# The Ionization Constants of Organic Compounds. I. The Microscopic Ionization Constants of Tyrosine and Related Compounds*

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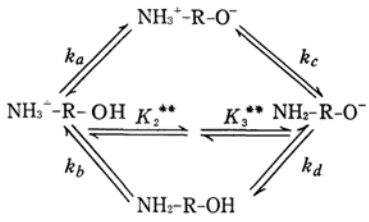
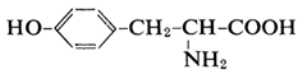
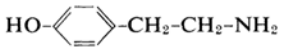
The object of this paper is to describe a method for evaluating microscopic ionization constants. Tyrosine (I), tyramine (II), 3-iodotyrosine (III), tyrosine methyl ester (IV), tyrosine ethyl ester (V), 3, 5-diiodotyrosine (VI), and 3, 5-dibromotyrosine (VII) will be investigated in the present work. The ionization of two protons from the phenolic and amino groups in these compounds may be represented by the following scheme:





(I)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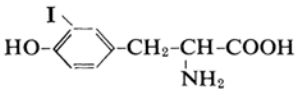
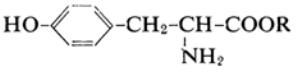
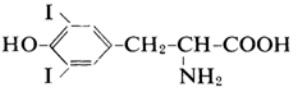
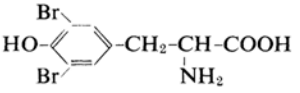

(III)

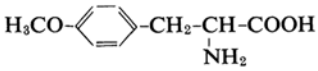

R=CH3 (IV)
R=C2H5 (V)


(VI)


(VII)

* Read in part at the Autumn Meeting of the Chemical Society of Japan, Tokyo, November, 1963, and at the 17th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1964.

** $K_2$ and $K_3$ should be properly replaced by $K_1$ and $K_2$ respectively for II, IV, and V.


(VIII)

The four compounds, I, III, VI, and VII, have a carboxyl group which corresponds to $pK_1$ as another ionizable group. However, as the $pK$ values of the carboxyl groups in these compounds are around 2, if we consider only the ionization which occurs at pH values above 4, it is safe to assume that the carboxyl group carries a negative charge in all the species under consideration.

The scheme is composed of two macroscopic constants, $K_2$ and $K_3$, and four microscopic constants, $k_a$, $k_b$, $k_c$ and $k_d$. Moreover, these constants are interrelated as follows:

$$K_2 = k_a + k_b \tag{1}$$

$$1/K_3 = 1/k_c + 1/k_d \tag{2}$$

$$K_2K_3 = k_ak_c = k_bk_d \tag{3}$$

The macroscopic constants of some of these compounds have been reported in the literature, but no discussion has been undertaken of the microscopic constants, except on I.[1]

We have reinvestigated the ultraviolet spectrum of I and confirmed that the maximum in the absorption spectrum shifts from 274 mμ to 294 mμ with an increase in the pH value of the solution (Fig. 1). On the other hand, *O*-methyltyrosine (VIII) displayed practically the same absorption at pH 6.10 and at pH 12.50 (see Experimental section). This behavior demonstrates that the ionization of the amino group does not affect the absorption spectra accordingly, it may be assumed that the molar extinction coefficient of the $NH_3^+$-R-$O^-$ form is identical with that of the $NH_2$-R-$O^-$ form. Furthermore, the molar extinction coefficients at 295 mμ and 300 mμ are practically zero for acidic media, where the OH group is un-ionized, whereas they are large in the alkaline media, where the OH group has been converted to $O^-$ ions. Therefore, the absorption at these wavelengths may be completely ascribed to $O^-$ species. The

1) J. T. Edsall, R. B. Martin and B. R. Hollingworth, *Proc. Natl. Acad. Sci.*, **44**, 505 (1958).

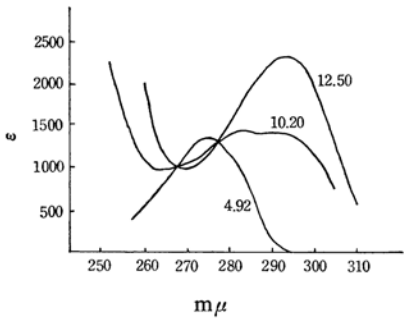



Fig. 1. Ultraviolet spectra of tyrosine at various pH (numbers besides curves).

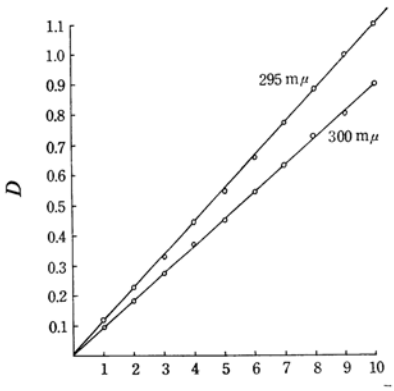



Fig. 2. Ultraviolet absorption of tyrosine at 295 mμ and 300 mμ in various dilution of 105 mg./l. solution at pH 12.50.

molar extinction coefficients of I in the completely ionized form (pH 12.50) were found to be independent of the concentration over the range less than 0.0005 M at these wavelengths (Fig. 2). Therefore, the fraction of all $O^-$ species in the solution at each pH can be determined from the extinction coefficient at 295 mμ and 300 mμ by dividing the maximum extinction coefficient at the same wavelength for the completely dissociated state (pH 12.50):

$$\alpha_{OH} = \varepsilon/\varepsilon_{max} \tag{4}$$

where $\varepsilon$ and $\varepsilon_{max}$ represent the molar extinction coefficient at each pH and that at pH 12.50 respectively.

On the other hand, the fraction $\alpha_{OH}$ can be expressed as follows by making use of Eqs. 1—3:

$$\alpha_{OH} = \frac{(NH_3^+\text{-R-}O^-) + (NH_2\text{-R-}O^-)}{\{(NH_3^+\text{-R-OH}) + (NH_3^+\text{-R-}O^-)\} + \{(NH_2\text{-R-OH}) + (NH_2\text{-R-}O^-)\}}$$

$$= \frac{k_a/(H^+) + K_2K_3/(H^+)^2}{1 + K_2/(H^+) + K_2K_3/(H^+)^2} \tag{5}$$

Once macroscopic constants have been determined by independent titration, each value of $(H^+)$ with an associated value of $\alpha_{OH}$ from the ultraviolet absorption should fix the value of $k_a$ from Eq. 6, which is a modification of Eq. 5;

$$k_a = \alpha_{OH}[(H^+) + K_2] - (1-\alpha_{OH})K_2K_3/(H^+) \tag{6}$$

A series of such $k_a$ values may be obtained and suitably averaged to obtain the most probable value of $k_a$. From this, the other microscopic constants may readily be determined from Eqs. 1—3. This method has been suggested by Edsall,[2] but no results have been reported. For the present work, the microscopic constants were studied by this procedure.

## Experimental and Calculations

**Materials.**—I, II, III, V, VI and VII were obtained from the Tokyo Kasei Co. and were recrystallized before use. IV was preared according to Fischer[3] from I. VIII was also prepared from I according to Beer[4] (monosulfate, $\lambda_{max}$: 274 mμ, log $\varepsilon$: 3.14 at pH 6.10, 3.16 at pH 12.50).

**Spectral Measurement.** — Spectral measurements were made with a Shimadzu QR-50 spectrophotometer and a Beckman DU spectrophotometer at 25±0.5°. For the measurement, 10 ml. of a 0.001 M solution of a compound were added to 10 ml. of buffer solution with an ionic strength of 0.01. The following buffers were used for the pH indicated: 6.10, 6.56, 7.13, 7.49, $Na_2HPO_4+NaH_2PO_4$; 8.10, $Na_2HPO_4+KH_2PO_4$; 8.60, $H_3BO_4+Na_2CO_3$; 9.15, 10.18, 10.60, 11.53, $Na_2CO_3+NaHCO_3$; 12.50, glycine +NaOH.—The absorption was determined immediately after mixing, the corresponding buffer being used as the blank.

**The Measurement of the pH.** — All pH measurements were carried out with a Horiba-Hitachi Model-P pH meter, equipped with a glass electrode and a double-junction sleeve-type caromel elctrode. The meter was calibrated with standard Horiba buffer solutions of pH 6.86 and 4.01 at 25°C.

**The Measurement of Macroscopic Constants.** — About a 0.001 M solution was titrated with 0.5 N sodium hydroxide. The titration solvent was a 0.05 M potassium nitrate solution in order to keep the ionic strength at 0.05. The vessel was maintained at 25±0.5°C in a water jacket with a stream from a thermo-circulator. The macroscopic constants were obtained from the titration curves, with reference to Albert.[5] The error is not greater than ±0.03 p$K$ units.

For the compounds other than I, it has also been assumed that the molar extinction coefficient of the $NH_3^+$-R-$O^-$ form is identical with that of the $NH_2$-R-$O^-$ form. The residual absorptions in acidic solutions, where the OH group is un-ionized, at a chosen wavelength were substantial in VI

2) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York (1958), p. 501.
3) E. Fischer and W. Scharanth, *Ann.*, **354**, 34 (1907).
4) L. D. Beer and H. F. Clark, *J. Am. Chem. Soc.*, **54**, 1630 (1934).
5) A. Albert, "Ionization Constants of Acid and Bases" Methuen & Co. (1962), Chap. II.

and VII. Therefore, the following formula was used instead of Eq. 4 to obtain $\alpha_{OH}$ in VI and VII:

$$\alpha_{OH} = (\varepsilon - \varepsilon_O)/(\varepsilon_{max} - \varepsilon_O)$$

Where $\varepsilon_O$ stands for the extinction coefficient in acidic media.

The values of $k_a$ were calculated in the region where $\alpha_{OH}$ is more than 0.1 and less than 0.9.

## Results and Discussion

**Tyrosine (I).**—The values of $\alpha_{OH}$ obtained at 295 m$\mu$ and 300 m$\mu$ appeared to be almost identical, supporting the validity of such an evaluation of $\alpha_{OH}$. The macroscopic and microscopic constants (Table I) agree with those of Edsall[1] when account is taken of the difference in the ionic strengths in the experiments. The values of $k_a$, $K_2$ and $K_3$ were used to construct a theoretical curve, which closely follows the experimental points (Fig. 3.) The ratio of $NH_3^+$–R–$O^-$ species to $NH_2$–R–OH species, $K_z$, becomes:

$$K_z = k_a/k_b \cong 1.1$$

This value shows that, in the course of the titration, comparable amounts of the two species are in equilibrium. Therefore, it is safe to say that $K_2$ and $K_3$ are almost completely hybridized and that, accordingly, a single assignment to the ionization of either the amino or the phenolic group is incorrect.

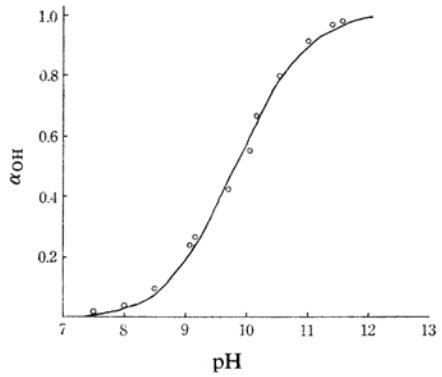



Fig. 3. Spectroscopic titration curve of tyrosine. The points are experimental, the line is calculated from the ionization constants.

The hydrogen ion concentration at $\alpha_{OH}=0.5$ is represented as follows from Eq. 5:

$$(H^+)_{\alpha=0.5} = \frac{2k_a - K_2 + [(K_2 - 2k_a)^2 + 4K_2K_3]^{1/2}}{2} \qquad (7)$$

This formula shows that, when a complete hybridization occurs in the ionization, i. e., $K_2 = 2k_a$, the value of pH at $\alpha_{OH}=0.5$ equals $(pK_2 + pK_3)/2$. The observed pH at $\alpha_{OH}=0.5$ for I was 9.82, which is approximately equal to $(pK_2+pK_3)/2$. In the literature on I, the ultraviolet spectrometric $pK_{OH}$[6-8] values are significantly lower than the titrimetric $pK_3$ values.[9,10] Apparently the former is a composite value of $pK_2$ and $pK_3$, since in the procedure $pK_{OH}$ is determined as the pH value at $\alpha=0.5$.

Also in support of the present method, it was attempted to prove that the $K_2$ for *O*-methyltyrosine (VIII) should be nearly equal to the $k_b$ for I. The ionization constants of *O*-methyl derivatives of fatty acids and amino acids with hydroxyl groups appeared essentially to confirm the above statement.[11] The $pK_2$ for VIII was determined as 9.50 at an ionic strength of 0.05, which agrees with the $pk_b$ for I within the range of experimental error.

Benesch[12] was remarkably successful in solving Eq. 5 for $k_a$, $k_b$, and $k_d$ for cysteine by the substitution of the ultraviolet spectral $\alpha_{SH}$ and $(H^+)$ values into three simultaneous equations. However, we have observed that in the case of I the ionization of the OH group was confined within a rather small pH range, and that the calculation error by his method became considerable.

**Tyramine (II).**—The values of $pK_1$ and $pK_2$ agree approximately with those obtained by Lewis[13] and by Ogston.[14] The microscopic constants are higher than the corresponding value for I by about 0.3 and 0.5 pH units respectively, as is to be expected from the elimination of the carboxyl group from I. From the results shown in Table I, the assignment of a single $pK_{OH}$ and a single $pK_{NH_3^+}$ is apparently unreasonable, as in the case of I, so each proton has an approximately equal chance to be ionized from either group.

**3-Iodotyrosine (III).** — The value of $K_z$, 5.0, shows that the hybridization in the ionization of the OH group and the $NH_3^+$ group is considerably less than those in I and II, and that the ionization of the OH group is mainly involved in $pK_2$. Herriot[15] has reported $pK'_{OH}$ as 8.20; he based his calculations on the ultraviolet absorption. The value is between $pK_2$ and $pK_3$, and it is obviously the composite constant of $pK_2$ and $pK_3$, Considerably lower values of $pK$ and $pk$, compared with those for I and II, apparently follow from the inductive effect of the iodine atom.

6) C. Tanford, *J. Am. Chem. Soc.*, **74**, 2509 (1952).
7) C. Fromageot, *Biochim. et Biophys. Acta*, **6**, 113 (1950).
8) A. Neuberger, *Biochem. J.*, **37**, 302 (1943).
9) H. Simms, *J. Gen. Physiol.*, **11**, 629 (1928).
10) A. Albert, *Bichem. J.*, **50**, 690 (1952).
11) Sec Ref. 2, p. 498.
12) R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955).
13) G. P. Lweis, *Brit. J. Pharmacol.*, **9**, 488 (1954).
14) A. G. Ogston, *J. Chem. Soc.*, **1936**, 1713.
15) R. M. Herriot, *J. Gen. Physiol.*, **31**, 18 (1947).

TABLE I

| | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|
| $\lambda_{max}$(OH), mμ | 274 | 273 | 281 | 273 | 273 | 285 | 286 |
| ($\varepsilon$) | (1380) | (1462) | (2499) | (1222) | (1354) | (2650) | (2577) |
| $\lambda_{max}$($O^-$), mμ | 294 | 293 | 304 | 292 | 291 | 308 | 308 |
| ($\varepsilon$) | (2330) | (2594) | (3961) | (2154) | (2408) | (5695) | (5736) |
| Chosen wavelength, mμ | 295 / 300 | 307 | 307 | 296 | 296 | 310 | 310 |
| p$K_1$ | — | 9.55 | — | 7.24 | 7.28 | — | — |
| p$K_2$ | 9.26 | 10.87 | 7.92 | 10.16 | 10.12 | 6.44 | 6.29 |
| p$K_3$ | 10.37 | — | 9.60 | — | — | 9.36 | 9.22 |
| p$k_a$ | 9.55 | 9.70 | 8.00 | 8.41 | 8.43 | 6.44 | 6.29 |
| p$k_b$ | 9.57 | 10.09 | 8.70 | 7.27 | 7.31 | $k_b \ll k_a$ | $k_b \ll k_a$ |
| p$k_c$ | 10.08 | 10.72 | 9.52 | 8.99 | 8.97 | 9.36 | 9.22 |
| p$k_d$ | 10.06 | 10.33 | 8.82 | 10.13 | 10.09 | $k_d \gg k_c$ | $k_d \gg k_c$ |
| $K_z$ | 1.1 | 2.4 | 5.0 | 0.07 | 0.08 | very large | very large |
| pH ($\alpha$=0.5) | 9.82 | 9.91 | 9.18 | 10.05 | 10.05 | 6.44 | 6.29 |

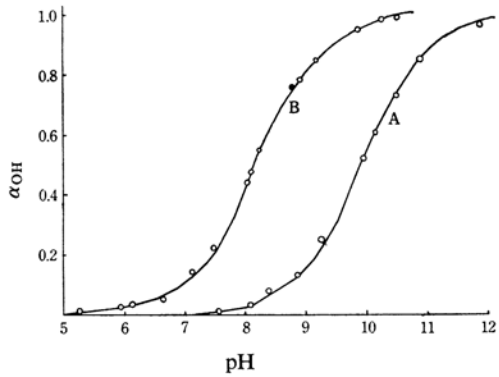



Fig. 4. Spectrophotometric titration curves of tyramine (A) and 3-iodotyrosine (B). The points are experimental, the lines are calculated from the constants given in Table I.

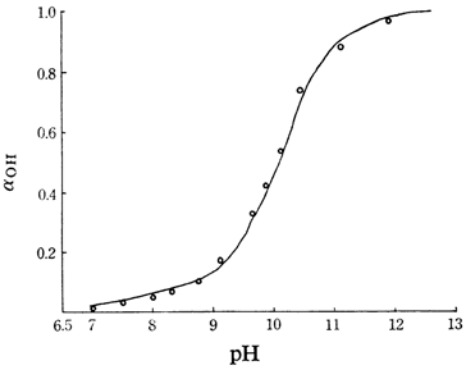



Fig. 5. Spectrophotometric titration curve of tyrosine methyl ester.

**Tyrosine Methyl Ester (IV) and Tyrosine Ethyl Ester (V).**—The ultraviolet spectra and ionization constants are identical within the range of experimental error in IV and V, as is to be expected from the nearly equal inductive effect of the methoxycarbonyl and ethoxycarbonyl groups. In these substances, $k_a \ll k_b$ and $k_b$ corresponds with $K_1$ within the range of experimental error. The Edsall method[15] is unsuitable for the cases where $k_a \ll k_b$ or where, conversely, $k_a \ll k_b$, but it is revealed that the present approach may be applied even in the former cases. In these compounds, p$K_1$ is reasonably shifted by nearly 2 pH units, compared with p$K_2$ of I, as a result of the removal of a negative charge on the carboxylate group. In conclusion, it is safe to say that $K_1$ and $K_2$ are mainly associated with the amino and the phenolic groups respectively in these compounds.

**3, 5-Diiodotyrosine (VI) and 3, 5-Dibromotyrosine (VII).**—In these compounds $k_a$ equals $K_2$ and corresponds to the pH value at which $\alpha_{OH}$ is exactly 0.5. It follows that the dissociation of the first proton is confined entirely to the OH group, and that $K_2$ and $K_3$ may be assigned exclusively to the ionization of the amino and phenolic groups respectively. It is revealed that when $k_a$ nearly equals $K_2$, $k_b$ and $k_d$ can not be estimated by the present method. The values of p$K_2$ are found to be lower than that of III, a natural result of the inductive effect of the second halogen atoms. Schmidt et al. determined the macroscopic constants from the solubility as p$K_2$=6.86, p$K_3$=7.82 for VI,[16] and p$K_2$=6.44, p$K_3$=7.58 for VII.[17] There is a significant discrepancy, especially in p$K_3$, between his values and those in Table I. However, the halogen atoms appear to be too separated from amino groups to exert such a large inductive effect as is shown by Schmidt's p$K_3$. It may be considered that the values of p$K_3$ in the present work are more reasonable.

16) J. D. Dalton, P. L. Kirk and C. L. A. Schmidt, *J. Biol. Chem.*, **88**, 589 (1933).
17) C. L. A. Schmidt and P. S. Winnek, *J. Gem. Physiol.*, **18**, 889 (1935).

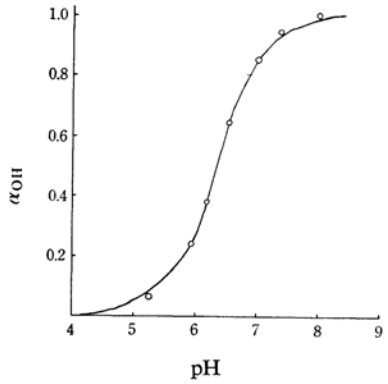



Fig. 6. Spectrophotometric titration curve of 3,5-dibromotyrosine

## Summary

A method has been formulated for the estimation of the microscopic ionization constants from spectroscopic data which measure the fractional ionization of one of the groups as a function of the pH value. This method has been applied at the ionic strength of 0.05 to the ionization of tyrosine (I), tyramine (II), 3-iodotyrosine (III), tyrosine methyl ester (IV), tyrosine ethyl ester (V), 3,5-diiodotyrosine (VI), and 3,5-dibromotyrosine (VII). The microscopic constants obtained are 9.55, 9.57, 10.08, 10.06 for I; 9.70, 10.09, 10.72, and 10.33 for II; 8.00, 8.70, 9.52 and 8.82 for III; 8.41, 7.27, 8.99 and 10.13 for IV, and 8.43, 7.31, 8.97 and 10.09 for V, in the order of $pk_a$, $pk_b$, $pk_c$, and $pk_d$. For VI and VII, the $pk_a$ values are 6.44 and 6.29, and the $pk_b$ values are 9.36 and 9.22, respectively. It has been illustrated that the method may be applied even in cases where $k_a \ll k_b$. This approach can probably be extended to other systems containing two dissociable protons, one of which affects the ultraviolet absorption and the other does not.

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