A NOVEL DIFFERENTIAL TITRATION TO DETERMINE pK VALUES OF PHENOLIC GROUPS IN TYROSINE AND RELATED AMINOPHENOLS[1]

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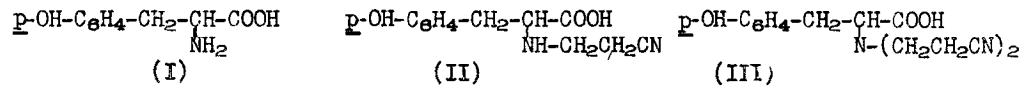
Acid-base equilibria of amino and hydroxy groups in tyrosine (I), N-cyanoethyltyrosine (II), and N,N-dicyanoethyltyrosine (III) were investigated by potentiometric and spectrophotometric titration procedures.



(I) (II) (III)

Cyanoethylation of the amino group in tyrosine causes a downward shift of the pK value of this functional group out of the range of the phenolic group. However, this modification does not alter the pK values of the phenolic groups in II and III. Based on these observations, a new procedure was developed for determining pK values of phenolic groups in aminophenols. The new differential potentiometric titration procedure for the estimation of dissociation constants of aminophenols complements the spectrophotometric methods and might have certain advantages when applied to proteins.

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[1] This is part IV in a series on "Reactions of Amino Acids, Peptides, and Proteins with α,β-Unsaturated Compounds." For part III see reference 7.

[2] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

MATERIALS AND METHODS

L-Tyrosine was obtained from Nutritional Biochemical;[3] N-monocyanoethyltyrosine and N,N-dicyanoethyltyrosine are the same compounds prepared by the Northern Laboratory (12).

Automatic potentiometric titrations were carried out in a TTT1C titrator with a Titrigraph (Radiometer-Copenhagen) standardized at 30° with three standards from the National Bureau of Standards at pH 4.01, 6.85, and 9.14 (1). Deionized, distilled water was used for all titrations and a 0.421 N carbonate-free KOH solution standardized with potassium acid phthalate was used as the base. Into a 20-ml. water-jacketed vessel was placed 10 ml. of a 0.0025 M solution of the amino acid in 0.15 M KCl adjusted to pH 2 with HCl. The temperature in the titration vessel was maintained at 30°. All titrations were carried out in duplicate under argon while the solution was being stirred magnetically.

Ultraviolet spectra of the compounds in solutions at various pH values were obtained on a Cary Model 14 spectrophotometer. Phosphate buffers were used at pH values 7-8.5; borate buffers, 9-10.4; and Na_2HPO_4-NaOH buffers, 10.4-11.9 (2). After concentrated aqueous solutions of the amino acids were diluted to a concentration of 8×10^{-5} M with the appropriate buffers which were equilibrated at 30°, ultraviolet spectra were determined in 5-cm. matched cells with the buffers as blanks. A separate aliquot was used to obtain the pH of the solution at the same temperature.

RESULTS

Representative ultraviolet spectra of N,N-dicyanoethyltyrosine at several pH values are shown in Fig. 1. Plots of extinction coefficients as a function of pH for I, II, and III at 290 mμ, 295 mμ, and 300 mμ were drawn from the experimental ultraviolet spectra. The plots for 295 mμ are

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[3] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

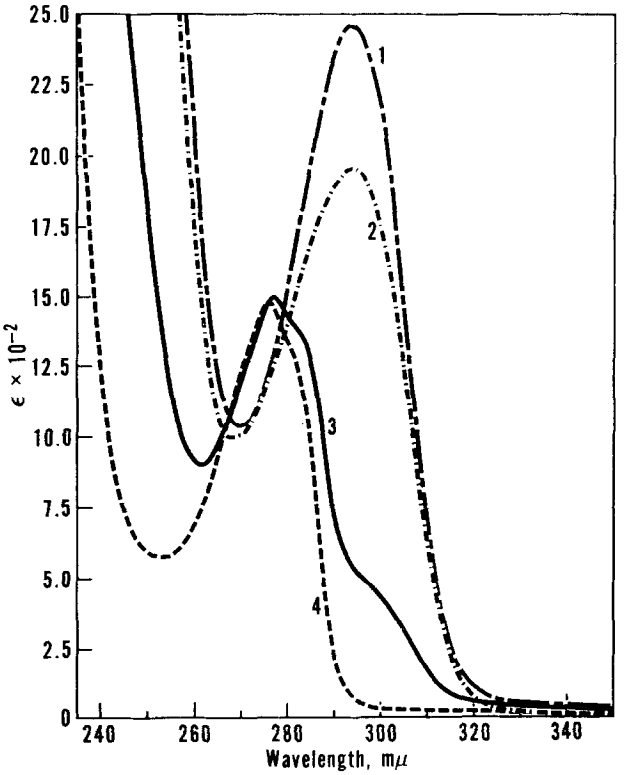

Fig. 1. Ultraviolet spectra of N,N-dicyanoethyltyrosine at different stages of ionization of the phenolic group: (1) in 0.1 N NaOH; (2) at pH 10.8; (3) at pH 9.50; (4) in 0.1 N HCl.

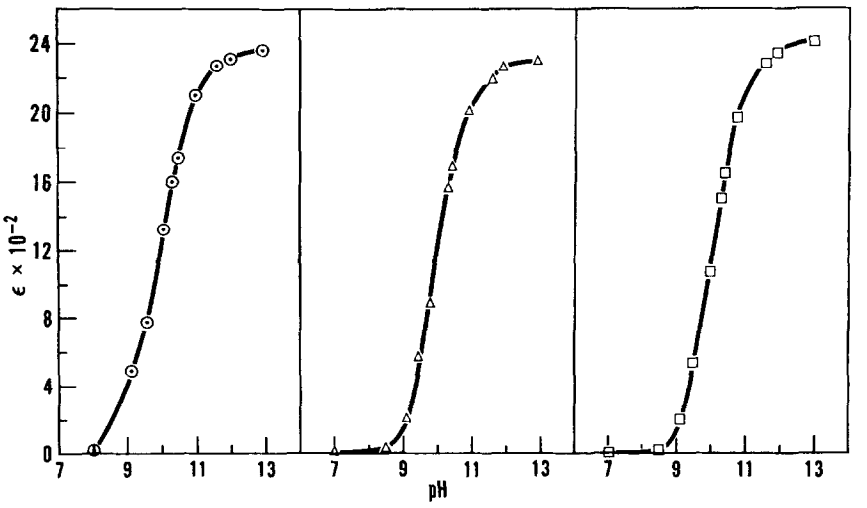

Fig. 2. Spectrophotometric titration curves at 295 mμ: ⊙ L-tyrosine; △ N-cyanoethyltyrosine; □ N,N-dicyanoethyltyrosine.

shown in Fig. 2. The spectrophotometric pK values of phenolic groups in II and III were determined graphically (3,9).

The pK value of the amino group in tyrosine was obtained via a graphical procedure based on the potentiometric titration data and the spectrophotometrically derived pK value of the phenolic group (11).

The accuracy of the pK values for the amino and hydroxy groups obtained by both methods is estimated to be ± 0.05 pK units.

All pK values from acid-base and spectrophotometric titrations are summarized in Table I.

Table I

Dissociation Constants of Tyrosine and Derivatives at 30° (μ = 0.15)

pK values determined via	Tyrosine	N-Cyanoethyl-tyrosine	N,N-Dicyanoethyl-tyrosine
Spectrophotometry: Hydroxy groups			
290 mμ	9.92	9.98	10.00
295 mμ	9.95	10.00	10.05
300 mμ	10.05	10.02	10.02
Potentiometry:			
Hydroxy groups		9.95	10.02
Amino groups	9.00	6.50	4.13

DISCUSSION

A combination of potentiometric, spectrophotometric, and differential titration methods is usually used to determine pK values of overlapping phenolic and amino groups in amino acids, in related physiologically active phenolamines such as tyramine, and in proteins. (3,4, 8-11, 13-18). The spectrophotometric method has an inherent disadvantage when applied to proteins because tryptophan and other residues absorb in the same

ultraviolet region as do ionized phenolic hydroxy groups of tyrosine side chains (13, 15-17). In the differential formol titration, many ill-defined products are formed between the formaldehyde and amino or other functional groups present in amino acids and proteins (4,14). Still another differential titration which involves the transformation of amino groups to guanidino groups by means of O-methylisourea has the disadvantage that only the ϵ-amino groups of lysine side chains in proteins appear to react, whereas α-amino groups appear to be inert to this reagent (15,16). The α-amino groups still interfere with the potentiometric titration of phenolic groups.

Acrylonitrile reacts with all types of amino groups in amino acids, peptides, and proteins and with sulfhydryl groups of aminothiols to form cyanoethyl derivatives (5,6). The introduction of one cyanoethyl group onto the amino group of an amino acid lowers the pK value of the amino group by around 2-2.5 units (5,12). This effect appears to be additive since a second cyanoethyl group causes an additional lowering of the pK value of the same order of magnitude as the first one.

The replacement of one or both hydrogens of the amino group in tyrosine by cyanoethyl groups shifts the pK value of the amino group out of the range of the corresponding value for the phenolic group, thereby permitting the direct determination of the dissociation constant of the phenolic hydroxyl group by potentiometric titration (Fig. 3). Since the experimental data listed in Table I indicate that the pK values of the hydroxy group in tyrosine and the two N-cyanoethyl derivatives are identical, the proposed differential potentiometric titration constitutes a new method for the determination of pK values of the phenolic group in tyrosine, and possibly in related aminophenols and proteins.

The suggested procedure for an aminophenol involves the modification of the amino group with acrylonitrile to an N-mono- or N,N-dicyanoethyl derivative followed by potentiometric titration of the phenolic group. If

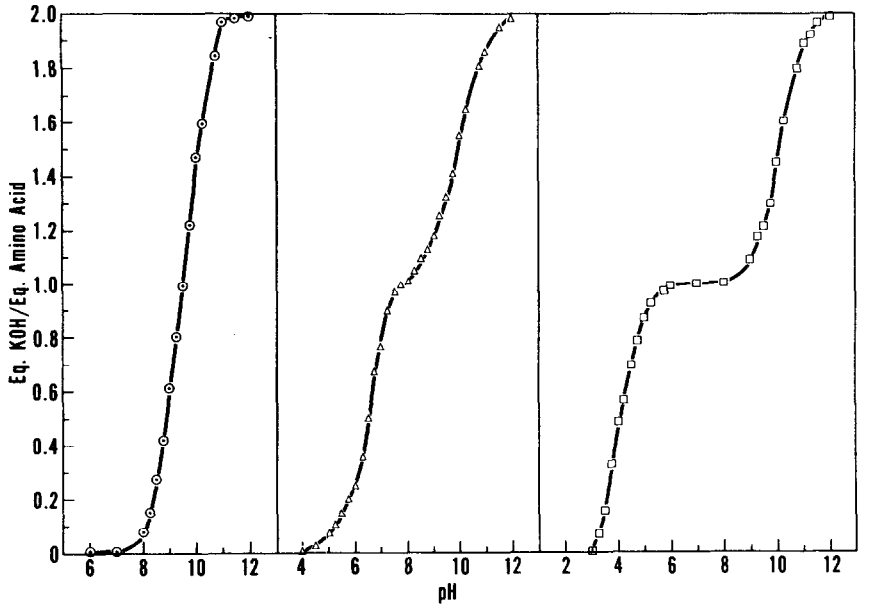

Fig. 3. Potentiometric titration curves: ⊙ L-tyrosine; △ N-cyanoethyltyrosine; □ N,N-dicyanoethyltyrosine.

the desired shift in the pK of the amino group is 2-2.5 units, then it is only necessary to prepare an N-monocyanoethyl derivative and if the desired shift is 4-5 pK units, then an N,N-dicyanoethyl derivative may be prepared.

Several problems that may arise during the use of this method require additional consideration. If the ultraviolet method is used to determine pK values of phenolic groups in aminophenols with overlapping pK values, it is implicitly assumed that the zwitterion NH_3^+-R-O^- and anion NH_2-R-O^- have identical extinction coefficients. In tyrosine, both species exist in the pH region where the hydroxy group ionizes, whereas only the anionic form exists in the cyanoethyl derivatives.

It is remarkable that a change in the dissociation constant of the amino group by around 10^5 in going from I to III does not affect that of the phenolic group. Evidently, inter- or intramolecular hydrogen bonding involving phenolic and amino groups must be negligible in these aminophenols because such hydrogen bonding would be a function of the basicities of the amino and phenolic groups, and in turn would influence their basicities.

Finally, if the new procedure is to be applied to proteins, it is expected that, by the time all amino groups have been modified with acrylonitrile, the free sulfhydryl groups present will also have completely reacted, since kinetic studies have shown that these groups react faster than amino groups with this reagent (6). This feature is desirable because then no sulfhydryl groups will interfere with potentiometric titrations of phenolic groups of tyrosine side chains. Preliminary kinetic and synthetic studies indicate that amino groups in proteins may be completely modified with α,β-unsaturated compounds under conditions which leave the phenolic groups unchanged.

Acid-base equilibria of proteins derivatized with acrylonitrile and other α,β-unsaturated compounds are under investigation.

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