peptides. The unusual growth-stimulating and growth-inhibiting effects observed for these peptides could be accounted for in terms of membrane transport and competition for transport by two peptides, *e.g.*, L-isoleucyl-L-phenylalanine and L-cyclopentaneglycyl-β-2-thienyl-L-alanine.

Kinetics and Mechanisms of Action of Drugs on Microorganisms. VIII. Quantification and Prediction of the Biological Activities of *meta*- and *para*-Substituted N_1-Phenylsulfanilamides by Microbial Kinetics

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The apparent first-order generation rate constants, k_{app}, were determined in the steady-state growth of *Escherichia coli* in presence of graded concentrations, S, of a series of systematically substituted N_1-phenylsulfanilamides. From the expression $k_{app} = k_0 - k_0k_bS/(1 + k_bS)$, where k_0 is the determined generation rate constant in absence of drug, the bacteriostatic activity parameters, k_b in l. μmole^{-1}, were calculated. A good linear relationship was obtained between log k_b values and modified Hammett substituent parameters, $\rho = 1.12 \pm 0.11$, with the exception of the parent compound and the N_1-3,5-dinitrophenylsulfanilamide. A systematic dependency of k_b values on pH in the range of 6.0–7.4, where k_0 was constant, was not observed. The k_{app} values obtained in the presence of sulfonamide when extrapolated to zero drug concentration yielded calculated values for k_0 that showed dependence on pH. The activity parameters were independent of the determined chloroform–water distribution coefficients and applications of these data to the Hansch equation did not improve the correlation of k_b with substituent constants.

A recent review article[1] has reported on the many investigations that have been made to evaluate relationships between the structure and antibacterial activity of substituted sulfonamides (SA). A linear correlation was observed between the logarithm of the minimum inhibitory concentration (MIC) for *Escherichia coli* and various physicochemical properties within the closely chemically related series of the substituted N_1-phenylsulfanilamides. The pertinent physicochemical parameters were Hammett substituent constants (σ), dissociation constants of the free amino (pK_{a_1}') and the sulfonamido groups (pK_{a_2}'),[2] and measures of the electron density of the nitrogen atom in the substituted anilines that were the SA precursors, determined from ir[2,3] and nmr[4] measurements. A recent analysis[5] of the structure activity relationships of a series of SA has indicated that these σ, pK_{a_2}' values, and partition coefficients estimated from a series of compounds with the same substituents[6] are statistically correlated with MIC data. This indicated a statistically significant effect not only of σ but of the fraction of the drug in the undissociated form in the nutrient media and its oil/water partition coefficient on these MIC values.

The action of SA on drug-equilibrated microbial growth in an individual cell has been described by a model that involves the partitioning of SA between the medium and the cell interior to establish a concentration of SA inside the cell (S') which can react with receptor sites (R).[7,8] The apparent first-order generation rate

$$S \underset{}{\overset{K_1}{\rightleftharpoons}} S' + R \overset{K_2}{\rightleftharpoons} RS' \qquad (1)$$

constant, k_{app}, in sec^{-1}, of a bacterial culture in steady-state growth and affected by SA concentration, S, adheres to the equation

$$k_{app} = k_0 - k_0k_bS/(1 + k_bS) \qquad (2)$$

where k_0 is the generation rate constant in absence of drug and k_b is defined as the product of the two equilibrium constants of eq 1. The parameter k_b may be calculated from positive k_{app} values observed at different concentrations of SA in the media and is independent of the SA concentration. The determination of MIC (μmole l.$^{-1}$), however, involves the observation of the absence of some degree of turbidity considered as a manifestation of an increase to a constant number of organisms after an arbitrarily fixed time interval. The activity parameters k_b, of two drugs can be related to their MIC values when the ideal condition of $k_{app} = 0$ is considered and where S_i might be equal to MIC_i. From simultaneous equations for each drug according to eq 2, the ratio of the activity parameters can be calculated as

$$k_{b_1}/k_{b_2} = MIC_2/MIC_1 \qquad (4)$$

$$k_b = K_1K_2 \text{ (l. μmole}^{-1}) \qquad (3)$$

Thus under the conditions specified the ratio of the k_b values is equal to the reciprocal of the ratio of their corresponding MIC values. However, the validity of the MIC value *per se* is limited by the sensitivity of the turbidimetric method and the normal error of the serial dilution techniques used. It is essentially a one-point method. In addition, at the dosage levels of SA which completely inhibit bacterial growth, as was shown for the specific case of sulfisoxazole-affected growth rates,[7] an additional phenomenon of kill or death of microorganisms occurs. Thus the MIC value might be based

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upon growth rate superimposed on death rate, whereas k_b is related to inhibition of growth only. An SA concentration calculated from eq 2 to obtain a given inhibition in a given time interval therefore will be underestimated by an MIC value obtained by the serial dilution technique. Thus the constant k_b may more readily characterize antibacterial activity than do MIC data. This activity constant may be related to molecular structure and the physicochemical properties of these structures.

If the hypothesis that partitioning affects antibacterial activity is valid and if it is related to oil–water distribution coefficients, it is possible that the k_b activity parameters may show a degree of correlation with the actual experimental partition coefficients obtained between chloroform and water.

Experimental Section

Test Organism.—Replicate slants were prepared from *E. coli* strain B/r isolated from a culture on an agar plate. These slants were then stored in a refrigerator at 4° and a new one was used for each experiment.

Sulfonamides.—The synthesis and properties of the substituted N_1-phenylsulfanilamides with the substituents 4-CH_3, 3-OCH_3, 3-Cl, 3-Br, 3-I, 4-$COCH_3$, 4-CN, and 4-NO_2 and of the unsubstituted N_1-phenylsulfanilamide have been described previously.[2] The 3-CF_3-, 3-NO_2-, and 3,5-$(NO_2)_2$-substituted compounds were synthesized from the respective substituted anilines by the method of Senner.[9] The corrected melting points for these compounds were 115, 173, and 215°, respectively.

Culture Media.—Anton's medium[10] was used as a culture medium. The molar ratio of KH_2PO_4 and K_2HPO_4 was varied to obtain different pH values between 6.0 and 7.4. The media were filtered through sterilized Millipore 0.45-μ HA filters. After filtration they were stored for 2 days in an incubator at 37.5° in order to detect any contamination.

Growth.—A broth culture was prepared from a slant and allowed to grow for 10 hr at 37.5°. The culture then was diluted 5000-fold into fresh broth and the growth was followed by the Coulter counter, Model B (Coulter electronics Co., Hialeah, Fla.) until it reached an organism concentration of 6.7×10^7 *E. coli*/ml in the logarithmic growth phase. Samples then were diluted 2500-fold in two steps into fresh broth. These were finally diluted 50-fold into replicate 50-ml volumes of broth in 125-ml loosely capped erlenmeyer flasks that contained the drugs in graded concentrations. Previous experiments had shown that the use of cotton plugs instead of metal caps or rapid stirring to assure saturation of the culture with ambient air throughout the time course of growth did not change the growth rate constant in absence of drug. The cultures were maintained at 37.5 ± 0.1° in a 50-gal constant-temperature water bath equipped with a shaker.

Cell Count Method.—Aliquots of 1.0 ml were taken at 20-min intervals from the growing cultures. They were appropriately diluted into a Millipore 0.45-μ HA filtered aqueous solution of 0.85% NaCl and 1% formaldehyde to obtain Coulter counts within 10,000–30,000 counts/50 μl. The instrument was equipped with a 30-μ orifice and the settings were an aperture current of 5, amplification of 8, gain of 10, lower threshold of 13, and upper threshold at maximum. Further details of this method have already been published.[11]

It has been shown previously[7] that for sulfisoxazole-affected growth rates at concentrations that diminish the growth rate constant by 90%, total cell counts were still coincident with viable counts.

Effect of Drug Concentration on Growth Rate.—The various SA were dissolved in 0.04 *N* NaOH. Aliquots (1.0 ml) of the appropriately diluted drug solutions were added to broth to achieve a volume of 50 ml and the appropriate drug concentrations in the media. A typical semilogarithmic plot of the total number of organisms subsequent to the lag period[7] with respect to time at graded concentrations of SA is shown in Figure 1.

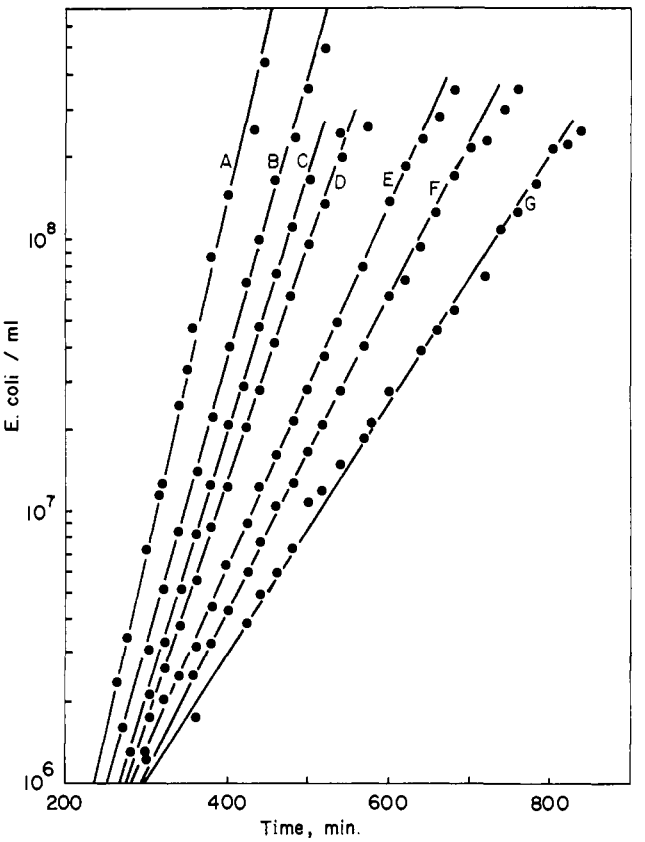


Figure 1.—Typical generation rate curves of *E. coli* B/r at 37.5° and pH 6.90 ± 0.05 in the presence of various concentrations of N_1-phenylsulfanilamide. The organism concentration at the addition of drug was 5×10^2 *E. coli*/ml and the plot shows the steady-state growth period subsequent to the lag phase. The curves and respective drug concentrations (10^5 *M*) were A, 0.0; B, 2.07; C, 3.32; D, 4.15; E, 6.64; F, 8.30; G, 11.1.

Acid Dissociation Constants (pK_{a_2}') and Substituent Constants (σ).—The pK_{a_2}' values of the N_1 atom were determined spectrophotometrically at the ionic strength of the medium, 0.12. The general procedure employed was as outlined by Yoshioka, *et al.*,[12] and the values are listed in Table I. The substituent constants (σ) used for the *para*-substituted N_1-phenylsulfanilamides were those of Yoshioka, *et al.*[12] The chosen values for σ for the 3-Br-, 3-CF_3-, and 3,5-$(NO_2)_2$-substituted compounds were after Jaffe.[13] The determined pK_{a_2}' values were linearly related to these values in accordance with the equation

$$pK_{a_2}' = 9.030 - 2.027\sigma \qquad (5)$$

where the standard error of the slope was ±0.084.

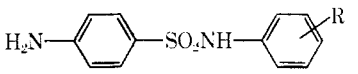
Partition Coefficients.—The partition coefficients of the compounds were determined as

$$P = HS_{CHCl_3}/HS_{H_2O} \qquad (6)$$

where *HS* is the concentration of the undissociated molecule, and are listed in Table I. The drugs were dissolved in 0.01 *N* NaOH and diluted into chloroform-saturated acetate buffer of pH 4.30 (of pH 4.00 for the 3,5-$(NO_2)_2$-substituted compound), ionic strength 0.12, to obtain concentrations within the range of 0.6 to 2×10^{-4} *M*. Aliquots of these solutions (75 ml) were placed into 125-ml separating funnels and 2–4 ml of buffer-saturated chloroform was added. The mixtures were shaken for 4 hr with constant agitation at 28°. A preliminary experiment had shown that equilibrium was well established within this time interval. The phases were separated, the aqueous phases were centrifuged for 5 min at approximately 3000 rpm, and the concentrations of the SA were determined spectrophotometrically, using 1- or 5-cm cells in a Cary recording spectrophotometer. A blank was run

(9) A. E. Senner, *J. Org. Chem.*, **11**, 376 (1946).
(10) A. H. Anton, *J. Pharmacol. Exptl. Therap.*, **129**, 383 (1960).
(11) E. R. Garrett and G. H. Miller, *J. Pharm. Sci.*, **54**, 427 (1965).
(12) M. Yoshioka, K. Hamamoto, and T. Kubota, *Bull. Chem. Soc. Japan*, **35**, 1723 (1962).
(13) H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).

TABLE I
PHYSICOCHEMICAL AND BACTERIOSTATIC ACTIVITY PARAMETERS OF VARIOUS SUBSTITUTED N_1-PHENYLSULFANILAMIDES IN MEDIA OF DIFFERENT pH



R	σ[a]	pK_{a_2}[b]		pH[c]	$10^{-4}k_{a_2}$[d] l. μmole^{-1}	$10\ k_a/k_{b_2}$[e] sec^{-1}	P[f]
4-CH_3	−0.170	...	(9.25)	6.90	0.258	6.13	99
H	0	9.20	(8.97)	6.80	1.317	5.33	28
				6.82	0.663	6.99	
				6.90	0.514	9.99	
					0.782	7.38	
					0.971	7.33	
					0.702	7.89	
					0.556	9.04	
					0.557	8.57	
				6.93	0.555	9.54	
					0.780	7.56	
3-OCH_3	0.115	8.95	(8.72)	6.84	0.338	7.55	44
				6.90	0.260	7.61	
					0.362	8.30	
					0.454	8.36	
				7.23	0.731	4.89	
3-I	0.352	8.84	(...)	6.90	0.592	6.21	295
3-Cl	0.373	8.38	(8.28)	6.90	0.411	7.96	94
					1.047	7.00	
3-Br	0.391	8.28	(...)	6.90	1.217	6.41	144
					1.980	4.69	
					0.958	5.84	
				7.25	0.978	4.75	
3-CF_3	0.420	7.95	(...)	6.84	1.18	2.73	...
3-NO_2	0.700	7.61	(7.67)	6.84	2.989	5.94	277
				6.88	1.481	8.33	
4-$COCH_3$	0.703	7.46	(7.61)	6.02	0.962	11.5	27
					1.035	11.4	
				6.84	2.467	6.15	
				6.87	2.192	7.13	
					1.897	7.14	
				6.90	2.239	4.87	
				7.25	2.983	5.37	
				7.39	2.700	5.23	
					3.704	4.55	
4-CN	0.804	...	(7.36)	6.90	2.769	5.95	..
					5.037	5.13	
					2.065	6.25	
4-NO_2	1.040	6.97	(6.97)	6.48	2.955	8.24	
				6.87	4.547	6.23	33
				6.90	6.338	5.34	
					3.082	8.82	
				6.95	4.914	6.80	
				7.24	4.062	4.53	
					2.683	5.11	
3,5-$(NO_2)_2$	1.47	6.16	(6.19)	6.42	0.903	14.8	81
				6.85	1.333	7.02	
					0.842	8.38	

[a] Values are after Jaffe[11] with the exception of those for the 4-$COCH_3$-, 4-CN-, and 4-NO_2-substituted compounds, which are after Yoshioka, *et al.*[12] [b] Values obtained spectrophotometrically by the method of Yoshioka, *et al.*,[12] at ionic strength 0.12. The standard deviation of the values, calculated from the average of the weighted variances of the various pK_a, was ±0.053. The values of Yoshioka, *et al.*,[12] are given in parentheses for comparison. [c] The pH values were observed in the growing cultures and were constant from 5×10^6 up to 10^8 organisms/ml. [d] The values given are those associated with the higher correlation coefficient in plots according to eq 8 or 9. [e] Calculated by linear regression according to eq 8 or 9 as the reciprocal of the intercepts and slopes, respectively. [f] Partition coefficients obtained in $CHCl_3$-H_2O.

in each set, treated in the same way and used in the reference cell of the spectrophotometer. The per cent standard deviation of the method was determined for the 3-Br-substituted compound and was ±4.2%.

Results and Discussion

Apparent generation rate constants, k_{app} in sec^{-1}, were obtained from the slopes of plots of log N *vs.* time (Figure 1) in accordance with the apparent first-order expression

$$\log N = kt/2.303 + \log N_0 \qquad (7)$$

where N_0 is the number of *E. coli*/ml extrapolated to $t = 0$, and t is in seconds.

Generation Rate Constants in Absence of Drug.—

The mean generation rate constant in absence of drug, k_0, calculated from 23 experiments performed on different days with different batches of media was $k_0 = 5.061 (\pm 0.18) \times 10^{-4}$ sec^{-1}. The same mean generation rate constant in absence of drug was obtained from five experiments performed on different days with media from the same batch and was $k_0 = 5.07 (\pm 0.19) \times 10^{-4}$ sec^{-1}. The k_0 values were obtained in media initially buffered at pH values between 6.0 and 7.4. The pH of the growth media changed only at high organism concentrations and was independent of the inital growth time and sulfonamide concentration (Figure 2). At an

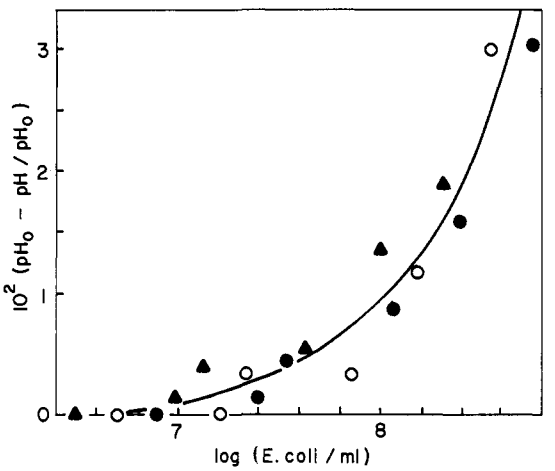


Figure 2.—Relative change of the pH of the culture media in per cent of the initial pH value with increasing logarithm of *E. coli*/ml. The buffered initial pH values were 6.40 (○), 6.91 (●), 7.36 (▲) in the growth media at an inoculum size of 5×10^2 *E. coli*/ml.

organism concentration of 10^8 *E. coli*/ml the mean deviation of pH from the inital value was −0.05 with a maximum of −0.08. The generation rate constants were obtained within 5×10^6 and 10^8 *E. coli*/ml and therefore observed at constant pH. The generation rate constant in absence of drug was independent of pH between pH 6.0 and 7.4. This result confirms earlier observations.[14,15] It implies that in absence of drug either the rate-limiting metabolic processes resulting in growth are independent of pH or that the pH inside the cell is kept constant by the buffer effects of the cell constituents.[16]

Effect of SA Concentration on Generation Rate Constants.—The reciprocal of the difference between the growth rate constant in the absence (k_0) and in the presence of SA (k_{app}) may be plotted against the reciprocal of its concentration (S, in mole l.$^{-1}$) in accordance with the rearrangement of eq 2.

$$1/(k_0 - k_{app}) = (1/k_a)(1/S) + k_b/k_a \quad (8)$$

An alternative method of plotting uses the expression

$$S/(k_0 - k_{app}) = 1/k_a + (k_b/k_a)S \quad (9)$$

where k_a and k_b are constants. The activity parameter k_b in l. μmoles^{-1} (Table I), was derived from the slope and intercept values, $1/k_a$ and k_b/k_a, obtained from eq 8 or 9 with the highest correlation coefficient. Typical plots according to eq 8 are shown in Figure 3.

(14) E. F. Gale and H. M. R. Epps, *Biochem. J.*, **36**, 600 (1942).
(15) E. R. Garrett and N. A. Dickinson, unpublished data.
(16) C. N. Hinshelwood, "The Chemical Kinetis of the Bacterial Cell," Oxford University Press, 1942, p 72.

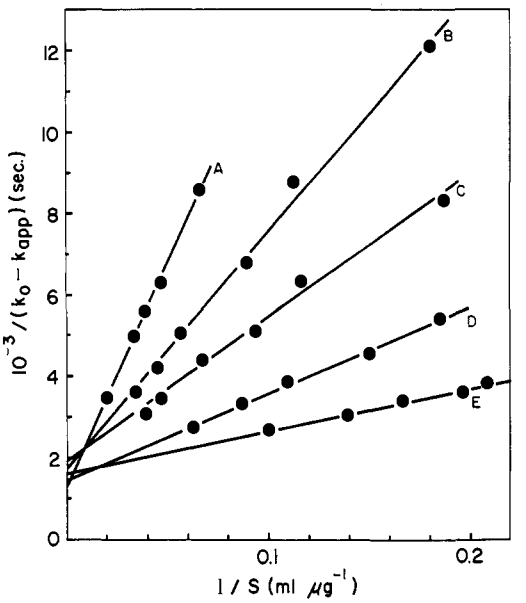


Figure 3.—Relation between apparent first-order generation rate constants, k_{app}, and sulfonamide concentration, S, plotted according to the equation $1/(k_0 - k_{app}) = (1/k_a)(1/S) + k_b/k_a$, where k_0 is the generation rate constant in absence of sulfonamide and k_a and k_b are constants. The curves and respective substituted N_1-phenylsulfanilamides were (pH 6.90 ± 0.05, 37.5°) A, 3-OCH_3; B, 3-Br; C, H; D, 4-$COCH_3$; E, 4-NO_2.

Reproducibility of the Derived Activity Constant k_b.—The functions on the left-hand side of eq 8 and 9 are excellently linearly related to the functions of the SA concentration on the right-hand side of these equations. The range of the correlation coefficients of these plots was 0.906–0.999 with an arithmetic mean of 0.993. The derived activity parameters k_b, however (Table I), showed a variability among runs. No significant difference was found between k_b values calculated according to either eq 8 [7.42 (±3.28) × 10^3 l. μmole^{-1}] or to eq 9 [8.04 (±2.40) × 10^3 l. μmole^{-1}] when four experiments were carried out at constant pH (6.90 ± 0.03) with N_1-phenylsulfanilamide in media from the same batch on different days.

The same mean activity constant (k_b from eq 8) was obtained from four experiments performed on different days with media from different batches, 6.39 (±1.07) × 10^3 l. μmole^{-1}.

Relationship between Physicochemical Parameters and Activity.—The relation between log k_b and the modified Hammett substituent parameters[12] is linear (Figure 4) in accordance with the Hammett equation

$$\log[(k_b)_X/(k_b)_H] = \log (K_1K_2)_X - \log (K_1K_2)_H = \rho\sigma \quad (10)$$

where $(k_b)_X$ is the activity constant for the compound with the substituent X and $(k_b)_H$ is that for the unsubstituted N_1-phenylsulfanilamide. The 3,5-$(NO_2)_2$-substituted compound, however, showed a large deviation from the plot. The reaction constant ρ, at pH 6.90 ± 0.05, calculated from the regression of the differences between the log $(k_b)_X$ and log $(k_b)_H$ values on σ when the disubstituted drug was excluded, was 1.12 ± 0.11 (r = 0.914). The mean value for log $(k_b)_H$ used was 3.821 ± 0.097. It is interesting to note that the k_b value can be modified[5] to $k_b' = k_b/f_{HS}$ by dividing by the undissociated fraction, f_{HS}, on the premise that the activity

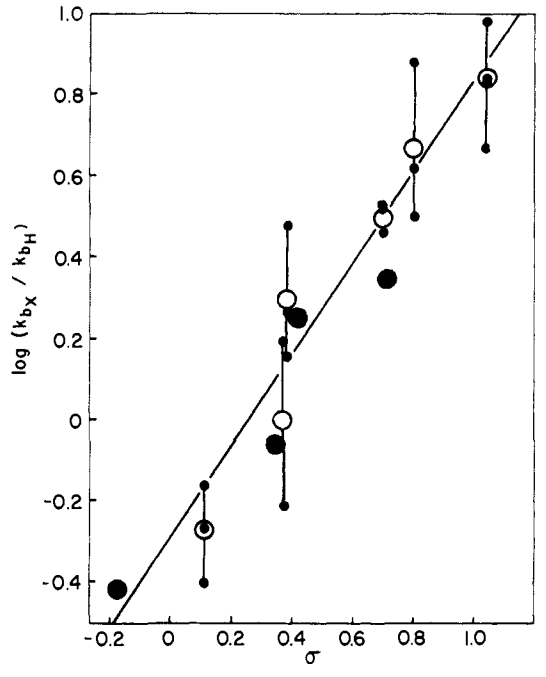


Figure 4.—Plot of log $[(k_b)_X/(k_b)_H]$, the ratio of the bacteriostatic activity constants of substituted N_1-phenylsulfanilamides to that of the parent compound, *vs.* the modified Hammett substituent constants, σ. The large open circles represent the mean values calculated from log k_b values (•) obtained at pH 6.90 ± 0.05 and 37.5° for each compound and the large closed circles are values for k_b from a single experiment for the particular compound.

parameter in pH 6.90 media is dependent solely on the concentration gradient established by the un-ionized fraction in the media. The log k_b' *vs.* σ regression (also with the exclusion of the disubstituted drug) yielded a higher correlation (r = 0.959 with ρ' = 1.32 ± 0.19). However, the difference between the correlation coefficients was statistically insignificant.[17]

The intercept of the plot log k_b *vs.* σ should be zero, if the unsubstituted drug rigorously adhered to the Hammett equation. However, the intercept value was statistically significantly different, *i.e.*, −0.294. The possibility of a technological mistake in that this compound was mislabeled was considered. However, ir and nmr spectra of the material used confirmed that it was the unsubstituted drug. When a substrate–enzyme complex is postulated as being involved in the rate-determining step, strict adherence to a Hammett relation should not be expected in all cases. It is not only linear free-energy relations that determine reactivity but the shape of both the enzyme surface and the reacting molecule which may influence the binding and the reaction at the active site.[18] The deviations of the 3,5-disubstituted and the unsubstituted drugs may be indicative of these limitations. In the former case the heavy substitution increases the volume of the anilide part of the SA molecule markedly and may inhibit the formation of the enzyme–substrate complex as visualized by the fitting of a key into a lock. An alternative explanation may be that 3,5-dinitro compounds may undergo ready reduction in such biological systems and the reduced compound has less intrinsic activity. In the latter case the unsubstituted benzene ring might so fit the enzyme surface that hyperconjugational effects with neighboring atomic groups of the enzyme can occur. An alternative model for the mode of action of SA does not assume competition between the drug and *p*-aminobenzoic acid (PABA) for the same enzyme.[19] The free amino group of SA may inhibit bacterial growth by formation of Schiff bases with an available aldehydic group of a folic acid precursor. If this reaction does not involve an enzyme, then a Hammett $\rho\sigma$ treatment might be applicable to the activity parameters. However, the formation of Schiff bases does not seem to follow simple free-energy relationships.[20] In neutral and mildly acid solutions the rate constants for both the formation of the carbinolamine and the subsequent loss of water have uncatalyzed and acid-catalyzed components. Therefore, the over-all rate constant may not adhere to the simple Hammett $\rho\sigma$ treatment. Furthermore, it has been shown in the very similar formation of semicarbazones,[21] that substituent effects were opposite in the separate steps. If one step was predominantly rate determining, the linear free-energy relations pertinent to that step may predominate but may be modified by the significance of the other. It is interesting to note that the k_b values of the various compounds can be normalized to an intrinsic k_b value (k_b*) when the ionized molecule is assumed to be the only active species. The negative charge on the molecule could provide an important inductive effect in the formation of the Schiff base. An alternative possibility would be that the negative charge on the molecule is essential for binding on an enzyme surface prior to the reaction. In both cases the negatively charged fraction could determine the concentration of the active species. At one particular pH of the reaction or receptor site compartment the k_b values corrected for the uncharged fraction would be identical with and equal to the intrinsic value k_b*. A calculation according to this concept was carried out by determining the pH at which log (k_b/f_s) would be invariant with σ. The pH was calculated as 8.1 with a k_b* of 3.32 (±0.94) × 10^4 l. μmole^{-1} when the N_1-3,5-dinitrophenylsulfanilamide and N_1-phenylsulfanilamide were omitted.

Influence of pH on the Activity of the Sulfonamides.—No systematic dependency of k_b on pH could be detected within this sulfonamide series for the studied pH range 6.0–7.4. A plot of log k_b *vs.* pH for the 4-$COCH_3$-substituted compound did show a linear relationship with a slope of +0.37 ± 0.04.

However, the other compounds did not show a statistically significant dependency (Table I) so that no definite conclusions can be made at this time. It has been postulated that the active concentration of SA inside the cell is determined by equilibration of the un-ionized fraction of the total concentration in the medium across the cell wall. Subsequent dissociation would then occur at the constant pH of the biophase, and the ionized fraction would react with the receptor.[22] On the basis of this hypothesis a tenfold increase in the apparent k_b would be predicted for a compound of pK_a' = 7.5 when

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the pH of the medium is changed from 7.4 to 6.0. The extent of the change was only fourfold for the 4-$COCH_3$ compound and could possibly be assigned to a fortuitous variation in the k_b values.

The receptor site model for the action of SA[7] assumes that the rate-limiting step in the metabolic processes resulting in growth is the same under drug-free as well as SA-affected growth. The receptor sites unbound by SA may be considered to have reacted with PABA. If the reaction of SA with the total number of receptor sites is necessary for complete cessation of microbial growth,[7] this should mean that the obtained

$$qk_m = k_a/k_b \ (\text{sec}^{-1}) \quad (11)$$

should have the same magnitude as k_0, the generation rate constant in the absence of SA. The k_m is a rate constant for the metabolism at the receptor sites and q is a proportionality constant relating the rate of metabolism to the number of organisms in the balanced growth culture. The ratio of the experimentally observed value, $k_0 = 5.06 \pm 0.18$, to the calculated value, $qk_m = 6.95 \pm 2.12$ (Table I), is 0.74. Thus, only about 74% of the receptor sites have to have reacted with SA to achieve complete cessation of growth within the series. The detailed analysis of this concept has been given previously.[8] The calculated constant log qk_m is linearly related to the pH with a slope of -0.223 ± 0.049. Since k_0 has been shown to be independent of pH within the studied range and qk_m is not, this implies that the rate-determining step has been modified under the action of the drug. The number of receptor sites available must then be some function of the pH of the medium.

Influence of Lipid Solubility on the Activity Parameters.—The parameter K_1 in eq 1 represents the over-all equilibrium constant for the equilibration of the SA molecules between the culture medium and the receptor site compartment including the possible partitions between hydrophilic and lipophilic phases within the biophase of the bacteria. Partition coefficients, P_i, for a given compound in two different solvent systems may be related[23] by

$$\log P_1 = a \log P_2 + b \quad (12)$$

where a and b are constants. It may be postulated that the log P values obtained experimentally in the solvent system chloroform–water are proportional to the logarithm of the over-all equilibrium constant K_1 in the biological system if it is dependent on oil–water partitioning and thus

$$\log K_1 = a \log P + b \quad (13)$$

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If the k_b values are considered as the products of the constants K_1 and K_2 where the former is dependent on partition and the latter on a Hammett $\sigma\rho$ relationship, the postulated dependency considering eq 13 and according to Hansch[6] would be

$$\begin{aligned} \log [(k_b)_X/(k_b)_H] &= \log [(K_1K_2)_X/(K_1K_2)_H] \\ &= \log [(K_1)_X/(K_1)_H] + \\ &\quad \log [(K_2)_X/(K_2)_H] \\ &= a \log [P_X/P_H] + \sigma\rho' \end{aligned} \quad (14)$$

which is one version of the Hansch equations.[24]

It is apparent from inspection of Figure 4 and eq 14 that increased correlation between log k_b and σ would result for a positive coefficient, a, if the log P value of the 3-Br (2.16) and the unsubstituted compound (1.45) were significantly greater and if the log P values for the 3-OCH_3 (1.64), 3-Cl (1.97), and 3,5-$(NO_2)_2$ (1.91) compounds were significantly smaller than those of the 4-CH_3 (1.99), 4-$COCH_3$ (1.43), and 4-NO_2 (1.52) compounds. For a negative coefficient, a, the converse would be true. The values in parentheses are the log P values from Table I and readily indicate no consistency that would permit this increased correlation. In addition, no correlation or trend of partition coefficients with k_b values alone is apparent. The application of the algebraic technique[25] to estimate the parameters of the expanded Hansch equation

$$\log k_b = a \log P + b + \sigma\rho' + d(\log P)^2 \quad (15)$$

permitted no consistent estimate of a, b, ρ', and d values whatsoever among the compounds. It is thus concluded that the only significant dependency of log k_b is on the $\sigma\rho$ product in accordance with eq 10 within the variability of the data and within the series of substituents studied. The solubility of compounds carrying more strongly electron-releasing substituents with much higher P values was not sufficient to permit the determination of their antibacterial activity in the system described.

Professor Corwin Hansch of Pomona College, Claremont, California, has kindly fit our data by his digital computer program and obtained the best correlation without considering partition. However, when MIC data obtained with these compounds and others with more lipophilic substituents were evaluated, a small influence of partition on activity was found.[5]

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