia and Walker 256 tumor tissue, whereas eq 3 is based on normal mammalian liver enzyme.

Enzymic space into which 6-substituents fall is not correlated by π and, since π-6 and MR-6 are reasonably orthogonal, this space is assumed to be polar in nature. Equation 1 also suggests polar character for comparable enzymic space. Optimal MR values are MR-4 of eq 1, 4.7 (4.2–5.6); MR-6 of eq 3, 6.4 (5.7–7.4). The agreement is not too bad, considering the quite different parent structures involved, the confidence limits, the fact that two different enzymes were employed, and that the work was done in two different laboratories.

Substituents in the 6 position of II may be producing their inhibitory effect by a combination of two factors. Such groups could bind inhibitors to the enzyme via dispersion forces; they could also produce an unfavorable conformational change in the enzyme. The initial slopes with the MR terms in eq 1 and 3 differ considerably. A possible explanation for this is that while the dispersion aspects of MR would be about the same in each case, 6-substituents of II might be more effective in producing conformational changes, hence, the higher weighting factor in eq 3.

The indicator variable *I*-4 shows considerable collinearity with MR-5 and π-5, especially MR-5. The congeners parameterized by this variable all have a large aryl group in the 5 position. The results embodied in Chart I which suggest an unfavorable steric interaction from ortho-substituted phenyltriazines lead us to think that *I*-4 may be accounting, in a crude way, for a deleterious steric interaction of large 5-substituents.

Indicator variables *I*-1, *I*-2, and *I*-3 all bring out the importance of having NH₂ groups in the 2 and 4 positions. *I*-1, which parameterizes 2-SH or 2-OH, shows an im-

Table I. Data Used in the Formulation of Eq 3–11 Correlating Inhibition of Dihydrofolate Reductase

No.	Group	Log 1/C Obsd[a]	Log 1/C Calcd[b]	Δ log 1/C	MR-6	π-5	I-1	I-2	I-3	I-4	MR-6·I-1	I-6	Ref
1	2,4-H	2.26	2.31	−0.05	0.10	0.00	0.0	1.0	1.0	0.0	0.00	0.0	4a
2	2-H, 4-NH$_2$	3.09	2.86	0.23	0.10	0.00	0.0	1.0	0.0	0.0	0.00	0.0	4a
3	2-H, 4-OH	3.62	2.31	1.30	0.10	0.00	0.0	1.0	1.0	0.0	0.00	0.0	4a
4	2-SH, 4-NH$_2$	3.72	4.25	−0.53	0.10	0.00	1.0	0.0	0.0	0.0	0.10	0.0	4a
5	2,4-(OH)$_2$	3.89	3.71	0.18	0.10	0.00	1.0	0.0	1.0	0.0	0.10	0.0	4a
6	2-NH$_2$, 4-OH, 5,6,7,8-H$_4$[c]	3.96	4.55	−0.59	0.21	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4a
7	2-OH, 4-NH$_2$	4.02	4.25	−0.23	0.10	0.00	1.0	0.0	0.0	0.0	0.10	0.0	4a
8	2,4-NH$_2$, 5-SO$_2$-C$_6$H$_3$-3,4-Cl	4.24	4.92	−0.68	0.10	1.69	0.0	0.0	0.0	1.0	0.00	0.0	4b
9	2-NH$_2$, 4-OH, 6-Me, 5,6,7,8-H$_4$[c]	4.26	4.89	−0.63	0.67	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4a
10	2,4-NH$_2$, 5-SO-C$_6$H$_3$-3,4-Cl	4.26	4.66	−0.40	0.10	1.35	0.0	0.0	0.0	1.0	0.00	0.0	4b
11	2-AcNH, 4-OH	4.28	3.71	0.57	0.10	0.00	1.0	0.0	1.0	0.0	0.10	0.0	4a
12	2-NH$_2$, 4-OH	4.31	4.46	−0.15	0.10	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4a
13	2-NH$_2$, 4-OH, 7-CF$_3$	4.35	4.46	−0.11	0.10	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4a
14	2,4-NH$_2$, 5-SO-2-C$_{10}$H$_7$	4.35	4.58	−0.23	0.10	1.25	0.0	0.0	0.0	1.0	0.00	0.0	4b
15	2-NH$_2$, 4-SH	4.37	4.46	−0.09	0.10	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4a
16	2-NH$_2$, 4-OH, 6-CH$_3$	4.44	4.82	−0.38	0.57	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4a
17	2,4,6-NH$_2$	4.57	5.34	−0.77	0.54	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
18	2,4-NH$_2$	4.66	5.00	−0.34	0.10	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
19	2-NH$_2$, 4-OH, 5-Cl	4.66	5.01	−0.35	0.10	0.71	0.0	0.0	1.0	0.0	0.00	0.0	4a
20	2-H, 4-NH$_2$, 6-S-2-C$_{10}$H$_7$	4.68	5.24	−0.56	4.97	0.00	0.0	1.0	0.0	0.0	0.00	0.0	4c
21	2-NH$_2$, 4-OH, 5-CH$_3$	4.80	4.89	−0.09	0.10	0.56	0.0	0.0	1.0	0.0	0.00	0.0	4a
22	2,4-NH$_2$, 5-SO$_2$-2-C$_{10}$H$_7$	4.82	4.85	−0.03	0.10	1.59	0.0	0.0	0.0	1.0	0.00	0.0	4b
23	2-H, 4-NH$_2$, 6-SO-2-C$_{10}$H$_7$	4.82	5.22	−0.40	4.88	0.00	0.0	1.0	0.0	0.0	0.00	0.0	4c
24	2-NH$_2$, 4-OH, 5-SO$_2$-2-C$_{10}$H$_7$	4.85	4.30	0.55	0.10	1.59	0.0	0.0	1.0	1.0	0.00	0.0	4b
25	2,4-NH$_2$, 6-CN	4.92	5.41	−0.49	0.63	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
26	2,4-NH$_2$, 6-CHO	5.00	5.45	−0.45	0.69	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
27	2-SH, 4-OH, 6-S-2-C$_{10}$H$_7$	5.00	5.13	−0.13	4.97	0.00	1.0	0.0	1.0	0.0	4.97	0.0	4c
28	2,4-NH$_2$, 6-CH$_2$NH$_2$	5.03	5.61	−0.58	0.91	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
29	2,4-SH, 6-S-2-C$_{10}$H$_7$	5.05	5.13	−0.08	4.97	0.00	1.0	0.0	1.0	0.0	4.97	0.0	4c
30	2,4-OH, 6-S-2-C$_{10}$H$_7$	5.07	5.13	−0.06	4.97	0.00	1.0	0.0	1.0	0.0	4.97	0.0	4c
31	2,4-NH$_2$, 5,6,7,8-H$_4$[c]	5.13	5.09	0.04	0.21	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
32	2-OH, 4-SH, 6-S-2-C$_{10}$H$_7$	5.24	5.13	0.11	4.97	0.00	1.0	0.0	1.0	0.0	4.97	0.0	4c
33	2,4-NH$_2$, 5-trans-CH=CH-2-C$_{10}$H$_7$[d]	5.28	6.75	−1.47	0.10	4.04	0.0	0.0	0.0	1.0	0.00	0.0	4b
34	2,4-NH$_2$, 6-Cl	5.40	5.39	0.01	0.60	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
35	2-H, 4-NH$_2$, 6-SO$_2$-2-C$_{10}$H$_7$	5.47	5.99	−0.52	4.86	0.00	0.0	1.0	0.0	0.0	0.00	1.0	4c
36	2-SH, 4-NH$_2$, 6-S-2-C$_{10}$H$_7$	5.52	5.67	−0.15	4.97	0.00	1.0	0.0	0.0	0.0	4.97	0.0	4c
37	2-NH$_2$, 4-OH, 5-CH$_3$, 6-COGlu(Et)$_2$[d,e]	5.57	7.02	−1.45	8.83	0.56	0.0	0.0	1.0	0.0	0.00	0.0	4e
38	2,4-NH$_2$, 6-Br	5.60	5.59	0.01	0.89	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
39	2,4-NH$_2$, 6-CH$_3$	5.66	5.36	0.30	0.57	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
40	2,4-NH$_2$, 6-CH$_3$, 5,6,7,8-H$_4$[c]	5.70	5.44	0.26	0.67	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a
41	2-OH, 4-NH$_2$, 6-S-2-C$_{10}$H$_7$	5.77	5.67	0.10	4.97	0.00	1.0	0.0	0.0	0.0	4.97	0.0	4c
42	2,4-NH$_2$, 6-CH$_2$NHCOCH$_2$C$_6$H$_3$-3,4-Cl[d]	5.82	7.44	−1.62	5.39	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d
43	2,4-NH$_2$, 5-CH$_3$	6.09	5.44	0.65	0.10	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4a
44	2-AcNH, 4-OH, 6-SO$_2$-2-C$_{10}$H$_7$	6.11	5.90	0.20	4.86	0.00	1.0	0.0	1.0	0.0	4.86	1.0	4c
45	2-NH$_2$, 4-OH, 5-S-2-C$_{10}$H$_7$	6.15	5.89	0.25	0.10	3.64	0.0	0.0	1.0	1.0	0.00	0.0	4b
46	2,4,6-NH$_2$, 5-Cl	6.18	5.89	0.29	0.54	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4a
47	2-NH$_2$, 4-OH, 6-COGlu(Et)$_2$[e]	6.24	6.58	−0.34	8.83	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4e
48	2,4-NH$_2$, 5-Cl	6.25	5.55	0.69	0.10	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4a
49	2-NH$_2$, 4-OH, 5-CH$_3$, 6-COGlu[f]	6.41	7.37	−0.96	6.98	0.56	0.0	0.0	1.0	0.0	0.00	0.0	4e
50	2,4-NH$_2$, 5-cis-CH=CH-2-C$_{10}$H$_7$	6.52	6.75	−0.23	0.10	4.04	0.0	0.0	0.0	1.0	0.00	0.0	4b
51	2,4-NH$_2$, 6-CH$_2$NHCO-C$_6$H$_4$-3-CF$_3$	6.59	7.24	−0.65	4.33	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d

No.	Substituents												
52	2-NH$_2$, 4-SH, 6-S-2-C$_{10}$H$_7$	6.64	6.84	-0.20	4.97	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4c
53	2-NH$_2$, 4-OH, 6-COGlu^f	6.68	6.94	-0.26	6.98	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4e
54	2,4-NH$_2$, 5,6-Cl	6.70	5.94	0.76	0.60	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4a
55	2,4-NH$_2$, 5-S-2-C$_6$H$_3$-3,4-Cl	6.80	6.52	0.28	0.10	3.74	0.0	0.0	0.0	1.0	0.00	0.0	4b
56	2-NH$_2$, 4-OH, 6-S-2-C$_6$H$_3$-3,4-Cl	6.92	6.72	0.20	4.43	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4c
57	2,4-NH$_2$, 5-S-2-C$_{10}$H$_7$	6.92	6.44	0.48	0.10	3.64	0.0	0.0	0.0	1.0	0.00	0.0	4b
58	2,4-NH$_2$, 5-Cl, 6-Br	7.00	6.14	0.85	0.89	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4a
59	2,4-NH$_2$, 6-CH$_2$NHCO-2-C$_{10}$H$_7$	7.00	7.45	-0.45	5.47	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d
60	2,4-NH$_2$, 6-CH$_2$NHCO-C$_6$H$_3$-3,4-Cl	7.00	7.37	-0.37	4.93	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d
61	2,4-NH$_2$, 6-NHCOCH$_2$-C$_6$H$_4$-3-CF$_3$	7.02	7.24	-0.22	4.33	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d
62	2-NH$_2$, 4-OH, 6-SO$_2$-C$_6$H$_3$-3,4-Cl	7.12	7.47	-0.35	4.32	0.00	0.0	0.0	1.0	0.0	0.00	1.0	4c
63	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH$_2$-C$_6$H$_4$-3-CF$_3$	7.23	7.68	-0.45	4.33	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
64	2,4-NH$_2$, 6-NHCO(CH$_2$)$_2$-C$_6$H$_4$-4-Cl	7.33	7.37	-0.04	4.89	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d
65	2-NH$_2$, 4-OH, 6-S-2-C$_{10}$H$_7$	7.35	6.84	0.51	4.97	0.0	0.0	0.0	1.0	0.0	0.00	0.0	4c
66	2-NH$_2$, 4-OH, 6-SO-2-C$_{10}$H$_7$	7.38	6.82	0.56	4.88	0.00	0.0	0.0	1.0	0.0	0.00	0.0	4c
67	2,4-NH$_2$, 6-S-C$_6$H$_4$-3-CF$_3$	7.44	7.09	0.34	3.83	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4c
68	2,4-NH$_2$, 5-Cl, 6-NHCO(CH$_2$)$_2$-C$_6$H$_4$-4-Cl	7.44	7.92	-0.48	4.89	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
69	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH$_2$-C$_6$H$_4$-3-Br	7.47	7.76	-0.29	4.71	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
70	2,4-NH$_2$, 5-CH$_3$, 6-NHCO(CH$_2$)$_2$-C$_6$H$_4$-4-Cl	7.48	7.80	-0.32	4.89	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
71	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH$_2$-C$_6$H$_4$-4-Cl	7.48	7.70	-0.22	4.43	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
72	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH$_2$-C$_6$H$_4$-4-Br	7.49	7.76	-0.27	4.71	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
73	2,4-NH$_2$, 6-NHCOCH$_2$-C$_6$H$_3$-3,4-Cl	7.51	7.37	0.14	4.93	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d
74	2,4-NH$_2$, 6-COGlu(Et)$_2$^{e}	7.54	7.12	0.42	8.83	0.00	0.0	0.0	0.0	0.0^e	0.00	0.0	4e
75	2,4-NH$_2$, 6-COGlu^f	7.59	7.48	0.11	6.98	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4e
76	2-NH$_2$, 4-SH, 6-SO$_2$-2-C$_{10}$H$_7$	7.60	7.59	0.01	4.86	0.00	0.0	0.0	1.0	0.0	0.00	1.0	4c
77	2,4-NH$_2$, 5-Cl, 6-NHCOCH$_2$-C$_6$H$_3$-3,4-Cl	7.62	7.93	-0.31	4.93	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
78	2,4-NH$_2$, 5-Cl, 6-NHCOCH$_2$O-C$_6$H$_3$-3,4-Cl	7.64	7.96	-0.32	5.16	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
79	2,4-NH$_2$, 6-NHCOCH$_2$-C$_6$H$_4$-4-Cl	7.64	7.27	0.37	4.43	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d
80	2,4-NH$_2$, 5-Cl, 6-NHCOCH$_2$-C$_6$H$_4$-3-CF$_3$	7.64	7.79	-0.15	4.33	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
81	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH[CH(CH$_3$)$_2$]S-2-C$_{10}$H$_7$	7.68	7.82	-0.14	7.76	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
82	2,4-NH$_2$, 5-CH$_3$, 6-COGlu(Et)$_2$^{e}	7.70	7.56	0.14	8.83	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4e
83	2,4-NH$_2$, 5-CH$_2$S-C$_6$H$_4$-4-Cl	7.70	7.53	0.17	0.10	3.25	0.0	0.0	0.0	0.0	0.00	0.0	4b
84	2,4-NH$_2$, 5-CH$_2$CH$_2$-2-C$_{10}$H$_7$	7.70	8.10	-0.40	0.10	3.98	0.0	0.0	0.0	0.0	0.00	0.0	4b
85	2,4-NH$_2$, 6-NHCH$_2$-C$_6$H$_3$-3,4-Cl	7.74	7.28	0.46	4.47	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4a,b
86	2,4-NH$_2$, 5-Cl, 6-NHCOCH(CH$_3$)C$_6$H$_5$	7.77	7.81	-0.04	4.39	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
87	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH$_2$-C$_6$H$_3$-3,4-Cl$_2$	7.77	7.81	-0.04	4.93	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
88	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH$_2$O-2-C$_{10}$H$_7$	7.82	7.91	-0.09	5.70	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
89	2,4-NH$_2$, 5-Cl, 6-NHCOCH$_2$O-2-C$_{10}$H$_7$	7.85	8.03	-0.18	5.70	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
90	2,4-NH$_2$, 5-Cl, 6-NHCOCH$_2$-C$_6$H$_4$-4-Br	7.89	7.88	0.01	4.71	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
91	2,4-NH$_2$, 5-Cl, 6-NHCOCH$_2$-C$_6$H$_4$-4-Cl	7.92	7.82	0.10	4.43	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
92	2,4-NH$_2$, 6-S-C$_6$H$_3$-3,4-Cl	7.96	7.27	0.69	4.43	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4c
93	2-NH$_2$, 4-OH, 6-SO$_2$-2-C$_{10}$H$_7$	7.96	7.59	0.37	4.86	0.00	0.0	0.0	1.0	0.0	0.00	1.0	4c
94	2,4-NH$_2$, 5-CH$_2$S-2-C$_{10}$H$_7$	8.00	8.27	-0.27	0.10	4.20	0.0	0.0	0.0	0.0	0.00	0.0	4b
95	2,4-NH$_2$, 6-SO$_2$-C$_6$H$_3$-3,4-Cl	8.05	8.01	0.03	4.32	0.00	0.0	0.0	0.0	0.0	0.00	1.0	4c
96	2,4-NH$_2$, 5-Cl, 6-NHCOCH$_2$-C$_6$H$_4$-3-Br	8.12	7.88	0.24	4.71	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
97	2,4-NH$_2$, 6-S-2-C$_{10}$H$_7$	8.15	7.38	0.77	4.97	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4c
98	2,4-NH$_2$, 6-SO-2-C$_{10}$H$_7$	8.15	7.36	0.78	4.88	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4c
99	2,4-NH$_2$, 5-CH$_3$, 6-COGlu^f	8.25	7.92	0.33	6.98	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4e
100	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH$_2$S-2-C$_{10}$H$_7$	8.27	7.94	0.33	6.36	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
101	2,4-NH$_2$, 6-NHCOCH$_2$-2-C$_{10}$H$_7$	8.30	7.45	0.84	5.47	0.00	0.0	0.0	0.0	0.0	0.00	0.0	4d
102	2,4-NH$_2$, 5-Cl, 6-NHCOCH$_2$-2-C$_{10}$H$_7$	8.30	8.01	0.29	5.47	0.71	0.0	0.0	0.0	0.0	0.00	0.0	4d
103	2,4-NH$_2$, 5-CH$_3$, 6-NHCOCH$_2$-2-C$_{10}$H$_7$	8.38	7.89	0.49	5.47	0.56	0.0	0.0	0.0	0.0	0.00	0.0	4d
104	2,4-NH$_2$, 6-SO$_2$-2-C$_{10}$H$_7$	8.40	8.14	0.26	4.86	0.00	0.0	0.0	0.0	0.0	0.00	1.0	4c

^{a} From ref 3. ^{b} Calculated using eq 3. ^{c} 5,6,7,8-H$_4$ = 5,6,7,8-tetrahydro. ^{d} These points omitted in deriving eq 3. ^{e} COGlu(Et)$_2$ = CH$_2$NH-C$_6$H$_4$-4-CONHCH(CO$_2$Et)CH$_2$-CH$_2$CO$_2$Et. ^{f} COGlu = CH$_2$NH-C$_6$H$_4$-4-CONHCH(CO$_2$H)CH$_2$CH$_2$CO$_2$H.

Table II. Stepwise Development of Eq 3

Intercept	MR-6	π-5	I-1	I-2	MR-6·I-1	(MR-6)²	I-4	I-3	I-6	r	s	$F_{1,x}{}^a$	Eq no.
5.16	0.37									0.640	1.167	68.7	4
4.53	0.46	0.67								0.761	0.990	39.6	5
5.54	0.36		−1.80	−2.44						0.819	0.881		6
4.17	1.07	0.73			−0.39	−0.088				0.869	0.763		7
4.41	1.05	0.64		−1.98	−0.42	−0.088				0.921	0.603	59.0	8
4.52	0.99	0.85		−2.00	−0.40	−0.083	−1.33			0.941	0.529	29.3	9
4.74	0.93	0.80		−2.00	−0.35	−0.076	−1.30	−0.45		0.949	0.495	14.5	10
4.80	0.86	0.80		−2.08	−0.35	−0.067	−1.35	−0.55	0.77	0.957	0.459	16.0	11
4.92	0.81	0.77	−0.73	−2.14	−0.20	−0.064	−1.39	−0.54	0.77	0.961	0.441	8.8	3

^{a} Where appropriate, the F test is made for the significance of the additional term. $F_{1,60;\,\alpha\,0.001} = 12; F_{1,60;\,\alpha\,0.005} = 8.5$.

Table III. Squared Correlation Matrix

|	MR-6	MR-5	(MR-6)²	π-6	π-5	MR-6·I-1	I-1	I-2	I-3	I-4	I-6
MR-6	1.00	0.17	0.89	0.23	0.11	0.03	0.00	0.00	0.01	0.14	0.03
MR-5		1.00	0.10	0.08	0.83	0.02	0.03	0.01	0.02	0.69	0.02
(MR-6)²			1.00	0.08	0.07	0.01	0.00	0.00	0.00	0.09	0.01
π-6				1.00	0.07	0.20	0.07	0.00	0.00	0.06	0.01
π-5					1.00	0.02	0.04	0.02	0.03	0.40	0.02
MR-6·I-1						1.00	0.62	0.00	0.07	0.01	0.01
I-1							1.00	0.01	0.07	0.01	0.00
I-2								1.00	0.00	0.01	0.01
I-3									1.00	0.00	0.03
I-4										1.00	0.01
I-6											1.00

portant interaction with MR-6 in the cross-product term MR-6·I-1. The negative coefficient with this term shows that the combination of 2-SH or 2-OH with a large 6-substituent produces less effective inhibitors. Since 2-SH or 2-OH are poor compared to 2-NH₂, one assumes that they bind the inhibitor to the enzyme less effectively. If these congeners are less firmly anchored, 6-substituents might not be able to produce their maximum conformational change in the enzyme. It would be interesting to check out this hypothesis by making a better selection of congeners than those of Table I.

No ready explanation for I-6 is at hand other than that the 6-SO₂ group might produce an inhibitory conformational change. I-4 in eq 1 brings out the increased activity of congeners with a properly placed –SO₂O-C₆H₅ which can act as a good leaving group. Nothing comparable to this type of structure is present in the molecules of Table I. In an academic sense, it would be interesting to build such a feature into II to see if the same effect occurs in both series.

We pointed out sometime ago[7] that in attempting to develop dihydrofolate reductase inhibitors into effective drugs, one must be concerned with designing molecules with optimum lipophilicity. The best approach with congeners of II would seem to be that of placing a large hydrophobic group such as CH₂S-C₆H₃-3,4-Cl₂ (π = 3.96) in the 5 position and a very hydrophilic group in the 6 position, having MR of about 3-6. Equation 3 predicts a log 1/C of about 10 for such compounds with amino groups in the 2 and 4 positions.

It might not be possible to realize such a large increase in activity (100 times greater than the most active in Table II) since there is a good chance of unfavorable interaction between such large groups adjacent to each other. Also, at 10⁻⁸–10⁻⁹ M inhibitor, one may be reaching the point of 1:1 reaction and higher log 1/C cannot be obtained under any conditions. For example, both may be producing conformational changes in the same general region of the enzyme. Equation 2 suggests that an 8-Br in II might increase activity by about 0.8 log units. Binding at 5- and 8-space might be more independent processes than binding in 5- and 6-space; hence, one might cut back on

MR-6 and recover the activity by 8-substitution. Equation 2 does indicate that a *large* hydrophobic pocket is not available for 8-substituents. The following two structures are offered as suggestions.
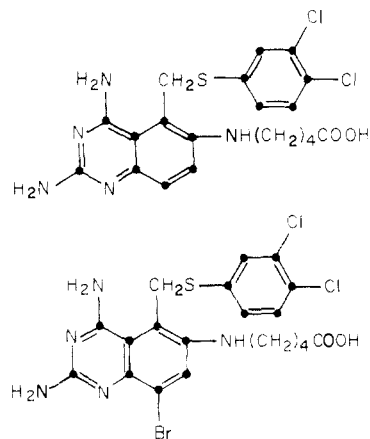
Three data points in Table I have not been employed in formulating eq 3-11. All of these show less than the expected activity. In the case of compound 33 this is very likely a steric effect since the corresponding cis isomer 50 is well fit. No obvious explanation is at hand to rationalize the poor fit of 37 and 42.

One of the most interesting aspects of our recent correlation studies with enzyme inhibitors[2,3,8] is the enormous assistance that indicator variables provide in the formulation of QSAR. Enough examples are now in hand to clearly establish the fact that many structural features provide an additive contribution to biological activity independent of other changes being made in a parent compound. There are of course exceptions where π, MR, or σ appears to have optimum values. However, the kind of exceptions which require cross-product terms does not seem to be very common; at least relatively few such examples have been reported.

The Furnival "leaps and bounds" regression technique constitutes a real breakthrough in regression analysis where many variables must be considered. For the first time we

have the tool necessary to systematically uncover subtle favorable or unfavorable interactions due to molecular features which, in the first approximation, appear to be additive. A new degree of sophistication in QSAR is now possible. We believe that indicator variables will play an increasingly important role in structuring large sets of congeners.

References and Notes

(1) This investigation was supported by U.S. Public Health Service Research Grant No. CA-11110 from the National Cancer Institute.
(2) C. Silipo and C. Hansch, *J. Am. Chem. Soc.*, 97, 6849 (1975).
(3) M. Yoshimoto and C. Hansch, *J. Med. Chem.*, 19, 71 (1976).
(4) (a) W. T. Ashton, F. C. Walker III, and J. B. Hynes, *J. Med. Chem.*, 16, 694 (1973); (b) W. T. Ashton and J. B. Hynes, *ibid.*, 16, 1233 (1973); (c) J. B. Hynes, W. T. Ashton, D. Bryansmith, and J. H. Freisheim, *ibid.*, 17, 1023 (1974); (d) J. B. Hynes and W. T. Ashton, *ibid.*, 18, 263 (1975); (e) J. B. Hynes and C. M. Garrett, *ibid.*, 18, 632 (1975).
(5) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, and E. J. Lien, *J. Med. Chem.*, 16, 1207 (1973).
(6) G. M. Furnival and R. W. Wilson, Jr., *Technometrics*, 16, 499 (1974).
(7) C. Hansch and C. Silipo, *J. Med. Chem.*, 17, 661 (1974).
(8) C. Hansch, C. Silipo, and E. E. Steller, *J. Pharm. Sci.*, 64, 1186 (1975).

Dependence of Hydrophobicity of Apolar Molecules on Their Molecular Volume

A. Leo,* Corwin Hansch, and Priscilla Y. C. Jow

Department of Chemistry, Pomona College, Claremont, California 91711. Received October 9, 1975

Cavity size is the primary determinant of the partition coefficient (P) of apolar solutes between octanol and water. Although the energy of cavity formation would be expected to be related to cavity *area*, older methods of area calculation give a poorer correlation with log P than does *volume*. Apolar solutes clearly fall into two classes based on their log P/volume relationship, the distinction possibly being whether the solute exposes mostly hydrogen atoms or unbonded electrons.

The great importance of hydrophobic interactions in biological and medicinal chemistry has stimulated much interest in delineating the determinants of this molecular property. Recently, a number of moderately successful efforts have been made to relate hydrophobicity of nonpolar solutes to the surface area or volume of the cavity necessary to contain them.[1-5] We wish to show that, for relatively apolar solutes,[6] the *nature* of the solutes surface and the molecular volume determine hydrophobicity, at least as it is measured by partitioning between octanol and water (coefficient = P).

Harris et al.[2] showed that the surface area of hydrocarbons, as determined by the attachment of small spheres representing water, correlated well with water solubility. Tanford[3] has refined this type of reasoning. Hermann,[4a] in a more sophisticated approach, showed that log S (S = molar solubility in water) of hydrocarbons was linearly related to the surface of a cavity which included the radius of the first layer of water molecules and that aromatic rings were more soluble but displayed nearly the same slope as the aliphatic series. Amidon et al.[4c] simplified Hermann's method of surface area calculation and extended it to include alcohols by inclusion of –OH surface area values. Moriguchi[5] has reported on factoring log P (octanol–water) into a hydrophilic effect of a polar group and a hydrophobic effect due to the free molar volume. He used Quayle's atomic parachor, molar refraction, and Exner's molar volume as parameters relating to molar volume.

We report a study which relates log P (octanol–water) to molecular volume as measured directly from CPK models. Our results show that a variety of apolar solutes[6] clearly separate into two classes (see Figure 1): one class has a surface of covalently bonded hydrogen atoms; the other class has a surface of noncovalently bonded electrons. Alkanes and $Si(CH_3)_4$ constitute the former class, while the rare gases, perhalogenated alkanes, aromatic hydrocarbons, and haloaromatic compounds comprise the latter (Table I).

Equations 1a and 1b show the relationship between log P for the two classes of solutes and surface area as cal-

culated by Bondi.[1] Equations 2a and 2b show that Bondi molar volume is clearly more closely related to log P than is Bondi area, while eq 3a and 3b show the volume taken directly from CPK molecular models is by far the best parameter for class II.

$$\log P_I = 0.513 \ (\pm 0.644) + 0.0207 \ (\pm 0.005) \ (\text{Bondi area}) \tag{1a}$$

$$\begin{array}{ccc} n & r & s \\ 11 & 0.952 & 0.206 \end{array}$$

$$\log P_{II} = -0.458 \ (\pm 0.220) + 0.0263 \ (\pm 0.002) \ (\text{Bondi area}) \tag{1b}$$

$$\begin{array}{ccc} n & r & s \\ 26 & 0.985 & 0.219 \end{array}$$

$$\log P_I = 0.568 \ (\pm 0.282) + 0.0283 \ (\pm 0.003) \ (\text{Bondi volume}) \tag{2a}$$

$$\begin{array}{ccc} n & r & s \\ 11 & 0.990 & 0.096 \end{array}$$

$$\log P_{II} = 0.007 \ (\pm 0.176) + 0.0288 \ (\pm 0.002) \ (\text{Bondi volume}) \tag{2b}$$

$$\begin{array}{ccc} n & r & s \\ 26 & 0.987 & 0.204 \end{array}$$

$$\log P_I = 0.728 \ (\pm 0.249) + 0.0281 \ (\pm 0.003) \ (\text{CPK volume}) \tag{3a}$$

$$\begin{array}{ccc} n & r & s \\ 11 & 0.991 & 0.090 \end{array}$$

$$\log P_{II} = -0.026 \ (\pm 0.076) + 0.0279 \ (\pm 0.001) \ (\text{CPK volume}) \tag{3b}$$

$$\begin{array}{ccc} n & r & s \\ 26 & 0.998 & 0.088 \end{array}$$

The number of data points used is represented by n, r is the correlation coefficient, s the standard deviation, and