Several of these compounds are closely related chemically to well-known sulfanilamido derivatives of the same type.

All of the sulfonamides showed some degree of bacteriostatic activity, but very little effect on experimental animal infections. Two compounds, in particular, were highly active *in vitro* and well absorbed. Possible explanations for these discrepancies and their bearing on the relation of molecular structure to chemotherapeutic activity, are discussed.

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[CONTRIBUTION FROM THE STAMFORD RESEARCH LABORATORIES OF THE AMERICAN CYANAMID COMPANY]

Studies in Chemotherapy. VII. A Theory of the Relation of Structure to Activity of Sulfanilamide Type Compounds[1]

BY PAUL H. BELL AND RICHARD O. ROBLIN, JR.

For the past three years we have been trying to find some relationship between the molecular structure and the chemotherapeutic activity of sulfanilamide type compounds. In spite of the many hundreds of derivatives which have been prepared and tested, no adequate explanation for the profound changes in therapeutic effect resulting from variations in structure has been proposed. Our approach to this problem has been through an attempt to utilize a fundamental physical property related to both structure and activity.[2] The present theory is based on the experimental observation that acid dissociation constants, which can be correlated with the structure of sulfanilamide derivatives, are also related to their bacteriostatic activity. The following is a description of this theory and a discussion of its implications.

I. Chemotherapeutic Activity and Mode of Action.—Before attempting to correlate the structure of sulfonamides with their chemotherapeutic activity, a reasonably accurate method for the determination of relative effectiveness is essential. Experimental animal tests for activity are frequently misleading because of the many factors, such as lack of absorption, rapid excretion, effect of diet and possible chemical changes in the compounds, as well as other less obvious variables,[3] which may affect the results. To a lesser degree, *in vitro* tests carried out in complex media are also somewhat confusing. In this investigation the term activity is used to indicate bacteriostatic activity against *E. coli* when the organisms are grown in a synthetic medium. This method of testing provides a more consistent and reproducible basis for the determination of relative activities by reducing the number of variables to a minimum. The relation of *in vitro* to *in vivo* results,[4] and the lack of any great degree of specificity among sulfanilamide derivatives,[5] appear to warrant this method of evaluation.

Another important prerequisite to this type of work is at least a partial understanding of the mechanism by which the compounds exert their bacteriostatic effects. None of the hypotheses advanced in recent years appeared to offer a very useful or convincing explanation, until Woods[6] demonstrated that *p*-aminobenzoic acid prevents the bacteriostatic action of sulfanilamide and sulfapyridine. This observation has since been extended by other investigators[7] to include sulfanilamide type compounds in general. Woods and Fildes[8] postulated that *p*-aminobenzoic acid is an essential metabolite associated with one or more of the enzymatic processes involved in bacterial growth. They pointed out the close structural relationship between the sulfonamides and this acid, and suggested that the former may act by blocking the enzyme system or systems with which *p*-aminobenzoic acid is involved and on which many bacteria depend for normal growth and development. Subsequent investigations have confirmed the essential nature of *p*-aminobenzoic acid,[9] and have shown experimentally

(1) Presented in part before the Divisions of Medicinal and Physical Chemistry, Buffalo Meeting of the American Chemical Society, September 9 and 10, 1942.

(2) Roblin and Bell, *Science*, **90**, 328 (1939).

(3) See, for example, Davis, *ibid.*, **95**, 78 (1942).

(4) White, Bratton, Litchfield and Marshall, *J. Pharmacol.*, **72**, 120 (1941).

(5) Wyss, Grubaugh and Schmelkes, *Proc. Soc. Exptl. Biol. Med.*, **49**, 618 (1942).

(6) Woods, *Brit. J. Expil. Path.*, **21**, 74 (1940).

(7) Landy and Wyeno, *Proc. Soc. Expil. Biol. Med.*, **46**, 59 (1941); Strauss, Lowell and Finland, *J. Clin. Investigation*, **20**, 189 (1941).

(8) Fildes, *Lancet*, **238**, I, 955 (1940).

(9) Rubbo and Gillespie, *Nature*, **146**, 838 (1940); Lampen and Peterson, THIS JOURNAL, **63**, 2283 (1941); Park and Wood, *Bull. Johns Hopkins Hosp.*, **70**, 19 (1942).

TABLE I

DISSOCIATION CONSTANTS AND BACTERIOSTATIC ACTIVITY OF SULFANILAMIDE TYPE COMPOUNDS

No.	Compound[a]	Acid constants pK_a	K_a	$K_a \times 10^{13}$ 1st	2nd	*In vitro* tests, C_R, Molar × 10^5	Ref.
1	*p*-Aminobenzoic acid	4.68	2.1×10^{-5}[b]	2.6[b]			
2	Sulfanilamide	10.43	3.7×10^{-11}[c]	2.3[c]		20.0	
3	N^1-Methylsulfanilamide	10.77	1.7×10^{-11}	1.6		30.0	*d*
4	N^1,N^1-Dimethylsulfanilamide	...	...	1.3		30.0	*d*
5	N^1-Hydroxyethylsulfanilamide	10.92	1.2×10^{-11}	2.0		50.0	*d*
6	Sulfanilylglycine	3.52	3.0×10^{-4}[e]	*f*		>90.0	*d*
7	N^1-Phenylsulfanilamide	9.60	2.5×10^{-10}[f]	1.4		3.0	*d*
8	N^1-*o*-Tolylsulfanilamide	9.96	1.1×10^{-10}[f]	1.1		10.0	*d*
9	N^1-*m*-Tolylsulfanilamide	9.74	1.8×10^{-10}[f]	1.3		5.0	*d*
10	N^1-*p*-Tolylsulfanilamide	9.82	1.5×10^{-10}[f]	1.4		5.0	*d*
11	N^3-Sulfanilylmetanilamide	8.23	5.9×10^{-9}[g]	1.6		2.0	*d*
12	N^4-Sulfanilylsulfanilamide	7.85	1.4×10^{-8}[g]	0.8		0.5	*d*
13	N^1-*p*-Aminophenylsulfanilamide	10.22	0.6×10^{-10}[f]	$>10^{-9}$	0.7	5.0	*d*
14	N^1-Furfurylsulfanilamide	10.88	1.3×10^{-11}[f]	1.8		20.0	*d*
15	Sulfapyridine	8.43	3.7×10^{-9}[g]	3.8	.1	0.6	*d*
16	3-Sulfanilamidopyridine	7.89	1.3×10^{-8}[f]	10	.4	.2	*d*
17	2-S-5-bromopyridine	7.15	7.1×10^{-8}[f]	0.8		.5	*h*
18	5-S-2-bromopyridine	7.12	7.6×10^{-8}[f]	1.0		.2	*h*
19	2-S-5-aminopyridine	8.47	0.34×10^{-8}	10	.3	.6	*h*
20	5-S-2-aminopyridine	8.82	$.15 \times 10^{-8}$	160[i]	.8	2.0	*h*
21	2-Sulfanilamidoimidazole	9.72	1.9×10^{-10}	*k*		40.0	*j*
22	3-Sulfanilamidopyridazine	7.06	0.87×10^{-7}	3.0	.2	0.08	*j*
23	Sulfadiazine	6.48	3.3×10^{-7}	1.0		.08	*l*
24	2-S-4-methylpyrimidine	7.06	0.87×10^{-7}	1.2		.2	*l*
25	2-S-4,6-dimethylpyrimidine	7.37	$.43 \times 10^{-7}$	2.3		.3	*m*
26	2-S-4-aminopyrimidine	9.44	3.6×10^{-10}	13.5[i]		20.0	*j*
27	4-S-pyrimidine	6.17	6.7×10^{-7}	22[i]	.2	0.1	*l*
28	5-S-pyrimidine	6.62	2.4×10^{-7}	0.8		.2	*m*
29	5-S-2-chloropyrimidine	5.80	1.6×10^{-6}	*k*		.1	*m*
30	2-Sulfanilamidopyrazine	6.04	0.91×10^{-6}[f]	0.6		.08	*n*
31	4-S-1,2,4-triazole	4.66	2.2×10^{-5}	.7		>80.0	*j*
32	2-Sulfanilamidoöxazole	6.5	3.2×10^{-7}	*k*		0.08	*j*
33	5-S-3-methylisoxazole	4.2	6.3×10^{-5}	*k*		.6	*j*
34	Sulfathiazole	7.12	7.6×10^{-8}[g]	2.3		.08	*d*
35	2-S-4-methylthiazole	7.79	1.6×10^{-8}	2.3		.2	*d*
36	3-S-4-methylfurazan	4.10	7.9×10^{-5}	0.8		1.0	*j*
37	3-S-5-methyloxadiazole	4.40	4.0×10^{-5}[f]	.5		2.0	*j*
38	2-S-1,3,4-thiadiazole	4.77	1.7×10^{-5}	1.4		0.6	*l*
39	2-S-5-methylthiadiazole	5.45	3.5×10^{-6}	1.6		.2	*p*
40	Sulfanilylcyanamide	2.92	1.2×10^{-3}	*f*		100	*q*
41	Sulfanilylurea	5.42	3.8×10^{-6}	0.6		10.0	*q*
42	Sulfanilylguanidine	...	...	5.6	.03	10.0	*h*
43	Sulfanilylaminoguanidine	...	...	3.0	.2	0.9	*q*
44	N^1-Acetylsulfanilamide	5.38	4.2×10^{-6}	0.6		.7	*d*
45	N^1-Chloroacetylsulfanilamide	3.79	1.6×10^{-4}	.4		10.0	*r*
46	N^1-Benzoylsulfanilamide	4.57	2.7×10^{-5}	.6		0.3	*d*
47	N^1-*p*-Aminobenzoylsulfanilamide	5.20	6.3×10^{-6}	2.7	.3	.5	*d*
48	N^1-Ethylsulfonylsulfanilamide	3.10	7.9×10^{-4}	0.3		1000	*d*
49	N^1-Sulfanilylsulfanilamide	2.89	1.3×10^{-3}	*f*		60.0	*d*
50	4,4'-Diaminodiphenylsulfone	...	...	3.1	.2	2.0	*s*

[a] S = Sulfanilamido; nomenclature according to Crossley, Northey and Hultquist, THIS JOURNAL, **60**, 2217 (1938). [b] Bjerrum, *Z. physik. Chem.*, **104**, 164 (1923), reported $pK_a = 4.8$; K_b, ref. 12. [c] Albert and Goldacre, *Nature*, **149**, 245 (1942), gave $K_a = 6.3 \times 10^{-11}$; $K_b = 1.6 \times 10^{-12}$. [d] See ref. 11. [e] First K_a represents carboxyl; second very weak. [f] Insol. glacial HAc. [g] Nielson and Wolffbrandt, *Dansk. Tids. Farm.*, **14**, 113 (1940); *J. Am. Pharm. Assoc. (Pharm. Abst.)*, **31**, 29 (1942), reported $pK_a = 8.7$. [h] Roblin and Winnek, THIS JOURNAL, **62**, 1999 (1940). [i] In water. [j] Anderson, Faith, Marson, Winnek and Roblin, *ibid.*, **64**, 2902 (1942). [k] No measurements made. [l] Roblin, Williams, Winnek and English, *ibid.*, **62**, 2002 (1940). [m] Roblin, Winnek and English, *ibid.*, **64**, 567 (1942). [n] Ellingson,

ibid., **63**, 2524 (1941). [o] Ref. (g) gave $pK_a = 7.6$. [p] Ganapathi, *Proc. Indian Acad. Sci.*, **13A**, 386 (1941); *Chem. Abs.*, **36**, 1022 (1942). [q] Winnek, Anderson, Marson, Faith and Roblin, THIS JOURNAL, **64**, 1682 (1942). [r] Ref. 23. [s] Fromm and Wittmann, *Ber.*, **41**, 2264 (1908). [t] Values in water calculated from measurements carried out in alcoholic solution (see Experimental).

that the type of inhibition produced by sulfanilamide derivatives is competitive with respect to *p*-aminobenzoic acid.[10]

If the bacteriostatic action of the sulfanilamide type compounds is due to a competition with the required *p*-aminobenzoic acid for an essential enzyme system, then the more closely the competitor compound resembles this acid, the greater should be its blocking or bacteriostatic effect. The characterizing groups in *p*-aminobenzoic acid are the carboxyl group and the aromatic amino group para to it. Aside from geometric configuration, probably the most important property of a group is its positive or negative character. This is reflected in an amino group by its basic, and in a carboxyl group by its acidic, properties. Since many of the sulfanilamide derivatives contain both an acidic sulfonamide, and a basic para amino group, this work was undertaken to compare their physical properties with the corresponding groups in *p*-aminobenzoic acid. Dissociation constants were selected because they provide a readily measurable property which furnishes a direct indication of the positive or negative character of the groups.

II. Acid and Base Dissociation Constants (Qualitative Discussion).—During this investigation, the acid and base constants of over one hundred sulfonamides and related compounds were determined. A number of the values obtained are recorded in Table I. To conserve space, only a fraction of the total number of compounds studied is listed in this table. The results on the derivatives which are not recorded confirmed the conclusions drawn from the reported data. Methods employed for the determination of acid and base constants are described under Experimental. For convenience, the compounds are listed according to the classification of Northey.[11] The term C_R in Table I represents the minimum molar concentration necessary to cause bacteriostasis of *E. coli* in a buffered (*p*H 7) synthetic medium under standardized condition; thus, the smaller the number, the greater the activity. It is difficult to obtain a high precision in such tests, and small differences in bacteriostatic activity (factor of 2) are within experimental error.

Only brief consideration need be given to the base constants. The range of basic strength of the aromatic para amino group of practically all the sulfonamides studied was small [$(0.5–2.3) \times 10^{-12}$]. However, in order to establish this small range, it was necessary to make base constant determinations on nearly all the compounds studied. Several of the first base dissociation constants listed in Table I are greater than 2.3×10^{-12}. In these cases it is possible to show, by comparing the basic strength of the corresponding acetyl and benzene sulfonamide derivatives, that the first base constant probably does not represent the aromatic para amino group. Neither is the second base constant for these compounds a true measure of the basic strength of the aromatic amino group, because of the presence of the ion resulting from the determination of the first base constant (see Experimental).

The ionization constant for the basic amino group in *p*-aminobenzoic acid is approximately 2.6×10^{-12}.[12] To the extent that all the active sulfanilamide derivatives have identical unsubstituted para amino groups with constants which are close to this value, the base constants may be an important factor in bacteriostatic activity. But due to the very small variations in basic strength, no relation between these constants and bacteriostatic activity could be found.

Acid dissociation constants, which vary over a wide range ($< 10^{-11}$–10^{-3}), present an entirely different picture. The relationship between the *in vitro* activity of N^1-substituted sulfanilamide derivatives and their acid strength is shown in Fig. 1 (data from Table I). For convenience in plotting the curve, log $1/C_R$ is used rather than C_R. An examination of this figure indicates that as the pK_a of the sulfonamides increases, the bacteriostatic activity passes through a maximum and then decreases. In order to account for this phenomenon, several factors must be considered.

p-Aminobenzoic acid has been shown to be in the "non-zwitter ion" form in solution.[13] Never-

(10) Wyss, *Proc. Soc. Exptl. Biol. Med.*, **48**, 122 (1941); Wood, *J. Exptl. Med.*, **75**, 369 (1942).

(11) Northey, *Chem. Revs.*, **27**, 85 (1940).

(12) Winkelblech, *Z. physik. Chem.*, **36**, 546 (1901), reported 2.3×10^{-12}.

(13) Harris, *Proc. Roy. Soc.* (London), **97B**, 364 (1925); **104B**, 412 (1929); *Biochem. J.*, **24**, 1080 (1930).

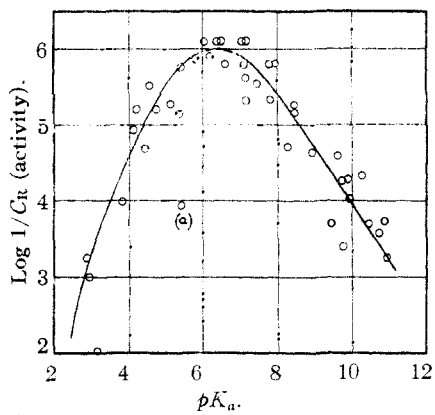


Fig. 1.—Relation of *in vitro* activity to acidity. Limits of error in log $1/C_R$ = ±0.3.

theless, it is a sufficiently strong acid so that in dilute solution in a medium buffered at *p*H 7, the carboxyl group is better than 99% ionized. The neutral solution is particularly important, since the form in which the compounds exist in bacterial culture media or in body fluids is of primary interest. Under these conditions, *p*-aminobenzoic acid consists of a benzene ring containing an NH_2 group, para to which is an ionic group having two very negative oxygens. Geometrically, these two oxygens are about 2.3 Å. apart,[14] while sulfones and sulfonamides both have a group similar to the CO_2 ion, namely, an SO_2 group, containing two negative oxygens[15] with an oxygen-oxygen distance of approximately 2.4 Å.[16] In general, the *p*-aminobenzene sulfonyl groups of the sulfones and sulfonamides are geometrically very similar in dimensions to the *p*-aminobenzoic ion as illustrated in Fig. 2.

Nearly all the amino groups of (a), (b) and (c), Fig. 2, have been shown to have base constants of the same order of magnitude. Consequently, the differences in the SO_2 groups of the various sulfonamides, compared with the CO_2 ion, must now be considered as a possible determining factor in the relative bacteriostatic activity of these compounds. Dipole moment studies have shown the SO_2 to be a relatively negative group.[15] However, the CO_2^- should be more negative than the SO_2, because the ion actually carries an electronic charge. It seems logical then, that the more negative the SO_2 group, the more closely it will resemble the CO_2 ion. On the basis of this reasoning, the theory which we shall attempt to develop may be stated as follows: *the more negative the SO_2 group of a sulfanilamide type compound, the greater the bacteriostatic activity of the compound.*

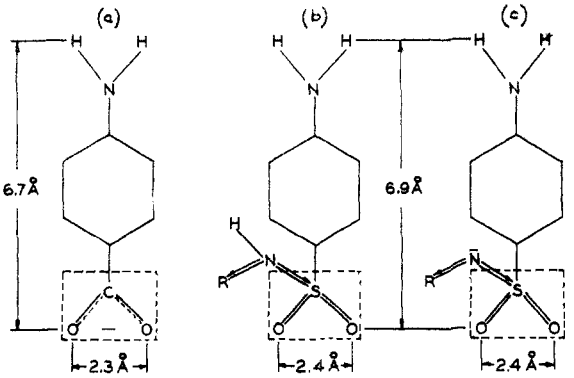


Fig. 2.—Geometric configurations.

The problem now becomes one of evaluating the relative negative character of the SO_2 group of the various sulfonamides in terms of their acid constants, to determine to what extent this property is related to bacteriostatic activity. Since the R group (Fig. 2) is the only variable involved in the N^1-substituted derivatives, it must be the factor controlling the acid constants of these compounds. For R to be acid strengthening, it must be an electronegative (electron attracting) group.[17] Under these conditions, R attracts electrons from the adjacent amide nitrogen so that the hydrogen can escape as a proton in solution, leaving the anion represented by form (c). The acid constants furnish an excellent indirect measure of the relative electronegative character of R, because any change in the acid strength of the sulfonamides should be proportional to the change in the electronegativity of the R group.

Since we are concerned with the form of the sulfanilamide type compounds in a medium buffered at *p*H 7, the effect of acid strength (as regulated by the properties of the R group) on the quantities of the molecular and ionic forms ((b) and (c), Fig. 2) existing at this *p*H must be considered. The fraction ionized, x, for any acid is given by the equation

$$x = \frac{K_a}{K_a + [H^+]} \text{ or at } p\text{H } 7 \quad x = \frac{K_a}{K_a + 10^{-7}} \qquad (1)$$

Using this equation, a plot of pK_a *versus* the fraction ionized may be made (Fig. 3). At *p*H 7, compounds which are strong acids (pK_a up to

(14) Zachariasen, *Phys. Rev.*, **53**, 917 (1938).

(15) Bergmann, *Ber.*, **65**, 457 (1932); Kumler and Halverstadt, THIS JOURNAL, **63**, 2182 (1941).

(16) Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 2nd ed., 1940

(17) The term electronegative refers throughout to the electron attracting power of a group.[16] Negative is used to indicate the relative electron density or negative charge around a group.

5–6) are practically completely ionized, weaker acids (pK_a = 6–12) are partly ionized, and the very weak acids (pK_a > 12) are almost entirely in the un-ionized form.

With the amounts of the molecular and ionic forms of any given sulfonamide known, the relative activities of the two species must be considered on the basis of the assumption that the bacteriostatic activity is proportional to the negative character of the SO_2 group. The ionic form has an electronic charge on the amide nitrogen, as shown in (c) (Fig. 2). This electronic (negative) charge *increases greatly* the negative character of the adjacent SO_2, since the SO_2 group is also electron attracting and acquires part of the ionic charge from the amide nitrogen. Consequently, *the SO_2 group of any sulfanilamide derivative in the ionized form (c) is much more negative than the SO_2 of the same compound in the un-ionized form (b). Therefore, the ionic form of any sulfonamide should be much more active than the molecular form.*[18]

The effect of the R group (Fig. 2) on ionization, and the relative activity of the ionic and molecular forms, have been discussed. Remaining to be considered is the effect of different R groups on the negative character of the SO_2 group. Consider first the ionic form (c). As the electron attracting power of R increases, the SO_2 group should become less negative because, under these conditions, a greater part of the ionic charge of the amide nitrogen will be taken by the R group which will be competing more strongly with the SO_2 for the ionic charge. The arrows in Fig. 2 illustrate the competition between the attracting forces of these two groups for the ionic charge on the adjacent amide nitrogen. As the SO_2 becomes less negative, its ion should be less active than an ion whose R is a weaker electron attracting group. Applying the same reasoning to the molecular form (b), we arrive at similar conclusions concerning the relative negativity of the SO_2 groups. Since the electron attracting power of R is proportional to the acid strength, it follows that, *the more acidic the sulfonamide, the less negative the SO_2 group of the ionic and molecular forms and the less the bacteriostatic activity of either form.* It should be recalled here that up to a certain point this decrease in activity with increasing acid strength is more than compensated for by the increasing proportion of highly active ions. Because the ions are much more active than the corresponding molecules, the over-all effect of increasing acid strength produces an increase in activity up to the point where the sulfonamides are largely ionized. Further increases in acid strength are not accompanied by a proportionate increase in the number of ions. The predominant effect beyond this point should be the decreasing negative character of the SO_2 group, accompanied by decreasing activity. Consequently, a maximum would be expected in the curve relating pK_a to bacteriostatic activity (compare Fig. 1).

It is also possible for the electron attracting power of R to change until it is actually an electron donor group. Under these conditions, R should repulse electrons toward the SO_2 group. As a result, the SO_2 group would become more negative. The acid strength also reflects the electron donor power of R. However, these compounds can become such weak acids that the effect of the highly active ions is negligible, and as a result the bacteriostatic effect should be of a lower order of magnitude. Even so, as pointed out above, the activity of the un-ionized forms should show a continuous increase as the acid strength

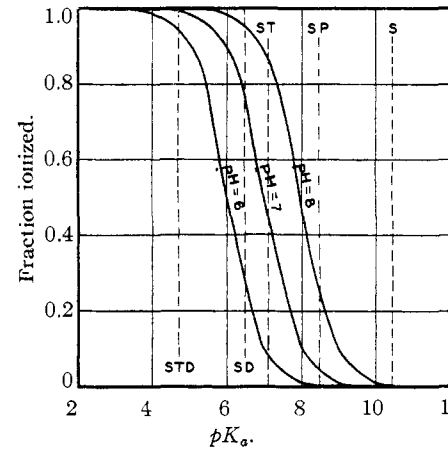


Fig. 3.—Acid ionization in buffers (nos. from Table I): S, sulfanilamide (2); sulfapyridine (15); ST, sulfathiazole (34); SD, sulfadiazine (23); STD, sulfathiadiazole (38).

(18) During the preparation of this manuscript, two papers appeared in which experimental evidence supporting this conclusion is reported. See Fox and Rose, *Proc. Soc. Expll. Biol. Med.*, **50**, 142 (1942); Schmelkes, Wyss, Marks, Ludwig and Sandskov, *ibid.*, **50**, 145 (1942). However, the present theory is not in accord with the conclusions of these authors that the ionic form of sulfanilamide derivatives is the only active form. For example, such a conclusion fails to account for the activity of sulfones, sulfaguanidine and N^1-disubstituted sulfanilamide derivatives, none of which exist as ions in solution, although they are also inhibited by *p*-aminobenzoic acid. Furthermore, we do not agree with Fox and Rose that the ions of different derivatives are equally active. Actually, the experimental data in both of these papers appear to support the view that the ions of the stronger acids are less active. Moreover, the idea of equal activity for all ions obviously is not in agreement with the experimental data for strong acids as shown in Fig. 1. This phase of the problem is considered in the subsequent discussion.

decreases. Thus, when the ionic form can be neglected, the curve relating pK_a to bacteriostatic activity should pass through a minimum and then increase as the acid strength decreases. Because compounds in which R is an electron donor group are extremely weak acids, it is not possible to determine their dissociation constants in aqueous solution. Evidence such as the relatively high bacteriostatic activity of sulfaguanidine, too weak an acid to be measured in aqueous solution, strongly suggests that a minimum may also be found in the experimental curve. Possibly studies in a basic solvent such as liquid ammonia would furnish quantitative data with which to fit these sulfonamides into this general picture.

III. Acid Constants (Quantitative Discussion).—In order to obtain optimum activity among acidic sulfanilamide derivatives, the problem is apparently one of obtaining a proper balance between the acid strengthening effect of the R group (Fig. 2) and the formal ionic charge on the sulfonamide nitrogen to give the maximum over-all negative character to the SO_2 group. Branch and Calvin[19] have shown that the dissociation constant of an organic acid can be predicted quantitatively by an equation of the type

$$\log K = \log K_a + \Sigma I_R \alpha^i \qquad (2)$$

where K_a is the acid constant of the parent acid, I_R the inductive constants for each atom or group other than hydrogen, α the fraction that reduces the inductive effect for the transmission across each bond, and i the number of bonds through which the effect must be transmitted. I_R multiplied by 2.3 RT then becomes a potential and has the units of free energy.

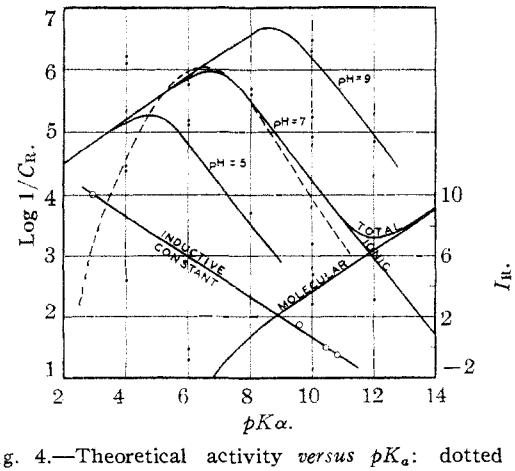


Fig. 4.—Theoretical activity *versus* pK_a: dotted line represents experimental activity from Fig. 1.

(19) Branch and Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1941.

Assuming that bacteriostatic activity is proportional to the potential of the SO_2 group, then, when the total activity is due almost entirely to the highly active ions (pK_a 2–11)

$$2.303\ RT \log (k/xC_R) = 2.303\ RT\ [\alpha(12.3 - I_R\alpha) - I_R\alpha^2] \qquad (3)$$

where C_R = minimum molar concentration of a sulfonamide required to exhibit a given bacteriostatic activity, attributing all activity to the ions, x = fraction of the total concentration of the compound in ionic form, k = proportionality constant (determined experimentally), to adjust the potential energy of the SO_2 to experimental conditions, I_R, α, as defined for equation (2), $(12.3 - I_R\alpha)$ = inductive effect of the ionic charge reduced by the effect of I_R on it, $I_R\alpha^2$ = inductive effect of R on SO_2 directly.

As a first approximation, resonance and polarization effects were neglected, and α was taken as $1/2.3$ (the value of Branch and Calvin for a covalent bond); then

$$\log \frac{k}{xC_R} = 4.04 - 0.255\ I_R \qquad (4)$$

Using Branch and Calvin's inductive constants for various radicals, it was found that I_R was a linear function of the pK_a values of the corresponding sulfanilamides.

From Fig. 4

$$I_R = -1.33\ pK_a + 13.88 \qquad (5)$$

also, for any acid at pH 7 (where the *in vitro* tests were made)

$$pK_a = 7 - \log x/(1 - x) \qquad (6)$$

Substituting (5) and (6) in equation (4), we have

$$\log 1/C_R + \log k = 3.23 + 0.661 \log x + 0.339 \log (1 - x) \qquad (7)$$

at conditions of maximum activity ($\log 1/C_R$ = max.)

$$\frac{d \log 1/C_R}{dx} = 0 = \frac{0.661}{x} - \frac{0.339}{1 - x}$$

$x = 0.661$ at maximum activity

This corresponds to a sulfanilamide derivative with a pK_a of 6.7 as calculated by equation (6), and agrees very well with the experimentally observed maximum (Fig. 1). This pK_a value of maximum activity is independent of the *in vitro* tests and depends only on the inductive effects of the R groups.

Using the experimental maximum activity, $\log 1/C_R = 6.1 \pm 0.3$, k may be evaluated by substi-

tuting $\log 1/C_R = 6.1$ and $x = 0.661$ in equation (7), and solving for k.

The final ion activity equation is then

$$\log 0.001/C_R = 3.23 + 0.661 \log x + 0.339 \log (1 - x) \quad (8)$$

A similar equation may be derived for the activity of the un-ionized form

$$\log \frac{0.001}{(1 - x)C_R} = -1.3\alpha - 2 I_R\alpha^2 \quad (9)$$

from which it can be shown that its contribution toward activity will be small except for very large pK_a values.

Combining equations (9) and (4) we may show that

$$\log \frac{(1 - x)\ C_R\ (\text{un-ionized})}{x\ C_R\ (\text{ionized})} = 4.85 \quad (10)$$

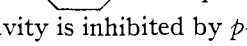
which means that any ion is approximately $10^{4.85}$ times more active than the corresponding molecule. Now, if C_R (un-ionized) = C_R (ionized)

$$\log (1 - x)/x = 4.85$$

and pK_a must be 11.85 to fulfil this condition. At this pK_a, log 2.0 should be added to $\log 1/C_R$ from equation (7). In this way the ion activity curve was corrected for the molecular activity at pK_a's greater than 10, and the total activity curve drawn as shown in Fig. 4.

The theoretical curves of Fig. 4 at pH 5 and 9 show how the pK_a of maximum activity may shift if the pH of the medium changes. At pH 7 the experimental and theoretical curves are in very good agreement from pK_a 10–5, but at lower pK_a values the compounds are less active than predicted. Probably the most important factor contributing to this deviation is the limitations in the development of the theory. As pointed out by Branch and Calvin, an exact equation of the type of equation (2) should contain summation terms for the polarization and resonance, as well as the inductive effect. While polarization is probably a function of pK_a, the relationship cannot be readily established experimentally. On the other hand, resonance is probably dependent on the specific character of the R group, and no general relationship between resonance and inductive effect can be established. In equation (3) polarization and resonance have been neglected, and therefore α is not equal to 1/2.8 (the simple covalent bond value) unless these effects are small. Where R is not too electronegative (weaker acids) the value for α probably is a good approximation; however, as R becomes strongly electronegative, the SO_2–N–R bonds should become more ionic, and α should be greater than 1/2.8. Such an increase in α would give better agreement between the theoretical and experimental curves in the low pK_a range.

This treatment also takes no account of the inductive effects on the p-amino group. The very acid compounds, in general, have less basic p-amino groups. In the extreme cases there appears to be considerable "zwitterion" formation, as indicated in the case of sulfanilylcyanamide by titrations in formaldehyde solution. Any large variation in the character of the p-amino group compared with the corresponding group in p-aminobenzoic acid may be expected to result in a decrease in activity.

IV. Relation of Structure to Activity.—The preceding discussion has been concerned primarily with an attempt to show how both structure and activity can be related to a common denominator, namely, the negative character of the SO_2 group, and how acid dissociation constants can be used to evaluate the relative negativity of the SO_2 group. Of necessity, this discussion has been limited largely to N^1-monosubstituted sulfanilamide derivatives, since they are the only active compounds on which measurements of the acid strength of the sulfonamide group can be made. Nevertheless, we believe that the principles outlined above apply to any substance of the type  , provided its bacteriostatic activity is inhibited by p-aminobenzoic acid. If the properties of the NH_2 are constant, the activity should depend on the relative negative character of the XO_2 group. In addition to sulfonamides and sulfones, the type formula should include compounds in which X is phosphorus, arsenic, selenium, or other elements.

The term "sulfanilamide type compounds," as used throughout this paper, is limited to derivatives of the type shown above. This limitation, of course, excludes N^4-substituted compounds; orthanilamide, metanilamide and their derivatives; nuclear substituted compounds; and substances in which the NH_2 or XO_2 groups are separated from the benzene ring by an alkyl, aryl or other radical. N^4-Substituted compounds have not been considered because it now appears to be generally accepted that these derivatives are active chemotherapeutic agents only after the N^4-substituent has been removed by some bio-

chemical process. First demonstrated by the Tréfouël's and their co-workers[20] for the original "Prontosil," this process has since been confirmed many times for a number of different N^4-substituents.

The mode of action of sulfanilamide type compounds proposed by Woods[5] and Fildes[6] should exclude all the types of compounds listed above on the basis of steric effects. The high degree of specificity of enzyme systems is well recognized. If the action of sulfonamides is based on the competitive inhibition of an enzyme system normally requiring *p*-aminobenzoic acid, then in order to be effective, a compound should have a spacial configuration as closely resembling that of the normally required substance as possible. Obviously, a substituted amino group no longer resembles a free NH_2 from this standpoint.[21] Similarly, orthanilamide and metanilamide derivatives, nuclear substituted compounds, and substances in which the groups are separated from the benzene ring, are much less closely related sterically to *p*-aminobenzoic acid than the compounds formulated above. Perhaps the most convincing argument in favor of this hypothesis is the experimental evidence that up to the present, so far as we are aware, no compounds of the types excluded have been reported to show bacteriostatic activity *per se*. In general, compounds not inhibited by *p*-aminobenzoic acid are considered to be outside the scope of the present theory.[22]

It should now be possible to generalize on the effects of various types of N^1-substituents on bacteriostatic activity, using sulfanilamide as the basis for comparison. Among these compounds all degrees of potency may be found, although none of them appears to be completely inactive. In the following discussion groups are referred to hydrogen which is considered as neutral. An electronegative substituent is one which tends to acquire electrons at the expense of the group to which it is attached. Such a group makes the resulting sulfanilamide derivative a stronger acid. By the same criterion, an electropositive substituent tends to donate electrons and form less acidic sulfonamides.

Alkyl groups are slightly electropositive and acid weakening. Hence, their effect in the region of weak acids should be to reduce the activity slightly (see Table I, nos. 3 and 5). Chain length should not alter the effect appreciably, as evidenced by the nearly constant acidities of the fatty acids. From a practical standpoint, of course, long chains may reduce solubility to a point where the bacteriostatic power can no longer be demonstrated. Introducing a strong electron attracting radical on the α-carbon atom of an aliphatic acid makes the group more electronegative, while further along the chain such a substituent has a much smaller effect.[19] Thus, an electronegative group (*e. g.*, halogen) in the α-position of an N^1-alkylsulfanilamide derivative should increase activity, but the effect in any other position should be relatively weak.

A second N^1-alkyl group should not exert much influence, since the monosubstituted compounds are too weakly acidic to ionize appreciably (*cf.* nos. 3 and 4). The effect of an alkyl group on another substituent such as a heterocyclic ring is also slightly acid weakening (*cf.* nos. 23, 24 and 25). For the purposes of this discussion, saturated rings (carbocyclic or heterocyclic) would be classified as alkyl substituents. Similarly, from the standpoint of their slightly electropositive character, aromatic or heterocyclic groups separated from the N^1-nitrogen by one or more methylene radicals should be considered as substituted alkyl groups (*e. g.*, no. 14).

Aromatic rings are slightly electronegative. Consequently, the activity of these derivatives, in general, should be somewhat greater than that of sulfanilamide, because they are relatively stronger acids. Moreover, since the electron attracting power of the ring may be increased or decreased considerably by substituents, the N^1-aromatic substituted compounds show rather large variations in bacteriostatic power. As might be anticipated, substituents in the ortho and para positions exert a more pronounced influence than in the meta position. For example, the electronegative sulfonamide group in N^4-sulfanilylsulfanilamide (no. 12) increases the activity over that of the unsubstituted phenyl, while in the meta position (no. 11) the effect is smaller. On the other hand, the electropositive amino group in the para position (no. 13) reduces activity.

Heterocyclic substituents show by far the greatest variation in electronegativity. As a result,

(20) Tréfouël, Tréfouël, Nitti and Bovet, *Compt. rend. soc. biol.*, **120**, 756 (1935).

(21) This effect is in contrast to an N^1-substituent which in most cases probably has very little influence on the spacial configuration of the SO_2 group.

(22) For example, *p,p'*-diaminodiphenyl sulfide has bacteriostatic activity, but its mode of action evidently is quite different since it is not inhibited by *p*-aminobenzoic acid (W. H. Feinstone, personal communication).

the potency of the N^1-heterocyclic sulfanilamide derivative varies widely. The bacteriostatic activity of this class of compounds increases to a maximum and then falls off as the acidity of the compounds increases (see Fig. 1). As demonstrated in Part II above, the effect of increasing ionization appears to be more than counterbalanced by a decrease in the negative character of the SO_2 group, when the sulfonamides become too strongly acidic. Consequently, compounds such as nos. 36 and 38 are stronger acids, but less potent than derivatives containing two hetero-atoms (nos. 32 and 34). Conversely, derivatives with only one hetero-atom are too weakly acidic, and again show less activity. In most cases, two hetero-atoms in the ring appear to promote optimum bacteriostatic power. Introducing substituents in the heterocyclic nucleus affects the activity differently, depending on the acidity of the unsubstituted derivative. If the compounds are too weakly acidic, an electronegative group (*e. g.*, halogen, as in the bromopyridines, nos. 17 and 18) increases their potency. But, an electropositive amino group in the pyridine ring reduces activity (nos. 19 and 20). On the other hand, when the unsubstituted N^1-heterocyclic derivatives are too strongly acidic, the presence of an electron donor group increases bacteriostasis (no. 39), while an electronegative group should cause the activity to decrease.

The carbonic acid derivatives such as sulfanilylurea (no. 41) and sulfaguanidine (no. 42) present interesting differences. The urea derivative is quite acidic, whereas sulfaguanidine is too weak an acid to be measured in aqueous solution. Sulfanilylurea is considered in the discussion of exceptions below. At first, the guanidines may also appear to be out of line with the theory. But, guanidine should be an electron donor group, since it is a very strong base. Consequently, this compound and others of the same type should fit somewhere beyond the minimum point on the high pK_a side of the total activity curve of Fig. 4.

Acyl and sulfonyl groups are strongly electronegative. Practically all of the sulfanilamide derivatives of this class are too acidic to show maximum bacteriostatic power. An electron attracting group in the α-position further reduces the activity (*cf.* nos. 44 and 45). The sulfonyl group, being more strongly electronegative than the acyl group, has an even greater tendency to reduce the bacteriostatic effect (*cf.* nos. 47 and 49).

A second N^1-substituent of any type, on a sulfanilamide derivative which is capable of appreciable ionization, should cause a pronounced decrease in the activity. Introducing such a group completely blocks the formation of the more potent ionic form, which in turn should result in a considerable reduction in the total activity. The *in vitro* data available in the literature appear to support this conclusion.[4,11]

A certain number of exceptions to any theory relating to biological phenomena which involve unknown variables are to be expected. So little is known about the mechanism of the inhibition of enzyme systems, that all the factors cannot be evaluated with our present knowledge. Considering the large group of compounds studied, the exceptions are not sufficiently numerous to detract from the general trend. Moreover, while positive exceptions (*i. e.*, compounds more active than predicted) would invalidate the preceding discussion, negative exceptions are less disconcerting. In Fig. 1 there is one point (a) which falls outside the possible limits of experimental error for the bacteriostatic tests. The compound sulfanilylurea (no. 41) is considerably more active during the first part of the test period than at the standard end-point. A plausible explanation for the low activity of the urea derivative is that it may be slowly broken down by the bacteria to sulfanilamide or some other less active substance. The fact that there is a biological mechanism for the breakdown of urea supports this supposition.

There are two other compounds listed in Table I (nos. 6 and 31) which, although definite values for bacteriostatic activity were not obtained, appear to be negative exceptions to the theory. In the case of sulfanilylglycine (no. 6), the first acid constant undoubtedly represents the carboxyl group. The second constant, which should correspond to the sulfonamide group, is too weak to be measured in aqueous solution. In general, it is possible that compounds with amino and carboxyl substituents may be less active than predicted. These substituents, in particular, may cause an improper orientation of the molecule in an enzyme system, due to their identity with the groups in *p*-aminobenzoic acid. No adequate explanation for the low activity of 4-sulfanilamido-1,2,4-triazole (no. 31) can be given at present. Undoubtedly there are other exceptions in addition to the ones encountered in this investigation, but it seems possible that practically all negative ex-

ceptions might find simple explanations if one could account for all the factors involved.

V. Implications of the Proposed Theory.—An example illustrates to what extent the proposed theory enables one to make predictions. When the curve in Fig. 1 was first plotted, there were no compounds available with a pK_a of approximately 4. Since there were fewer points on this side of the curve, it seemed desirable to strengthen the evidence by the preparation of a sulfonamide of this type. From the well-known electronegative character of the chloroacetyl group, it was possible to predict, before the compound had been synthesized, that N^1-chloroacetylsulfanilamide[23] would have a pK_a of about 4, and consequently a bacteriostatic activity of approximately 10×10^{-5} (*cf.* Table 1).

In general, we believe that sufficient data are now available so that, without the aid of physical measurements, the relative electronegativity of any substituent group can be approximated from its molecular structure. Thus, for the first time, a relationship between structure and activity is established which enables one to predict the bacteriostatic effect of any new N^1-substituted sulfanilamide derivative. Furthermore, it is possible that the approach used in this study may find application to similar investigations among other types of chemotherapeutic agents, particularly those depending on enzyme inhibitions for their activity as proposed by Fildes.[8]

From the biological standpoint, a number of interesting implications are evident. It should be possible, for instance, to show a reversal in the order of activity of a selected series of compounds with changes in *p*H. This effect would be predicted on the basis of the relative change in the proportion of ions to molecules. In going from a lower to a higher *p*H this ratio does not change appreciably for a strong acid, while a weaker acid shows an appreciable increase in the ratio of ions to molecules. Conversely, passing to a lower *p*H has the opposite effect on this ratio. If organisms such as *E. coli* can be grown from *p*H 5–9 on a synthetic medium, it is possible to design an experiment to test this prediction. The relative activity of the three compounds, sulfathiadiazole (no. 38, $pK_a = 4.77$), sulfathiazole (no. 34, $pK_a = 7.12$) and sulfapyridine (no. 15, $pK_a = 8.44$), at *p*H 7 is in the order sulfathiazole > sulfathiadiazole = sulfapyridine (Table I). But at *p*H 5 the relative activities should change so that sulfathiadiazole > sulfathiazole > sulfapyridine, and at *p*H 9 the order should be sulfapyridine > sulfathiazole > sulfathiadiazole (Fig. 4). Smaller *p*H ranges probably would not be sufficient to overcome the rather large limits of error inherent in the bacteriostatic tests.[24]

Perhaps the most important implication based on both experimental and theoretical considerations is that the optimum in bacteriostatic activity of N^1-substituted sulfanilamide derivatives appears to have been reached. The maximum in the experimental curve, and the limitations on the negative character of the SO_2 group imposed by the conflicting effects of increasing acidity, both point to such a conclusion. This, of course, does not mean that better chemotherapeutic agents of this type, from the standpoint of lower toxicity or differences in absorption and excretion and other factors of practical importance, are not possible, but it does suggest that inherently more active sulfanilamide derivatives are not likely to be found.

Experimental

Materials.—All inorganic and common organic chemicals were of "Analytical Reagent" grade. Aniline was freshly distilled from zinc dust (water white, b. p. 94–95° (25–30 mm.)). Hydrogen gas (electrolytic, from pressure tanks) was further purified by passing it through an alkaline pyrogallol solution, dilute potassium permanganate and concentrated sulfuric acid. Chloranil, m. p. 298–300°; *m*-nitroaniline, m. p. 112–113°; *p*-nitroaniline, m. p. 146–147°; tetrachlorohydroquinone, m. p. 234–236°; *p*-toluidine, m. p. 44–45°; and triethylamine, b. p. 88–90°, were all obtained from Eastman Kodak Co., and further purified when necessary. The compounds listed in Table I were prepared in these Laboratories or obtained from the Calco Chemical Division, American Cyanamid Co.

Apparatus.—Electromotive force (e. m. f.) measurements were made with a Leeds and Northrup no. 7660 vacuum-tube meter. Acid constants in 50% ethyl alcohol were measured with a Beckman (Model G) *p*H meter, employing a glass electrode and a saturated potassium chloride-calomel electrode. Hydrogen and calomel electrodes were of standard type. Measurements in acetic acid were made using a chloranil electrode[25] and a calomel reference electrode. The two electrodes were connected by a salt bridge, fitted with ground glass joints, and containing acetic acid saturated with lithium chloride.[26] Two smooth platinum inert electrodes were used for the chloranil part of the cell. These electrodes were flamed before each immersion and all readings discarded in which the two electrodes failed to give the same e. m. f.

(23) English, Chappell, Bell and Roblin, THIS JOURNAL, **64**, 2516 (1942).

(24) Work is now in progress in these Laboratories to determine whether or not such an experiment can be carried out.

(25) Hall and Werner, THIS JOURNAL, **50**, 2367 (1928).

(26) Hall and Conant, *ibid.*, **49**, 3047 (1927).

The conductance bridge was made by using a Kohlrausch bridge, a Leeds and Northrup no. 4750 resistance box, and a Freas type conductance cell. This bridge was balanced by amplifying the signal with a General Radio no. 814-A amplifier and obtaining the null point from the minimum observed on a General Radio no. 726-A vacuum-tube voltmeter

Acid Constant Determination.—The N^1-substituted sulfanilamide derivatives are amphoteric, since they contain a basic amino, as well as the acid sulfonamido, group. Formaldehyde titrations indicated that all the sulfanilamides used in this study, except the very acidic ones, were in the "non-zwitterion" form. Hence, the usual weak acid and weak base theories could be applied without considering the other group. The acid constants were determined from the pK_a values obtained from 0.05 N NaOH electrometric titration curves (hydrogen electrode). Experimentally, it was impossible to measure all the compounds by this method, since some were extremely insoluble in water, and, because of their high molecular weights, the pK_a values were not significant.

For the very insoluble sulfanilamides it was possible to use 50% ethanol as the solvent. It has been shown that in methanol–water and ethanol–water mixtures, acid constants, within each class of acids, exhibit roughly equal changes in any given solvent mixture.[27,28] It was found that compounds in the sulfanilamide series, which were measurable in water, when measured in 50% ethanol gave a smooth curve of $pK_{a(H_2O)}$ versus $pK_{a(50\%\ EtOH)}$ as shown in Fig. 5. From this curve, it was possible to determine the acid constants, for compounds in the same series, from $pK_{a(50\%\ EtOH)}$ measurements. The alcohol values in Table I have all been corrected, by means of Fig. 5, so that they may be compared directly with the values obtained in water.

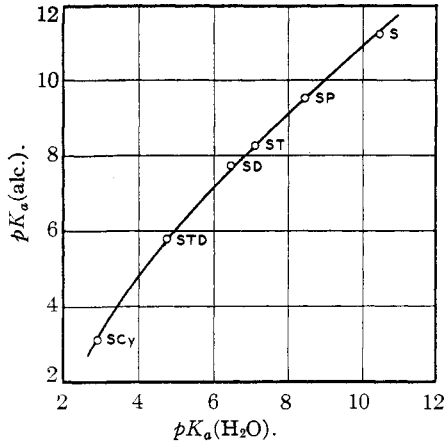


Fig. 5.—Standardization curve for 50% ethanol titrations (nos. from Table I): S, sulfanilamide (2); SP, sulfapyridine (15); ST, sulfathiazole (34); SD, sulfadiazine (23); STD, sulfathiadiazole (38); SCy, sulfanilylcyanamide (40).

Using these methods, it was possible to determine the acid constants for sulfanilamides more acid than approximately $K_a = 2 \times 10^{-11}$. Titration of acids weaker than this did not give curves sufficiently different from a blank titration to be reliable.

Base Constant Determination.—The basic groups of the sulfanilamides were all weak (pK_b = 11–13) and the results of 0.05 N HCl titrations were not significant unless the compounds were quite water soluble. Sulfanilamide, metanilamide and p-aminobenzoic acid were carefully studied by this method, using a hydrogen electrode. The base constants of sulfanilamide and p-aminobenzoic acid were also determined from conductance measurements on their hydrochlorides.[29] These results agreed very well with the water titration values. It was not possible to obtain the base constants of other sulfanilamides by this method, because of the low solubility of the free bases in water.

Using an acidic solvent such as 100% acetic acid, it was possible to increase the basic properties of these very weak bases and titrate them with a very strong acid. The chloranil electrode, which is reversible in 100% acetic acid,[26] was used to follow a titration of the base with 0.0835 N perchloric acid. Because of the low dielectric constant of the solvent, it was necessary to maintain a constant ionic strength. All the solvent had enough neutral triethylammonium perchlorate added to give an ionic strength of 0.2.[30] Employing the method of Hall[31] a standardization curve of $pK_{b(H_2O)}$ *versus* e. m. f. of chloranil electrode, at the $pK_{b(HAc)}$ point in the titration, was made as shown at Fig. 6.

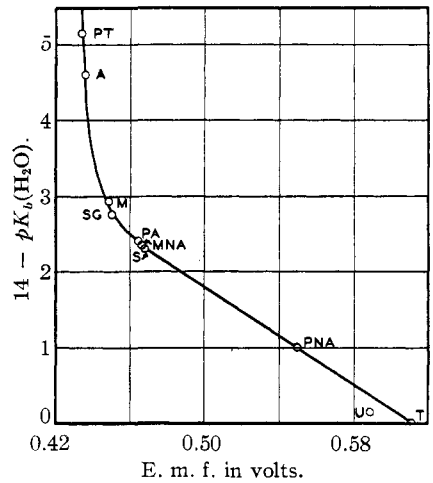


Fig. 6.—Standardization curve for acetic acid titrations (nos. from Table I): e. m. f. is that of a chloranil electrode *vs.* satd. calomel at pK_b (HAc) point of the titration: PT, p-toluidine; A, aniline; M, metanilamide; SG, sulfaguanidine (42); PA, p-aminobenzoic acid (1); MNA, m-nitroaniline; S, sulfanilamide (2); PNA-p-nitroaniline; U, urea; T, theory.

The $pK_{b(H_2O)}$ values for the compounds used to establish the points of the curve in Fig. 6 are all

(27) Michaelis and Mizutani, *Z. physik. Chem.*, **116**, 135–159 (1925).

(28) Mizutani, *ibid.*, **118**, 318–326 (1925).

(29) MacInnes, "The Principles of Electro-chemistry," Reinhold Publishing Corp., New York, N. Y., 1939.

(30) Conant and Werner, THIS JOURNAL, **52**, 4436–4450 (1930).

(31) Hall, *ibid.*, **52**, 5115–5128 (1930).

from the literature[32,33] except sulfanilamide, metanilamide and sulfaguanidine which were soluble enough to be measured in water, as outlined above. By means of the curve in Fig. 6, the K_b values in Tables I and II were obtained: the first K_b from the e. m. f. given when one-half equivalent of perchloric acid had been added and the second K_b when one and one-half equivalents had been added.

It should be pointed out that the second K_b obtained in this manner is not a measure of the group's basic character as it normally exists in a neutral solution, unless the stronger basic group is strong enough to be ionized at *p*H 7. The effect of ionic charge, depending on its sign, may either increase or decrease the apparent basic properties of the group being measured. A positive ion should decrease the negative character and the basic strength of the group, while a negative ion should have the reverse effect.

As pointed out above, the carboxyl group of *p*-aminobenzoic acid is better than 99% ionized at *p*H 7. The presence of the carboxyl ion may increase the basicity of the *p*-amino group somewhat over the value given in Table I, since this result was obtained in an acid medium where the carboxyl was not ionized. It is possible to show a relationship between the basic strength of substituted anilines and the acid constants of the N^1-sulfanilamides derived from them. From this relationship and the second K_a of N^1-*p*-carboxyphenylsulfanilamide (2nd pK_a = 8.24, for the sulfonamido group para to the completely ionized carboxyl) it may be possible to estimate the basic strength of the amino para to the carboxyl ion. Such indirect evidence suggests that the carboxyl ion of *p*-aminobenzoic acid increases the para amino basicity approximately threefold over the value obtained when the carboxyl group was un-ionized. If this is true, it is evident that the influence of a carboxyl ion on the base constant of *p*-aminobenzoic acid is relatively small.

The para amino groups of the sulfanilamide derivatives are the basic groups of primary interest. However, a number of the sulfanilamides contain two or more basic groups. The first K_b of Table I is only a true measure of the *p*-amino base constant when that group is the strongest in the molecule. When there was any question as to whether or not the paraamino was the strongest basic group in the polybasic compounds, it was checked by blocking the para amino group or removing it. In a large number of cases the benzenesulfonamido and N^4-acetyl derivatives (see Table II) were so much weaker bases than the corresponding *p*-amino compounds, that there was little doubt but that the para amino was the strongest basic group.

(32) Urea, *m*-nitroaniline, aniline, and *p*-toluidine: Landolt-Börnstein, "Tabellen," 5th ed., 1936.

(33) *p*-Nitroaniline: Farmer and Warth, *J. Chem. Soc.*, **85**, 1726 (1904).

TABLE II

BASE CONSTANTS OF SULFONAMIDE COMPOUNDS

Compounds[a]	$K_b \times 10^{13}$	Ref. (Table 1)
2-B-pyridine	5.0	*r*
2-A-pyridine	11.0	*d*
2-B-pyrimidine	0.14	*r*
2-B-4-methylpyrimidine	0.4	*r*
2-A-4-methylpyrimidine	0.8	*l*
4-A-1,2,4-triazole	3.2	*j*
2-B-thiazole	0.04	*r*
2-B-thiadiazole	very weak	*r*
Acetylsulfanilylguanidine[b]	26.0	

[a] B = benzenesulfonamido; A = acetylsulfanilamido. [b] Marshall, Bratton, White and Litchfield, *Bull. Johns Hopkins Hosp.*, **67**, 163 (1940).

In each of these cases the *p*-amino was a weaker basic group than the *p*-amino of sulfanilamide. This is consistent with the corresponding acid constants, since any N^1-substituent which is more electronegative than hydrogen should be acid strengthening and base weakening. Because of these facts it did not seem likely that the first K_b of Table I was the base constant of the desired *p*-amino group for compounds nos. 13, 15, 16, 19, 20, 22, 26, 27, 42, 43 and 47 (nos. from Table I).

Acknowledgment.—We are greatly indebted to Dr. W. Harry Feinstone, Dr. Herbert Florestano and Mr. Roger D. Williams for the bacteriostatic results reported in this paper.

Summary

Based on the experimental observation that the acid dissociation constants of N^1-substituted sulfanilamide derivatives are related to their chemotherapeutic activity, a theory of the relation of structure to activity of these compounds is proposed.

The acid and base constants of a large number of sulfanilamide type compounds have been determined. A plot of acid constants *versus* bacteriostatic activity, gives a smooth curve which passes through a maximum as the acid strength

increases. This correlation between acidic dissociation and activity is shown to be directly associated with the negative character of the SO_2 group. In brief, the theory may be stated as follows: the more negative the SO_2 group of an N^1-substituted sulfanilamide derivative, the greater is its bacteriostatic power.

The inductive constants of the various N^1-substituents have been evaluated. Based on this method, a quantitative treatment of the theory has been developed. The calculated value of the acid constant for optimum activity agreed very well with the experimental results. The relative activity of the ionic and molecular forms of the sulfonamides has also been predicted by this treatment.

Since acid constants are related to both the structure of the N^1-substituent and the activity of the derivative, an indirect correlation between structure and chemotherapeutic activity is established. Knowing something about the relative electron attracting power of the N^1-substituent, it is possible for the first time to predict the bacteriostatic power of any new sulfanilamide derivative of this type. A discussion of the relation of structure to activity, and a description of the proposed theory and its implications are given.

STAMFORD, CONN. RECEIVED JULY 31, 1942

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE JOHNS HOPKINS UNIVERSITY]

Mixed Heteropoly Acid Catalysts for the Vapor Phase Air Oxidation of Naphthalene[1]

BY HENRY TRUEHEART BROWN[2] AND J. C. W. FRAZER

Catalysts prepared from heteropoly acids were first used for the partial oxidation of naphthalene in the vapor phase by Marisic.[3] The chief products from this reaction were phthalic and maleic anhydrides together with traces of naphthoquinone and benzoic acid. The reader is referred to Marisic's paper for a discussion of other catalysts used in this reaction and of the reasons for investigating heteropoly acid catalysts.

The present investigation arose from a consideration of the catalyst prepared from ammonium phospho-vanado-tungstate,[3] which gave considerably higher conversions of naphthalene to phthalic anhydride than either vanadium pentoxide or tungsten oxide alone.

In view of the fact that (1) heteropoly acid ions have a cage-like structure[4] into which only groups of the right size can fit, *i. e.*, octahedrally coördinated molybdenum, tungsten, and vanadium oxide complexes, and that (2) mixed compounds of the phospho-vanado-tungstate type exist, it seemed desirable to attempt the preparation of mixed heteropoly acids which should vary in composition from 12-molybdosilicic acid to 12-tungstosilicic acid wherein the mixed anions should contain both tungsten and molybdenum. It was expected that a series of catalysts prepared from such acids should exhibit coactivation similar to that in ammonium phospho-vanado-tungstate.

(1) Condensed from a dissertation submitted by H. T. Brown to the faculty of The Johns Hopkins University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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(3) Marisic, THIS JOURNAL, **62**, 2312 (1940).

(4) Keggin, *Proc. Roy. Soc.* (London), **A144**, 75 (1934).

Experimental

Preparation of the Catalysts.—The general method employed in preparing the "mixed heteropoly acids" was a stepwise acidification of a solution containing tungstate, molybdate and silicate ions in the desired proportions and then an ether extraction of the product. This method was adapted from North's[5] preparations of 12-molybdosilicic acid with careful attention to all of the precautions which he mentions.

Catalysts were prepared from carefully purified acid crystals of 8–14 mesh size. These crystals were slowly heated in a stream of air to 400° and maintained at that temperature for one hour.

Preparations of the Heteropoly Acids.—Because of the importance of this step in the work, detailed directions will be given for preparing a typical "mixed acid" while directions for the others, together with their analyses, are summarized in Table I.

6-Molybdo-6-tungsto-silicic acid was prepared by dissolving 12.5 g. sodium molybdate and 17.1 g. sodium tungstate in 100 ml. of water heated to 65°. Then 5 ml. of concentrated hydrochloric acid was added dropwise with mechanical stirring, followed by 2.8 g. of sodium silicate solution (d. 1.375) diluted with a little water. Seventeen ml. of concentrated hydrochloric acid was added dropwise with vigorous stirring and the hot solution was filtered through asbestos to remove a slight precipitate of silica. After cooling, 22 ml. of concentrated hydrochloric acid was added and the clear solution was extracted with ether and the ether layer was purified as directed by North.[5] The

(5) North, in Booth "Inorganic Syntheses," McGraw-Hill Book Co., Inc., New York, N. Y., 1939, Vol. I, pp. 127–129.