tem. At 1:1–1:3 ratios, the equilibrium lies in favor of the bis-cysteinate but as the cobalt: cysteine ratio is increased above 1:3 progressively more of the green complex is formed. At ratios greater than about 1:5 the equilibrium is shifted totally in favor of the tris-cysteinate. Above *p*H 11, a 1:3 ratio gives pure tris-cysteinate showing that chelation of the third cysteine residue is strongly *p*H-dependent. From these observations it is clear that the third cysteine molecule is not so tightly bound to cobalt as are those in the bis-cysteinate, which is not decomposed in strong acid. Lowering the *p*H from 11 to 8, even in concentrated solutions, causes immediate dissociation of the tris-cysteinate to the bis-complex and cysteine. The corresponding cobalt(III) chelates with 2-mercaptoethylamine behave in the same manner. Comparison of the spectra of the bis- and green tris-complexes at *p*H 8 and 11 show that these compounds are quantitatively interconvertible. The marked instability toward oxygen of the green tris-complexes of cysteine and 2-mercaptoethylamine may be explained by the ease of dissociation of the third ligand, which is then oxidized to the corresponding disulfide and does not recombine with the cobalt.

The very close similarity in the spectra of the bis- and green tris-complexes of cysteine and 2-mercaptoethylamine, together with the quantitative interconversion data, lead to the formulation of the bis- and green tris-cysteinates as sulfhydryl- and amino-coördinated complexes (Figs. 1 and 2).[13] In N-formylcysteine, the formyl group renders the nitrogen atom less basic than it is in cysteine resulting in sulfhydryl- and carboxyl-coördination. The similarity in spectra of the tris-complexes of cysteine and N-formylcysteine indicates that this mode of coördination occurs in the red triscysteinate (Fig. 3).

Treatment of the red tris-cysteinate with hot sodium cysteinate at *p*H 11–12 results in rapid formation of the green isomer. Lowering the *p*H to 6 gives the bis-cysteinate. The red tris-cysteinate is actually prepared at *p*H 6, from cobalt(II) ion and cysteine, but the above observation shows that, once formed, sulfhydryl- and amino-coördination is favored. The red and green tris-cysteinates are thus not interconvertible.

Acknowledgment.—We wish to express our thanks to the Research Corporation for financial assistance in this work.

[13] Paper I of this series discusses the structure of the bis-cysteinate.

EUGENE, OREGON

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MALAYA]

The Constitution of the Pyridine Monocarboxylic Acids in their Isoelectric Forms

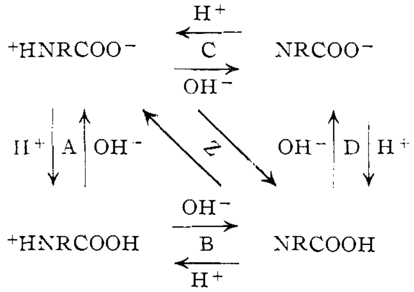
BY R. W. GREEN AND H. K. TONG

RECEIVED APRIL 17, 1956

Absorption spectra of the pyridine monocarboxylic acids, their methyl esters and N-methylbetaines demonstrate that in aqueous solutions of the acids near the isoelectric point the predominant species is the dipolar ion. From the dissociation constants of the methyl esters, it is found that less than 10% of the isoelectric form is present as uncharged molecules.

Introduction

In an aqueous solution of a pyridine monocarboxylic acid, NRCOOH, the following equilibria must be considered



The cationic species behaves as a dibasic acid for which two thermodynamic dissociation constants K_1 and K_2 can be measured by the usual methods. These are related to the constants of the above equilibria by

$$K_1 = K_A + K_B \qquad (1)$$

$$\frac{1}{K_2} = \frac{1}{K_C} + \frac{1}{K_D} \qquad (2)$$

$$K_Z = \frac{K_A}{K_B} = \frac{K_D}{K_C} \qquad (3)$$

Values of K_1 and K_2 have been determined for all three acids,[1,2] and several workers[1-3] have shown that the changes in absorption spectra with *p*H of the aqueous solutions can be correlated with the two dissociation constants.

The equilibrium Z is independent of *p*H, and none of the individual constants K_A, K_B, K_C, K_D or K_Z can be evaluated from *p*H measurements on the acids alone. However, two conflicting views have recently been expressed as to the constitution of the predominant species in isoelectric solutions. Hughes, Jellinek and Ambrose[3] state that nicotinic acid is not present as zwitterion in solution; Evans, Herington and Kynaston[2] support that opinion by referring to the dissociation of the carboxyl group in terms of K_2. This implies a very small value for K_Z. On the other hand Jaffé[4] has concluded from

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[1] H. H. G. Jellinek and J. R. Urwin, *J. Phys. Chem.*, **58**, 548 (1954).

[2] R. F. Evans, E. F. G. Herington and W. Kynaston, *Trans. Faraday Soc.*, **49**, 1284 (1953).

[3] E. B. Hughes, H. H. G. Jellinek and B. A. Ambrose, *J. Phys. Colloid Chem.*, **53**, 414 (1949).

[4] H. H. Jaffé, THIS JOURNAL, **77**, 4445 (1955).

an application of the Hammett equation[5] that the acids exist predominantly as zwitterions with only 2–10% in the uncharged form, so that K_Z would lie between 10 and 50.

In this paper we find support for Jaffé's view in a comparison of the absorption spectra of the acids with those of their methyl esters and methyl betaines; we evaluate K_Z by measuring dissociation constants of the esters.

Experimental

Materials.—Picolinic, nicotinic and isonicotinic acids were sublimed at 100° *in vacuo*. Methyl esters of the three acids were prepared by the method of Engler[6] and purified by redistillation *in vacuo*, only the central fraction being used. The boiling points were: picolinic 55–56° (0.5 mm.); nicotinic 86° (6 mm.); isonicotinic 46° (0.5 mm.). N-Methylbetaines were prepared from the corresponding methiodides.[7] However, the action of moist silver oxide on the methiodides of picolinic and isonicotinic acids produced solutions which darkened rapidly during evaporation, even in the cold. The possibility that this instability was connected with the presence of traces of silver led to an attempt to use an ion-exchange resin in place of silver oxide. An aqueous solution of the crude methiodide was passed through a column of Deacidite FF, a quaternary ammonium resin, in the hydroxide form. The effluent and washings were colorless, neutral in reaction, and free from iodide. Although the solutions were much more stable than those derived from the silver oxide reaction, a little darkening was still noticeable with the picolinic acid derivative. The solutions were therefore frozen and the water was removed by sublimation *in vacuo* at −15° leaving colorless crystals. After one recrystallization from alcohol, the picolinic acid betaine was obtained anhydrous, while the betaines of nicotinic and isonicotinic acids crystallized as monohydrates. Their identity was confirmed by m.p. determinations and by conversion to the hydrochloride to determine the equivalent weight.

Methods.—In determining pK values, 0.1 M solutions in CO_2-free water were titrated with 0.1 M NaOH or HCl in an atmosphere of nitrogen at room temperature (22°). The only exception was isonicotinic acid, whose low solubility necessitated the use of 0.05 M solutions. In titration with alkali, the end-point was determined from the slope of the pH titration curve. All pH values were measured with a Beckman Model G pH meter and a glass electrode standardized with $M/20$ potassium hydrogen phthalate solution (pH 4.00).[8] For absorption spectra at different pH values, a solution about 0.02 M in CO_2-free water was first prepared by direct weighing of the solute. Care was necessary in weighing the esters and betaines, several of which are hygroscopic. The stock solution, which was prepared fresh every two days in the case of the acids and used immediately in the case of esters or betaines, was further diluted to give a solution with optical density between 0.4 and 0.9. During this dilution, sufficient sodium hydroxide or hydrochloric acid was added to produce the required final pH, which was measured with the pH meter. Acidified solutions of the esters were not perceptibly hydrolyzed during the time needed to measure the absorption spectrum. This was established by measuring the optical density at a wave length where the difference in molar extinction coefficient between ester and free acid was a maximum. No change in optical density was observed in the duration of the experiment. In a few instances sulfuric acid was used instead of hydrochloric acid. Each reported absorption spectrum is compounded of data from three solutions of different concentrations chosen to have optical densities (0.4–0.9) in the region where the measurements were most reproducible.

Results and Discussion

In calculating thermodynamic dissociation constants, it was assumed that the activity coefficient of the isoelectric form was unity, and the activity coefficients of the other species were estimated from Güntelberg's[9] approximation

$$\log \gamma_{H} = \log \gamma_{^+HNRCOOH} = \log \gamma_{NRCOO^-} = -\frac{0.5\sqrt{I}}{1+\sqrt{I}} \tag{4}$$

where I is the ionic strength. The pyridine carboxylic acids were then treated as dibasic acids with fairly widely separated pK's, and each pK was calculated for at least 20 points distributed over almost the whole course of the titration. Near the isoelectric point the calculated values of the two constants were refined alternately by successive approximations.

The esters and nicotinic acid N-methylbetaine in their cationic forms were treated as monobasic acids, with similar assumptions about the activity coefficient, and constants were determined for the equilibria

$$^+NHRCOOCH_3 \rightleftarrows NRCOOCH_3 + H^+$$

$$CH_3\overset{+}{N}RCOOH \rightleftarrows CH_3\overset{+}{N}RCOO^- + H^+$$

The results of these calculations appear in Table I. The values of pK_1 and pK_2 for picolinic, nicotinic and isonicotinic acids agree well with those reported by other workers from spectroscopic[2] or potentiometric measurements.

TABLE I

THERMODYNAMIC DISSOCIATION CONSTANTS OF PYRIDINE MONOCARBOXYLIC ACIDS AND DERIVATIVES

	pK_1	pK_2
Picolinic acid	1.01	5.32
methyl ester	2.21	
Nicotinic acid	2.07	4.81
methyl ester	3.13	
methyl betaine	2.04	
Isonicotinic acid	1.84	4.86
methyl ester	3.26	

The values of pK for the betaine and the esters show that at pK values a little below 1.0 they will be almost entirely in the cationic form, $CH_3\overset{+}{N}R$-$COOH$ or $^+HNRCOOCH_3$, while in neutral solution they will have the respective formulas CH_3-$\overset{+}{N}RCO\overset{-}{O}$ and $NRCOOCH_3$. Figs. 1, 2, 3 show absorption spectra of the three esters and the three methylbetaines in both acid and neutral solution.

The absorption spectra of the acids themselves are more complex, as the cationic, isoelectric and anionic species all have their own absorption curves and may all be present in some solutions. Spectra of all three acids over a wide pH range have been measured both by Evans, Herington and Kynaston[2] and by Jellinek and Urwin.[1] In alkaline solution, the spectrum is that of the pure anionic form, $NRCOO^-$. If the spectrum of the pure cationic form $^+HNRCOOH$ could be obtained directly from acidified solutions, the spectrum of the isoelectric form could then be calculated from K_1, K_2 and the observed spectrum near pH 3–4.

However, in its first ionization (A + B), picolinic acid is quite a strong acid, so that it is not en-

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[5] L. P. Hammett. "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York. N. Y., 1940, Chapter VII.

[6] C. Engler, *Ber.*, **27**, 1784 (1894).

[7] H. P. Sarett, W. A. Perlzweig and E. D. Levy, *J. Biol. Chem.*, **135**, 483 (1940).

[8] W. J. Hamer, G. D. Pinching and S. F. Acree, *J. Research Natl. Bur. Standards*, **36**, 47 (1946).

[9] E. Güntelberg, *Z. physik. Chem.*, **123**, 199 (1926).

tirely cationic even in 1 *M* HCl; in this region anomalous changes have been observed[2] in its spec-

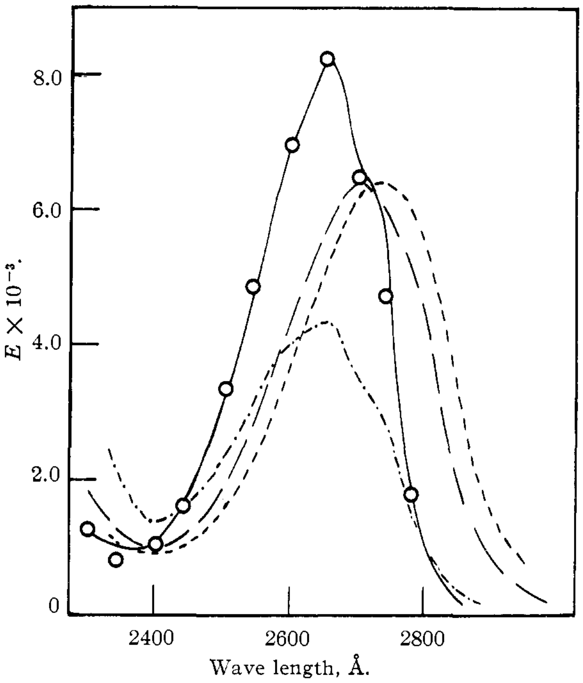

Fig. 1.—Absorption spectra of picolinic acid derivatives: ——, methyl ester, *p*H 0.5; —·—, methyl ester, *p*H 6; — —, methyl betaine, *p*H 1; ----, methyl betaine, *p*H 6; O, cationic form of free acid.

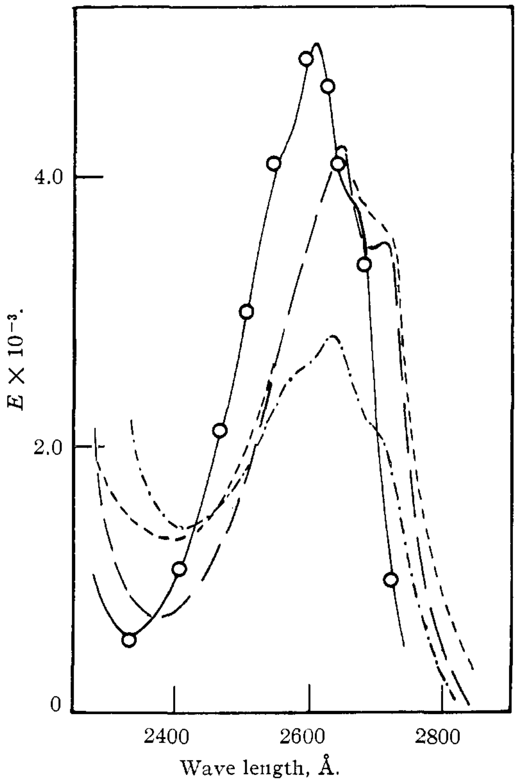

Fig. 2.—Absorption spectra of nicotinic acid derivatives: ——, methyl ester, *p*H 1; —·—, methyl ester, *p*H 6; — —, methyl betaine, *p*H 1; ----, methyl betaine, *p*H 6; O, cationic form of free acid.

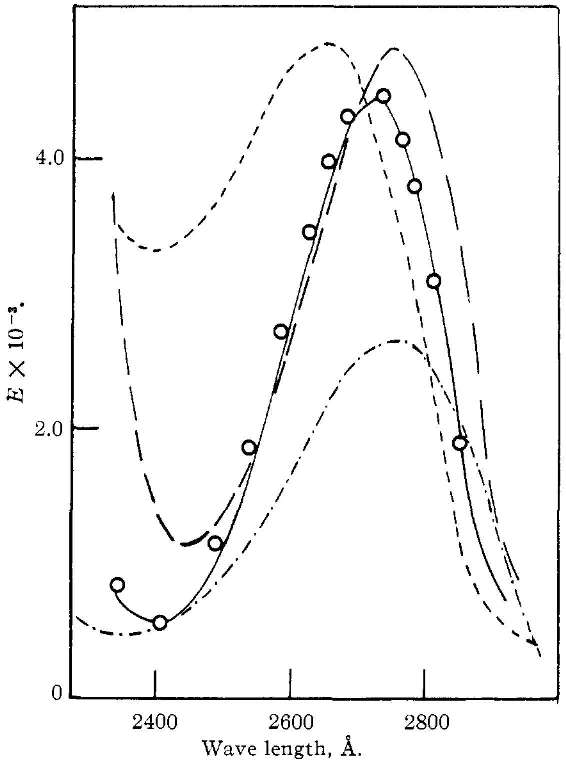

Fig. 3.—Absorption spectra of isonicotinic acid derivatives: ——, methyl ester, *p*H 1; —·—, methyl ester, *p*H 6; — —, methyl betaine, *p*H 1; ----, methyl betaine, *p*H 6; O, cationic form of free acid.

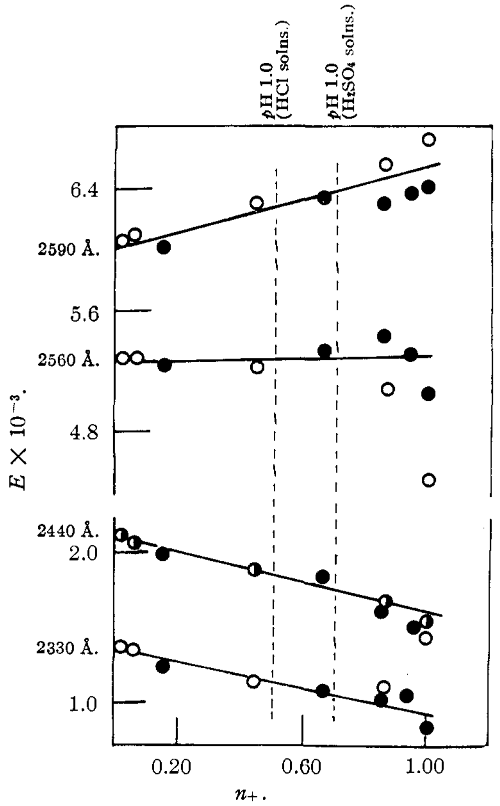

Fig. 4.—Estimation of E_+ for picolinic acid by extrapolation: O, ◑, solutions acidified with HCl; ●, solutions acidified with H_2SO_4.

trum. Evans, *et al.*,[2] suggest that "undissociated hydrochloride" may be formed and state that solutions acidified with sulfuric acid behaved normally. At some wave lengths we have observed abnormalities even with sulfuric acid and to some extent also in solutions of nicotinic and isonicotinic acids, although the latter are not as strong as picolinic acid in the first dissociation. It therefore appears that E_+, the molar extinction coefficient of the cationic form is not directly accessible, and we have adopted the following method for its determination.

Absorption spectra were measured for a number of solutions acidified with either hydrochloric or sulfuric acid to *p*H values in the range 0–3.

If n_+, n_i and n_- are the relative proportions of the cationic, isoelectric and anionic forms of the ampholyte, and if E_+, E_i, E_- are the molar extinction coefficients, n_- is negligible below *p*H 3 and

$$\begin{aligned} n_i &= 1 - n_+ \\ E_{\text{obs.}} &= E_+ n_+ + E_i(1 - n_+) \\ &= E_i + n_+(E_+ - E_i) \end{aligned}$$

The value of n_+ can be calculated from the expression

$$n_+ = a^2_{\text{H}}/(a^2_{\text{H}} + \gamma K_1 a_{\text{H}} + K_1K_2)$$

where $\log a_{\text{H}} = -p\text{H}$. The activity coefficient γ is assumed to be equal to the mean ionic activity coefficient of the hydrochloric[10] or sulfuric acid[11] used at that concentration. It is then possible to plot $E_{\text{obs.}}$ against n_+, when any abnormal behavior will appear as a deviation from linearity. The examples in Fig. 4 show no significant difference between hydrochloric and sulfuric acid solutions less acid than *p*H 1.0, but beyond that point quite

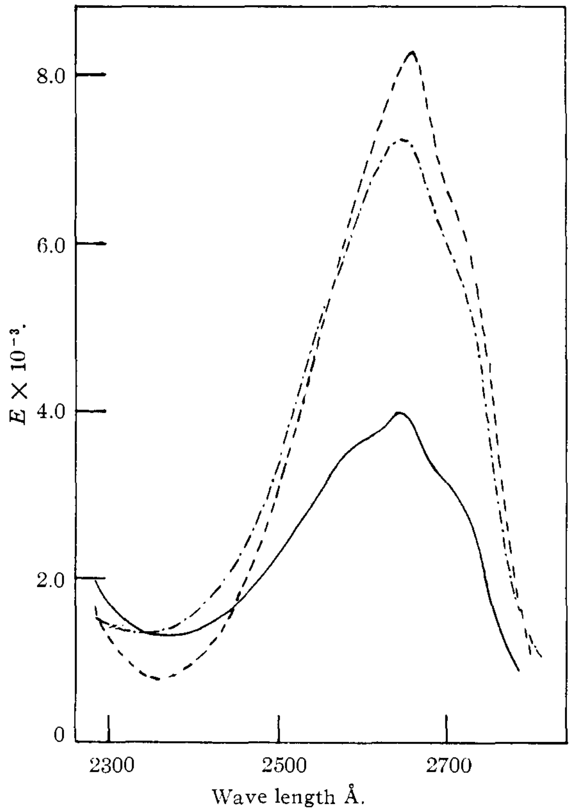

Fig. 5.—Absorption spectra of picolinic acid: – – –, E_+; —·—, E_i; —— E_-.

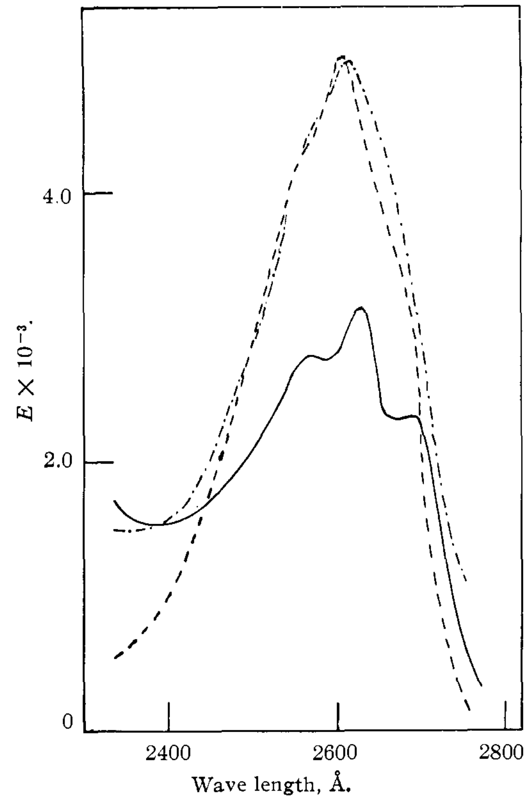

Fig. 6.—Absorption spectra of nicotinic acid: – – –, E_+, —·—, E_i; ——, E_-.

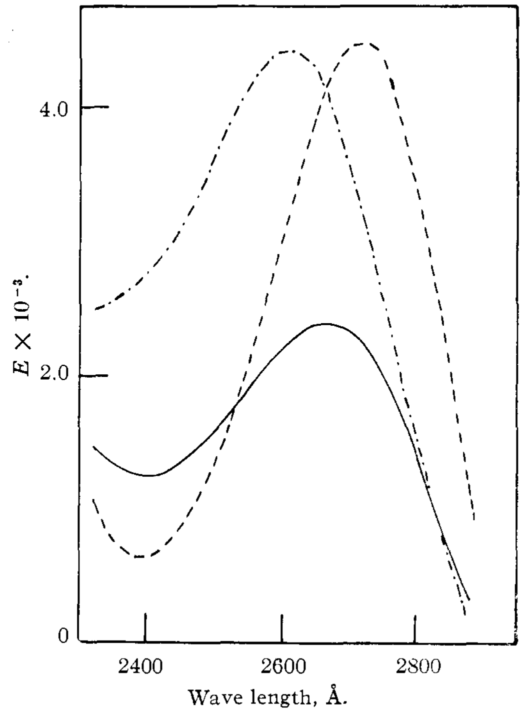

Fig. 7—Absorption spectra of isonicotinic acid: – – –, E_+; —·—, E_i; ——, E_-.

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(10) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworths Scientific Publications, London, 1955, pp. 466, 476.

(11) Ref. 10, pp. 462, 466.

marked departures from linearity sometimes appear. We have therefore drawn the best straight line through points above *p*H 1 and have extrapolated this to $n_+ = 1$ to give E_+. Although this is a rather long extrapolation, it appears from Fig. 4 to be preferable to any other means of determining E_+.

Figures 5, 6 and 7 show the extrapolated spectra of the cationic form of the three acids, together with the observed spectra of the anionic and the calculated spectra of the isoelectric species. For comparison, the extrapolated values of E_+ have also been plotted in Figs. 1, 2 and 3 where it will be seen that they fall very close to the absorption curve for the cationic form of the ester. That is the two formulas $^+HNRCOOH$ and $^+HNRCOOCH_3$ have almost identical absorption spectra, indicating that the replacement of a methyl ester group by an undissociated carboxyl produces a negligible change in spectrum. It is therefore reasonable to suppose that the uncharged molecule NRCOOH should have almost the same absorption spectrum as the neutral form, $NRCOOCH_3$, of the ester. The totally different forms of the absorption curves for the neutral ester and the isoelectric acid, strongly suggest that the latter is principally in the form of dipolar ions $^+HNRCOO^-$ and not uncharged molecules.

This view is strengthened by a comparison of the spectra of the acids with those of the corresponding betaines. The latter contain a positive nitrogen atom at all *p*H values, the only change with *p*H being ionization of the carboxyl group; the only effect of *p*H on the spectrum is a slight wave length shift with no change in the maximum extinction coefficient. Hence ionization of a carboxyl group attached to the pyridine ring appears to have little influence on the absorption spectrum, while a large decrease in *E* accompanies the dissociation of the NH^+ group. The small change in *E* of a pyridine monocarboxylic acid in passing from the cationic to the isoelectric form, and the much greater decrease from the isoelectric to the anionic form indicate clearly that the principal species at intermediate *p*H values is the dipolar ion.

TABLE II

EQUILIBRIUM CONSTANTS IN AQUEOUS SOLUTIONS OF PYRIDINE MONOCARBOXYLIC ACIDS

Acid	K_Z	% uncharged molecules	pK_A	pK_B	pK_C	pK_D
Picolinic	15	6	1.04	2.21	5.29	4.12
Nicotinic	10	9	2.11	3.13	4.77	3.75
Isonicotinic	25	4	1.86	3.26	4.84	3.44

Interpretation of spectroscopic measurements, while providing qualitative proof of the structure of the predominant species in the isoelectric acid, is not sufficiently precise to enable us to evaluate K_Z. This can be done, however, if we assume the dissociation constant of the NH^+ group in the acid to be equal to that in the methyl ester. This assumption was first used by Ebert,[12] who showed K_Z for the aminobenzoic acids to be not far from unity, and again by Edsall and Blanchard,[13] who found values of K_Z between 10^4 and 10^6 for the simple aliphatic amino acids. Our observation that the two substances have identical absorption spectra gives added support to Ebert's assumption. If K_E is the acid dissociation constant of the ester, and if $K_E = K_B$, then

$$K_Z = (K_1 - K_B)/K_B = (K_1 - K_E)/K_E = K_1/K_E - 1$$

With the reservation that this may not be exact, we present in Table II values of the several calculated equilibrium constants. They confirm the impression gained from the absorption spectra that the isoelectric form consists mainly of dipolar ions. The values of pK_A show that a carboxyl group attached to the pyridinium nucleus is a very much stronger acid than benzoic acid[14] and considerably stronger than the *A* dissociation of the aminobenzoic acids.[12] Similarly the acidic function of the NH^+ group of the isoelectric acids is of the order of ten times stronger than in the corresponding methylpyridines.[15]

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

(13) J. T. Edsall and M. H. Blanchard, THIS JOURNAL, **55**, 2337 (1933).

(14) "Handbook of Chemistry & Physics," Chemical Rubber Publishing Company, Cleveland, Ohio.

(15) R. J. L. Andon, J. D. Cox and E. F. G. Herington, *Trans Faraday Soc.*, **50**, 918 (1954).