Journal of Medicinal Chemistry

VOLUME 10, NUMBER 6 OCTOBER 26, 1967

Analysis of the Structure–Activity Relationship of the Sulfonamide Drugs Using Substituent Constants[1]

TOSHIO FUJITA

Department of Agricultural Chemistry, Kyoto University, Kyoto, Japan

AND CORWIN HANSCH

Department of Chemistry, Pomona College, Claremont, California

Received May 15, 1967

A recently developed method for the correlation of biological activity and chemical structure of dissociable compounds under physiological conditions using the Hammett σ constant and the hydrophobicity constant π with a correction for the effect of dissociation has been applied to the analysis of the bacteriostatic activity and protein binding of the sulfonamide drugs. The hydrophobicity of the drugs is found to play a definite role on the activity, and the optimal hydrophobic character for the activity is deduced from the relationships. Other factors being equal, logarithmic plots of the apparent activity against the dissociation constant are shown to be expressed by two straight lines, the intersection of which corresponds sometimes to the maximal activity for a series of sulfanilamides. The most favorable dissociation constant for the maximum activity and the optimal hydrophobicity for a series of sulfanilamides have been suggested to help in the designing of new sulfonamide drugs.

Much work has been done to elucidate the relation between physicochemical properties and bacteriostatic activity of the sulfonamide drugs. Bell and Roblin[2] found that a logarithmic plot of the bacteriostatic activities of a series of sulfonamides against their dissociation constants exhibits a parabolic relationship. Considering that the activity increases with the negative character of the SO_2 group, they postulated that the electron-attracting power of the N^1 substituent should be in an optimal range for the maximal activity so that the ionization constant of the SO_2NH group is about 10^{-6}–10^{-7}. An alternative explanation for the parabolic relationship was proposed by Cowles[3] who assumed that the negative ion which is responsible for the bacteriostatic action penetrates with difficulty to the site of action inside the cell so that there should be an optimal dissociation constant where the balance between the intrinsic activity and the penetration is most favorable to the bacteriostatic process. Seydel and his co-workers,[4] from a correlation between ir spectra and activity of a number of sulfonamides, emphasized that the amount of negative charge on the aromatic amino group is a significant factor for the activity. In spite of these efforts and others,[5] generally considering a single physicochemical parameter, the structure–activity studies on the sulfonamides still leave much to be desired.

Recently,[6] we have developed a method for the correlation of biological activity and chemical structure using substituent constants such as the Hammett σ constant and a hydrophobicity constant π defined as $\pi = \log P_X - \log P_H$, where P_X and P_H are the partition coefficients, determined in the system 1-octanol–water, of the substituted and unsubstituted compounds, respectively. The contributions of the electronic and hydrophobic characters of a substituent to a specific biological activity of a series of substituted compounds can be analyzed simultaneously by eq 1. In eq 1, C is

$$\log (1/C) = a\pi - b\pi^2 + \rho\sigma + c \qquad (1)$$

the equieffective molar concentration, *i.e.*, the concentration causing a standard response such as CD_{50}, ED_{50}, minimum inhibitory concentration, etc., and a, b (≥ 0), ρ, and c are constants which are determined by the method of least squares. The value of $1/C$ is proportional to the magnitude of biological activity. We have applied this method[7] to compounds which are dissociated under physiological conditions such as a series of substituted phenols. For these compounds, the biological activity can be expressed by either eq 2 or 3 regardless of whether the sites of action are located inside or outside the cell. In eq 2 and 3, $[H^+]$ is the hy-

(1) Studies on Structure–Activity Relationship. II.

(2) P. H. Bell and R. O. Roblin, Jr., *J. Am. Chem. Soc.*, **64**, 2905 (1942).

(3) P. B. Cowles, *Yale J. Biol. Med.*, **14**, 599 (1942).

(4) J. K. Seydel, E. Krüger-Thiemer, and E. Wempe, *Z. Naturforsch.*, **15b**, 628 (1960).

(5) See, *e.g.*, (a) H. G. Ing, "Organic Chemistry, An Advanced Treatise," Vol. III, H. Gilman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1953, p 436; (b) W. A. Sexton, "Chemical Constitution and Biological Activity," 3rd ed, D. Van Nostrand Co., Inc., Princeton, N. J., 1963, p 160; (c) G. Zbinden, "Molecular Modification in Drug Design," F. W. Schuler, Ed., American Chemical Society, Washington, D. C., 1964, p 25.

(6) (a) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964); (b) C. Hansch and A. R. Steward, *J. Med. Chem.*, **7**, 691 (1964); (c) C. Hansch, E. W. Deutsch, and R. N. Smith, *J. Am. Chem. Soc.*, **87**, 2738 (1965); (d) C. Hansch, K. Kiehs, and G. L. Lawrence, *ibid.*, **87**, 5770 (1965); (e) K. Kiehs, C. Hansch, and L. Moore, *Biochemistry*, **5**, 2602 (1966).

(7) T. Fujita, *J. Med. Chem.*, **9**, 797 (1966).

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = a\pi - b\pi^2 + \rho\sigma + c \quad (2)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = a\pi - b\pi^2 + \rho'\sigma + c' \quad (3)$$

drogen ion concentration of the extracellular phase and K_A is the dissociation constant. ρ' and c' are constants for the ionized form having the same significance as ρ and c for the neutral molecule. Equation 2 describes the structure–activity correlation when the action of a series of compounds is solely due to the neutral molecule. Likewise, eq 3 holds when the ionic form is regarded as the active form. However, if the structure–activity correlation is considered on the basis of the activity data obtained at a single extracellular pH, eq 2 and 3 are interrelated by eq 4 and 5. Therefore, whether the

$$\rho - \rho' = \rho_A \quad (4)$$

$$c' = c + pK_A^0 - pH \quad (5)$$

active form is the neutral or ionized form or both, eq 2 and 3 should hold simultaneously. In these equations, ρ_A is the Hammett reaction constant for the ionization of the dissociable group in the molecule and pK_A^0 is the pK_A of a standard compound (in most cases, the unsubstituted compound). When $\Delta pK_A = (\log K_A^x - \log K_A^0)$ is used for the analysis in place of the Hammett σ constant, ρ_A becomes 1.

The purpose of this paper is to apply this approach to the sulfonamide drugs which exist partly in the dissociated form under conditions of physiological pH. In this way we can get further insight into the significance of electronic and hydrophobic modifications of the molecule on the biological activity. We have found[6a,7] in some cases that equations where one or two terms on the right side of eq 1–3 are deleted are sufficient for rationalizing the physiological actions. Thus, for a series of sulfanilamides, equations such as 6a–c and 7a–c as well as eq 2 and 3 are derived from the apparent biological activity, pH of the test medium, and physicochemical parameters for substituents by the method of least squares. By examining the correlation coefficients and standard deviations of these equations, an equation of the best fit is chosen for discussion of the structure–activity relationship.

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = a\pi + c \quad (6a)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = \rho\sigma + c \quad (6b)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = a\pi + \rho\sigma + c \quad (6c)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = a\pi + c' \quad (7a)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = \rho'\sigma + c' \quad (7b)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = a\pi + \rho'\sigma + c' \quad (7c)$$

Results and Discussion

Bacteriostatic Activity of the Substituted Sulfanilanilides.—The bacteriostatic activity of various substituted sulfanilanilides was tested by Schmidt and Sesler[8] against gram-positive pneumococcus and gram-negative Friedländer's bacillus and recently by Seydel[9] against gram-negative *E. coli*. They did not consider either the effect of ionization under the experimental conditions or the hydrophobic effect of the substituents; however, they did notice that the activity was highly influenced by the position and the nature of the substituent. In fact, Seydel,[9] who had earlier pointed out the significance of the aromatic amino group for the activity, showed that there is an approximately linear relationship between the logarithm of the minimum inhibitory concentration for *E. coli* and the physicochemical parameters of the substituted aniline moiety of the drugs such as the Hammett σ constant and ir spectral data.

Recently, Yoshioka[10] and his associates have determined the acid dissociation constant of this series of compounds and found that a modified Hammett relationship nicely rationalizes the effect of the substituent on the acid dissociation as shown in eq 8. Using the

$$\log K_A = 1.883[\sigma + 0.54(\sigma^- - \sigma)] - 8.942 \quad (8)$$

dissociation constants obtained by these authors and those calculated by eq 8 when the constant is lacking, we have analyzed the structure–activity relationship for the sulfanilanilides.

While Schmidt and Sesler studied 35 substituted sulfanilanilides, 20 compounds are included in Table I omitting those where steric effects of the substituent(s) make the estimation of the dissociation constant difficult. For regression analysis of the activity against pneumococcus, 18 compounds (3,4-disubstituted derivatives omitted) are used. Equations 9a–c and 10a–c are those for the *meta* derivatives and eq 11a–c and 12a–c are for the *para* derivatives (the unsubstituted sulfanilanilide is included in each case). In these equations, A is the *in vitro* activity relative to that of sulfanilamide calculated from the original value corrected to a molar basis and $[H^+]$ is $10^{-7.8}$.[11] n is the number of points used in the regression, s is the standard deviation, and r is the correlation coefficient. For the values of π, those derived from substituted anilines are used.[12] Factoring the groups into *meta* and *para* derivatives is found to give a much better correlation. When both groups of derivatives are mixed together for the correlation, the activity of the 4-CF_3 derivative is only poorly predicted. The addition of a π^2 term does not improve the correlation.

meta derivatives

	n	s	r	
$\log A + \log \frac{K_A + [H^+]}{[H^+]} =$				
$0.446\pi + 1.274$	12	0.542	0.612	(9a)
$\log A + \log \frac{K_A + [H^+]}{[H^+]} =$				
$1.372\sigma + 0.866$	12	0.248	0.933	(9b)
$\log A + \log \frac{K_A + [H^+]}{[H^+]} =$				
$1.204\sigma + 0.239\pi + 0.767$	12	0.136	0.982	(9c)

(8) L. H. Schmidt and C. L. Sesler, *J. Pharmacol. Exptl. Therap.*, **87**, 313 (1946).

(9) (a) J. K. Seydel, *Mol. Pharmacol.*, **2**, 259 (1966); (b) *Arzneimittel Forsch.*, **16**, 1447 (1966).

(10) M. Yoshioka, K. Hamamoto, and T. Kubota, *Bull. Chem. Soc. Japan*, **35**, 1723 (1962).

(11) L. H. Schmidt, C. L. Sesler, and H. B. Hughes, *J. Pharmacol. Exptl. Therap.*, **81**, 43 (1944).

(12) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

TABLE I

BACTERIOSTATIC ACTIVITY OF SUBSTITUTED SULFANILANILIDES

			—Against pneumococcus—				—Against Friedländer's bacillus—				—Against *E. coli*—			
			Log A + log $\frac{K_A + [H^+]}{[H^+]}$		Log A + log $\frac{K_A + [H^+]}{K_A}$		Log A + log $\frac{K_A + [H^+]}{[H^+]}$		Log A + log $\frac{K_A + [H^+]}{K_A}$		Log $(1/C)$ + log $\frac{K_A + [H^+]}{[H^+]}$		Log $(1/C)$ + log $\frac{K_A + [H^+]}{K_A}$	
Substituent	σ[a]	π[b]	Obsd	Calcd[c]	Obsd	Calcd[d]	Obsd	Calcd[e]	Obsd	Calcd[f]	Obsd	Calcd[g]	Obsd	Calcd[h]
H	0	0	0.8	0.77	2.0	1.91	0.5	−0.03	1.7	1.16	−1.19	−1.20	0.6	0.57
3-CH_3	−0.07	0.50	...	...	...	...	...	...	...	...	−1.34	−1.37	0.5	0.47
3-$N(CH_3)_2$	−0.05*	0.08	0.9	0.73	2.1	1.96	−0.3	−0.04	0.9	1.19	...	...	...	...
3-OCH_3	0.12	0.03	1.0	0.92	1.9	1.83	0.1	0.14	1.0	1.05	−1.04	−1.05	0.5	0.53
3-OC_2H_5	0.12	0.53	...	...	...	...	...	...	...	...	−1.11	−1.12	0.4	0.41
3-I	0.35	1.32	...	...	...	...	...	...	...	...	−0.87	−0.94	0.2	0.16
3-Cl	0.37	0.98	1.3	1.45	1.8	1.90	0.7	0.25	1.2	0.69	−0.87	−0.86	0.2	0.23
3-Br	0.39	1.13	1.4	1.51	1.8	1.92	0.5	0.25	0.9	0.66	−1.01	−0.86	0.0	0.19
3-CF_3	0.43	1.28	1.4	1.59	1.7	1.93	−0.1	0.26	0.2	0.60	...	...	...	...
3-CN	0.56	−0.02	1.4	1.44	1.4	1.52	0.5	0.60	0.5	0.68	...	...	...	...
3-NO_2	0.71	0.47	1.6	1.74	1.5	1.54	1.1	0.67	0.9	0.48	−0.18	−0.35	0.3	0.26
3,5-Br_2	0.78	2.26	2.4	2.25	2.1	1.93	0.6	0.47	0.3	0.15	...	...	...	...
3,5-$(CF_3)_2$	0.86	2.56	2.5	2.42	2.0	1.95	0.4	0.50	−0.1	0.04	...	...	...	...
3,5-$(CN)_2$	1.12	−0.04	2.2	2.11	1.2	1.14	0.7	1.16	−0.3	0.20	...	...	...	...
3,5-$(NO_2)_2$	1.42	0.94	2.7	2.70	1.2	1.18	1.5	1.32	0.0	−0.20	...	...	...	...
H	0	0	0.8	0.74[i]	2.0	1.88[i]	...	...	...	...	...	...	...	...
4-$N(CH_3)_2$	−0.27*	−0.15	0.6	0.65	2.3	2.28	−0.3	−0.22	1.4	1.41	−1.65	−1.53	0.6	0.68
4-OCH_3	−0.27	−0.21	0.6	0.65	2.1	2.28	−0.3	−0.21	1.2	1.42	−1.54	−1.53	0.6	0.69
4-OC_2H_5	−0.24	0.29	...	...	...	...	...	...	...	...	−1.51	−1.56	0.7	0.57
4-*t*-Bu	−0.20	1.68	0.6	0.67	2.1	2.18	−0.6	−0.43	0.9	1.08	...	...	...	...
4-*t*-Am	−0.20	2.18	0.6	0.67	2.1	2.18	−0.9	−0.51	0.6	1.01	...	...	...	...
4-CH_3	−0.17	0.49	...	...	...	...	...	...	...	...	−1.43	−1.49	0.6	0.50
4-Cl	0.23	0.80	1.3	0.81	2.1	1.54	−0.7	0.14	1.5	0.84	−1.18	−1.02	0.2	0.31
4-Br	0.23	1.12	...	...	...	...	...	...	...	...	−1.03	−1.06	0.3	0.24
4-I	0.28	1.39	...	...	...	...	...	...	...	...	−0.98	−1.04	0.2	0.16
4-CF_3	0.54	1.28	0.6	0.91	0.7	1.08	0.0	0.38	0.1	0.50	...	...	...	...
4-$COCH_3$	0.87	−0.11	...	...	...	...	...	...	...	...	0.00	−0.06	0.4	0.35
4-NO_2	1.27	0.49	...	...	...	...	...	...	...	...	0.27	0.38	0.1	0.10
3-Br-4-*t*-Bu	0.19	2.81	1.7	1.43[j]	2.5	2.20[k]	0.2	−0.21	1.0	0.58	...	...	...	...
3,4-Cl_2	0.60	1.78	2.1	1.50[j]	2.1	1.54[k]	0.0	0.36	0.0	0.38	...	...	...	...

[a] Taken from D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, **23**, 420 (1958), except for those marked with an asterisk which are taken from ref 10. The values for the polysubstituted compounds are obtained by summing values for the individual substituents. [b] Taken from ref 12 or estimated by eq 16, 17, and/or 19 of ref 12 and simply summed to get a figure for the polysubstituted compounds. [c] Calculated by eq 9c for the *meta* derivatives and eq 11b for the *para* derivatives. [d] Calculated by eq 10c for the *meta* derivatives and eq 12b for the *para* derivatives. [e] Calculated by eq 13c. [f] Calculated by eq 14c. [g] Calculated by eq 15c. [h] Calculated by eq 16c. [i] Calculated by eq 11b and 12b. [j] Calculated using $\log A + \log [(K_A + [H^+])/[H^+]] = (1.204\sigma + 0.239\pi)_{meta} + (0.323\sigma)_{para} + 0.751$ which is derived by combining eq 9c and 11b. [k] Calculated using $\log A + \log [(K_A + [H^+])/K_A] = (-0.676\sigma + 0.245\pi)_{meta} + (-1.486\sigma)_{para} + 1.892$ which is derived by combining eq 10c and 12b.

$$\log A + \log \frac{K_A + [H^+]}{K_A} = 0.129\pi + 1.621 \quad n = 12 \quad s = 0.322 \quad r = 0.353 \quad (10a)$$

$$\log A + \log \frac{K_A + [H^+]}{K_A} = -0.505\sigma + 2.007 \quad 12 \quad 0.251 \quad 0.685 \quad (10b)$$

$$\log A + \log \frac{K_A + [H^+]}{K_A} = -0.676\sigma + 0.245\pi + 1.906 \quad 12 \quad 0.134 \quad 0.930 \quad (10c)$$

para derivatives

$$\log A + \log \frac{K_A + [H^+]}{[H^+]} = -0.029\pi + 0.752 \quad 7 \quad 0.286 \quad 0.105 \quad (11a)$$

$$\log A + \log \frac{K_A + [H^+]}{[H^+]} = 0.323\sigma + 0.736 \quad 7 \quad 0.267 \quad 0.378 \quad (11b)$$

$$\log A + \log \frac{K_A + [H^+]}{[H^+]} = 0.353\sigma - 0.050\pi + 0.777 \quad 7 \quad 0.292 \quad 0.418 \quad (11c)$$

$$\log A + \log \frac{K_A + [H^+]}{K_A} = -0.144\pi + 2.029 \quad 7 \quad 0.576 \quad 0.253 \quad (12a)$$

$$\log A + \log \frac{K_A + [H^+]}{K_A} = -1.486\sigma + 1.878 \quad 7 \quad 0.322 \quad 0.841 \quad (12b)$$

$$\log A + \log \frac{K_A + [H^+]}{K_A} = -1.454\sigma - 0.054\pi + 1.922 \quad 7 \quad 0.354 \quad 0.846 \quad (12c)$$

For the activity of the *meta* derivatives in terms of the concentration of the neutral form, it seems that the role of the electronic effect of the substituent is most significant, whereas that of the hydrophobic character appears to be supplementary to the activity. An F test indicates, however, that the π term in eq 9c is justified at better than 0.995 level of significance when compared with eq 9b ($F_{1,9} = 24.03$; $F_{1,9,0.005} = 13.61$). The somewhat lower correlation coefficient of eq 10c for the activity in terms of concentration of the ionized form is attributed to the smaller variance in the values of $\log A + \log [(K_A + [H^+])/K_A]$. As expected from eq 4 and 5, eq 9c and 10c are related by the difference between ρ values: $\rho - \rho' = 1.88$ which is essentially equal to ρ_A in eq 8 and that between constant terms which is approximately equal to $pK_A^0 - pH = 9.0 - 7.8 = 1.2$.

For the *para* derivatives, the situation is quite different. Here, the much poorer correlations obtained for eq 11a–c are partly attributable to the much smaller variance in the values of $\log A + \log [(K_A + [H^+])/[H^+]]$ than those for the *meta* derivatives. However, eq 12b, a counterpart of 11b, shows a moderately good fit for the values of $\log A + \log [(K_A + [H^+])/K_A]$. The most important inference from the result obtained by factoring is that the hydrophobic bonding of the *para* substituents as measured by π plays practically no role in the relative activity. The introduction of a π term into eq 11b and 12b does not yield a better correla-

tion. The *para* isomers are quite active regardless of the type of substituent, at least within the limits studied. This result could be taken to mean that inhibition of a metabolic change at this point (*e.g.*, hydroxylation) by the bacteria is responsible for the higher activity.

For the activity against the gram-negative bacteria, we have derived eq 13a–e and 14a–e for Friedländer's bacillus and eq 15a–e and 16a–e for *E. coli*. In eq 13a–e and 14a–e, A and $[H^+]$ have the same meaning as in eq 9a–e and 10a–e. In eq 15a–e and 16a–e, C is the minimum inhibitory concentration in μmole/l. and $[H^+]$ is $10^{-7.2}$ (Sauton medium[13]). It is noteworthy that the equations for the two gram-negative bacteria are very similar to each other, although the correlations obtained for Friedländer's bacillus are not very good.

against Friedländer's bacillus

	n	s	r	
$\log A + \log \frac{K_A + [H^+]}{[H^+]} =$				
$-0.071\pi + 0.316$	20	0.587	0.120	(13a)
$\log A + \log \frac{K_A + [H^+]}{[H^+]} =$				
$0.955\sigma - 0.105$	20	0.369	0.781	(13b)
$\log A + \log \frac{K_A + [H^+]}{[H^+]} =$				
$1.009\sigma - 0.153\pi + 0.027$	20	0.347	0.822	(13c)
$\log A + \log \frac{K_A + [H^+]}{K_A} =$				
$-0.217\pi + 0.910$	20	0.556	0.363	(14a)
$\log A + \log \frac{K_A + [H^+]}{K_A} =$				
$-0.908\sigma + 1.028$	20	0.404	0.736	(14b)
$\log A + \log \frac{K_A + [H^+]}{K_A} =$				
$-0.857\sigma - 0.147\pi + 1.155$	20	0.388	0.775	(14c)

against *E. coli*

$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$0.109\pi - 1.038$	17	0.555	0.106	(15a)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$1.256\sigma - 1.270$	17	0.117	0.978	(15b)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$1.298\sigma - 0.141\pi - 1.204$	17	0.094	0.987	(15c)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} =$				
$-0.293\pi + 0.533$	17	0.145	0.736	(16a)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} =$				
$-0.351\sigma + 0.457$	17	0.151	0.711	(16b)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} =$				
$-0.280\sigma - 0.239\pi + 0.568$	17	0.088	0.919	(16c)

F tests indicate that the π term in eq 13c is justified at better than 0.90 level of significance when compared with eq 13b ($F_{1,17} = 3.41$, $F_{1,17,0.10} = 3.03$) and that in eq 15c at better than 0.975 level of significance compared with eq 15b ($F_{1,14} = 9.35$, $F_{1,14,0.025} = 6.30$). For the gram-negative bacteria, factoring the groups into the *meta* and *para* derivatives does not improve the correlation. Equations 13c and 14c are approximately related by eq 4 and 5. Equations 15c and 16c are not related by eq 4 and 5 since σ^- values are used for the *p*-nitro and *p*-acetyl derivatives in eq 15a–c and 16a–c where $\log K_X$ is not a linear function of σ^- but expressed by eq 8. In this case, the difference between ρ and ρ' is approximately equal to ρ'_X expressed by eq 17 where σ^- and σ are those for the *p*-nitro group, *i.e.*, $\rho'_X = 1.883[0.78 + 0.54(1.27 - 0.78)]^{-1}1.27 = 1.56$.

$$\rho'_X\sigma^- = \rho_X[\sigma + 0.54(\sigma^- - \sigma)] \quad (17)$$

As to the active form of sulfonamide drugs, some workers[14] have concluded that the activity is due to the ionized form from the nature of the function relating the activity of several sulfonamides to the hydrogen ion concentration. Other workers[15] have considered that the negative ion is not itself a prerequisite for activity since the same activity is observed in such nonacidic compounds as 4,4′-diaminodiphenyl sulfone and sulfanilguanidine. By our procedure, however, even if the true active agent is unknown and whatever the active form may be, the activity data obtained at a specific pH can be accommodated by either eq 2 or 3 because of the relationship expressed by eq 4 and 5. In this situation, therefore, one seems justified in discussing the structure–activity relation using the equation with the higher correlation coefficient unless the standard deviations of the correlations are quite different.

Schmidt and Sesler[5] also studied the antagonistic effect of *p*-aminobenzoic acid on the antibacterial activity of these compounds. The determinations could not be made for all the compounds included in Table I, but they found that *p*-aminobenzoic acid blocked the activity against Friedländer's bacillus in every instance where the determinations could be made, whereas against pneumococcus, *p*-aminobenzoic acid exerted the antagonistic effect on only some of the *meta* and *para* derivatives. This fact might indicate that some of the sulfanilanilides exert antipneumococcal activity through a different mechanism from others of the same series of compounds. For the *meta* and *para* derivatives, it is possible to consider different mechanisms as indicated by the different equations (eq 9c and 11b). However, the good correlation coefficients obtained for eq 9b and eq 9c would indicate that the antibacterial action against pneumococcus is brought about by the same mechanism, at least throughout the *meta* derivatives. The close similarity in ρ values observed in eq 9c, 13c, and 15c suggests that the electronic demands for the antibacterial activity are the same in these three instances. In this respect, it should be noted that the activity of sulfonamide drugs has been postulated in some cases to occur through effects on metabolites other than *p*-aminobenzoic acid.[5,6]

The plus sign of the coefficient of the π term in eq 9c infers that the higher the π value, the higher the antipneumococcal activity, while the minus sign in eq 13c and 15c indicates that the reverse is true for the gram-negative bacteria. If we consider that the linear de-

(13) "Biseibutsugaku-Handobukku (Handbook of Microbiology)," Gihodo Publishing Co., Tokyo, 1962, p 1371.

(14) (a) C. C. Cox, Jr., and D. M. Rose, *Proc. Soc. Exptl. Biol. Med.*, **50**, 112 (1942); (b) F. C. Schaefkes, O. Wyss, H. C. Marks, B. J. Ludwig, and F. B. Strandskov, *ibid.*, **50**, 145 (1942); (c) I. M. Klotz, *J. Am. Chem. Soc.*, **66**, 459 (1944).

(15) W. D. Kumler and T. C. Daniels, *ibid.*, **65**, 2190 (1943).

pendence of the antibacterial activity on the π value obtained in these equations comes from the "linear portion" of the parabolic relationship expressed by eq 1,[6a] the above results would indicate that, for the gram-positive pneumococcus, increasing the π value beyond the limit of the sulfanilanilides tested in this research would yield an optimal π for the highest activity. On the other hand, for the gram-negative bacteria, decreasing the π value below the limit of the series of compounds, an optimal π would be found. That the optimal π value in this series is larger with the gram-positive bacteria than with the gram-negative bacteria is quite consistent with data obtained earlier for the antibacterial activities of the chloramphenicol analogs[16] and the substituted phenols.[6a]

For the sulfanilanilides for which $K_A << [H^+]$, eq 9c, 13c, and 15c reduce to eq 18, 19, and 20, respectively. For those for which $K_A >> [H^+]$, eq 10c, 14c, and 16c reduce to eq 21, 22, and 23, respectively.

$$\log A = 1.204\sigma + 0.239\pi + 0.767 \quad (18)$$

$$\log A = 1.009\sigma - 0.153\pi + 0.027 \quad (19)$$

$$\log (1/C) = 1.298\sigma - 0.141\pi - 1.204 \quad (20)$$

$$\log A = -0.676\sigma + 0.245\pi + 1.906 \quad (21)$$

$$\log A = -0.857\sigma - 0.147\pi + 1.155 \quad (22)$$

$$\log (1/C) = -0.280\sigma - 0.239\pi + 0.568 \quad (23)$$

From this discussion, it is apparent that for the antibacterial activity of this series of compounds, the position and the hydrophobicity of the substituents on the aniline moiety are significant as well as the dissociation of the SO_2NH group and/or the electronegativity of the substituent. However, keeping the hydrophobic parameter constant in eq 18–23, the apparent activity [log A or log $(1/C)$] is expressed by biphasic plots with respect to σ of which the slopes are 1.204 and -0.676 for the *meta* derivatives against pneumococcus, 1.009 and -0.857 for Friedländer's bacillus, and 1.298 and -0.280 for *E. coli* according to the conditions of $[H^+] >> K_A$ and $[H^+] << K_A$, respectively. Thus, Bell and Roblin's parabolic relationship between log $(1/C)$ and pK_A can be nicely interpreted by these biphasic plots, the intersection of which corresponds approximately to the maximal activity. The maximum contribution from the σ term to the apparent potency of the drugs would be made by setting $\partial \log A/\partial\sigma = 0$ in eq 2, 6b, or 6c.

Equations 2, 6b, and 6c can be rearranged to the form of eq 24 where $f(\pi)$ is either zero, $a\pi$, or $a\pi - b\pi^2$. By taking the partial differential of eq 24, we obtain eq 25.

$$\log A = f(\pi) + \rho\sigma + \log [H^+] - \log ([H^+] + K_A) + c \quad (24)$$

$$\frac{\partial \log A}{\partial\sigma} = \rho - \frac{1}{K_A + [H^+]}\frac{dK_A}{d\sigma} = \rho - \frac{1}{K_A + [H^+]}\frac{d \log K_A}{d\sigma}\Big/\frac{d \log K_A}{dK_A} = \rho - \frac{K_A}{K_A + [H^+]}\frac{d \log K_A}{d\sigma} = \rho - \frac{K_A}{K_A + [H^+]}\rho_A \quad (25)$$

Similarly, from eq 3, 7b, and 7c we can derive eq 26

$$\frac{\partial \log A}{\partial\sigma} = \rho' - \frac{[H^+]}{K_A + [H^+]}\rho_A \quad (26)$$

which relates to eq 25 by eq 4. Substituting eq 4 into eq 25 or 26 and setting the resultant equation to zero yields eq 27 or 28 for the ideal dissociation constant,

$$K_A = -(\rho/\rho')[H^+] \quad (27)$$

$$pK_A = -\log(-\rho/\rho') + pH \quad (28)$$

regardless of the value of ρ_A. Thus, the most favorable dissociation constants under the experimental conditions are found to be $K_A \sim 10^{-7.5}$ for the *meta* derivatives against pneumococcus, $K_A \sim 10^{-7.7}$ against Friedländer's bacillus, and $K_A \sim 10^{-6.5}$ against *E. coli*.

Bacteriostatic Activity of the Substituted N^1-Benzoylsulfanilamides.—Seydel and Wempe[17] examined the relationship between antibacterial activity and physicochemical properties of the N^1-benzoylsulfanilamides and argued that the activity is correlated to the difference between pK_A values of a certain substituted N^1-benzoylsulfanilamide and the corresponding N^4-acetyl derivative. We have analyzed their activity data obtained at pH 7.2 (Sauton medium[13]) against gram-negative *E. coli* and gram-positive *Mycobacterium smegmatis* with the correction for ionization. From the data in Table II, eq 29a–d and 30a–d are obtained for the activity against *E. coli* and eq 31a–d and 32a–d for that against *M. smegmatis*.

against *E. coli*

	n	s	r	
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$-0.913\pi + 8.240$	15	0.502	0.787	(29a)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$3.499\Delta pK_A + 8.346$	15	0.383	0.882	(29b)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$2.644\Delta pK_A - 0.334\pi + 8.442$	15	0.365	0.902	(29c)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$-0.291\pi^2 + 0.465\pi +$				
$2.636\Delta pK_A + 8.036$	15	0.312	0.936	(29d)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} =$				
$-0.685\pi + 5.668$	15	0.421	0.751	(30a)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} =$				
$2.481\Delta pK_A + 5.702$	15	0.386	0.796	(30b)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} =$				
$1.657\Delta pK_A - 0.321\pi + 5.795$	15	0.371	0.830	(30c)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} =$				
$-0.289\pi^2 + 0.474\pi +$				
$1.650\Delta pK_A + 5.390$	15	0.320	0.887	(30d)

against *M. smegmatis*

$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$-0.940\pi + 8.571$	14	0.605	0.750	(31a)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} =$				
$3.654\Delta pK_A + 8.742$	14	0.512	0.828	(31b)

(16) C. Hansch, R. M. Muir, T. Fujita, P. P. Maloney, F. Geiger, and M. Streich, *J. Am. Chem. Soc.*, **85**, 2817 (1963).

(17) J. K. Seydel and E. Wempe, *Arzneimittel-Forsch.*, **14**, 705 (1964).

TABLE II

BACTERIOSTATIC ACTIVITY OF SUBSTITUTED N^1-BENZOYLSULFANILAMIDE

			Against *E. coli*				Against *M. smegmatis*			
			$\log (1/C) + \log \frac{K_A + [H^+]}{[H^+]}$		$\log (1/C) + \log \frac{K_A + [H^+]}{K_A}$		$\log (1/C) + \log \frac{K_A + [H^+]}{[H^+]}$		$\log (1/C) + \log \frac{K_A + [H^+]}{K_A}$	
Substituent	ΔpK_A[a]	π[b]	Obsd	Calcd[c]	Obsd	Calcd[d]	Obsd	Calcd[e]	Obsd	Calcd[f]
H	0	0	7.9	7.91	5.25	5.39	7.9	8.12	5.33	5.52
2-CH_3	−0.35	0.68	6.9	7.40	4.57	5.00	7.3	7.63	5.02	5.36
3-CH_3	−0.20	0.52	7.8	7.77	5.40	5.23	8.2	7.98	5.77	5.56
4-CH_3	−0.15	0.42	7.9	7.84	5.40	5.29	8.2	8.07	5.70	5.60
4-C_2H_5	−0.15	0.92	8.1	7.77	5.62	5.33	8.2	8.28	5.70	5.79
4-C_3H_7	−0.21	1.42	7.6	7.63	5.17	5.13	8.2	8.06	5.77	5.62
4-*i*-C_3H_7	−0.15	1.40	7.9	7.70	5.40	5.24	8.2	8.25	5.70	5.74
2,4-$(CH_3)_2$	−0.45	1.10	6.8	6.97	4.65	4.82	7.2	7.42	5.10	5.24
2,5-$(CH_3)_2$	−0.50	1.20	6.7	6.73	4.57	4.72	7.1	7.27	5.02	5.14
3,4-$(CH_3)_2$	−0.31	0.94	7.7	7.48	5.44	5.07	7.9	7.82	5.55	5.50
2,4,6-$(CH_3)_3$	−0.55	1.78	6.3	6.40	4.19	4.41	6.3	6.88	4.19	4.80
2,4,5-$(CH_3)_3$	−0.55	1.62	6.9	6.42	4.80	4.49	7.6	6.99	5.47	4.90
2,3,4,5,6-$(CH_3)_5$	−0.70	2.82	5.2	5.32	3.29	3.27	5.2	5.14	3.29	3.22
3-CH_3-4-OCH_3	−0.35	0.60	7.6	7.41	5.25	4.99	8.0	7.59	5.70	5.33
3-CH_3-4-SCH_3	−0.15	1.12	7.2	7.74	4.70	5.31				

[a] Calculated from the values of pK_A in Table II of ref 17. [b] Taken from ref 12 and simply summed to get figures for the polysubstituted compounds. [c] Calculated by eq 29d. [d] Calculated by eq 30d. [e] Calculated by eq 31d. [f] Calculated by eq 32d.

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = 2.724\Delta pK_A - 0.342\pi + 8.811 \quad 14 \quad 0.509 \quad 0.846 \quad (31c)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = -0.519\pi^2 + 1.128\pi + 2.909\Delta pK_A + 8.119 \quad 14 \quad 0.350 \quad 0.937 \quad (31d)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = -0.728\pi + 6.039 \quad 14 \quad 0.516 \quad 0.717 \quad (32a)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = 2.653\Delta pK_A + 6.112 \quad 14 \quad 0.496 \quad 0.742 \quad (32b)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = 1.669\Delta pK_A - 0.362\pi + 6.186 \quad 14 \quad 0.488 \quad 0.776 \quad (32c)$$

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = -0.500\pi^2 + 1.055\pi + 1.847\Delta pK_A + 5.519 \quad 14 \quad 0.333 \quad 0.912 \quad (32d)$$

In these equations, C is the minimum inhibitory concentration in μmole/l. and ΔpK_A is obtained using the pK_A value of the unsubstituted N^1-benzoylsulfanilamide as a standard. For the π values, those obtained for the substituted benzoic acids are used.[12] Comparison of these equations would indicate that the activity of this series of compounds is not linearly related to π. Both π and π^2 terms in eq 29d and 31d are justified at better than 0.95 ($F_{2,11} = 4.64$, $F_{2,11,0.05} = 3.98$) and 0.99 ($F_{2,10} = 7.87$, $F_{2,10,0.01} = 7.56$) level of significance compared with eq 29b and 31b, respectively. For this series of compounds, hydrophobic characteristics of the substituents are sufficiently varied to cover the optimal value for the maximum activity. The optimal π values are calculated by setting $\partial \log (1/C)/\partial\pi = 0$, *i.e.*, $\pi_0 = 0.80$ for *E. coli* and $\pi_0 = 1.08$ for *M. smegmatis*. The larger π_0 value for the gram-positive bacterium than for the gram-negative one is in accord with the finding obtained in the case of the sulfanilanilides as described above. Equations 29d and 30d and eq 31d and 32d are related by eq 4 and 5 as theoretically expected. Thus, the structure–activity correlation of this series of compounds is neatly rationalized in terms of electronic and hydrophobic characters of the substituents instead of the complex parameter (a pK_A difference) described by the original authors.[17]

Since all the dissociation constant values for the compounds studied in this work are at least 100 times larger than the value of the hydrogen ion concentration of the test medium ($10^{-7.2}$), the correction term, $\log [(K_A + [H^+])/K_A]$, becomes practically zero. For such compounds where $K_A >> [H^+]$, the apparent potency of the drugs is predicted by eq 33 and 34. Likewise, for those where $K_A << [H^+]$, the apparent potency is described by eq 35 and 36 which are obtained from eq 29d and 31d with the $\log [(K_A + [H^+])/[H^+]]$ term deleted. Thus, if the hydrophobicity of the substituent is kept constant, the biphasic plots for the apparent drug activity [$\log (1/C)$] with respect to ΔpK_A can be expressed by two straight lines of which the slopes are 1.650 and 2.636 for *E. coli* and 1.847 and 2.909 for *M. smegmatis* according to $K_A >> [H^+]$ and $K_A << [H^+]$, respectively. In this series of compounds, the apparent potency, $\log (1/C)$, seems to keep increasing with increasing ΔpK_A or the electron-attracting ability of the substituent, regardless whether $K_A << [H^+]$ or $K_A >> [H^+]$ even though the slopes are different. However, the compounds used in deriving the equations are mostly alkyl-substituted derivatives for which ΔpK_A values do not vary significantly so that the ρ values obtained by means of the least-squares method are not highly reliable. Therefore, a definite conclusion on the pK_A dependence of the *in vitro* activity could not been drawn before the derivatives possessing electron-withdrawing substituents are tested in this series of com-

$$\log (1/C) = -0.289\pi^2 + 0.474\pi + 1.650\Delta pK_A + 5.390 \quad \text{(against } E. \text{ coli)} \quad (33)$$

$$\log (1/C) = -0.500\pi^2 + 1.055\pi + 1.847\Delta pK_A + 5.519 \quad \text{(against } M. \text{ smegmatis)} \quad (34)$$

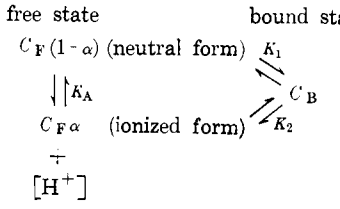
$$\log (1/C) = -0.291\pi^2 + 0.465\pi + 2.636\Delta pK_A + 8.036 \quad \text{(against } E. \text{ coli)} \quad (35)$$

$$\log (1/C) = -0.519\pi^2 + 1.128\pi + 2.909\Delta pK_A + 8.119 \quad \text{(against } M. \text{ smegmatis)} \quad (36)$$

pounds. At any rate, it appears that the Bell and Roblin's parabolic relationship does not necessarily hold in some types of sulfonamide drug where both ρ and ρ' are positive or negative in eq 27 or 28.

Plasma Protein Binding and Bacteriostatic Activity of the N¹-Heterocyclic Sulfanilamides.—While protein binding inactivates the sulfonamide drugs,[18] it was found that the protein bound drugs are only slowly metabolized at the liver, and the binding is reversible so that the active free form can be liberated gradually as the levels in the blood are lowered.[19] Thus, protein binding could be a significant factor for the duration of action of drugs. There has been considerable controversy as to correlations of physicochemical properties of sulfonamides with their protein binding.[5c,20] Rieder has recently studied a number of sulfonamide drugs and determined their binding to human plasma protein.[21] His analysis of this phenomenon in terms of physicochemical properties such as acid dissociation constants and oil–water partition coefficients, however, did not consider the effect of ionization of the drugs under physiological conditions.

If we assume that the process of binding of dissociable compounds is as shown below, *i.e.*, in the free state, they exist as two different species, the neutral and ionized form of which concentrations are $C_F(1 - \alpha)$ and $C_F\alpha$, respectively, but in the bound state they exist as only one form, C_B, then the equilibrium constants for the binding of the two species are expressed by eq 37 and 38.



$$K_1 = C_B/C_F(1 - \alpha) \quad (37)$$

$$K_2 = C_B/C_F\alpha \quad (38)$$

The effective binding constant, C_B/C_F, is thus described in eq 39.

$$\frac{C_B}{C_F} = \frac{K_1K_2}{K_1 + K_2} \quad (39)$$

If we take the ratio of the effective binding constants of a series of substituted derivatives to that of the unsubstituted standard compound, eq 40 is obtained.

$$\frac{C_B}{C_F}\Big/\left(\frac{C_B}{C_F}\right)_0 = \frac{K_1K_2}{K_1^0K_2^0}\,\frac{K_1^0 + K_2^0}{K_1 + K_2} \quad (40)$$

Taking the logarithms of both sides yields eq 41.

$$\log \frac{C_B}{C_F} = \log \frac{K_1}{K_1^0} + \log \frac{K_2}{K_2^0} + \log \frac{K_1^0 + K_2^0}{K_1 + K_2} + \log \left(\frac{C_B}{C_F}\right)_0 \quad (41)$$

The ratios, K_1/K_1^0 and K_2/K_2^0, can be considered to be functions of the hydrophobic and electronic character of the substituent so that they can be expressed as in eq 42 and 43 where a_1, b_1, c_1, a_2, b_2, and c_2 are constants.

$$\log \frac{K_1}{K_1^0} = a_1\pi + b_1\sigma + c_1 \quad (42)$$

$$\log \frac{K_2}{K_2^0} = a_2\pi + b_2\sigma + c_2 \quad (43)$$

The logarithm of the binding constant of a series of phenols to serum albumin and to mitochondrial protein determined under conditions where the dissociation can be ignored has been recently shown to be correlated by a linear combination of free-energy-related parameters such as π and pK_A.[6d] Substituting eq 37, 38, 42, and 43 into eq 41 and collecting the terms yields eq 44.

$$\log \frac{C_B}{C_F} = (a_1 + a_2)\pi + (b_1 + b_2)\sigma + \log \frac{\left(\frac{C_B}{C_F}\right)_0\left(\frac{1}{1 - \alpha_0} + \frac{1}{\alpha_0}\right)}{\left(\frac{C_B}{C_F}\right)\left(\frac{1}{1 - \alpha} + \frac{1}{\alpha}\right)} + \log \left(\frac{C_B}{C_F}\right)_0 + c_1 + c_2 \quad (44)$$

Since $\alpha = K_A/(K_A + [H^+])$ and $1 - \alpha = [H^+]/(K_A + [H^+])$, eq 44 can be converted to eq 45 and further to eq 46.

$$2 \log \frac{C_B}{C_F} = (a_1 + a_2)\pi + (b_1 + b_2)\sigma + \log \frac{\alpha}{\alpha_0} + \log \frac{1 - \alpha}{1 - \alpha_0} + 2 \log \left(\frac{C_B}{C_F}\right)_0 + c_1 + c_2 \quad (45)$$

$$2 \log \frac{C_B}{C_F} = (a_1 + a_2)\pi + (b_1 + b_2)\sigma + \log \frac{K_A}{K_A^0} + 2 \log \frac{K_A^0 + [H^+]}{K_A + [H^+]} + 2 \log \left(\frac{C_B}{C_F}\right)_0 + c_1 + c_2 \quad (46)$$

By substituting $\log (K_A/K_A^0) = \rho_A\sigma$ into eq 46 and collecting terms, eq 47 is obtained, where $k = (a_1 + a_2)/2$, $\rho = (b_1 + b_2 + \rho_A)/2$, and $c = \log [(C_B/C_F)_0] + (c_1 + c_2)/2$.

$$\log \frac{C_B}{C_F} + \log \frac{K_A + [H^+]}{K_A^0 + [H^+]} = k\pi + \rho\sigma + c \quad (47)$$

Equation 47 can be modified into eq 48a and 48b where

$$\log \frac{C_B}{C_F} + \log \frac{K_A + [H^+]}{[H^+]} = k\pi + \rho\sigma + c' \quad (48a)$$

$$\log \frac{C_B}{C_F} + \log \frac{K_A + [H^+]}{K_A} = k\pi + \rho'\sigma + c'' \quad (48b)$$

$c' = c - \log [[H^+]/(K_A^0 + [H^+])]$, $\rho' = \rho - \rho_A$, and $c'' = c - \log [K_A^0/(K_A^0 + [H^+])]$.

Equation 48a is the expression for the binding constant K_1, when only the neutral form of the free molecule is responsible for the binding, and eq 48b is that for K_2 where only the ionized form is considered in the binding process. Klotz and Walker[20a] examined the binding of some sulfonamides by bovine serum albumin and postulated that the binding is attributable mainly to the ionized form of the drugs. Since the expressions are interrelated *a priori*, the correlation of the protein binding of a series of dissociable compounds to their hydrophobic and electronic properties can be analyzed by the same procedure, whichever form of the free molecule, the neutral or ionic or both, might be associated with the binding process.

As a criterion of the plasma-binding ability, Rieder[21] has determined Langmuir's α constant which is inversely proportional to the effective binding constant, C_B/C_F. Langmuir's β constant, which reflects the total number of binding sites per molecule of protein, is nearly constant; *i.e.*, the mechanism of binding is considered to be the same throughout the series of com-

(18) A. H. Anton, *J. Pharmacol. Exptl. Therap.*, **129**, 282 (1960).
(19) B. B. Newbould and R. Kilpatrick, *Lancet*, **1**, 887 (1960).
(20) (a) I. M. Klotz and F. M. Walker, *J. Am. Chem. Soc.*, **70**, 943 (1948); (b) W. Scholtan, *Arzneimittel-Forsch.*, **14**, 348 (1964); (c) M. Nakagaki, N. Koga, and H. Terada, *Yakugaku Zasshi*, **83**, 586 (1963); **84**, 516 (1964); (d) O. Jardetzky and N. G. W. Jardetzky, *Mol. Pharmacol.*, **1**, 214 (1965); (e) L. B. Holder and S. L. Hayes, *ibid.*, **1**, 266 (1965).
(21) J. Rieder, *Arzneimittel-Forsch.*, **13**, 81 (1963).

TABLE III

PLASMA PROTEIN BINDING AND BACTERIOSTATIC ACTIVITY OF N^1-HETEROCYCLIC SULFANILAMIDES

			Protein binding			Bacteriostatic activity			
				Log $(1/\alpha) + \log \frac{K_A + [H^+]}{[H^+]}$		Log $(1/C) + \log \frac{K_A + [H^+]}{[H^+]}$		Log $(1/C) + \log \frac{K_A}{K_A + [H^+]}$	
Compound	ΔpK_A[a]	π[b]	Log $(1/\alpha)$	Obsd	Calcd[d]	Obsd	Calcd[e]	Obsd	Calcd[f]
Sulfanilamide	0	0	−2.98	−2.98	−2.90	−2.11	−2.09	0.77	0.79
N^1-Acetylsulfanilamide	4.30	0.62	−3.15	−1.56	−1.87	0.90	1.01	−0.50	−0.40
2-Sulfanilamidopyrimidine	3.56	0.79	−2.99	−2.04	−1.59	0.70	0.66	0.00	−0.01
2-Sulfanilamido-4-methylpyrimidine	3.10	0.88	−2.23	−1.69	−1.44	0.44	0.42	0.24	0.22
2-Sulfanilamido-5-methylpyrimidine	3.31	1.17	−2.11	−1.41	−0.96	0.60	0.66	0.21	0.24
2-Sulfanilamido-4,6-dimethylpyrimidine	2.38	0.76	−2.28	−2.10	−1.64	0.23	−0.07	0.73	0.44
4-Sulfanilamido-2,6-dimethylpyrimidine	2.51	0.29	−1.78	−1.57	−2.42	−0.03	−0.31	0.36	0.07
5-Sulfanilamido-2,4-dimethylpyrimidine	2.83	1.05	−1.54	−1.19	−1.16	...	...	...	...
4-Sulfanilamido-6-methoxypyrimidine	4.14	1.49	−1.78	−0.27	−0.44	1.65	1.23	0.35	−0.03
2-Sulfanilamido-5-methoxypyrimidine	3.06	0.72	−1.90	−1.36	−1.71	0.11	0.32	−0.09	0.15
4-Sulfanilamido-2,6-dimethoxypyrimidine	3.76	1.62	−0.81	0.32	−0.22	1.06	1.01	0.16	0.13
2-Sulfanilamido-4,5,6-trimethoxypyrimidine	3.54	1.76	−1.00	−0.05	0.01	...	...	...	...
3-Sulfanilamido-6-methoxypyridazine	2.88	0.92	−1.75	−1.34	−1.38	0.30	0.31	0.30	0.32
3-Sulfanilamido-6-chloropyridazine	3.98	1.28	−2.00	−0.68	−0.78	...	...	...	...
3-Sulfanilamido-5-methylisoxazole	4.03	1.27	−2.51	−1.19	−0.80	1.19	1.12	0.09	−0.02
5-Sulfanilamido-3,4-dimethylisoxazole	5.08	2.23	−1.73	0.67	0.79	1.78	1.71	−0.45	−0.50
2-Sulfanilamido-4,5-dimethyloxazole	2.68	0.68	−2.16	−1.86	−1.77	−0.32	0.07	−0.12	0.28
3-Sulfanilamido-2-phenylpyrazole	3.99	1.97	−1.00	0.32	0.36	0.98	1.12	−0.12	0.01
2-Sulfanilamidothiazole	2.83	0.69	−2.45	−2.10	−1.76	0.07	0.16	0.17	0.22
2-Sulfanilamido-5-ethyl-1,3,4-thiadiazole	4.43	1.84	−1.18	0.53	0.14	1.18	1.40	−0.33	−0.15

[a] Calculated from the values of pK_A in ref 21. [b] Calculated from the "Übergangszahlen" in ref 21 with correction for ionization in the aqueous phase; see text. [c] The value of pH under which the binding experiments were performed is 7.4. [d] Calculated by eq 49a. [e] Calculated by eq 52d. [f] Calculated by eq 53d.

pounds,[22] so that the analysis of the binding constant in terms of Langmuir's α constant with the use of π and σ seems justified.

Fitting the data in Table III to eq 48a, eq 49a–c are obtained. To derive the equations we omitted 3-sulfanilamido-4,5-dimethylpyrazole from the original work by Rieder[21] since it was found to be very poorly correlated. This seems to be due to its somewhat anomalous value for Langmuir's β constant when compared with those of others. The values of π are calculated

$\log \frac{1}{\alpha} + \log \frac{K_A + [H^+]}{[H^+]}$	n	s	r	
$= 1.651\pi - 2.896$	20	0.365	0.938	(49a)
$= 0.740\Delta pK_A - 3.533$	20	0.670	0.769	(49b)
$= 0.092\Delta pK_A + 1.519\pi - 3.056$	20	0.370	0.940	(49c)

from the partition coefficient obtained in the isobutyl alcohol–water system with the correction for dissociation in the aqueous phase (pH 7.4), assuming that any dissociation and association in the organic phase can be ignored. Instead of σ, ΔpK_A values are used for the analysis. The unsubstituted sulfanilamide is taken as the standard so that the π value indicates the hydrophobicity of the whole N^1 substituent. The ΔpK_A value is assumed to be proportional to the electron-withdrawing ability of the N^1 substituent. Rieder[21] also measured the partition coefficients of the drugs with toluene, $CHCl_3$, and ethylene dichloride as the organic phase. A good correlation is found only with the π values obtained with the isobutyl alcohol–water system.

Comparison of eq 49a–c would indicate that the modified binding constant is determined mainly by the hydrophobicity of the N^1 substituent, and nothing is to be gained by the introduction of a ΔpK_A term. Conversion of eq 49a into eq 50 shows that for the sulfonamides, for which the value of K_A is much larger than that of $[H^+]$, the larger the hydrophobicity of the N^1 substituent and the smaller the dissociation constant, the more firmly the sulfonamides are bound to the protein. For those for which K_A is much smaller than $[H^+]$, eq 50 indicates that the protein binding is only

$$\log \frac{1}{\alpha} = 1.651\pi - \log (K_A + [H^+]) - 12.896 + pH \quad (50)$$

dependent on the hydrophobicity of the N^1 substituent. Klotz and Walker[20a] recognized that the larger dissociation constant of sulfonamides tends to reduce their ability to combine with the protein. Equations 49a and 50 clearly indicate that for a series of sulfonamides of closely related structure where the dissociation constant is not appreciably varied, the binding is governed mostly by the hydrophobicity of the N^1 substituent. This is supported by the work of Scholtan[20b] who has shown that for series of 5-alkyl- and 5-alkoxy-2-sulfanilaminopyrimidines, the free-energy change for the binding is linearly related to the carbon number of the side chain. This important role for hydrophobic forces in holding sulfonamide drugs to serum protein is in line with our earlier findings on the binding of organic compounds to albumin and hemoglobin.[6d,e] However, the slope of eq 49a indicates an even greater dependence on this property for sulfonamide drugs than for simple aromatic compounds. Another inference of eq 50 is that for a particular sulfonamide drug, the plots of log $(1/\alpha)$ against variation of the experimental pH consist of two phases; *i.e.*, when $pH << pK_A$, the binding is almost unchanged with variation of the pH, while when $pH >> pK_A$, the drug is less firmly bound to the protein with increasing the pH. This is in accord, at least qualitatively, with the finding of Nakagaki and his co-workers[20c] who have shown for several sulfonamides that

(22) P. Spring, *Arzneimittel-Forsch.*, **16**, 346 (1966).

the plots show a maximum approximately at the point of pH = pK_A and the slope in the region of pH >> pK_A is steeper than that where pH << pK_A. Even though the sulfanilamido moiety may be of considerable importance for the protein binding as argued by Jardetzky[20d] from nmr studies, it is important to note that the N^1 substituent contributes strongly to the binding since log $(1/\alpha)$ is linearly related to the hydrophobicity of the substituent.

The assumptions underlying eq 49a–c can be justified, since, when the values of log $1/\alpha$ are analyzed without correction for the ionization with ΔpK_A and π, the correlations are much worse than eq 49a–c in terms of both the correlation coefficient and the standard deviation as shown in eq 51a–c. Moreover, none of these equations is capable of describing the pH dependence of the binding constant.

	n	s	r	
$\log \frac{1}{\alpha} = 0.799\pi - 2.846$	20	0.491	0.696	(51a)
$= 0.215\Delta pK_A - 2.680$	20	0.642	0.343	(51b)
$= -0.321\Delta pK_A + 1.258\pi - 2.285$	20	0.452	0.761	(51c)

Recently, Krüger-Thiemer and Bünger[23] determined the minimum inhibitory concentration of this series of drugs for *E. coli*. For 17 out of 29 compounds in their paper, the physicochemical parameters, ΔpK_A, and π obtained from Rieder's work, can be directly used for the analysis of the structure–activity relationship. Fitting the data in Table III to eq 2, 3, 6a–c, and 7a–c, eq 52a–d and 53a–d are obtained. In these equations,

	n	s	r	
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = 1.207\pi - 0.760$	17	0.536	0.816	(52a)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = 0.761\Delta pK_A - 1.995$	17	0.244	0.965	(52b)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = 0.181\pi + 0.684\Delta pK_A - 1.932$	17	0.242	0.968	(52c)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} = -0.296\pi^2 + 0.985\pi + 0.605\Delta pK_A - 2.090$	17	0.223	0.975	(52d)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = -0.300\pi + 0.421$	17	0.318	0.509	(53a)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = -0.240\Delta pK_A + 0.896$	17	0.238	0.764	(53b)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = 0.167\pi - 0.312\Delta pK_A + 0.955$	17	0.238	0.783	(53c)
$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = -0.308\pi^2 + 1.005\pi - 0.393\Delta pK_A + 0.790$	17	0.216	0.839	(53d)

C is the minimum inhibitory concentration in μmole/l. and $[H^+]$ is taken as $10^{-7.2}$ (Sauton medium[13]). Although the electronic effect of the N^1 substituent seems most significant for the activity, an F test indicates that both the π and π^2 terms of eq 52d are justified at almost 0.90 level of significance compared with eq 52b ($F_{2,13}$ = 2.42, $F_{2,13,0.10}$ = 2.76, $F_{2,13,0.25}$ = 1.54), so that the antibacterial activity of this series of compounds is not linearly related to π. The optimal π value is calculated by setting $\partial \log (1/C)/\partial\pi = 0$ in eq 52d or 53d, *i.e.*, $\pi_0 = 1.67$. The plots of log $(1/C)$ against pK_A, with a fixed hydrophobicity, would consist of two straight lines expressed by eq 54 and 55 according to conditions of $K_A << [H^+]$ and $K_A >> [H^+]$, respectively, where the π and π^2 terms are collected and set constant.

$$\log \frac{1}{C} = 0.605\Delta pK_A - 2.090 + \text{constant}\ (\pi) \qquad (54)$$

$$\log \frac{1}{C} = -0.393\Delta pK_A + 0.790 + \text{constant}\ (\pi) \qquad (55)$$

The optimal pK_A value for the apparent activity, log $(1/C)$, is obtained by setting $\partial \log (1/C)/\partial\Delta pK_A = 0$ in eq 52d or 53d; *i.e.*, $pK_A = 7.0$ is calculated as the most favorable value for these heterocyclic sulfonamide series. These findings on the optimal physicochemical properties should be kept in mind in designing new sulfa drugs, especially since many of the clinically accepted sulfa drugs belong to this class of N^1-heterocyclic sulfanilamides.

One might assume that the optimal hydrophobic nature of the sulfonamide drug molecule is not significantly different from series to series, regardless of the type of the N^1 substituent, at least for activity against a particular microorganism. Thus, for the activity of the sulfanilanilides against *E. coli*, π_0 for the whole N^1 substituent is estimated with the aid of the additive character of π as less than the π value for the N^1-phenyl moiety with the least hydrophobic substituent, *i.e.*, $\pi_0 < 1.9 \cong [2.13\ (\pi \text{ for benzene}) - 0.21\ (\pi \text{ for 4-OCH}_3 \text{ group})]$.[12] Activity is decreasing with increasing π of the substituent in this series as shown in eq 15c. For the N^1-benzoylsulfanilamides against the same bacteria, π_0 for the N^1-benzoyl moiety is calculated similarly, *i.e.*, $\pi_0 \cong 1.9 \cong [0.8\ (\pi_0 \text{ for the substituent on the benzene ring}) + 1.58\ (\log P \text{ for acetophenone}) - 0.5\ (\pi \text{ for CH}_3)]$.[12] Since the log P value for the unsubstituted sulfanilamide is -0.78,[24] the log P_0 value, the optimal hydrophobic parameter of the whole drug molecule for these two series of compounds, is estimated as log $P_0 \leqq 1.1$ on the octanol–water scale. The value for the N^1-heterocyclic derivatives cannot be compared on the same basis with those for the above two series since the π values are determined using partition coefficients obtained in an isobutyl alcohol–water system. However, the π_0 value, 1.68, for the N^1 heterocycles and the log P_0 value for the N^1-heterocyclic sulfanilamides, $\cong 1.6 \cong [1.67\ (\pi_0) - 0.07\ (\log P \text{ for sulfanilamide determined in isobutyl alcohol–water system}^{21})]$, seems not inconsistent with values for the other series. Since the solubility of the highly polar sulfanilamides is expected to be greater in the more hydrophilic and polar solvent, isobutyl alcohol, than in the less so 1-octanol, the value of the partition coefficient of a sulfonamide would be larger in isobutyl alcohol–water than in the 1-octanol–water system.

The optimal pK_A value for the apparent potency of the sulfonamide drugs, except for the N^1-benzoyl deriva-

(23) E. Krüger-Thiemer and P. Bünger, *Chemotherapia*, **10**, 61, 129 (1965/1966).

(24) C. Hansch and S. M. Anderson, *J. Org. Chem.*, **32**, 2583 (1967).

tives, is always located a little below the value of experimental pH (*i.e.*, between pK_A = 6.5 and 7.7) depending on the experimental conditions and test organisms. In spite of the exception of the N^1-benzoyl derivatives, our over-all results indicate that the pK_A value should be as close to body pH as possible in order to obtain a maximal chemotherapeutic activity.

Although the ρ value for the N^1-heterocyclic derivatives, 0.605, is very similar to that obtained for the sulfanilanilides against *E. coli*, 0.7 (ρ value in eq 15c divided by ρ_A = 1.88), the ρ value for the N^1-benzoylsulfanilamides against the same *E. coli*, 2.6, is considerably larger than the other two. As described above, the ρ value for the latter is not highly reliable so that the difference in ρ may not be worth trying to rationalize. However, in our procedure, the ΔpK_A or σ term cannot be assigned only to the contribution of an electronic demand of the drug molecule at the site of action. If the transfer process from outside the cell to the intracellular site of action through many partitionings and adsorption and desorption processes *via* biological membranes is governed to some extent by an electronic effect of the substituent, this effect is contained in the ρ value together with the effect at the site of action. Since we are unable to separate the roles of the ΔpK_A term, the difference in ρ values for different series would not necessarily indicate the difference in the essential electronic demand of the drugs at the site of action.

The above analyses provide another illustration of the great practical advantage of the use of the extrathermodynamic approach[25] to structure–activity problems. The role of the hydrophobic property of the molecule in the bacteriostatic activity and the protein binding is nicely delineated by means of π. The analysis, where the effects of substituent on ionization are separated from other electronic effects of substituents, is able to describe the pK_A dependence of the bacteriostatic activity. It also shows, in a procedure independent from those of earlier workers,[2,3] that the maximal antibacterial activity is exerted by drugs having an optimal pK_A value. This procedure should help in designing new sulfonamide drugs with optimal pK_A and π_0. It should also aid in understanding the pharmacokinetic mechanism underlying sulfonamide chemotherapy when a comprehensive set of biological data and physicochemical constants for *in vivo* properties are available, and an appropriate model can be chosen for *in vivo* phenomena such as curative effect, metabolic process, and renal excretion. Thus, if this procedure could be combined with the recently developed method by Krüger-Thiemer and Bünger,[23] a relationship between dosage schedule and molecular structure of the sulfonamides could be integrated so that an ideal dosage schedule for a new drug could be predicted from structural parameters such as log P and ΔpK_A.

Acknowledgment.—The authors wish to express their sincere thanks to Professors Tetsuo Mitsui and Minoru Nakajima for their support of this work.

(25) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1963.

Relationships among Current Quantitative Structure–Activity Models[1]

JUDITH A. SINGER AND WILLIAM P. PURCELL

Department of Medicinal Chemistry, University of Tennessee College of Pharmacy, Memphis, Tennessee 38103

Received June 28, 1967

Structure–activity models falling into two categories are compared. One category includes those models in which the observed biological activity is expressed as a function of group contributions to the activity and the other includes the Hansch substituent constant model. It is demonstrated that, if the biological activity is a parabolic function of Hansch's substituent constant, π, the model assuming additive and constant contribution from each group is not appropriate, but a model previously successful in a specific instance is analogous to the Hansch equation. If the π^2 term is not significant, however, the model assuming additive and constant contribution is appropriate when the biological activity is dependent on π and/or σ.

The recent success of attempts to express quantitatively the relationship of chemical structure to biological activity is most encouraging to the medicinal chemist who wishes to approach drug design rationally. The quantitative models for structure–activity relationships of related series of molecules fall into two broad categories. (A) There are mathematical models in which the observed biological activity is expressed as a function of parameters assigned to each substituent group and/or the parent portion of the molecule; the values of these parameters are obtained, after a particular model has been selected, by fitting the experimentally observed activities of a series of molecules using the method of multiple regressions. (B) The second category is comprised of linear free-energy relationships which ascribe the biological activity of a molecule to contributions from various free-energy-related physicochemical parameters of the substituents, the constants associated with each physicochemical parameter being generated by regression analysis for the biologically tested molecules.

Examples of the first approach include those of Free and Wilson[2] and Kopecký and co-workers.[3,4] The method of Free and Wilson[2] is based upon an additive mathematical model in which a particular substituent in a specific position is assumed to make an additive and constant contribution to the biological activity of a molecule in a series of chemically related

(1) This research is being supported by the U. S. Army Medical Research and Development Command (DA-49-193-MD-2779) and the National Science Foundation (GB-4453). This paper is Contribution No. 222 from the Army Research Program on Malaria.

(2) S. M. Free, Jr., and J. W. Wilson, *J. Med. Chem.*, **7**, 395 (1964).

(3) K. Bočck, J. Kopecký, M. Krivucová, and D. Vlachová, *Experientia*, **20**, 667 (1964).

(4) J. Kopecký, K. Bočck, and D. Vlachová, *Nature*, **207**, 981 (1965).