The Linear Free-Energy Relationship between Partition Coefficients and the Binding and Conformational Perturbation of Macromolecules by Small Organic Compounds*

Friederike Helmer, Karl Kiehs, and Corwin Hansch†

ABSTRACT: The binding of a wide variety of organic compounds of miscellaneous structure to bovine serum albumin is correlated with their octanol–water partition coefficients. The simple linear relation brings out the nonspecific nature of the process. The hydrophobic bonding of the bovine serum albumin is shown to be quite similar to that of other proteins such as hemoglobin and ribonuclease. The linear relation appears to hold until about 3 moles of small molecules are bound/mole of protein. It is also shown that the change caused in the optical rotation of bovine serum albumin by the adsorbate is proportional to the logarithm of its partition coefficient. Using partition coefficients, a quantitative comparison of the results of Gordon and Jencks indicates that the dependence of the denaturation of T4 phage DNA upon the lipophilic character of the denaturant closely parallels the hydrophobic binding of organic compounds by macromolecules.

The importance of the binding of various organic compounds by proteins has long been recognized to be of concern to the biochemist (Klotz *et al.*, 1958; Némethy, 1967) as well as the pharmacologist (Goldstein, 1949; Ariëns, 1964). We have been studying the interaction of organic compounds with proteins (Hansch *et al.*, 1965c; Kiehs *et al.*, 1966) to better understand the structure–activity relationship between enzyme and substrate in drug action. We have considered this problem from several points of view in different reference systems such as whole animals (Hansch and Fujita, 1964), plants (Muir *et al.*, 1967), bacteria (Lien *et al.*, 1968), isolated organs (Hansch and Anderson, 1967a), and purified enzymes (Hansch *et al.*, 1965a). Our work has been directed to finding suitable physicochemical parameters such that structure–activity discussions could be carried on in mathematical terms. The very complex process of drug action can be factored into two processes: (1) movement of the drug from the point of application to the site of action in the biological system; and (2) the occurrence of a rate-limiting physical or chemical reaction at the site of action. The question arises, which factors do influence the two individual processes which are quite complex in themselves?

As a basic working hypothesis, it has been postulated (Hansch *et al.*, 1965c, 1968) that drugs find their sites of action by a random walk process. In this process they will encounter and interact with various proteins. The freedom of movement of biologically active compounds will hence depend upon how firmly they are bound by the protein or lipids they encounter. Once they have reached the site of action, they will often be adsorbed onto a critical enzyme or membrane on which a rate-limiting process may occur. Thus we might think of three steps, any one of which might or might not be critical in the action of a given set of congeneric drugs: (1) movement to site of action; (2) partitioning onto enzyme or membrane; and (3) causing a particular perturbation or conformational change in the enzyme or membrane. Ever since the work of Meyer and Overton it has been recognized that there are many instances in which a linear relationship exists between log $1/C$ (the molar concentration of drug causing a standard biological response) and log P (the partition coefficient of the drug between a lipid and an aqueous phase). If the partition coefficient is taken as a measure of the hydrophobic character of the drug, then one must anticipate a linear relationship between hydrophobic character and each or any of the above three processes (Hansch, 1968). We have been studying the binding of organic compounds by pure bovine serum albumin (BSA)[1] in order to better understand these processes and especially to formulate mathematical relationships. We have been using octanol–water partition coefficients, P, to serve as a measure of relative hydrophobicity. The results of our own studies as well as the work of other investigators which we have placed in mathematical terms are summarized in Table I.

Equations 1–7 show that although the binding of organic compounds by macromolecules involves a complex set of interactions, a rather good general description can be obtained for neutral molecules or those with

* From the Department of Chemistry, Pomona College, Claremont, California. *Received February 26, 1968.* Supported by Grants GM-07492 from the National Institutes of Health.

† To whom to address requests for reprints.

[1] Abbreviation used that is not listed in *Biochemistry 5*, 1445 (1966), is: BSA, bovine serum albumin.

TABLE I: Linear Free-Energy Relationship between Log P and the Binding of Organic Compounds by Macromolecules.

Compounds	Macromolecular System	Equation	n^a	r^b	s^c
Phenols	BSA	Log $1/C$ = 0.681 (±0.08) log P + 2.489 (1)[d]	19	0.962	0.133
Barbiturates	BSA	Log $1/C$ = 0.582 (±0.35) log P + 2.397 (2)[e]	4	0.961	0.137
Miscellaneous	Bovine hemoglobin	Log $1/C$ = 0.713 (±0.11) log P + 1.512 (3)[f]	17	0.950	0.160
ROH	Ribonuclease	Log K_B = 0.504 (±0.04) log P − 1.560 (4)[g]	4	0.999	0.012
$RCOO^-$	BSA	Log K = 0.594π (±0.22) − 6.514 (5)[h]	5	0.966	0.213
Barbiturates	Homogenized rabbit brain	Log % bound = 0.526 (±0.14) log P + 0.467 (6)[i]	4	0.992	0.056
Penicillins	Human serum	Log (B/F) = 0.488$\Sigma\pi$ − 0.628 (7)[j]	79	0.924	0.134

[a] The number of compounds employed in the study. [b] Correlation coefficient. [c] Standard deviation from regression. The figures in parentheses are the 95% confidence intervals. [d] Hansch *et al.* (1965b). C in eq 1–3 represents the molar concentration of compound necessary to produce a 1:1 complex of protein compound. [e] The experimental results are those of Goldbaum and Smith placed in mathematical context by Hansch (1966a). [f] Kiehs *et al.* (1966). [g] Experimental results of Schrier, Ingwall, and Scheraga placed in equation form by Hansch (1968). K represents the binding constants in eq 4 and 5. [h] Experimental results of Teresi and Luck (Hansch, 1968). [i] Experimental results of Goldbaum and Smith (Hansch, 1968). % bound represents the per cent barbiturate bound by the rabbit tissue. [j] In eq 7, B stands for per cent bound penicillin and F for per cent free penicillin from the work of Brid and Marshall (1967). $\Sigma\pi$ represents the sum of π values for attached substituents. π is defined (Hansch and Fujita, 1964) as: π = log P_X − log P_B, where P_X is the partition coefficient of a derivative and P_B that of a parent compound.

a constant charge with a simple linear relationship. Of greatest interest is the narrow range of slopes (~0.60 ± 0.13) found for the widely diverse systems. Often in biochemical studies considerable significance is attached to the fact that a particular organic molecule is bound by a macromolecular system. The above results indicate that caution must be used in such studies since any sufficiently lipophilic compound will be bound by a variety of macromolecules in a nonspecific way.

Three important questions not answered by our first work are: (1) Can simple aliphatic as well as large bulky molecules be accommodated in a single equation such as 1? (2) How many molecules can be bound before the linear relationship fails? (3) Do the bound molecules produce conformational changes in the protein in proportion to their lipophilic character? This report discusses our work on these three questions.

Method

The procedure for measuring the binding affinity of organic compounds to proteins has been previously described (Hansch *et al.*, 1965b). In the present work, ultraviolet spectroscopy and gas–liquid partition chromatography were used for the quantitative analysis of the dialyzed compounds. The amount of compound bound by BSA (5 ml of 3.0×10^{-5} M solution used) was determined for four or five different concentrations of compound. A plot of these data yielded r (r = moles of bound compound/mole of BSA). In this way log $1/C$ values were obtained for $r = 1$, $r = 2$, etc. These concentrations were then correlated with the partition coefficients of the compounds by the method of least squares using an IBM 360/40 computer. The data and the results are summarized in Table II.

In a second set of experiments of the effect of three different concentrations of a variety of organic compounds on the optical rotation of BSA was determined. In this way the per cent change in levorotation compared with a standard BSA solution at pH 7 (phosphate buffer) was made. The rotation was found to change slowly with time. The values used in this work were those taken at the end of 6 hr. From the per cent change in rotation caused by 1×10^{-3} M solutions of adsorbate the correlation equation relating this effect to log P was derived.

Results and Discussion

From the data in Table II we have derived eq 8. In eq 8, C is the concentration of compound producing a 1:1

$$\text{Log}\frac{1}{C} = 0.751\ (\pm 0.07)\ \log P + 2.301\ (\pm 0.15)$$

$$n = 42 \quad r = 0.960 \quad s = 0.159 \qquad (8)$$

complex with BSA; in effect, C is an equilibrium constant. The constants of eq 1 and 8 are the same within experimental error although the correlation with eq 8 is slightly less good (compare values of s). This shows that no special steric interactions not contained in our octanol–water model occur in the binding of very bulky molecules such as 1-hydroxyadamantane, neopentanol, or camphorquinone. Also, the relatively un-ionized amines and phenols are accommodated by the same equation with no special correction for degree of ionization. Aliphatic compounds without extensive π electron systems, such as neopentyl alcohol, 1-hexanol, and 2-

TABLE II: Concentrations of Organic Compounds Necessary to Form a 1:1 Molar Complex with BSA.

Compound	Log P	Obsd Log $1/C$	Calcd[a] Log $1/C$	$\lvert\Delta \log 1/C\rvert$
Phenol	1.46	3.32	3.397	0.08
3-Fluorophenol	1.93	3.86	3.750	0.11
4-Fluorophenol	1.77	3.52	3.630	0.11
3-Chlorophenol	2.50	4.30	4.178	0.12
4-Chlorophenol	2.39	4.00	4.095	0.01
4-Bromophenol	2.59	4.22	4.245	0.03
4-Iodophenol	2.91	4.40	4.485	0.09
4-Methylphenol	1.94	3.70	3.757	0.06
3-Ethylphenol	2.40	4.22	4.103	0.12
3-Trifluoromethylphenol	2.95	4.52	4.515	0.01
3-Cyanophenol	1.22	3.26	3.217	0.04
3-Hydroxyphenol	0.80	3.15	2.902	0.25
3-Methoxyphenol	1.58	3.54	3.487	0.05
4-Methoxyphenol	1.34	3.40	3.307	0.10
3-Nitrobenzonitrile	1.17	2.94	3.179	0.24
4-Methoxybenzyl alcohol	1.10	2.94	3.127	0.19
Benzonitrile	1.56	3.23	3.472	0.24
Acetophenone	1.58	3.31	3.487	0.18
Nitrobenzene	1.85	3.58	3.690	0.11
4-Bromoacetanilide	2.18[b]	4.00	3.937	0.06
4-Nitroanisole	2.03[b]	4.00	3.825	0.18
4-Chloronitrobenzene	2.39	4.07	4.095	0.03
2,4-Dichloronitrobenzene	2.93[b]	4.59	4.500	0.09
Naphthalene	3.37	4.91	4.831	0.08
Azobenzene	3.82	5.29	5.168	0.12
Anisole	2.11	4.00	3.885	0.12
3-Fluoroaniline	1.30	3.09	3.277	0.19
4-Chloroaniline	1.83[b]	3.68	3.675	0.01
4-Methoxyaniline	0.78[b]	2.92	2.887	0.03
4-Bromoaniline	2.03[b]	4.06	3.825	0.24
4-Methylaniline	1.39	3.30	3.344	0.04
1-Naphthylamine	2.23[b]	3.94	3.975	0.04
Indole	2.14	4.07	3.907	0.16
Neopentanol	1.36	3.47	3.322	0.15
Camphorquinone	1.52	3.17	3.442	0.27
1-Hydroxyadamantane	2.14	3.94	3.907	0.04
Thymol	3.30	4.66	4.778	0.12
unsym-Methylphenylthiourea	0.85[b]	3.30	2.939	0.36
n-Hexyl alcohol	1.84[b]	3.94	3.682	0.26
Phenylethyl carbamate	2.30	3.83	4.027	0.20
Ethylamylbarbituric acid	2.24	3.66	3.982	0.33
2-Nonanone	2.79[b]	4.33	4.395	0.07

[a] Calculated using eq 8. [b] These values were calculated by taking advantage of the additivity of π values (Fujita *et al.*, 1964; Hansch and Anderson, 1967b). For example, to obtain log P for substituted anilines, π values of substituents from phenols were added to log P of 0.90 for aniline. For naphthylamine, π for $(CH)_4$ of 1.42 was added to log P of aniline. The value of 1.42 was found by subtracting log P for phenol from log P for 1-naphthol.

nonanone, are also well fit. These findings are important for enzyme chemistry as well as pharmacology since they show that a linear free-energy relationship exists between the binding of organic compounds or drugs by protein and their partition coefficients obtained from the octanol–water reference system. Although we were not surprised to find that eq 1 would correlate a set of closely related congeners such as the phenols, we were surprised to find as good a correlation as that contained in eq 8 for such a great diversity of structures as those in Table II. This constitutes needed support for quantitative correlations in the study of enzyme–substrate

TABLE III: Concentrations of Compounds Forming 2:1, 3:1, and 4:1 Complexes with BSA.

Compound	Obsd Log $1/C_2$	Calcd[a] Log $1/C_2$	Obsd Log $1/C_3$	Calcd[b] Log $1/C_3$	Obsd Log $1/C_4$	Calcd[c] Log $1/C_4$
3-Trifluoromethylphenol	3.96	4.002	3.71	3.767	3.56	3.571
3-Ethylphenol	3.65	3.658	3.41	3.443	3.25	3.394
3-Chlorophenol	3.76	3.721	3.54	3.502	3.39	3.426
3-Fluorophenol	3.56	3.364	3.42	3.166	3.28	3.243
4-Methoxyphenol	3.21	3.146				
4-Bromophenol	3.92	3.777	3.73	3.555	3.60	3.455
4-Chlorophenol	3.70	3.652	3.52	3.437	3.40	3.391
4-Methylphenol	3.44	3.371				
4-Chloronitrobenzene	3.57	3.652	3.32	3.437		
Nitrobenzene	3.29	3.314	3.15	3.119		
Acridine	4.24	4.283				
Indole	3.63	3.496	3.40	3.290		
Acetophenone	3.01	3.146	2.82	2.959		
Benzonitrile	3.02	3.133	2.88	2.948		
Anisole	3.46	3.477				
4-Nitroanisole	3.54	3.427	3.33	3.225		
4-Bromoacetanilide	3.70	3.683	3.42	3.467		
3-Fluoroaniline	2.87	2.970	2.72	2.794		
4-Bromoaniline	3.52	3.777	3.29	3.555		
4-Chloroaniline	3.33	3.333	3.14	3.136		

[a] Calculated using eq 9. [b] Calculated using eq 10. [c] Calculated using eq 11.

interactions (Hansch *et al.*, 1965a; Hansch, 1966b). Various authors, in studying the interaction of proteins with organic compounds, have invoked steric hindrance and charge transfer complexes to rationalize the different degrees of binding of different compounds. Until rather recently hydrophobic interactions were not given sufficient consideration. The above linear relations constitute a base from which one can work using regression analysis to evaluate the relative importance of electronic, steric, and hydrophobic interactions in the binding of small molecules by proteins.

Considering the large difference in the molecular weights of the organic compounds and the BSA, it was of interest to see how many moles of small compounds could be adsorbed per mole of BSA within the framework of eq 8. Equations 9–11 come from the data of Table III. In eq 9, where we are considering the case of 2 moles of small molecules/mole of protein, the situation is not much different from that of eq 8. A reasonable correlation is still found in eq 10 for the 3:1 case. However, in eq 11, the dependence of binding upon log P drops greatly as indicated by the lower slope and the correlation is quite poor, even for a closely related set of phenols. Still poorer correlations are found when one attempts to encompass a wider variety of molecules in eq 11. The good correlations with eq 8–10 indicate that the free-energy change in binding of the small molecules by BSA parallels quite closely their free energy of transfer from water to octanol. The main driving force for water to octanol partitioning would appear to be the entropic change in the shearing off of an ordered water layer around the small molecules (Némethy, 1967; Kauzmann, 1959). Once in the octanol phase, one would expect very little restriction on the movement of the partitioned molecule and no special orientation. The linear relationship of eq 8–10 indicates that binding to the BSA of up to about three molecules must be a similar process. The coefficient in eq 8 is not 1, but about 0.7. Thus for a given increment in hydrophobicity, one finds more molecules moving from water to octanol than from water to BSA; this is to be expected. One would expect a greater energy requirement to force a molecule into a crevice or pouch in the BSA molecule than into the fluid octanol phase. The precise reasons for the coefficients of 0.7 and the break at about three molecules per mole of BSA are at present not clear. It is of interest to note that the bactericidal action of a wide

$$\text{Log}\frac{1}{C_2} = 0.625\ (\pm 0.11)\ \log P + 2.158\ (\pm 0.23)$$

$$n \quad r \quad s$$
$$20 \quad 0.947 \quad 0.111 \qquad (9)$$

$$\text{Log}\frac{1}{C_3} = 0.590\ (\pm 0.16)\ \log P + 2.027\ (\pm 0.36)$$

$$n \quad r \quad s$$
$$16 \quad 0.900 \quad 0.133 \qquad (10)$$

$$\text{Log}\frac{1}{C_4} = 0.332\ (\pm 0.39)\ \log P + 2.621\ (\pm 0.98)$$

$$n \quad r \quad s$$
$$6 \quad 0.749 \quad 0.106 \qquad (11)$$

TABLE IV: Per Cent Decrease in Optical Rotation Correlated with Log P.[a]

Compound	Log P	Obsd % Decrease	Calcd[a] % Decrease	$\lvert\Delta$ % Decrease$\rvert$
Phenol	1.46	0.67	0.560	0.11
4-Fluorophenol	1.77	1.25	1.252	0.00
4-Chloroaniline[b]	1.83	0.86	1.386	0.53
4-Bromoaniline[b]	2.03	2.40	1.833	0.57
1-Hydroxyadamantane	2.14	1.95	2.078	0.13
1-Naphthylamine[b]	2.42	3.30	2.704	0.60
4-Bromophenol	2.59	2.80	3.083	0.28
Thymol	3.30	4.18	4.668	0.49
Chloropromazine	5.35	9.40	9.244	0.16

[a] Calculated using eq 12. These values calculated assuming additivity of π and log P (Fujita *et al.*, 1964; Hansch and Anderson, 1967b).

variety of different sets of congeners showed (Lien *et al.*, 1968) a similar dependence upon log P.

We have, of course, been interested in the effect of binding of small molecules on the macromolecule. Conformational changes in the protein have important consequences for specific activity of biologically active proteins or membranes. In a preliminary study to see if any change in physical property of the protein is correlated with the hydrophobic character of small molecules, we have measured the change in optical rotation of BSA brought about by the binding of small molecules. From the data in Table IV we have derived eq 12. In eq 12,

$$\% \Delta\alpha_D = 2.232\ (\pm 0.31) \log P - 2.699\ (\pm 0.86)$$

$$n \quad r \quad s$$
$$9 \quad 0.988 \quad 0.435 \qquad (12)$$

$\% \Delta\alpha_D$ is the per cent decrease in levorotation (sodium D line) obtained by comparing a 10-cm tube of pure BSA at pH 7 with a similar tube containing BSA plus 1×10^{-3} M compound. The effect of organic compounds on the optical rotation of proteins has received attention (Gordon and Jencks, 1963; Levine *et al.*, 1963). However, to our knowledge, eq 12 is the first quantitative relation between $\Delta\alpha$ and hydrophobic character. Exactly how $\Delta\alpha$ is brought about by the small molecules is, of course, not known. From the three different types of molecules, acidic phenols, basic amines, and neutral 1-hydroxyadamantane, one would be inclined to expect that conformation change causes $\Delta\alpha$ rather than a specific reaction between adsorbate and a particular optically active center. It should be noted that in the case of chloropromazine we have used log P of the un-ionized species.

Jencks and Gordon studied the denaturation of BSA by a large variety of chemicals. However, in their work very high concentrations of (1–8 M) substances were studied and hence are not comparable with ours. In summarizing work on the effect of compounds on changes in optical rotation, Gordon and Jencks point out three possible mechanisms: (1) changes in helical content; (2) other changes in conformation; and (3) effects which do not involve a change in conformation. They defined the first of the above two as "denaturation" and the third as a "solvent effect." They concluded that their results were best considered as denaturation. This would also be true of ours since we have worked at very low "solvent" concentrations. Exactly what kind of conformational perturbation is involved remains to be seen.

The above hydrophobic interactions of small molecules with proteins appear to be closely related to interactions with other macromolecules such as DNA. Levine, Gordon, and Jencks studied the denaturization action of various small molecules on bacteriophage DNA. Using a specific immunochemical technique they determined the molar concentration of molecules causing 50% denaturation of T4 bacteriophage DNA in aqueous solution at 73°. From their data in Table V we have derived eq 13 and 14. The slope of eq 13 is remarkably close to eq 7. Again the slope of 0.7 brings to mind the dependence of bactericidal action of a variety of different sets of congeners (Lien *et al.*, 1968) upon log P. While the amide group seems to be inherently more potent (compare intercepts in eq 13 and 14) than the hydroxy compounds, the slope of eq 14 is not as high as eq 13. This could be interpreted to mean that amides act at a somewhat different site than the hydroxy com-

Denaturation by Alcohols and Phenols

$$\text{Log } \frac{1}{C} = 0.702\ (\pm 0.16) \log P + 0.031\ (\pm 0.13)$$

$$n \quad r \quad s$$
$$12 \quad 0.951 \quad 0.168 \qquad (13)$$

Denaturation by Amides

$$\text{Log } \frac{1}{C} = 0.412\ (\pm 0.06) \log P + 0.451\ (\pm 0.06)$$

$$n \quad r \quad s$$
$$5 \quad 0.997 \quad 0.037 \qquad (14)$$

TABLE V: Correlation of 50% Denaturation T_4-Phage DNA with Log P.

Compound	Log P	Obsd Log $1/C$	Calcd[a] Log $1/C$	$\|\Delta$ Log $1/C\|$
1. Methanol	−0.66	−0.54	−0.433	0.11
2. Ethanol	−0.16[b]	−0.08	−0.082	0.00
3. Isopropyl alcohol	0.14[b]	0.05	0.129	0.08
4. Propanol	0.34	0.27	0.269	0.00
5. Allyl alcohol	0.04[b]	0.30	0.059	0.24
6. 2-Butanol	0.61	0.21	0.459	0.25
7. *t*-Butyl alcohol	0.37	0.22	0.291	0.07
8. Cyclohexanol	1.23	0.66	0.894	0.23
9. Benzyl alcohol	1.10	1.05	0.803	0.25
10. Phenol	1.46	1.10	1.056	0.04
11. 4-Methoxyphenol	1.34	1.05	0.972	0.08
12. Acetonitrile	−0.34	−0.08	−0.208	0.13
13. Formamide	−1.71[b]	−0.28	−0.253	0.03
14. Acetamide	−1.21[b]	−0.04	−0.047	0.01
15. Propionamide	−0.71[b]	0.21	0.159	0.05
16. Butyramide	−0.21	0.34	0.365	0.03
17. Hexanamide	0.79[b]	0.77	0.777	0.01

[a] Values for 1–12 obtained using eq 13; values for 13–17 obtained using eq 14. [b] See Table II.

pounds and thus have a somewhat different hydrophobic effect.

It is, of course, most interesting to the field of drug design that eq 7 and 13 have such similar slopes. As a first rough approximation, a given increment in hydrophobic character appears to cause similar conformational changes in both proteins and DNA.

The results in this report constitute further evidence for the value of octanol–water partition coefficients as a reference system of use for correlating the interaction of small molecules with macromolecules. Using physicochemical parameters with regression analysis, we should be able to gain further insight into the action of organic molecules with biochemical systems.

Acknowledgment

We wish to thank Professor Hermann Stetter of the Technischen Hochschule of Aachen for a generous sample of 1-hydroxyadamantane.

References

Ariëns, E. J. (1964), Molecular Pharmacology, Vol. 1, New York, N. Y., Academic, p 29.
Brid, A. E., and Marshall, A. C. (1967), *Biochem. Pharmacol. 16*, 2275.
Fujita, T., Iwasa, J., and Hansch, C. (1964), *J. Am. Chem. Soc. 86*, 5175.
Goldstein, A. (1949), *Pharmacol. Rev. 1*, 102.
Gordon, J. A., and Jencks, W. P. (1963), *Biochemistry 2*, 47.
Hansch, C. (1966a), *Proc. Intern. Congr. Pharmacol., Sao Paulo*.
Hansch, C. (1966b), *Ann. Rept. Med. Chem.*, 347.
Hansch, C. (1968), *Farmaco 23*, 293.
Hansch, C., and Anderson, S. M. (1967a), *J. Med. Chem. 10*, 745.
Hansch, C., and Anderson, S. M. (1967b), *J. Org. Chem. 32*, 2583.
Hansch, C., Deutsch, E. W., and Smith, R. N. (1965a), *J. Am. Chem. Soc. 87*, 2738.
Hansch, C., and Fujita, T. (1964), *J. Am. Chem. Soc. 86*, 1616.
Hansch, C., Kiehs, K., and Lawrence, G. (1965b), *J. Am. Chem. Soc. 87*, 5770.
Hansch, C., Steward, A. R., Anderson, S. M., and Bentley, D. L. (1968), *J. Med. Chem. 11*, 1.
Hansch, C., Steward, A. R., Iwasa, J., and Deutsch, E. W. (1965c), *Mol. Pharmacol. 1*, 205.
Kauzmann, W. (1959), *Advan. Protein Chem. 14*, 37.
Kiehs, K., Hansch, C., and Moore, L. (1966), *Biochemistry 5*, 2602.
Klotz, I. W., Ayers, J., Ho, J. Y. C., Horowitz, M. G., and Heiney, R. E. (1958), *J. Am. Chem. Soc. 80*, 2132.
Levine, L., Gordon, J. A., and Jencks, W. P. (1963), *Biochemistry 2*, 168.
Lien, E. J., Hansch, C., and Anderson, S. M. (1968), *J. Med. Chem. 11*, 430.
Muir, R. M., Fujita, T., and Hansch, C. (1967), *Plant Physiol. 42*, 1519.
Némethy, G. (1967), *Angew. Chem. 6*, 195.