s-butylcarbinol (Eastman Kodak Co. "Synthetic") and 40 g. (0.29 mole) of *n*-butyl bromide.

Summary

1. Butenylmagnesium bromide couples with allylic bromides to give mixtures of diolefins, whereas with allylic chlorides individual products tend to predominate.

2. The principal products of the coupling reactions of allylic chlorides with butenylmagnesium bromide, like the products from carbonyl-addition reactions, may be considered to be derived from the secondary form of the Grignard reagent.

3. The reaction of butenylmagnesium bromide with *n*-butyl chloromethyl ether gives *s*-butenylcarbinyl *n*-butyl ether.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Isoelectric Nature of Sulfanilamide and *p*-Aminobenzoic Acid

BY IRVING M. KLOTZ AND DIETER M. GRUEN

It has been assumed generally[1] in correlating the acid–base properties of sulfonamides with their mode of action that these substances, as well as their inhibitor, *p*-aminobenzoic acid, exist in neutral and not in zwitterionic form in their isoelectric state in aqueous solution. Conflicting evidence[17,18] is available for *p*-aminobenzoic acid but none is known for a sulfonamide. One possible approach to the question is the comparison of the ionization constants of the amino group in the acid and in its corresponding esters for the case of *p*-aminobenzoic acid, and in the amide and the corresponding N^1-alkyl-substituted amides for the case of sulfanilamide. The basis of this procedure was developed by Adams[2] and by Ebert[3] and has been applied widely to the amino acids by Edsall.[4] Data are presented in this paper which allow the necessary comparisons to be made for sulfanilamide and *p*-aminobenzoic acid. The results together with the ultraviolet absorption spectra from which they are derived substantiate the assumption that these substances are primarily neutral in their isoelectric state.

Experimental

The compounds examined were prepared as follows.

Sulfanilamide.—A commercial sample was recrystallized from water; m. p. 165°.

N^1,N^1-Dimethylsulfanilamide.—*p*-Acetaminobenzenesulfonyl chloride was treated with dimethylamine, the resultant acetyl compound hydrolyzed with 15% hydrochloric acid and the free amine obtained by neutralization with sodium bicarbonate. The solid was recrystallized first from acetone and then from ethyl alcohol; m. p. 170–171°.

N^1,N^1-Diethylsulfanilamide.—The same procedure was used as for the corresponding methyl compound, except that diethylamine replaced dimethylamine; m. p. 103–104°.

***p*-Aminobenzoic Acid.**—A commercial sample was decolorized twice with charcoal and then recrystallized from water; m. p. 187°.

Methyl *p*-Aminobenzoate.—A solution of *p*-aminobenzoic acid in methyl alcohol was saturated with hydrochloric acid and refluxed for six hours. The free base was liberated and then recrystallized three times from methyl alcohol; m. p. 110–111°.

Ethyl *p*-Aminobenzoate.—This compound was prepared in a manner analogous to that of the corresponding methyl ester; m. p. 86–87°.

Ultraviolet absorption spectra in the region of 2200–3200 Å. were obtained with a Beckman quartz spectrophotometer. Aqueous solutions approximately 5×10^{-5} molar in concentration were made by quantitative dilution of a more concentrated solution prepared from a weighed quantity of solute dissolved in the solvent in a volumetric flask. Two matched, silica absorption cells were used, one being filled with solvent and the second with solution. The cells were each 1 cm. in length. The temperatures of the *p*-aminobenzoic acid and ester solutions were between 25–27°, those of the sulfonamides between 23–25°.

For each of the six compounds investigated, three spectra were collected, one in pure distilled water, the second in an aqueous solution of approximately 0.005 *N* hydrochloric acid (exact concentration known) and the third in an aqueous solution of approximately 0.50 *N* hydrochloric acid.

Results and Calculations

If two isoelectric forms of sulfanilamide are in equilibrium with each other, the equilibrium constant

$$K_Z = \frac{{}^+H_2NC_6H_4SO_2NH^-}{H_2NC_6H_4SO_2NH_2} \qquad (1)$$

should be evaluated from an equation analogous to that used by Edsall,[4] that is

$$K_Z = \frac{K_S}{K_M} - 1 \qquad (2)$$

where K_S is the acid ionization constant of ${}^+H_3NC_6H_4SO_2NH_2$ and K_M is the acid ionization constant of the corresponding N^1,N^1-dimethyl or diethyl-substituted compound. Either of these constants may be represented by the equation

$$K = (H^+)(S)/({}^+HS) \qquad (3)$$

where (S) represents the total concentration of isoelectric sulfonamide.

The equations for *p*-aminobenzoic acid are identical in form but K_S represents the acid ionization constant of ${}^+H_3NC_6H_4CO_2H$ and K_M the corresponding constant for the methyl or ethyl ester.

Since most of the sulfonamides, especially the

(1) Bell and Roblin, THIS JOURNAL, 64, 2905 (1942); Klotz, *ibid.*, 66, 459 (1944).

(2) Adams, *ibid.*, 38, 1503 (1916).

(3) Ebert, *Z. physik. Chem.*, 121, 385 (1926).

(4) Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, pp. 96–99.

alkyl-substituted ones, are sparingly soluble in water, a simple electrometric titration to determine the ionization constant was not feasible. On the other hand, the ultraviolet absorption spectra of these compounds are intense even in very dilute solution and differ markedly in acid and neutral media.

Ionization constants may be determined from absorption spectra by finding (S) and ($^+$HS) in a solution of known (H^+) by solving the following two simultaneous equations

$$(^+HS) + (S) = C \qquad (4)$$

$$\log (I_0/I) = \epsilon_{^+HS}(^+HS)d + \epsilon_S(S)d \qquad (5)$$

where C is the total concentration of sulfonamide, $\log (I_0/I)$ is the density of absorption in the solution of known (H^+), and $\epsilon_{^+HS}$ and ϵ_S are the extinction coefficients of the molecules represented by the subscripts. The procedure for calculating the constants is illustrated best by the following example.

Sample Calculation.—As a first approximation assume that none of the isoelectric form is present in the solution of 0.5 N hydrochloric acid. Substituting the known quantities from the data listed in Table I, for sulfanilamide as an example, into equations (4) and (5), one obtains at 2650 Å.

$$(^+HS) + (S) = 6.28 \times 10^{-5}$$

$$0.629 = \frac{0.095}{6.28 \times 10^{-5}}(^+HS) + \frac{0.950}{6.28 \times 10^{-5}}(S)$$

Solution of these equations leads to the following results

$$(S) = 3.92 \times 10^{-5}$$
$$(^+HS) = 2.36 \times 10^{-5}$$
$$(H^+) = 0.00507 - 0.00002 = 0.00505$$

Therefore the first approximation to the ionization constant is

$$K = \frac{(H^+)(S)}{(^+HS)} = 0.00839$$

It is now possible to correct the value of $\log (I_0/I)$ in the solution of 0.5 N hydrochloric acid for the absorption due to the small amount of S present.

$$(S) = \frac{K(^+HS)}{(H^+)} = \frac{0.00839}{0.50}[6.28 \times 10^{-5} - (S)]$$

$$(S) = 1.04 \times 10^{-6}$$

$$\log (I_0/I) \text{ due to } S = 1.04 \times 10^{-6}\,\frac{0.950}{6.28 \times 10^{-5}} = 0.016$$

Equations (4) and (5) must now be solved again with a corrected value for $\epsilon_{^+HS}$.

$$(^+HS) + (S) = 6.28 \times 10^{-5}$$

$$0.629 = \frac{0.079}{6.28 \times 10^{-5}}(^+HS) + \frac{0.950}{6.28 \times 10^{-5}}(S)$$

The results obtained are

$$(S) = 3.97 \times 10^{-5}$$
$$(^+HS) = 2.31 \times 10^{-5}$$
$$(H^+) = 0.00505$$
$$K = 0.00868$$

A third approximation may be carried out in the same fashion but, in all cases reported here, one obtains the same value of K as in the second approximation.

Table I contains the pertinent experimental data and the calculated values of the ionization constants for the six compounds investigated. The column labeled "K (uncor.)" lists the first approximation to the constant, that labeled "K (cor.)" the final approximation.

The method of successive approximations to determine the absorption of the hydrochloride form of the compounds mentioned is preferable to determination by extrapolation of data in solutions of increasing acidity because in the latter method specific ion effects on the absorption curve become more and more pronounced.[5]

The use of very dilute solutions avoids a number of errors which might otherwise occur in this type of measurement. Beer's law has been shown to be applicable for *p*-aminobenzoic acid[6] and for sulfanilamide and sixteen other sulfonamides.[7] Similarly in such dilute solutions activity coefficients contribute a negligible correction for, at the concentrations used, the Debye–Hückel limiting law would be applicable, and for an ionization of the type encountered here, the ratio of activity coefficients would be one.

It is necessary to observe a further precaution in the calculation of the ionization constant of *p*-aminobenzoic acid, in contrast to the others, because in this case the neutral aqueous solution contains some *p*-aminobenzoate ion as well as the un-ionized acid. Since the ion has an absorption spectrum slightly different from that of the undissociated acid, the ionization constants calculated at two different wave lengths will differ. Nevertheless, the true ionization constant can be calculated accurately if a wave length is chosen at which the absorption coefficients of the ion and acid, respectively, are essentially identical. The value (2675 Å.) listed in Table I has been found to satisfy this condition in that the absorption in water was identical with that in 0.05 N sodium hydroxide. This value is very close to that estimated from the curves of Dede and Rosenberg (about 2700 Å.).[8] and differs by only 15 Å. from that determined by Kumler and Strait.[9]

By far the largest source of error in these constants is the relative inconstancy of the temperature. The ionization of the R–NH_3^+ ion is quite sensitive to temperature. In the case of glycine[10] and similar amino acids the variation in ionization constant is about 2% per degree. If the *o*-chloroanilinium ion is chosen as more representative of the compounds of interest here, a temperature coefficient of about 4% per degree is indicated.[11]

(5) Kortüm, *Z. physik. Chem.*, **B30**, 317 (1935).

(6) Marchlewski and Mayer, *Bull. intern. acad. Polonaise*, **No. 3A**, 169 (1929); *C. A.*, **24**, 2052 (1930).

(7) Ciminera and Wilcox, *J. Am. Pharm. Assoc.*, **33**, 85 (1944).

(8) Dede and Rosenberg, *Ber.*, **67**, 147 (1934).

(9) Kumler and Strait, THIS JOURNAL, **65**, 2349 (1943).

(10) Owen, *ibid.*, **56**, 24 (1934).

(11) Pedersen, *Kgl. Danske Videnskab. Selskab, Skrifter, Naturvidenskab. math. Afdel.*, **16**, 3 (1937).

TABLE I

IONIZATION CONSTANTS

Compound	Concn., moles/liter	Exact concn. of 0.005 *N* HCl, moles/liter	Wave length, Å.	Log (I_0/I) in water	Log (I_0/I) in 0.5 *N* HCl	Log (I_0/I) in 0.005 *N* HCl	*K* (uncor.)	*K* (cor.)	*pK*, av.
Sulfanilamide	6.28×10^{-5}	0.00507	2650	0.950	0.095	0.629	0.00839	0.00868	2.058
	6.28×10^{-5}	.00507	2800	.375	.025	.245	.00856	.00880	
Dimethylsulfanilamide	4.81×10^{-5}	.00507	2650	.791	.130	.689	.0278	.0297	1.535
	4.81×10^{-5}	.00507	2700	.682	.108	.591	.0270	.0287	
Diethylsulfanilamide	4.24×10^{-5}	.00507	2650	.698	.061	.550	.0166	.0175	1.752
	4.24×10^{-5}	.00507	2800	.355	.013	.277	.0171	.0179	
p-Aminobenzoic acid	7.53×10^{-5}	.00439	2675	.960	.005	.402	.00309	.00313	2.504
Methyl *p*-aminobenzoate	6.30×10^{-5}	.00439	2700	.800	.025	.386	.00379	.00386	2.404
	6.30×10^{-5}	.00439	2800	.940	.019	.457	.00396	.00402	
Ethyl *p*-aminobenzoate	5.71×10^{-5}	.00439	2700	.740	.057	.395	.00429	.00435	2.366
	5.71×10^{-5}	.00439	2800	.888	.043	.457	.00419	.00426	

Nevertheless, the maximum possible error would not affect the relative order of the ionization constants listed in Table I and hence would not change any of the conclusions which may be reached.

The acidity constant of the anilinium ion of sulfanilamide has been determined by Redlich and Maranville,[12] who found a *pK* value of 2.04, and by Albert and Goldacre,[13] who reported a *pK* of 2.20.

The ionization constants of the anilinium ions of *p*-aminobenzoic acid and its methyl and ethyl ester were reported by Johnston[14] and are not in agreement with ours. However, they were determined by an indirect and less precise method (catalysis of hydrolysis of methyl acetate) and are probably less reliable than those listed in this paper. Albert and Goldacre[13] find a *pK* of 2.49 for the hydrochloride of *p*-aminobenzoic acid, a value which is in good agreement with ours.

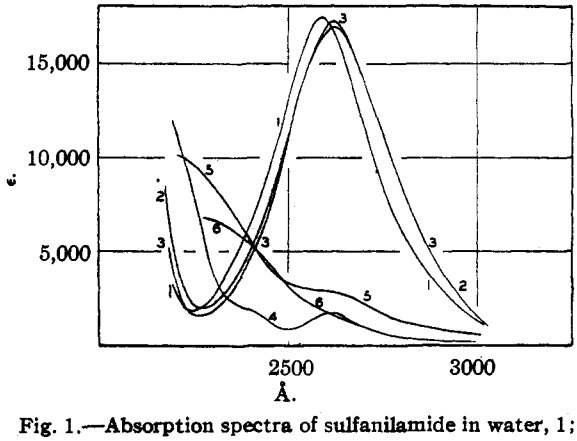

Fig. 1.—Absorption spectra of sulfanilamide in water, 1; N^1,N^1-dimethylsulfanilamide in water, 2; N^1,N^1-diethylsulfanilamide in water, 3; sulfanilamide in 0.5 *N* HCl, 4; N^1,N^1-dimethylsulfanilamide in 0.5 *N* HCl, .5; N^1,N^1-diethylsulfanilamide in 0.5 *N* HCl, 6.

(12) Redlich and Maranville, *Northwest Sci.*, **17**, No. 1, 4 (1943).
(13) Albert and Goldacre, *Nature*, **149**, 245 (1942).
(14) Johnston, *Proc. Roy. Soc.* (London), **A78**, 82 (1906).

Full spectral curves have been determined for each compound in the aqueous and 0.5 *N* acid solutions, and are shown in Figs. 1 and 2. Extinction coefficients were calculated from the familiar equation $\epsilon = (1/cd) \log_{10} (I_0/I)$, where I_0 is the intensity of the light passing through the solvent, I, the intensity of the light passing through the solution, c, the concentration of the solute in moles per liter, and d, the thickness of the cell in centimeters.

The absorption spectrum of sulfanilamide has been examined by a number of investigators.[7,9,15] The curve presented here is in good agreement with those of other laboratories. So far as we are aware, no previous data have been reported on the N^1-disubstituted compounds which are also included in Fig. 1.

Riegel and Buchwald[16] have compared the spectra of *p*-aminobenzoic acid and its methyl and ethyl esters. Their data deviate somewhat from those presented here, particularly as to the magni-

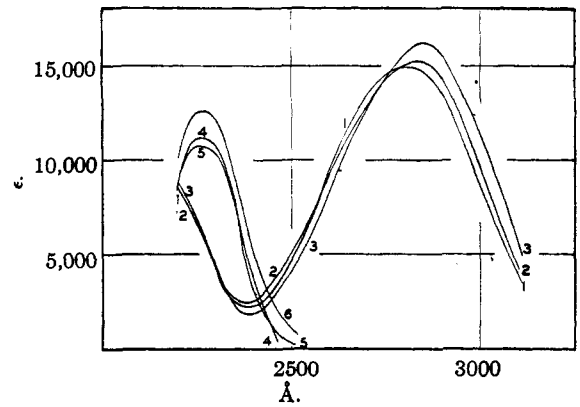

Fig. 2.—Absorption spectra of *p*-aminobenzoic acid in water, 1; methyl *p*-aminobenzoate in water, 2; ethyl *p*-aminobenzoate in water, 3; *p*-aminobenzoic acid in 0.5 *N* HCl, 4; methyl *p*-aminobenzoate in 0.5 *N* HCl, 5; ethyl *p*-aminobenzoate in 0.5 *N* HCl, 6.

(15) Vandenbelt and Doub, THIS JOURNAL, **66**, 1633 (1944).
(16) Riegel and Buchwald, *ibid.*, **51**, 484 (1929).

tude of the maximum extinction coefficients, but inasmuch as theirs was a photographic method of measurement it is likely to be in greater error in measuring intensities than a photoelectric method would be.

Discussion

For both *p*-aminobenzoic acid and sulfanilamide, the ionization constants of the methyl- and ethyl-substituted compounds are larger than those of the parent substance. Reference to equation (2) shows that Kz in every case would be negative. A negative value of Kz is, of course, meaningless, since no equilibrium constant can be less than zero. The difficulty arises from the necessity of assuming in the derivation of equation (2) that the presence of the alkyl group at one end of the molecule affects the opposite NH_3^+ group exactly as does a hydrogen atom. Such an assumption is an approximation and is obviously not strictly true, for the slight differences in the absorption curves (Figs. 1 and 2) show that the alkyl group has a very definite, though small effect on the resonance states of the molecules. Nevertheless, within the limitations of the method used, there is no indication of any zwitterion in the isoelectric state.

A comparison of the spectra of the methyl and ethyl derivatives with that of the corresponding parent compound leads to similar conclusions. It has been noted by many workers[9] that the conversion of an $-NH_2$ group, attached to a benzene ring, to an $-NH_3^+$ group is accompanied by a very marked reduction in the absorption of radiation near 2600–2800 Å. Figures 1 and 2 show a number of examples of this phenomenon, for in the ethyl- and methyl-substituted compounds the changes in spectra in going from neutral to acid solution must be due to the formation of anilinium-type ions. The behavior of sulfanilamide in going from neutral to acid solution is analogous to that of its alkyl derivatives and is exactly what should occur if the isoelectric form is neutral, that is, if the change is from an $-NH_2$ to an $-NH_3^+$ group. Furthermore, if an appreciable fraction of the isoelectric form were dipolar in nature, that is, had an $-NH_3^+$ substituent, one would expect the absorption peak to be somewhat below that of the methyl or ethyl compound, which must have $-NH_2$ substituents in neutral solution. Actually the peak is higher for sulfanilamide.

Similar behavior is observed with *p*-aminobenzoic acid, the addition of acid being accompanied by a marked reduction in absorption at 2800 Å. In this case the parent acid in neutral solution has a slightly lower maximum absorption than its esters. If the difference is attributed entirely to the presence of some dipolar ions in a neutral solution of *p*-aminobenzoic acid, a comparison of the absorption of the acid with that of its methyl ester leads to an estimate of about 3% dipolar ions. The corresponding comparison with the ethyl ester leads to a maximum of 9% in the form of zwitterions. In any event only a small fraction of the isoelectric form may be dipolar ions.

In the case of *p*-aminobenzoic acid, the magnitude of the dielectric increment as well as that of the electrostriction effect, may indicate a small amount of dipolar form.[17] In these phenomena, however, it would be desirable to evaluate approximately the effect of the contribution of resonance forms with a separation of charge. Evidence from formol titrations, in contrast to that from dielectric constants and molal volumes, indicates the absence of dipolar ions,[18] but this method of analysis might disturb an equilibrium between the two possible isoelectric forms.

Since the isoelectric behavior of sulfonamides more acid than sulfanilamide (in which the pK of the SO_2NH_2 group is 10.85) should parallel that of *p*-aminobenzoic acid and since the latter is at least predominantly neutral in nature, it seems reasonable to conclude that if any of the common sulfa compounds are predominantly dipolar in nature they must be among those with acid pK's smaller than 4.5, the pK of the COOH group in *p*-aminobenzoic acid. Among the most active drugs, *i. e.*, those with pK's between 4.5 and 10, the neutral form is at least the predominant form in the isoelectric state.

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Summary

Acidity constants have been determined for the hydrochlorides of sulfanilamide, N^1,N^1-dimethylsulfanilamide, N^1,N^1-diethylsulfanilamide, *p*-aminobenzoic acid, methyl *p*-aminobenzoate and ethyl *p*-aminobenzoate from the ultraviolet spectra of these compounds in solutions of different *p*H.

A comparison of these acidity constants gives no indication of zwitterions in the isoelectric states of sulfanilamide and *p*-aminobenzoic acid.

EVANSTON, ILLINOIS RECEIVED OCTOBER 30, 1944

(17) Edsall, *loc. cit.*, pp. 159–160.

(18) Harris, *Biochem. J.*, **24**, 1080 (1930).