[CONTRIBUTION NO. 1547 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Ionization of Amino Acids in the Solvent Sulfuric Acid

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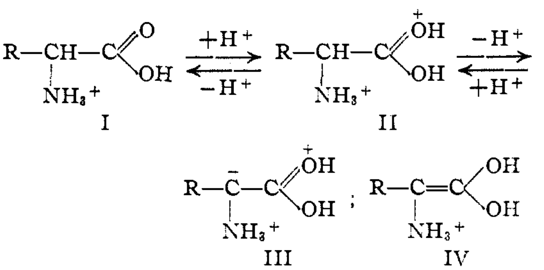
The degree of ionization of eight aliphatic and aromatic amino acids in sulfuric acid has been determined by cryoscopic measurements and the factors influencing the process of multiple protonation, in sulfuric acid, are discussed. Formation of the double charged cation, $RCHNH_3CO_2H_2^{++}$, occurs to the extent of only 20–30% with the α-amino acids, but increases in a uniform manner as the distance between the polar groups increases. The second ionization of ϵ-amino-*n*-caproic acid is complete, this compound accepting two protons from sulfuric acid. The resonance effects which largely determine the dissociation of the isomeric aminobenzoic acids in aqueous solution do not influence their ionization in sulfuric acid. A mechanism for the acid-catalyzed racemization of α-amino acids is considered in the light of the above results.

The reaction between an amino acid and a strong acid such as sulfuric acid is of considerable theoretical and practical interest. Numerous examples are known[2] in which the racemization of an α-amino acid has been achieved by heating the amino acid in aqueous solution in the presence of a strong acid (sulfuric or hydrochloric) catalyst. It appears that as the acidity of the solvent is increased, complete racemization can be attained at relatively low temperatures, *i.e.*, about 100°. Accordingly, these racemizations have been postulated to take place in the following manner.[2]



(1)

It is argued[2] that the positive charges on the doubly charged cation (II) will promote ionization of the hydrogen on the α-carbon atom and also that the resulting ion will be a resonance hybrid of III and IV and thus be stabilized. The sequence of steps would transform the asymmetric cation (I) into a cation {III, IV} which has lost its asymmetry. The latter, on reversal of the prototropic changes, would revert to the racemized amino acid cation.

The first experimental evidence for the existence of a doubly charged amino acid cation (II) is that of Thomas and Niemann,[3] who found that L-leucine had a van't Hoff *i*-factor[4] of 2.2 in 100% sulfuric acid. This indicates that although the reaction

$$RCHNH_3CO_2^{\pm} + H_2SO_4 \rightleftarrows RCHNH_3CO_2H^+ + HSO_4^- \quad (2)$$

is essentially complete, there is relatively little but yet a significant amount of ionization of the type

$$RCHNH_3CO_2H^+ + H_2SO_4 \rightleftarrows RCHNH_3CO_2H_2^{++} + HSO_4^- \quad (3)$$

It is apparent that the positively charged ammonium group is responsible for the lack of extensive protonation of the carboxyl group of L-leucine cation in 100% sulfuric acid.[5] In view of the optical stability of L-leucine in 100% sulfuric acid at 25° for periods exceeding one month,[3] one must conclude that the rate of racemization of the doubly charged cation is extremely slow under these conditions. It should be remembered that if the concentration of water is low enough to be considered negligible, the only proton acceptors in such a system are the amino acid cation (I) and bisulfate ion. These very weak bases would not be expected to show much tendency to promote the loss of a proton from the α-carbon atom.[6] It is also indicated that the concentration of the doubly charged cation in a solution of an α-amino acid in aqueous acid must be very small. In any event, it is clear that quantitative data on the kinetics of the acid-catalyzed racemization in aqueous systems confirming the indicated first-order hydroxonium ion catalysis must be obtained before the mechanism shown in equation (1) can be accepted.

The formation of the doubly charged cation (II) in sulfuric acid is interesting from another point of view, for the possibility of polyfunctional compounds accepting more than one proton in this solvent appears to have been largely ignored by recent workers.[7] This may be due in part to the recognition of the inherent errors in the procedure used by Hantzsch[8] who had concluded that this type of ionization does occur. The *i*-factors obtained by Hantzsch[8c] for a number of polynitrogenous bases, although indicative of multiple protonation, are all much lower than would be expected for the degree of ionization postulated. Thus his work cannot be regarded as having quantitative significance.

In the present investigation van't Hoff *i*-factors of 2.2 for glycine and 2.3 for L-leucine in sulfuric acid have been observed, confirming the results reported previously.[3] Differences of 0.1 unit in the *i*-factor must be regarded as being of questionable significance.

The process of multiple protonation in sulfuric acid was clearly demonstrated with the determination of the degree of ionization of the β-, γ- and ϵ-amino acids. It was found that the effectiveness

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(1) To whom inquiries regarding this article should be sent.

(2) A. Neuberger in M. L. Anson and J. T. Edsall, "Advances in Protein Chemistry," Vol. IV, Academic Press, Inc., New York, N. Y., 1948, p. 298.

(3) D. W. Thomas and C. Niemann, *J. Biol. Chem.*, **175**, 241 (1948).

(4) (a) L. P. Hammett and A. J. Deyrup, THIS JOURNAL, **55**, 1900 (1933); (b) H. P. Treffers and L. P. Hammett, *ibid.*, **59**, 1708 (1937).

(5) The results of A. Hantzsch, *Z. physik. Chem.*, **61**, 257 (1908), and of G. Oddo and A. Casalino, *Gazz. chim. ital.*, **47**, II, 200 (1917), indicate that the ammonium group of an α-amino acid cation is more effective in preventing protonation of the carboxyl group than are the two chlorine atoms in dichloroacetic acid.

(6) The condensations for racemization imply the necessity of the presence of water, and the application of heat.

(7) M. S. Newman, H. G. Kuivila and A. B. Garrett, THIS JOURNAL, **67**, 704 (1945).

(8) (a) A. Hantzsch, *Z. physik. Chem.*, **61**, 257 (1908); (b) **65**, 41 (1908); (c) *Ber.*, **63B**, 1782 (1930).

of the positively charged ammonium group in preventing protonation of the carboxyl group of the amino acid cation diminishes as the distance between the groups increases. The *i*-factors obtained were: β-alanine, 2.7; γ-amino-*n*-butyric acid, 2.9; and ε-amino-*n*-caproic acid, 3.0. The last value indicates that the di-protonation of the ε-amino acid is essentially complete.[9]

One may evaluate the results for those amino acids in which the second ionization (equation 3) is incomplete as follows. From equations (2) and (3) it is seen that in a solution which is one molal in amino acid

$$i = \Sigma\{[RCHNH_3CO_2^{\pm}] + [RCHNH_3CO_2H^+] + [RCHNH_3CO_2H_2^{++}] + [HSO_4^-]_{(2)} + [HSO_4^-]_{(3)}\} \quad (4)$$

Since the data indicate that reaction (2) is complete, $[RCHNH_3CO_2^{\pm}] = 0$ and $[HSO_4^-]_{(2)} = 1$. From the relationships $[RCHNH_3CO_2H_2^{++}] = [HSO_4^-]_{(3)}$ and $[RCHNH_3CO_2H^+] = 1 - [RCHNH_3CO_2H_2^{++}]$ it follows that

$$i = 2 + [RCHNH_3CO_2H_2^{++}] \quad (5)$$

The dissociation constant of the doubly charged cation is

$$K = [RCHNH_3CO_2H^+]/[RCHNH_3CO_2H_2^{++}][HSO_4^-]_{(total)} \quad (6)$$

where $[HSO_4^-]_{(total)}$ includes not only the bisulfate ion produced by the ionization of the amino acid (equations 2 and 3) but also that formed by the ionization of the water used to suppress the self-dissociation of the solvent. However, we do not have an accurate enough knowledge of the total bisulfate ion concentration to evaluate K.[10] If it is assumed that $[HSO_4^-]_{(total)}$ is approximately the same in all cases, a second quantity, K', may be defined as the ratio of the concentration of singly charged cation to that of doubly charged cation, *i.e.*

$$K' = [RCHNH_3CO_2H^+]/[RCHNH_3CO_2H_2^{++}] \quad (7)$$

and K' be taken as a measure of the dissociation of the doubly charged cation, *i.e.*, as a kind of empirical dissociation constant. Values of K' were calculated from equation (5) and the relationship $RCHNH_3CO_2H^+ = 1 - RCHNH_3CO_2H_2^{++}$ and are given in Table I with approximate values of log K'.

TABLE I

Amino acid	i	K'	log K'
Glycine	2.2	4.0	0.6
β-Alanine	2.7	0.4	−0.4
γ-Amino-*n*-butyric acid	2.9	0.1	−0.1

A plot of log K' as a function of the reciprocal distance[11] between the polar groups is approximately linear, *cf.*, Fig. 1. The same relation has been shown to exist for the logarithmic dissociation constants (pK_1) of the amino acids in aqueous solution.[12] Surprisingly, a direct plot of i *versus* $1/d$ shows a better approximation to linearity (*cf.* Fig. 1), but this is perhaps fortuitous.

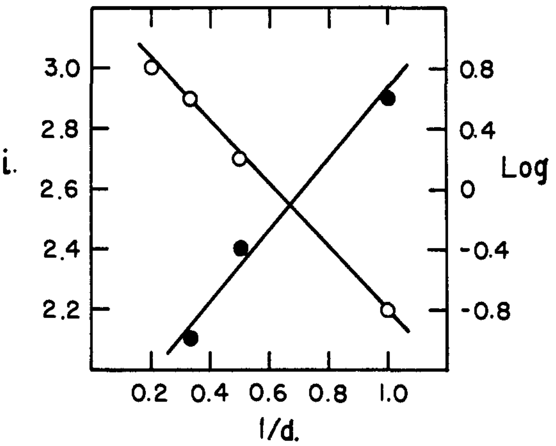

Fig. 1.—Ionization of aliphatic amino acids in sulfuric acid; ○, plot of i *versus* $1/d$; ●, plot of log K' *versus* $1/d$).

In the determination of the ionization of the aminobenzoic acids in sulfuric acid it was found that anthranilic acid behaved very much like an α-amino acid, giving an *i*-factor of 2.3. The meta and para compounds were similar in their behavior, the *i*-factors observed being 2.7 and 2.8, respectively. Consideration of the second ionization of the para acid reveals why resonance effects[13] would be expected to have little or no influence on the basicity of the singly charged cation. This is indeed the case, for the data indicate that the degree of ionization of the isomeric aminobenzoic acids in sulfuric acid, like that of the aliphatic amino acids, is primarily determined by the electrostatic interaction of the ammonium group and the protonated carboxyl group.

Experimental

Apparatus and Procedure.—The cell used for the freezing point depression measurements was similar to that described by Newman, Kuivila and Garrett,[7] except that a Beckmann thermometer (calibrated by the National Bureau of Standards) was employed. The finely powdered solutes were introduced by means of a weight buret.[7] Control experiments showed that all the operations could be carried out with the required precision. Drift in the freezing point due to entrance of moisture into the cell was found to be negligible over the period required for a series of measurements. When 50 ml. of stock sulfuric acid (slightly fuming, prepared by mixing 1 lb. of 20–30% fuming acid, C.P., Baker, with 380 ml. of 96% acid, C.P., Baker) was allowed to drain into the cell in 35 minutes, the weight delivered was found to be 91.7 ± 0.1 g. Sufficient water was then added to make the acid just less than 100%,[4a] and the resulting acid allowed to stand in the sealed apparatus for at least 12 hours before freezing point determinations were begun. Other details of technique were similar to those already described.[7] As recommended by the previous workers,[4] corrections for supercooling and disappearance of solvent in the ionization

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[9] This result, from a mechanistic point of view, is in accord with the observation that ε-amino-*n*-caproic acid gives a satisfactory yield (70%) of pentamethylenediamine in the Schmidt reaction (H. Wolff, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 307). See also M. S. Newman and H. L. Gildenhorn, THIS JOURNAL, **70**, 317 (1948), and Peter A. S. Smith, *ibid.*, **70**, 320 (1948).

[10] It is only since the experimental part of this paper had been completed that the papers of (a), R. J. Gillespie, E. D. Hughes and C. K. Ingold, *J. Chem. Soc.*, 2473 (1950), and (b), R. J. Gillespie, *ibid.*, 2493 (1950), on cryoscopic measurements in sulfuric acid became available to us. The refined methods introduced by these authors, involving very accurate control of the concentration of water and knowledge of its basicity constant, were therefore unknown to us at the time our measurements were made. A critical analysis of our results in the light of the findings of the above authors is given in the Experimental section.

[11] The distance, d, is taken as the number of carbon atoms separating the charged groups.

[12] E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 101.

[13] Reference 12, p. 127.

reactions, as well as for errors in the scale, for exposed stem and setting factor of the thermometer, were applied.

Solutes.—All solutes were carefully purified, dried at 100°, and stored in desiccators over concentrated sulfuric acid until used. Melting points observed compared favorably with literature values in all cases. L-Leucine was a highly purified sample[3] kindly furnished by Dr. D. W. Thomas. In order to standardize the experimental procedure, potassium sulfate, barium sulfate and benzoic acid were also investigated. The sulfates were ignited to a dull red heat.

Tabulation of Data.—The results of the cryoscopic measurements are summarized in Table II where T is the initial freezing point of the sulfuric acid; Δ_m, the increment in molality of the solution; ΔT, the resultant freezing point depression; and i, the van't Hoff factor calculated from the relation $i = \Delta T/\Delta m \times 6.154\ (1 - 0.0047t)$ where 6.154 is the molal freezing point depression constant of sulfuric acid and t the mean depression, which is the difference between the freezing point of pure sulfuric acid, taken as 10.46°,[8a] and the mean of the initial and final values which determine ΔT.

Evaluation of the Cryoscopic Data.—The proximity of the observed i-factors for the potassium and barium salts to the integers 2 and 3, respectively, attests to the general accuracy of the method. However, our results with these solutes differ from those of Hammett and Deyrup[4a] in one significant respect. For both solutes, our initial value of i, at molalities of about 0.02–0.03, is low by about 0.15–0.20. The i-factors then increase with increasing solute molality, finally approaching the theoretical values. This phenomenon, at first difficult to explain, is understandable in the light of the recent work of Gillespie, Hughes and Ingold.[10] In a very careful study of cryoscopic measurements in sulfuric acid, these authors found that the depression of the freezing point which follows the addition of an inorganic sulfate is smaller than it should (theoretically) be because the degree of ionization of the water used to suppress the self-dissociation of the solvent is lessened by the added sulfate.[14] At higher solute molalities this effect is counterbalanced by solvation of the inorganic sulfate, leading to higher values of the i-factor.[15] Thus one can now appreciate the reasons behind the apparent concentration dependence of the i-factors observed for benzoic acid[4b] and the amino acids (*cf.*, Table I). It appears that these results are due to the very same properties of the solvent system discussed above, *i. e.*, incomplete ionization of water and solvation, and need not be ascribed to any deviations from ideality peculiar to organic solutes. The dependence of this effect upon the nature of the solute, deduced by Treffers and Hammett,[4b] is probably due to differences in the degree of solvation,[10a] which one might expect to be influenced by differences in molecular structure.

In order to compare i-factors obtained for different solutes, therefore, it is necessary first of all that

TABLE II

T	Δm	ΔT	
	$KHSO_4$		
10.0	0.0195	0.221	1.84
	.0468	.553	1.93
	.0552	.689	2.04
	.0566	.692	2.01
	$Ba(HSO_4)_2$		
9.8	0.0299	0.392	2.80
	.0172	.310	2.95
	Benzoic acid		
9.9	0.0263	0.302	1.87
	.0346	.411	1.93
	.0436	.531	1.99
	Glycine		
9.6	0.0477	0.565	1.94
	.0548	.709	2.12
	.0461	.593	2.12
9.8	.0583	.703	1.96
	.0709	.920	2.13
	.0468	.625	2.20
10.4	Depressed with K_2SO_4		
9.2	0.0630	0.794	2.06
	.0528	.715	2.23
	....	.718	..
	.0380	.511	2.22
	L-Leucine		
9.9	0.0411	0.489	1.94
	....	.485	..
	.0480	.671	2.28
	.0437	.610	2.29
	β-Alanine		
10.2	0.0538	0.838	2.54
	.0538	.867	2.64
	....	.868	..
	.0370	.602	2.67
	γ-Amino-*n*-butyric acid		
9.9	0.0337	0.559	2.70
	.0459	.788	2.81
	....	.792	..
	.0545	.966	2.91
	ε-Amino-*n*-caproic acid		
9.9	0.0346	0.582	2.75
	.0352	.624	2.90
	....	.617	..
	.0350	.641	3.00
	Anthranilic acid		
10.0	0.0435	0.536	2.01
	.0492	.624	2.07
	.0388	.539	2.28
	....	.543	..
	.0370	.515	2.29
	m-Aminobenzoic acid		
10.1	0.0220	0.334	2.47
	.0339	.547	2.64
	....	.543	..
	.0588	.962	2.68
	p-Aminobenzoic acid		
10.1	0.0286	0.447	2.55
	.0411	.655	2.61
	....	.657	..
	.0505	.853	2.77

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(14) Contrary to previously held opinion, it is now found[10b] that water is not completely ionized in the solvent sulfuric acid. It has been computed that, at 0.1 molal concentration, about 7% of water remains non-ionized.

(15) Because of the lack of a sufficient number of measurements and in view of certain approximations involved in our experimental procedure it does not appear feasible to attempt recalculation of our data according to the precise methods of Gillespie, Hughes and Ingold.[10a]

the concentration of water in the solvent be approximately the same in each case, as shown by nearly equal values of T in Table II.[16] Secondly, one must find a concentration value, $\Sigma(\Delta m)$, at which the effects of incomplete ionization of water and solvation of the solute are canceled. For carboxylic acids, this concentration appears to be about 0.1 molal.[17] Furthermore, there appears to be little tendency for the i values to increase significantly above the theoretical value ($i = 2$) at higher concentrations, at least in the case of benzoic acid[4b,7] and potassium sulfate.[4a] That one is justified in extending these conclusions to the amino acids may be seen by examining the results obtained for ε-amino-*n*-caproic acid. Here the theoretical value, 3.00, is obtained at $\Sigma(\Delta m) = 0.1$. Therefore, in deriving the values of the van't Hoff i-factor for the amino acids listed in Table III, extra weight has been given to those values obtained at $\Sigma(\Delta m) \geqslant 0.1$. The initial value of a series is always low and has been excluded; therefore the values given in Table III are *not* averages of all the i-factors obtained in a given series. Since the rounded values are probably accurate to ±0.05, differences of 0.1 unit in the i-factor are of questionable significance.

TABLE III

Solute	i-Factor
$KHSO_4$	2.0
$Ba(HSO_4)_2$	3.0
Benzoic acid	2.0
Glycine	2.2
L-Leucine	2.3
β-Alanine	2.7
γ-Amino-*n*-butyric acid	2.9
ε-Amino-*n*-caproic acid	3.0
Anthranilic acid	2.3
m-Aminobenzoic acid	2.7
p-Aminobenzoic acid	2.8

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[16] That the i-factor of an amino acid is not independent of the water concentration may be concluded from the results with glycine, where the values observed appear to increase slightly as the water concentration decreases, as shown by a higher value of T. In the last series of measurements with this solute the self-dissociation of nearly anhydrous sulfuric acid was suppressed by the addition of potassium sulfate instead of water. Recently, R. J. Gillespie, *J. Chem. Soc.*, 2997 (1950), has employed sulfuric acid of maximum freezing point (pure H_2SO_4) instead of the slightly aqueous acid used here, by previous workers[4,7] and in his own earlier papers.[10] With the anhydrous solvent, correction for the repression of the autoprotolysis can easily be made by using the autoprotolysis constant (R. J. Gillespie, *ibid.*, 2516 (1950)

$$K = [H_3SO_4^+][HSO_4^-] = 0.00017$$

The accuracy of Gillespie's results indicates that there is no longer any justification for the use of slightly aqueous sulfuric acid for cryoscopic measurements.

[17] Treffers and Hammett[4b] report an i-factor of 1.98 for benzoic acid at this concentration; we find a value of 1.99 (*cf.* Table II).

[CONTRIBUTION FROM THE NATIONAL BUREAU OF STANDARDS]

The Relation Between the Absorption Spectra and the Chemical Constitution of Dyes. XXIV. Absorption Spectra of Some Thioindigo Dyes in Sulfuric Acid

BY WALLACE R. BRODE AND GEORGE M. WYMAN

The absorption spectra of nine thioindigo dyes, containing mostly methyl and ethoxyl groups or halogens as substituents, in concentrated sulfuric acid solution were determined over the wave length range 210–800 mμ. It was observed that in this medium, most of these dyes undergo a slow, irreversible change in their absorption spectra, probably due to sulfonation and/or decomposition. Thioindigo dyes containing substituents in the 4- or 6-position were found to be particularly unstable, especially when irradiated with blue or red light; whereas the spectra of dyes having substituents in the 5- and 7-positions showed little or no change under these conditions. A comparison of the absorption spectra of most of these dyes in sulfuric acid with their spectra in organic solvents reveals an extensive shift of the absorption to lower frequencies and an increase in the separation between the first (long-wave) absorption band and the absorption peak in the near ultraviolet (300–340 mμ) region. This was attributed to the formation of two hydrogen-bonded rings (similar to what had been postulated for indigo) by the addition of two protons by the sulfuric acid. The introduction of methyl groups or halogens into the thioindigo nucleus shows a normal bathochromic effect; the absorption curve undergoes a complete change, however, when ethoxy groups are introduced in the 6,6′-positions or, in the case of bis-4,5-benzothioindigo.

Introduction

Two earlier papers in this series[1,2] discussed the absorption spectra of some purified thioindigo dyes in benzene and chloroform solutions. It was observed that the absorption spectra were a reversible and reproducible function of the wave-length range of the illumination to which the solutions had been exposed prior to the measurement. This phenomenon was attributed to the existence of a dynamic equilibrium between the *cis* and *trans* isomers in each dye solution. In support of this explanation the pairs of isomers of two of these dyes (thioindigo and 5,5′-dichloro-4,4′,7,7′-tetramethylthioindigo) were separated chromatographically.

The measurement of the absorption spectra of dyes of this type[3] in sulfuric acid solution, as a sequel to the earlier work on solutions in organic solvents, was considered to be of interest because of both theoretical and practical considerations. From a theoretical standpoint, a comparison of the spectra in this and other solvents was likely to provide some indication concerning the chemical species actually present in sulfuric acid solutions of these dyestuffs. In addition, it is of great practical importance to determine the solubility and stability of these dyes in concentrated sulfuric acid and, if soluble and stable, measure their absorption spectra in this medium, because of the widespread use of this acid as a solvent in the dyestuff industry.

Experimental

(a) Preparation of Solutions.—Approximately 0.01 g. of each dye was weighed accurately and dissolved in 500–600

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[1] G. M. Wyman and W. R. Brode, THIS JOURNAL, 73, 1487 (1951).

[2] W. R. Brode and G. M. Wyman, *J. Research, Natl. Bur. Standards*, in press.

[3] The dyes studied in this investigation are listed in Figs. 1–3.