for the heats of formation of liquid water and gaseous carbon dioxide.

Discussion of Results

Karrer and Fioroni[11] reported a value for the heat of combustion of glucose pentaacetate. On the basis of the 1942 value for the heat of combustion of benzoic acid and in units of the defined calorie, Karrer and Fioroni's value is 1722.4 kcal. per mole for $-\Delta U_B$. It is not clearly stated that these authors were dealing with α-D-glucose pentaacetate, but this is assumed to be the case. No value has been reported previously for β-D-glucose pentaacetate.

The following equation indicates that the acetylation of glucose with acetic acid is an endothermic reaction for both isomers.

D-glucose (s) + 5 acetic acid (l) = D-glucose pentaacetate (s) + $5H_2O$ (l)

α-isomer. ΔH = 6.32 kcal./mole
β-isomer, ΔH = 8.92 kcal./mole

The use of acetic anhydride, however, produces the acetylation with a considerable evolution of energy.

D-Glucose (s) + 5/2 acetic anhydride (l) = D-glucose pentaacetate (s) + $5/2H_2O$ (l)

α-isomer. ΔH = −30.88 kcal./mole
β-isomer. ΔH = −28.29 kcal./mole

The rather large exothermic value for ΔH in the latter reaction suggests that the use of the acetic anhydride will produce a favorable yield of the pentaacetates since the ΔF value will probably have the same order of magnitude. This is in harmony with experience.

In making the indicated calculations the following thermal values were employed. The values employed have been recalculated by the authors in terms of the defined calorie and based on the 1942 value for the heat of combustion of benzoic acid. This places all values secured by and utilized by the authors on a comparable basis. The heat of combustion of α-D-glucose is given as $-\Delta H_R$ = 669.81 kcal./mole.[12] Employing the values for carbon dioxide and water previously mentioned, the heat of formation was calculated to be −304.28 kcal./mole. The authors used the value obtained by Huffman and Fox for the heat of combustion of β-D-glucose of $-\Delta H_R$ = 671.33 kcal. per mole. Huffman and Fox state that this value is not of the highest precision, but it is probably very near to the true value. While its agreement with a value calculated from solution data is good, Huffman and Fox point out that the same type of irregularities may have complicated both measurements. The calculated heat of formation from the data of Huffman and Fox for β-D-glucose is −302.76 kcal. per mole. The heat of formation of acetic acid was calculated to be −116.20 kcal./mole, from the heat of combustion reported by Schjanberg.[13] The heat of formation of acetic anhydride was calculated to be −149.20 kcal./mole from the heat of combustion by Thomsen.[14]

While the conversion of β- to α-D-glucose is accompanied by a heat evolution of about 1.5 kcal./mole,[12] the conversion of β- to α-D-glucose pentaacetate is accompanied by a heat evolution of about 4.0 kcal./mole. How much of this heat effect is to be attributed to the β- to α-shift about the saccharide carbon cannot be estimated at present on the basis of information now available

Summary

Values for the heats of combustion at 25° and 1 atm. pressure are given for α- and β-D-glucose pentaacetate. The value for the specific heat at 25° is also reported. From these and other data, the heats of formation and heats of acetylation were computed.

(11) Karrer and Fioroni, *Helv. Chim. Acta*, **6**, 396 (1923).

(12) Huffman and Fox, THIS JOURNAL, **60**, 1403 (1938).

(13) Schjanberg, *Svensk. Kem. Tids.* **44**, 227–231 (1932).

(14) Thomsen, *Z. physik. Chem.*, **52**, 343 (1905).

PITTSBURGH, PA. RECEIVED MAY 14, 1943

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Mode of Action of Sulfonamides

BY IRVING M. KLOTZ

Since the discovery that *p*-aminobenzoic acid inhibits the action of sulfonamides,[1] attempts have been made to correlate the activity of these drugs with their structure. Some investigators[2] soon observed that the sulfonamide potency seemed to be directly related to the amount of the ionic form of the drug. The work of Cowles[3] indicated that sulfonamide activity increased as the acid pK of the drug decreased toward 7, but that further increase in acid strength resulted in diminished activity. A more comprehensive study by Bell and Roblin[4] has shown that bacteriostatic activity is a parabolic type of function of acid strength of the sulfonamide, with maximum potency being obtained, in a solution of pH 7, with drugs whose pK's are approximately 6.7.

Inasmuch as current opinion attributes sulfonamide activity to the blocking of an enzyme system,[1] it was considered advisable to examine

(1) Woods, *Brit. J. Exptl. Path.*, **21**, 74 (1940).

(2) Fox and Rose, *Proc. Soc. Exptl. Biol. Med.*, **50**, 142 (1942); Schmelkes, Wyss, Marks, Ludwig and Strandskov, *ibid.*, **50**, 145 (1942).

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

(4) Bell and Roblin, THIS JOURNAL, **64**, 2905 (1942).

the available data from the point of view of the general concepts of acidity and the law of mass action. A brief qualitative description of the approach has been given previously.[5] A detailed account of the treatment and results is presented in this paper.

Bacteriostatic Effect of Sulfonamides

Consider a buffer solution with a fixed hydrogen ion concentration, (H^+), a fixed total concentration, T, of the enzyme or protein, P, and a given total concentration, S, of the sulfonamide, HD. Since the buffer equilibrium and the ionization product of water are fixed and known we need consider only the following equilibria in the solution.

$$H^+ + D^- = HD \quad (1)$$
$$H^+ + P = PH^+ \quad (2)$$
$$P + OH^- = POH^- \quad (3)$$
$$P + D^- = PD^- \quad (4)$$

(For basic sulfonamides HD would be replaced by HD^+, D^- by D and PD^- by PD.) For each of these equilibria we may set up the corresponding mass law expression.

$$(H^+)(D^-)/(HD) = K_{HD} \quad (5)$$
$$(P)(H^+)/(PH^+) = K_{PH^+} \quad (6)$$
$$(P)(OH^-)/(POH^-) = K_{POH^-} \quad (7)$$
$$(P)(D^-)/(PD^-) = K_{PD^-} \quad (8)$$

Since the total concentration of sulfonamide must equal the sum of the concentrations of the various forms in which the drug is present in solution, we can write the equation

$$S = (D^-) + (HD) + (PD^-) \quad (9)$$

Similarly for the protein

$$T = (P) + (PH^+) + (POH^-) + (PD^-) \quad (10)$$

It is now possible to derive an expression for S in terms of variables having experimental significance. For convenience in algebraic manipulation, the following definitions have been made.

$$\frac{(D^-)}{(HD)} = \frac{K_{HD}}{(H^+)} = \lambda \quad (11)$$

$$1 + \frac{(H^+)}{K_{PH^+}} + \frac{(OH^-)}{K_{POH^-}} = \sigma \quad (12)$$

Using (6) and (7) to eliminate (PH^+) and (POH^-) from (10) and re-grouping terms one obtains the expression

$$T = \sigma(P) + (PD^-) \quad (13)$$

Similarly, making use of (11) and (8) one can eliminate (D^-) and (HD) in (9) and then use (13) to replace (P) in the resultant expression and obtain

$$S = \left[\left(1 + \frac{1}{\lambda}\right)\frac{\sigma K_{PD^-}}{T - (PD^-)} + 1\right](PD^-) \quad (14)$$

It seems reasonable to assume that bacteriostasis occurs when a given amount of the sulfonamide–enzyme complex, PD^-, has been formed. If S is the quantity of sulfonamide just sufficient to produce bacteriostasis, (PD^-) in (14) must be a constant. To find the drug of maximum potency at a given pH one must minimize S with respect to K_{HD} while (PD^-) and the pH are held constant.

$$\frac{\partial S}{\partial K_{HD}} = \frac{1}{(H^+)}\frac{\partial S}{\partial \lambda} = \frac{(PD^-)\,\sigma K_{PD^-}}{(H^+)[T - (PD^-)]}\left\{\left(1 + \frac{1}{\lambda}\right)\left(\frac{1}{K_{PD^-}}\right)\left(\frac{\partial K_{PD^-}}{\partial \lambda}\right) - \frac{1}{\lambda^2}\right\} = 0 \quad (15)$$

Inspection shows quickly that the factor within the brackets must be the vanishing one. Rearrangement of terms within the brackets and use of (11) to replace λ, leads to the following form of the minimizing condition

$$\frac{d \ln K_{PD^-}}{d \ln K_{HD}} = \frac{(H^+)}{K^0_{HD} + (H^+)} \quad (16)$$

where K^0_{HD} is the acid ionization constant of the drug which is active at a minimum S. Using the symbol f to represent the left-hand side of (16) and then converting the latter equation into its logarithmic form, one obtains

$$pK^0_{HD} = p\mathrm{H} - \log\left(\frac{1 - f}{f}\right) \quad (17)$$

Were f known for the bacterium–sulfonamide complex, one could calculate K^0_{HD}, and for *E. coli*, for example, the value so obtained could be compared with that observed by Bell and Roblin.[4] In the absence of the pertinent data, one can still test (17) by using the known value of pK^0_{HD}, calculating f for the *E. coli*-sulfonamide complex, and comparing the value so obtained with that which may be derived from the work of Davis and Wood[6] for the plasma protein–sulfonamide complex.

Bell and Roblin[4] found that a plot of bacteriostatic effectiveness versus pK for about a hundred sulfonamides in a solution at pH 7 showed a maximum at a pK of about 6.7. Substituting the appropriate values into (17) we find $f = 0.3$.

Davis and Wood,[6] using a dialysis technique, have determined the amounts of sulfonamide bound to plasma protein for a series of seven drugs. They have expressed their results "as the percentage of the value of the unbound drug which is bound per gram of protein."[6] If we designate this percentage by 100α, and in view of the approximate nature of the following calculation consider P directly proportional to T (see equation (13)), then

$$(\text{Bound drug}) = \alpha\sigma(P)\,(\text{Unbound drug}) \quad (18)$$

where the parentheses represent concentrations. Since the unbound drug may consist of HD and D^-, (18) becomes

$$(PD^-) = \alpha\sigma(P)[(HD) + (D^-)] \quad (19)$$

Making use of (5) one obtains

$$(PD^-) = \alpha\sigma(P)\left[(D^-)\frac{(H^+)}{K_{HD}} + (D^-)\right] \quad (20)$$

Since the pH of the solutions used by Davis and Wood was 7.4, one may insert the numerical value of (H^+) and transform (20) into

(5) Klotz, *Science*, **98**, 62 (1943).

(6) Davis and Wood, *Proc. Soc. Exptl. Biol. Med.*, **51**, 283 (1942).

$$\frac{(PD^-)}{(P)(D^-)} = \alpha\sigma\left[\frac{4.0 \times 10^{-8}}{K_{HD}} + 1\right] \qquad (21)$$

The left-hand side of (21) is the inverse of K_{PD^-}, the dissociation constant of the serum protein–sulfonamide complex (P being expressed in grams, the other concentrations in moles). Hence, one may write

$$\sigma K_{PD^-} = \frac{1}{\alpha\left[\frac{4.0 \times 10^{-8}}{K_{HD}} + 1\right]} \qquad (22)$$

Using values of α given by Davis and Wood[6] and those of K_{HD} in the tables of Bell and Roblin[4] we have calculated σK_{PD^-} for six of the seven drugs for which data are available. (The results for sulfaguanidine have been omitted, because of the conflicting data in the literature on its acid ionization constant. If the acidity is actually too small to be measured in water then the ionization constant of the acid form of sulfaguanidine, HD^+, should be used in the following graph.) Figure 1 shows that a straight line is a sufficiently good approximation to the relationship between log (σK_{PD^-}) and log K_{HD}, for the available data are not very precise. The slope of the line is f and is equal to 0.5. Thus if there is any similarity in behavior of the proteins in *E. coli* to those in blood plasma, the value of 0.3 obtained for the former case is apparently a very reasonable one.

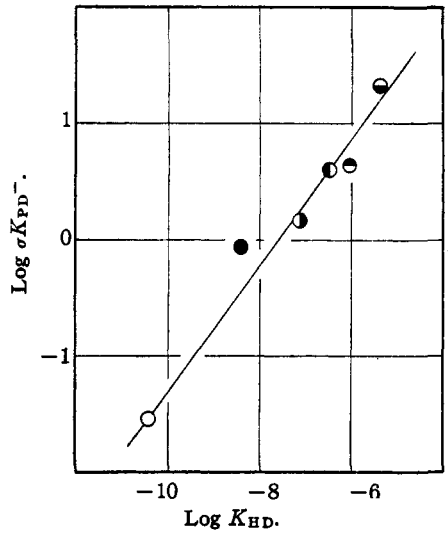

Fig. 1.—Dissociation constants of the plasma protein-sulfonamide complexes: ◒, sulfacetamide; ◓, sulfapyrazine; ◐, sulfadiazine; ◑, sulfathiazole; ●, sulfapyridine; ○, sulfanilamide.

Equation (17) also predicts that as the pH of the medium is increased, the pK of the sulfonamide of maximum potency should also increase. This is in agreement with the results reported by Cowles[3] for *E. coli*. His data, summarized in Fig. 2, show a definite displacement of the potency curves toward higher pK's as the pH is increased. Similar results have been obtained by Brueckner[7] for *Staph. aureus*, *B. subtilis* and *Ps. pyocyanea*.

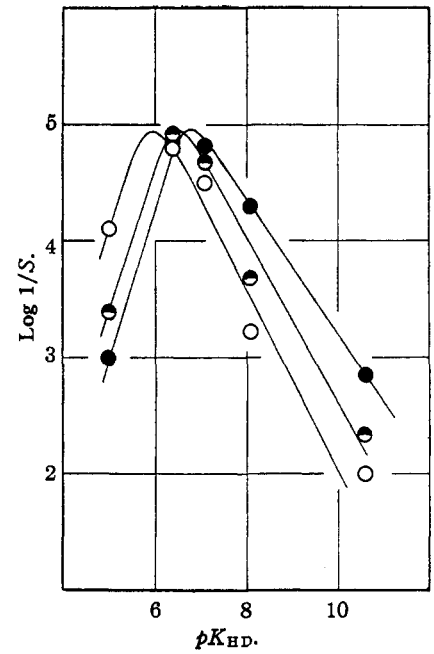

Fig. 2.—Activity as a function of acidity at various pH's for *E. coli*: ○, pH 6.0; ◓, pH 6.8; ●, pH 7.6.

These results indicate that a reasonable first approximation to the relationship between K_{PD^-} and K_{HD} would be

$$K_{PD^-} = kK_{HD}^{f} \qquad (23)$$

the integrated form of

$$\frac{d \ln K_{PD^-}}{d \ln K_{HD}} = f \qquad (24)$$

f and k being constants. This equation may be substituted into (14) to give an explicit expression for S, which in turn may be converted into logarithmic form

$$\log [S - (PD^-)] = \log k' + \log\left(1 + \frac{H^+}{K_{HD}}\right) + f \log K_{HD} \qquad (25)$$

k' is a constant for a fixed pH and is given by the equation

$$k' = \frac{\sigma k(PD^-)}{T - (PD^-)} \qquad (26)$$

In general, the amount of bound drug, (PD^-), will be small compared to the total amount of sulfonamide. Hence, we may use log S instead of log $[S - (PD^-)]$ in equation (25), and obtain the following expression after a simple rearrangement

$$\log S - \log\left(1 + \frac{H^+}{K_{HD}}\right) = \log k' + f \log K_{HD} \qquad (27)$$

This relationship affords an alternative and more reliable method of evaluating f from the entire range of data, as well as an opportunity for evaluating k'.

(7) Brueckner, *Yale J. Biol. Med.*, **15**, 813 (1943).

Using the data of Bell and Roblin,[4] one can plot the sum of the terms on the left-hand side of equation (27) as a function of log K_{HD} and thus obtain a value of f for *E. coli*. f turns out to be 0.37. Substituting this value for f and -3.46 for log k', the intercept of (27) for *E. coli*, one obtains an expression for log S in terms of pK_{HD}, which has been plotted in Fig. 3. The experimental curve is shown also, for purposes of comparison. The agreement is quite satisfactory, except possibly for drugs with pK_{HD}'s below 3.5. It may well be that in this region of high acidity constants an additional limiting factor is the inability of the anions to penetrate the cell wall.[8] Diffusion experiments in this Laboratory may soon give some information on this factor.

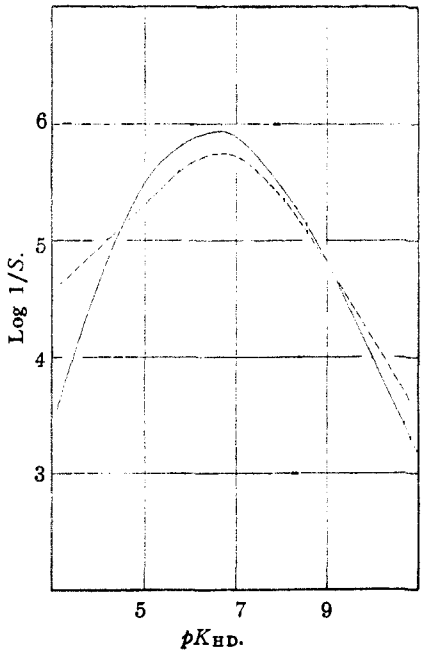

Fig. 3.—Bacteriostatic acivity as a function of acidity. for *E. coli*: - - -, calculated: ——, experimental.

Inhibition of Bacteriostasis by *p*-Aminobenzoic Acid

The inhibitory action of *p*-aminobenzoic acid on sulfonamide activity is also amenable to the mass law treatment. Consider a buffer solution containing a total concentration, T, of enzyme and a concentration, R, of *p*-aminobenzoic acid, HA. To this solution the sulfonamide is added until one reaches a concentration, S, beyond which bacteriostasis will set in. For the solution described one must consider two more equilibria in addition to those of equations (1) to (4).

$$H^+ + A^- = HA \quad (28)$$
$$P + A^- = PA^- \quad (29)$$

In addition to equations (5), (6), (7), (8) and (9) which the system must satisfy we can set up the following mass law expressions

$$(H^+)(A^-)/(HA) = K_{HA} \quad (30)$$
$$(P)(A^-)/(PA^-) = K_{PA^-} \quad (31)$$

and the following stoichiometric relationships

$$R = (A^-) + (HA) + (PA^-) \quad (32)$$
$$T = (P) + (PH^+) + (POH^-) + (PD^-) + (PA^-) \quad (33)$$

With these new equations one can derive a suitable expression for R. Rearrangement of (9) gives the equation

$$(D^-) = \frac{S - (PD^-)}{1 + 1/\lambda} \quad (34)$$

A similar expression, obviously, can be written for (A^-)

$$(A^-) = \frac{R - (PA^-)}{1 + 1/\rho} \quad (35)$$

where

$$\rho = \frac{K_{HA}}{(H^+)} = \frac{(A^-)}{(HA)} \quad (36)$$

The quotient of (34) by (35) becomes

$$\frac{(D^-)}{(A^-)} = \frac{S - (PD^-)}{R - (PA^-)} \frac{1 + 1/\rho}{1 + 1/\lambda} \quad (37)$$

Making use of (8) and (31) to obtain another expression for the ratio $(D^-)/(A^-)$

$$\frac{(D^-)}{(A^-)} = \frac{(PD^-)K_{PD^-}}{(PA^-)K_{PA^-}} \quad (38)$$

we can obtain the following equation

$$[R - (PA^-)] = \frac{\beta}{K_{PD^-}(1 + 1/\lambda)}[S - (PD^-)] \quad (39)$$

where

$$\beta = \frac{(PA^-)}{(PD^-)} K_{PA^-}(1 + 1/\rho) \quad (40)$$

We assume that the bacteriostatic effect of a sulfonamide is inhibited when the (PA^-) reaches a value such that $(PA^-)/(PD^-)$ is equal to some fixed number. Hence, β is constant at a fixed pH. Within the reproducibility of growth experiments, we can assume that $[S - (PD^-)]$ does not differ significantly from S and that $[R - (PA^-)]$ does not differ significantly from R. Thus, we arrive at the equation

$$R = \frac{\beta}{K_{PD^-}(1 + 1/\lambda)} S \quad (41)$$

It is immediately obvious that for a given sulfonamide acting at a fixed pH, the concentration of *p*-aminobenzoic acid which will counteract the sulfonamide is proportional to the concentration of the latter. Such is the case experimentally as has been found by Strauss, Lowell and Finland,[8] by Wood,[9] and by Brueckner.[7]

Furthermore, the following equation can be derived readily from (41) and (23)

$$\log \frac{R}{S} = \log \frac{\beta}{k} - 0.37 \log K_{HD} - \log\left(1 + \frac{H^+}{K_{HD}}\right) \quad (42)$$

The data of Rose and Fox[10] have been used to test this relationship. Only one constant, log β/k, has been evaluated from their data, and the calcu-

(8) Strauss, Lowell and Finland, *J. Clin. Invest.*, **20**, 189 (1941).
(9) Wood, *J. Exp. Med.*, **75**, 369 (1942).
(10) Rose and Fox, *Science*, **95**, 412 (1942).

lated values of log R/S have been compared, in Fig. 4, with the logarithm of the observed ratios. The experimental points do not deviate from the theoretical line to a degree greater than the reproducibility of the results.

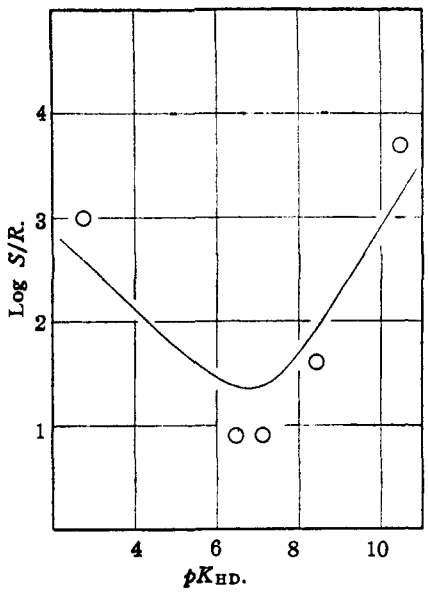

Fig. 4.—Ratios of *p*-aminobenzoic acid to sulfonamide. The solid line is the calculated curve, the circles the experimental points.

Brueckner[7] has published recently some very interesting data on the variation in the sulfonamide–p.a.b. ratio as the *p*H is changed. His work shows that for sulfanilamide, S/R falls continuously as the *p*H range from 6.5 to 8.9 is covered. In contrast, for sulfathiazole, S/R falls from *p*H 6.0 to 7.0 but remains constant thereafter until *p*H 8.9.

Such behavior is a direct consequence of equation (41). Algebraic manipulation leads one readily to the expression

$$\frac{S}{R} = \frac{K_{PD^-}(PD^-)K_{HA}[K_{HD} + (H^+)]}{K_{PA^-}(PA^-)K_{HD}[K_{HA} + (H^+)]} \qquad (43)$$

In the *p*H range studied by Brueckner, $(H^+) << K_{HA}$, and hence one may simplify (43) into the relationship

$$S/R = \epsilon[K_{HD} + (H^+)] \qquad (44)$$

where ϵ is a constant for a given sulfonamide. The reason for the very different behavior of sulfanilamide and sulfathiazole now becomes obvious. In the former case ($pK = 10.43$), $K_{HD} << (H^+)$ over the entire *p*H range, and hence S/R should be directly proportional to the (H^+) concentration. For sulfathiazole ($pK = 7.12$), however, (H^+) is larger than K_{HD} only for *p*H's quite a distance below 7, and at *p*H's appreciably above 7, (H^+) becomes negligible with respect to K_{HD}. Hence, S/R falls uniformly down to a *p*H of about 7, but beyond that becomes constant, because the right-hand side of (44) becomes essentially constant.

That the experimental data agree satisfactorily with the quantitative predictions of equation (44) is shown in Fig. 5. The results have been plotted in logarithmic fashion to expand the region of low hydrogen-ion concentration, but it should be noted that this form of representation also magnifies the discrepancies for small S/R ratios.

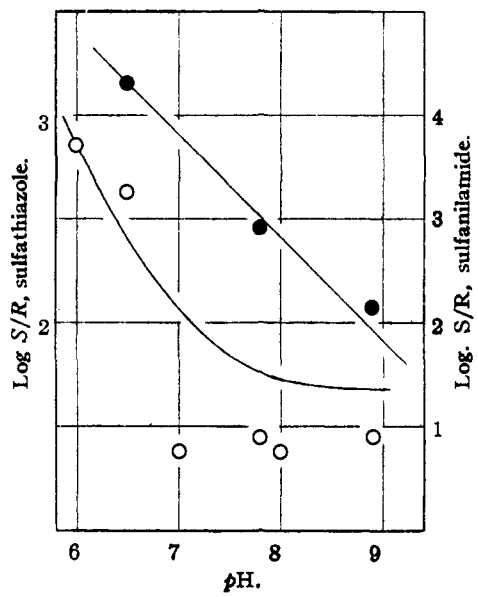

Fig. 5.—Ratios of *p*-aminobenzoic acid to sulfonamide as a function of *p*H for *Staph. aureus:* ●. sulfanilamide; O. sulfathiazole: ——. calculated values.

Conclusion

These calculations show that on the basis of the simple assumption that sulfonamide inhibition of bacterial growth is due to a reversible combination between the basic molecule or anion and an enzyme one can account quantitatively for the various aspects of bacteriostasis and for the resumption of growth by the addition of *p*-aminobenzoic acid. Other more detailed hypotheses[3,4] are unnecessary, and do not give as comprehensive a picture as that derived from the law of mass action.

It should be pointed out, however, that the equations which have been derived may express only a necessary condition for activity but not a sufficient one. It is quite likely that some compounds combine with the enzyme and, nevertheless, are not chemotherapeutically active. Thus, Davis and Wood[8] have found that metanilamide and various inactive substituted sulfonamides, as well as active sulfonamides, combine with blood plasma proteins. Perhaps some structural factor is included in the conditions for activity, but the nature of this additional factor will probably be uncertain until the enzyme system or systems involved are identified clearly.

The equations derived here are based on very

general fundamental principles which may be applicable to many other chemotherapeutic or biological actions. Anionic antiseptics in general, if their action is reversible, may fit equations analogous to those derived above. The equations describing the action of cationic reagents can also be derived very readily. When comprehensive quantitative data on drug activities in these various cases are available, it may be possible to throw some light on the mechanism of their action by analyzing the results from the point of view described above.

Acknowledgment.—The author is indebted to Professor Arthur A. Frost and to Dr. Helmut Gutmann for their careful examination of the manuscript. This investigation was supported by a grant from the Abbott Fund of Northwestern University.

Summary

The inhibition of bacterial growth by sulfonamides may be accounted for quantitatively by assuming that the action is due to a reversible combination between the basic form of the drug and the neutral form of the protein, and that the law of mass action is applicable. Equations may be derived which relate drug potency to the acid ionization constant of the sulfonamide and to the *p*H of the solution.

The reversal of sulfonamide bacteriostasis by addition of *p*-aminobenzoic acid may be considered from the same point of view. Expressions may be obtained which account for variations in the ratio of sulfonamide to *p*-aminobenzoic acid from drug to drug and from one *p*H to another.

EVANSTON, ILLINOIS RECEIVED DECEMBER 10, 1943

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, No. 292]

The Structure of Styracitol[1]

BY ROBERT C. HOCKETT AND MARYALICE CONLEY[2]

In a recent paper,[3] it was indicated that the behavior of styracitol when oxidized by lead tetraacetate in acetic acid under standard conditions points clearly to the 1,5-anhydro-D-mannitol structure assigned to this compound by Zervas and Papadimitriou.[4] It was stated further that because the configuration of styracitol has been the subject of so much controversy[5] and because this oxidation method is relatively new, we would seek other evidence concerning the configuration of this substance. The rate-of-oxidation measurement would appear to offer an extremely simple and rapid method of solving such structural problems as this, provided that it can be depended upon invariably to give the right answer. Hence the establishment of the styracitol structure beyond question was essential to any extension of the oxidation procedure to other problems.

In 1931, Asahina and Takimoto[5] reported the complete methylation of this anhydro-alcohol and claimed the isolation of *d*-dimethoxysuccinic acid from the products of oxidation of tetramethyl styracitol by nitric acid. The formation of this acid is entirely incompatible with the 1,5-anhydromannitol structure assigned by Zervas and Papadimitriou[4] and provided the basis for Asahina's claim that styracitol is 1,5-anhydro-D-sorbitol. Zervas' synthesis of the compound[5] appears to establish the position of the ring and to limit the structural possibilities to the two alternatives mentioned.

We have undertaken to repeat the work of Asahina and Takimoto in order to discover the cause of this disagreement. Styracitol has been prepared synthetically by the method of Zervas.[5] Methylation by the procedure of West and Holden[6] yielded a tetramethyl ether of the expected composition and whose properties agreed well with those reported by the Japanese workers and by Freudenberg and Sheehan.[5] On oxidation of this ether with nitric acid, we obtained two substances that were definitely identified by means of well-known derivatives. These were oxalic acid and *l*-(−)-dimethoxysuccinic acid. None of the *d*-(+)-dimethoxysuccinic acid was found and the mother liquor left after separation of the two acids described above was somewhat levorotatory. Therefore, the experimental observations of Asahina and Takimoto were erroneous.

It should be noted that while the formation of the *d*-dimethoxysuccinic acid would be definitive, the levo isomer could be produced from either structure proposed for styracitol. Hence, the present work must be regarded as only removing a puzzling discrepancy and not in itself a structure proof. The isolation of *meso*-dimethoxysuccinic acid, D-arabotrimethoxyglutaric acid or of D-xylotrimethoxyglutaric acid would be necessary to complete the proof. Since, however, Richtmyer

(1) This paper is taken from a thesis submitted by Maryalice Conley to the graduate School of the Massachusetts Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy in January, 1943.

(2) Ellen H. Richards Memorial Fellow from 1939–1942. Now Mrs. James F. Moore, Pigments Department, E. I. du Pont de Nemours and Company, Newport, Delaware.

(3) Hockett, Dienes and Ramsden, THIS JOURNAL, **65**, 1474 (1943).

(4) Zervas and Papadimitriou, *Ber.*, **73**, 174 (1940).

(5) Asahina and Takimoto, *ibid.*, **64**, 2032 (1931); Freudenberg and Rogers, THIS JOURNAL, **59**, 1602 (1937); Freudenberg and Sheehan, *ibid.*, **62**, 559 (1940); *cf.* Zervas, *Ber.*, **63**, 1689 (1930).

(6) West and Holden, THIS JOURNAL, **56**, 930 (1934).