Notes

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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 potentiomeric titration and absorption spectra at 25° and $\mu=0.1$ ($NaClO_4$).

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_2 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_2 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_1 in tyramine.

Accurate evaluation of acid dissociation of catecholamines is indispensable to an understanding of their pharmacological effect and biological reactions.[2-4] 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,[5] 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,[6-8] and the third is a modification of the complementary tristimulus colorimetry (CTS method).[9,10] The CTS method is simple in operation and is found to have a wide application compared with other methods. The compounds examined were β-4-hydroxyphenylalanine (tyrosine)[11] (I), β-3-hydroxyphenylalanine (*m*-tyrosine) (II), β-4-hydroxyphenylethanolamine (octopamine) (III), and β-4-hydroxyphenylamine (tyramine)[5,12] (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——β-4-Hydroxyphenylalanine (tyrosine), β-4-hydroxyphenylethanolamine (octopamine), β-4-hydroxyphenylamine (tyramine), 3,4-dimethoxyphenylalanine, β-phenethylamine, and 3,4-dimethoxy-

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1) Location: *a) Nakauchicho, Misasagi, Yamashina, Higashiyama, Kyoto, 607, Japan; b) Sho-machi-1-chome, Tokushima, 770, Japan.*
2) Symposium on Catecholamines, *Pharmacol. Rev.*, **11**, 241 (1959).
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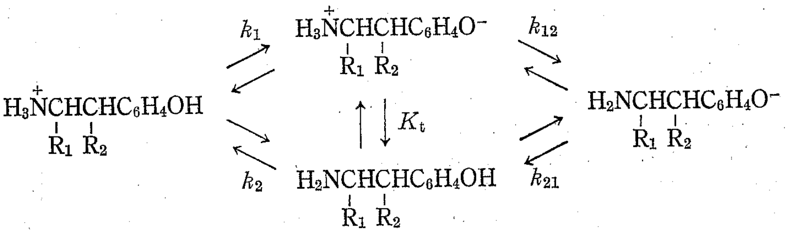
phenylamine were purchased from Nakarai Chemicals Co., Kyoto. β-3-Hydroxyphenylalanine (*m*-tyrosine) and β-hydroxyphenylamine were obtained from Sigma Chemical Co., U.S.A.

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 5×10^{-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°±0.1°.

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.



	R_1	R_2	C_6H_4OH
I	COO^-	H	*p*-
II	COO^-	H	*m*-
III	H	OH	*p*-
IV	H	H	*p*-

$$K_1 = k_1 + k_2 \tag{1}$$

$$K_2 = k_{12}^{-1} + k_{21}^{-1} \tag{2}$$

$$K_1K_2 = k_1k_{12} = k_2k_{21} \tag{3}$$

$$K_t = \frac{k_2}{k_1} = \frac{k_{12}}{k_{21}} \tag{4}$$

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

Method A: Since in alkaline region, phenoxide anion markedly increases absorption at 295 nm,[5] the micro-constants of compounds were calculated according to the method of Edsall and Blanchard[6] and Benesch and Benesch.[7]

Method B: In Chart 1, the dissociation constants of dimethoxyphenyl[16,17] or phenyl derivatives[8] were expediently used as substitution values for k_2, which corresponds to the dissociation of NH_3^+.

Method C: By use of absorption spectra in three different proton concentrations, k_1, k_2, and k_{21} values were calculated from equation (5).

$$\left.\begin{aligned} &k_1[H^+] - M_{OH}k_2 + k_2k_{21} - M_{OH}[H^+] = 0 \\ &M_{OH} = \frac{[H^+]q_{OH}}{(1-q_{OH})} = \frac{k_1[H^+]+k_2k_{21}}{[H^+]+k_2} \end{aligned}\right\} \tag{5}$$

q_{OH} defined by the CTS method[9] equals α_{OH} in the Edsall method.[17] From these three values (k_1, k_2, and k_{21}), the remaining k_{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

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agree with those previously reported values.[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_{COOH}	pK_1	pK_2		pK_1	pK_2	pK_{COOH}	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
							2.34	9.11 ±0.01	10.13 ±0.01	11
								9.21	10.13	19
m-Tyrosine	2.33 ±0.06	8.94 ±0.03	10.21 ±0.03	10.03 ±0.02	8.85 ±0.03	10.19 ±0.03		9.09	10.11	19
Octopamine		8.91 ±0.01	9.99 ±0.03	9.88 ±0.04	8.88 ±0.03	10.04 ±0.01		8.89 ±0.06	9.94 ±0.07	20
								9.57	9.66	21
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	10.78 ±0.02	22
								9.77 ±0.06		22
								9.37	10.70	12

(μ=0.1 ($NaClO_4$), 25°)

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}	$K_t(k_2/k_1)$	Ref.
Tyrosine	A	9.51	9.18	9.66	10.05	2.2	
	A	9.63±0.02	9.28±0.04	9.69±0.05	10.04±0.03	2.3	11)
	B	9.54	9.16[a]	9.63	10.02	2.4	
	B	9.63	9.13[b]	9.55	10.05	3.1	
	B	9.62	9.27	9.62	9.97	2.2[c]	
	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[a]	9.81	9.99	1.5	
	B	9.38	9.13[b]	9.76	10.02	1.8	
	C	9.44±0.02	9.12±0.04	9.72±0.02	10.00±0.04	2.1	
Octopamine	A	9.48	9.06	9.43	9.85	2.7	
	B	9.54	9.04[d]	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	
	A	9.59	9.66	10.03	9.97	0.9	12)
	A	9.66	10.11	10.65	10.20	0.4	5)
	B	9.76	9.99[e]	10.49	10.26	0.6	
	B	9.83	9.89[f]	10.42	10.36	0.8	
	C	9.74±0.01	9.99±0.04	10.44±0.04	10.18±0.01	0.06	

(μ=0.1 ($NaClO_4$), 25°)

a) dissociation constant of phenylalanine (9.16±0.01)

b) dissociation constant of 3,4-dimethoxyphenylalanine (9.13±0.01)

c) k_2=9.27, K_1=9.11 and K_2=10.13 (ref. 11)

d) dissociation constant of β-hydroxyphenethylamine (9.04±0.03)

e) dissociation constant of β-phenylethylamine HCl (9.99±0.01)

f) dissociation constant of 3,4-dimethoxyphenethylamine (9.89±0.01)

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of the dissociation constants by titration and Method C with those by the CTS method shows that the pK_2 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_1. 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.[5,11,12] The values of tautomeric constant K_t (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 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.

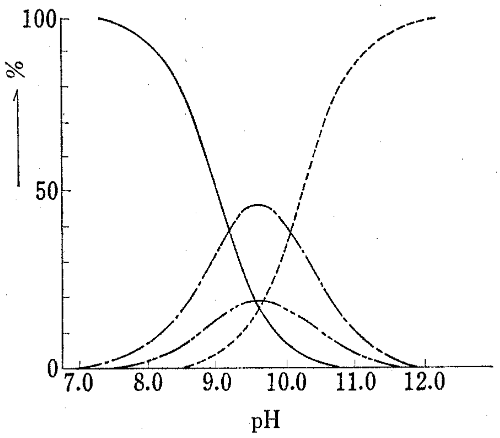
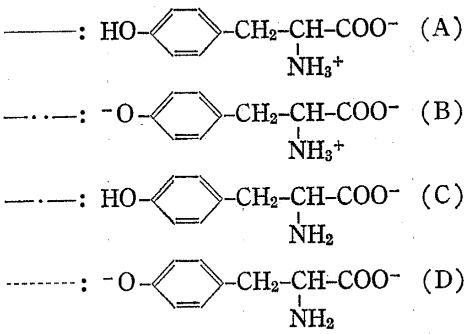


Fig. 1. Relative Concentration of Various Ionic Forms of Tyrosine



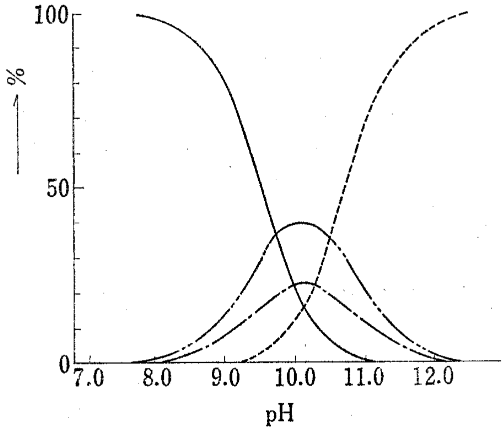


Fig. 2. Relative Concentration of Various Ionic Forms of Tyramine