Acid Dissociation of Tyrosine and Its Related Compounds

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The acid dissociation equilibria in aqueous solution of tyrosine and its related compounds were determined by potentiometric titration and absorption spectra at 25°C and \( \mu = 0.1 \) (NaClO₄).

Microscopic equilibrium constants were calculated by three different methods.

In method A, the absorbance at 295 nm is a measure of the total concentration of the dissociated phenol.

Method B is based on the assumption that \( k_3 \) is the same as the dissociation constants of dimethoxy-phenyl or phenyl derivatives.

Method C is a modification of the Edsall method.

Tyrosine, \( m \)-tyrosine, and octopamine were found to be present in maximum amount as the amino-phenol form at pH 9.5, whereas tyramine was found to be present in about 30% as a zwitter-ionic form at pH 10. From the values obtained, \( pK_a \) values seem to contribute to the dissociation of phenol group in tyrosine, \( m \)-tyrosine, and octopamine, while the dissociation of phenol group may contribute to the value of \( pK_a \) in tyramine.

Accurate evaluation of acid dissociation of catecholamines is indispensable to an understanding of their pharmacological effect and biological reactions. In the course of studies on acid dissociation of 3,4-dihydroxyphenylalanine (DOPA), whose microscopic acid dissociation constants (micro-constants) have not all been reported, we have applied three methods to obtain the micro-constants in each equilibrium (Chart 1) of tyrosine and its related compounds. Two of the three methods were previously reported, and the third is a modification of the complementary tristimulus colorimetry (CTS method). The CTS method is simple in operation and is found to have a wide application compared with other methods. The compounds examined were \( \beta \)-4-hydroxyphenylalanine (tyrosine) (I), \( \beta \)-3-hydroxyphenylalanine (\( m \)-tyrosine) (II), \( \beta \)-4-hydroxyphenylethanolamine (octopamine) (III), and \( \beta \)-4-hydroxyphenylamine (tyramine) (IV). The micro-constants of (I) and (IV) have been reported previously, but those of (II) and (III) are not known so far.

Experimental

Materials—\( \beta \)-4-Hydroxyphenylalanine (tyrosine), \( \beta \)-4-hydroxyphenylethanolamine (octopamine), \( \beta \)-4-hydroxyphenylamine (tyramine), 3,4-dimethoxyphenylalanine, \( \beta \)-phenethyamine, and 3,4-dimethoxy-

1) Location: a) Nahauchiho, Misasagi, Yamashina, Higashiyama, Kyoto, 607, Japan; b) Shio-machi-Ichome, Tohushima, 770, Japan.
phenylamine were purchased from Nakarai Chemicals Co., Kyoto. \( \beta \)-3-Hydroxyphenylalanine (\( m \)-tyrosine) and \( \beta \)-hydroxyphenylalanine were obtained from Sigma Chemical Co., U.S.A.

**Measurements of Absorption Spectra** — The absorption spectra of aqueous solutions were measured in the UV-region with a Shimadzu UV-200S recording spectrophotometer.

**pH Titration** — The pH titration was carried out with a Hiranuma Titrator TB-101 and Titrigraph Rat 101 equipped with a Denkikagaku Keiki MC-30 combination electrode. The combination electrode was adjusted with standard solutions (pH 4.01, 6.86, 9.18). Solutions containing 5 \( \times \) \( 10^{-3} \) m catecholamine were titrated with carbonate-free 0.1N KOH. The solution was stirred by a magnetic stirrer and CO\(_2\)-free nitrogen gas was introduced slowly into the solution during the titration. The ionic strength was adjusted to 0.1 with NaClO\(_4\). The temperature of the solution was maintained at 25\(^\circ\) \( \pm \) 0.1\(^\circ\).

**Determination of Dissociation Constants** — The dissociation constants of the four compounds were calculated according to the method of Schwarzenbach,\(^{13}\) by the CTS method,\(^{14,15}\) and Method C described below.

**Determination of Micro-constants** — The acid dissociation of compounds may be expressed as shown in Chart 1. The micro-constants were obtained by the use of following three methods.

\[
\begin{align*}
H_2N\text{CHCHC}_2\text{H}_2\text{O}^- & \rightleftharpoons R_1 R_2 \rightarrow H_2N\text{CHCHC}_2\text{H}_2\text{O}^- \\
R_1 R_2 & \rightleftharpoons K_4 \rightarrow \text{H}_2N\text{CHCHC}_2\text{H}_2\text{O}^- \\
R_1 R_2 & \rightleftharpoons K_4 \rightarrow \text{H}_2N\text{CHCHC}_2\text{H}_2\text{O}^- \\
\end{align*}
\]

\[
\begin{align*}
K_1 &= h_1 + h_2 \\
K_2 &= k_{12}^{-1} + k_{21}^{-1} \\
K_1 K_2 &= h_1 h_{12} = h_2 k_{21} \\
K_1 &= \frac{h_2}{h_1} = \frac{k_{21}}{k_{12}}
\end{align*}
\]

**Chart 1.** Scheme of Ionization Equilibrium of Tyrosine and Its Related Compounds

Method A: Since in alkaline region, phenoxy anion markedly increases absorption at 295 nm,\(^{9}\) the micro-constants of compounds were calculated according to the method of Edsall and Blanchard\(^{16}\) and Benesch.\(^{7}\)

Method B: In Chart 1, the dissociation constants of dimethoxyphenyl\(^{14,17}\) or phenyl derivatives\(^{8}\) were expediently used as substitution values for \( h_7 \), which corresponds to the dissociation of NH\(_2\)\(^+\).

Method C: By use of absorption spectra in three different proton concentrations, \( h_1 \), \( h_2 \), and \( h_{12} \) values were calculated from equation (5).

\[
\begin{align*}
2 h_1 [H^+] - M_{0H} h_2 & = h_7 h_{21} - M_{0H}[H^+] = 0 \\
M_{0H} = \frac{[H^+] q_{OH}}{1 - q_{OH}} = \frac{h_1 [H^+] + h_7 h_{21}}{[H^+] + h_2}
\end{align*}
\]

\( q_{OH} \) defined by the CTS method\(^{9}\) equals \( M_{0H} \) in the Edsall method.\(^{17}\) From these three values (\( h_1 \), \( h_2 \), and \( h_{12} \)), the remaining \( h_{12} \) and the dissociation constants, \( pK_1 \) and \( pK_2 \), were calculated from equations (1) to (4) in Chart 1.

**Results and Discussion**

The dissociation constants of four compounds are listed in Table I. The values of \( pK_1 \) and \( pK_2 \) correspond to the dissociation of amino and/or phenol group, and the dissociation constants of carboxyl groups are represented by \( pK_{COOH} \). The result obtained in this study

agree with those previously reported values.\textsuperscript{11,12,18–22} Further, the values measured by the three different methods, i.e., titration, CTS method and Method C agree well. Comparison

| Table I. Dissociation Constants of Tyrosine and Its Related Compounds |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Compound                        | Titration                        | CTS-method                       | Method C                        | Reference                       |
|                                 | $pK_{\text{coh}}$ | $pK_1$ | $pK_2$ | $pK_{\text{coh}}$ | $pK_1$ | $pK_2$ | $pK_{\text{coh}}$ | $pK_1$ | $pK_2$ | No. |
| Tyrosine                        | 2.46±0.02                        | 9.01±0.02                        | 10.17±0.02                      | 10.05±0.03                      | 9.01±0.03                      | 10.19±0.03 | 2.24 | 9.21 | 10.28 | 18 |
| m-Tyrosine                      | 2.33±0.06                        | 8.94±0.06                        | 10.21±0.06                      | 10.03±0.02                      | 8.85±0.03                      | 10.19±0.03 | 2.34 | 9.11 | 10.13 | 11 |
| Octopamine                      | 8.91±0.01                        | 9.99±0.01                        | 9.88±0.04                       | 9.88±0.04                       | 10.04±0.03                      | 8.89±0.06 | 8.89 | 9.94 | 20 |
| Tyramine                        | 9.56±0.01                        | 10.69±0.03                      | 9.65±0.02                       | 9.54±0.03                       | 10.63±0.03                      | 9.53±0.03 | 9.57 | 9.66 | 21 |
|                                 |                                 |                                 |                                 |                                 |                                 |                                 |      |      |      |    |

($\mu$=0.1 (NaClO$_4$), 25\degree)

| Table II. Microscopic Acid Dissociation Constants and Tautomeric Constants of Tyrosine and Its Related Compounds |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Compound                        | Method | $k_1$ | $k_2$ | $k_{12}$ | $k_{21}$ | $K_1(k_{21}/k_1)$ | Ref. |
| Tyrosine                        | A      | 9.51  | 9.18  | 9.66     | 10.05    | 2.2               | 11) |
|                                 | B      | 9.54  | 9.63  | 9.55     | 9.62     | 2.2               | 2.4 |
|                                 | C      | 9.56±0.03 | 9.16±0.04 | 9.66±0.04 | 10.04±0.02 | 2.5               |      |
| m-Tyrosine                      | A      | 9.38  | 9.13  | 9.76     | 10.02    | 1.8               |      |
|                                 | B      | 9.34  | 9.16(b) | 9.81     | 9.99     | 1.5               |      |
|                                 | C      | 9.38  | 9.13(b) | 9.76     | 10.02    | 1.8               |      |
| Octopamine                      | A      | 9.48  | 9.06  | 9.43     | 9.85     | 2.7               |      |
|                                 | B      | 9.54  | 9.04(c) | 9.37     | 9.87     | 3.2               |      |
|                                 | C      | 9.44±0.02 | 9.04±0.01 | 9.49±0.03 | 9.89±0.01 | 2.5               |      |
| Tyramine                        | A      | 9.76  | 9.99  | 10.49    | 10.26    | 0.6               | 12) |
|                                 | B      | 9.59  | 9.66  | 10.03    | 9.97     | 0.9               | 5)  |
|                                 | A      | 9.66  | 10.11 | 10.65    | 10.20    | 0.4               |      |
|                                 | B      | 9.76  | 9.99(c) | 10.49    | 10.26    | 0.8               |      |
|                                 | C      | 9.83  | 9.89(f) | 10.42    | 10.36    | 0.8               |      |
|                                 |                                 | 9.74±0.01                        | 9.99±0.04                       | 10.44±0.04                      | 10.18±0.01                      | 0.06 |

(a) dissociation constant of phenylalanine (9.16±0.01)
(b) dissociation constant of 3,4-dimethoxyphenylalanine (9.13±0.01)
(c) $k_2=9.87$, $K_{21}=9.11$ and $K_{12}=10.12$ (ref. 11)
(d) dissociation constant of $\beta$-hydroxyphenylalanylamine (9.94±0.00)
(e) dissociation constant of $\beta$-phenylethylalanylamine (9.94±0.00)
(f) dissociation constant of 3,4-dimethoxyphenethylamine (9.89±0.01)

18) H.S. Simms, J. Gen. Physiol., 11, 629 (1928).
of the dissociation constants by titration and Method C with those by the CTS method shows that the pK\textsubscript{a} values in tyrosine, \( m \)-tyrosine, and octopamine seem to be affected by the dissociation of phenol groups, while in tyramine the dissociation of phenol group may contribute to the value of pK\textsubscript{a}. To obtain further detailed information on the acid dissociation of the four compounds, micro-constants were calculated by the three different methods and values for the four compounds are shown in Table II. The values by the three methods agree well, and the values in tyrosine and \( m \)-tyrosine agree with those reported in the literature\textsuperscript{5,11,12}. The values of tautomeric constant \( K_4 \) (\( k_2/k_1 \)) were 1.5—3.2 in tyrosine, \( m \)-tyrosine, and octopamine and 0.4—0.9 in tyramine. The relative concentrations of various ionic forms in tyrosine and tyramine as a function of pH are shown in Figs. 1 and 2. In tyrosine the relative

![Fig. 1. Relative Concentration of Various Ionic Forms of Tyrosine](image)

![Fig. 2. Relative Concentration of Various Ionic Forms of Tyramine](image)

amount of the amino-phenol form (C) (Fig. 1) reached maximum at pH 9.5, and almost the same results were obtained in \( m \)-tyrosine and octopamine. On the other hand, in the case of tyramine, the concentration of zwitter-ionic form (B) (Fig. 2) is about 30% or above in the range of pH 9.5—10.5. These results indicate that tyrosine, \( m \)-tyrosine, and octopamine dissociate preferentially at the amino group, whereas tyramine dissociates at the phenol group. The calculation of micro-constants of DOPA by these method is now in progress and will be reported subsequently.