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Microscopic Acid Dissociation Constants of 3,4-Dihydroxyphenethylamine (Dopamine)

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The acid dissociation equilibria in aqueous solution of dopamine were determined by potentiometry and complementary tristimulus colorimetry (CTS method), at 25° and $\mu=0.1$ ($NaClO_4$).

From these data, microscopic equilibrium constants were calculated by the use of the substitution method.

At physiological pH region (pH 7.2—7.4), dopamine is present as an ammonium species (I), and at pH 9.75 as zwitter-ionic species, (III) and (IV) in about 30% and 20%, respectively.

The concentration of mono-phenolate anionic forms, (V) and (VI), are about 40% and 30%, respectively, at pH 11.0.

Keywords——dopamine; tyramine; titration; CTS method; dissociation constant; micro-constant

3,4-Dihydroxyphenethylamine (dopamine) which is found in various tissues of animals, is a decarboxylated metabolic product of 3,4-dihydroxyphenylalanine (DOPA). In an explanation of physiological effects caused by dopamine and DOPA, it is always indicated the participation of chelations of these products with various bivalent metal ions.[2,3] It is indispensable to evaluate the dissociation constants in order to understand the chemical forms of these compounds and further the mode of coordination of dopamine- or DOPA-metal complexes in aqueous solution.

However, there are few reports on the acid dissociation phenomena of the sympathomimetic catecholamines.[4] Recently we have reported the accurate microscopic acid dissociation constants (micro-constants) and the variation of relative concentrations of various species against pH both in 3,4-dihydroxyphenylpropionic acid and DOPA.[5] In this report, we have investigated the dissociation phenomena in aqueous solutions of dopamine and related compounds on the basis of micro-constants and thermodynamic parameters.

Experimental

Materials——3,4-Dihydroxyphenethylamine (dopamine), 3,4-dimethoxyphenethylamine, β-4-hydroxyphenethylamine (tyramine), and β-phenethylamine were purchased from Nakarai Chemical Co., Kyoto. 3-Methoxy-4-hydroxyphenethylamine was obtained from Calbiochem, USA. These materials were of analytical grade and used without further purification. β-3-Hydroxyphenethylamine (*m*-tyramine) was synthesized by the method of Axelrod *et al.*[6] and 3-hydroxy-4-methoxyphenethylamine was prepared by the method of Beke *et al.*[7]

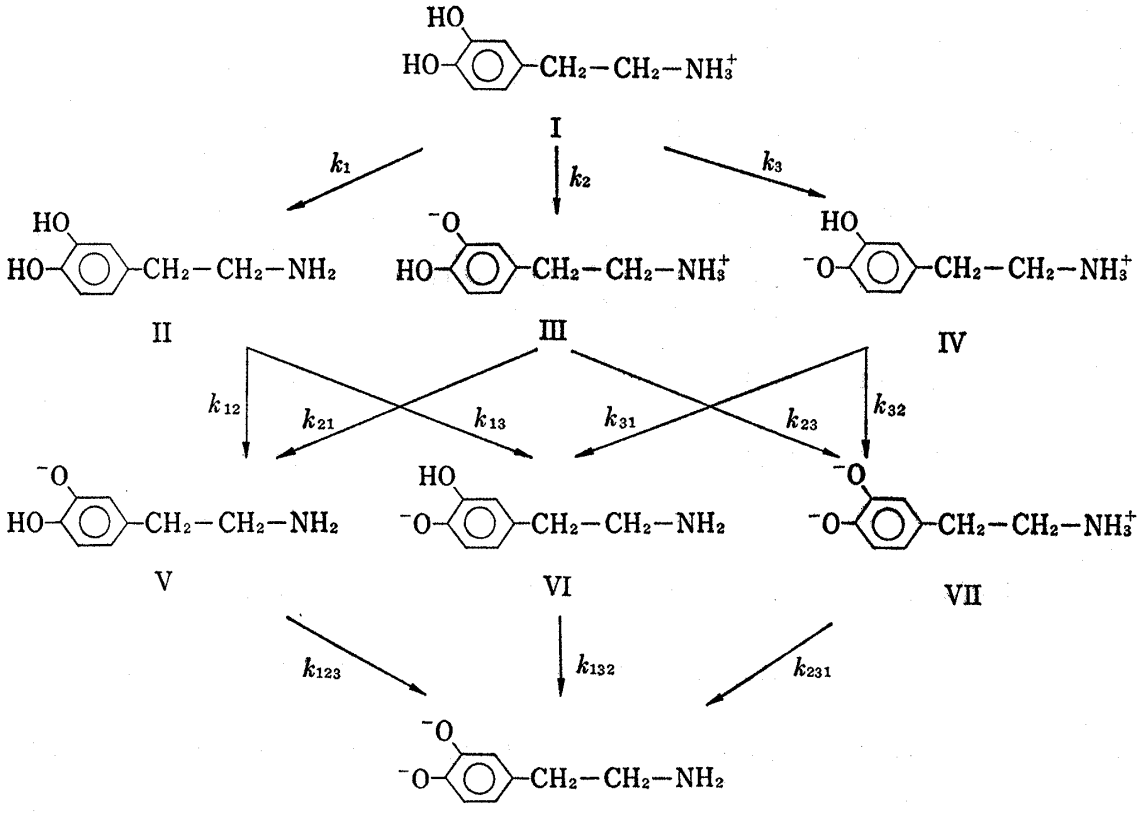
1) Location: *a) Nakauchicho, Misasagi, Yamashina, Kyoto, 607, Japan; b) Sho-machi-1-chome, Tokushima, 770, Japan.*
2) B. Grgas-Kuznar, VL. Simeon, and O.A. Weber, *J. Inorg. Nucl. Chem.*, **36**, 2151 (1974).
3) K.S. Rajan, J.M. Davis, and R.W. Colburn, *J. Neurochem.*, **18**, 345 (1971).
4) R.B. Martin, *J. Phys. Chem.*, **75**, 2657 (1971).
5) T. Ishimitsu, S. Hirose, and H. Sakurai, *Talanta*, **24**, 555 (1977).
6) J. Axelrod, S. Senoh, and B. Witkop, *J. Biol. Chem.*, **233**, 697 (1958).
7) D. Beke and Cs. Szantay, *Acta Chim. Acad. Sci. Hung.*, **14**, 325 (1958).

Measurement of Absorption Spectra——The absorption spectra of aqueous solutions in the UV-region were measured in an atmosphere of nitrogen gas according to the method of previous paper.[8] The spectra of dopamine and its related compounds were measured at a concentration of 2×10^{-4} M.

pH Titration——The pH titration procedure described in the previous paper[8] was employed. Dopamine is relatively stable in acidic solution but decomposes slowly in solutions of high pH values, as is indicated by the development of a brown colour. Therefore, the titrations were carried out in an atmosphere of nitrogen gas to minimize the oxidation.

Determination of the Dissociation Constants——The dissociation constants of compounds were calculated according to the both method of Schwarzenbach[9] and the complementary tristimulus colorimetry (CTS method) reported previously.[8]

Determination of the Micro-constants——The micro-constants of dopamine were calculated by the method of Edsall *et al.*[10] which was employed previously in the calculation of micro-constants of DOPA.[5] The micro-constants of dopamine are related to the dissociation constants (pK_1, pK_2, and pK_3) by equations (1), (2), and (3) in Chart 1. Since the deprotonation processes related to k_2 and k_{21}, do not involve the deproto-



$$K_1=k_1+k_2+k_3 \quad (1)$$

$$K_1K_2=k_1k_{12}+k_1k_{13}+k_2k_{23}=k_2k_{21}+k_3k_{31}+k_3k_{32} \quad (2)$$

$$K_1K_2K_3=k_1k_{12}k_{123}=k_2k_{21}k_{123}=k_2k_{23}k_{231}=k_1k_{13}k_{132}=k_3k_{31}k_{132}=k_3k_{32}k_{231} \quad (3)$$

Chart 1. Scheme of Ionization Equilibrium of Dopamine

nation of 4-hydroxy group (*p*-phenol), the four micro-constants of 3-hydroxy-4-methoxyphenethylamine were assumed to be the same as k_1, k_2, k_{12}, and k_{21}. Also, the values of k_1, k_3, k_{13}, and k_{31} can be evaluated to be the same as the micro-constants of 3-methoxy-4-hydroxyphenethylamine. In the above two systems, the ammonium dissociation constant of 3,4-dimethoxyphenethylamine was used in place of k_1, since the effect of deprotonation of the phenol groups can be excluded. The other constants were calculated by equations (2) and (3).

Thermodynamic Parameters——The values of thermodynamic parameters were obtained in the same manner as previous paper.[5] Plots of pK_a^T ($a=1, 2, 3$) against $1/T$°K were linear or slightly curved (15°±0.1°, 25°±0.1°, 35°±0.1°). From the empirical equations base on the method of least squares, the values of

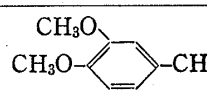
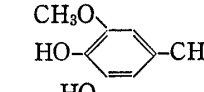
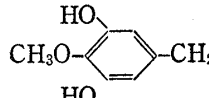
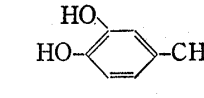
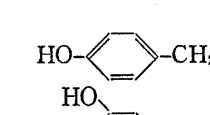
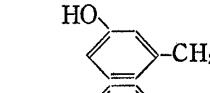
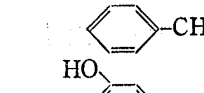
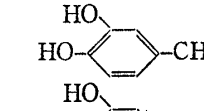
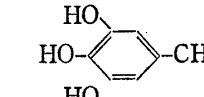
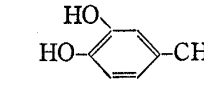
8) T. Ishimitsu, S. Hirose, and H. Sakurai, *Chem. Pharm. Bull.* (Tokyo), **24**, 3195 (1976).
9) G. Schwarzenbach, *Helv. Chim. Acta*, **33**, 947 (1950).
10) J.T. Edsall, R.B. Martin, and B.R. Hollingworth, *Proc. Natl. Acad. Sci. U.S.A.*, **44**, 505 (1958).

free energy (ΔG kcal/mol), enthalpy (ΔH kcal/mol), and entropy (ΔS e.u.) of acid dissociation were calculated.[11-13] Materials subjected were phenethylamine, tyramine, *m*-tyramine, and dopamine.

Results and Discussion

Dissociation Constants and Thermodynamic Parameters——The values of the dissociation constants of all the compounds investigated obtained from both pH titration and CTS method, at 25°, are listed in Table I. Since the Qr plot, which is obtained from the change in absorbance in ultraviolet region due to the dissociation of phenol groups, showed two straight lines (Fig. 1), the dissociation constants of phenol groups of dopamine were calculated by CTS method. Nevertheless some of the compounds studied are rather unstable as mentioned in experimental section, the pK values obtained from the both methods agreed satisfactorily. Also, these values obtained in this work are in the close agreement with those reported previously by other authors.[2,3,14] Although the macroscopic acid dissociation constants of dopamine are composites of the dissociation of all acidic groups, it can be assigned roughly that pK_1 and pK_3 values are mainly contributed from the dissociation of phenol group, from the comparison of the pK_a values of related compounds. The pK value of ammonium group of phenethylamine, 3,4-dimethoxyphenethylamine, and phenylalanine are approximately 10, and those of dopamine obtained by CTS method which concerns only with the dissociation

Table I. Dissociation Constants of Dopamine and Its Related Compounds

Compound	Titration			CTS method		Ref.
	pK_1	pK_2	pK_3	p$K_{1st\text{-}phenol}$	p$K_{2nd\text{-}phenol}$	
	9.89±0.01					
	9.45±0.01	10.58±0.02		9.56±0.01		
	9.34±0.01	10.49±0.02		9.43±0.02		
	9.05±0.04	10.52±0.02	11.98±0.06	9.00±0.03	11.90±0.07	
	9.06±0.01	10.60±0.01	12.05±0.01			2)
	8.96±0.03	10.50±0.04				3)
	8.86	10.31				14)
	9.56±0.01	10.69±0.03		9.65±0.02		
	9.45±0.03	10.58±0.03		9.49±0.03		
	9.99±0.01					
	9.58±0.02	12.15±0.03		9.61±0.04	12.03±0.02	5)
	9.69±0.02	12.11±0.04		9.76±0.03	11.99±0.02	5)
	8.76±0.06	9.96±0.02	12.14±0.08	9.78±0.04	11.97±0.06	5)

μ=0.1 ($NaClO_4$), 25°.

11) F.S. Feats and D.J.G. Ives, *J. Chem. Soc.*, **1956**, 2798.
12) G.H. Nancollas, "Interaction in Electrolyte Solutions," Elsevier, New York, 1966.
13) R. Fujishiro, G. Wada, and R. Tamamushi, "Yoeki no Seishitsu," Tokyo Kagaku Dozine, 1968, p. 121.
14) P.J. Antikainen and U. Witikainen, *Acta Chim. Scand.*, **27**, 2075 (1973).

of phenol groups lie in 9.00 and 11.90. On the other hand, it was pK_2 and pK_3 that were ascribed to the dissociation of phenol groups of DOPA in the same condition, as described previously.[5] It is presumed that the acidity of amino group in DOPA is strengthed (pK_1=8.76) owing to the electrostatic effect (or direct effect) of adjacent carboxylate anion.[15] The similar consideration has been shown in the dissociation constant of such compound as tryptophan[16] or tyrosine.[8] These assignments were also corroborated by thermodynamic consideration. Standard free energy (ΔG kcal/mol), enthalpy (ΔH kcal/mol), and entropy (ΔS e.u.) changes for the acid dissociations were calculated in dopamine and its related compounds, and the thermodynamic parameters are summarized in Table II. Fig. 2 shows the plots of ΔH against $T\Delta S$ for the first phenol group dissociation (ΔH_1 *vs.* $T\Delta S_1$), for the amino group dissociation (ΔH_2 *vs.* $T\Delta S_2$) and for second phenol dissociation (ΔH_3 *vs.* $T\Delta S_3$) in dopamine and its related compounds. The slopes of these three kinds of plots were significantly different from each other. The first phenol group dissociation of dopamine was correlated well both with the acid dissociation of mono-phenol derivatives and the first acid dissociation of catechols, 3,4-dihydroxyphenylpropionic acid, 3,4-dihydroxyphenylacetic acid, and DOPA.[5] This fact shows that the first ionizable phenol group of dopamine has the same thermodynamic behaviour as phenol and hence it can be permitted to use the dissociation constants of such methoxy derivatives as 3-hydroxy-4-methoxyphenethylamine and 3-methoxy-4-hydroxyphenethylamine in the calculation of the micro-constants of dopamine. The second phenol group dissociation of dopamine gave a different linear-correlation, prob-

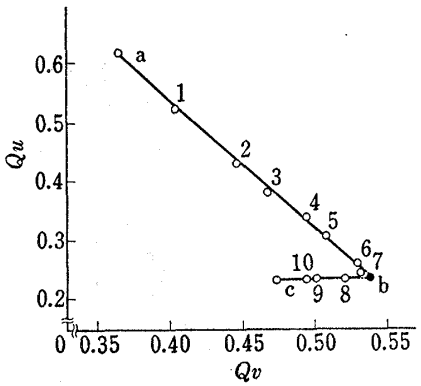
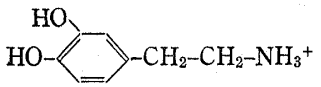
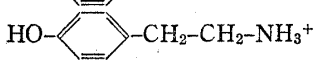
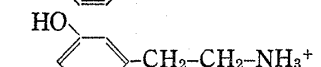
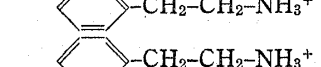
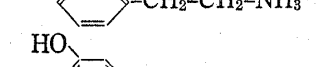
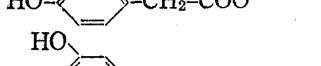
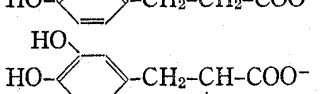


Fig. 1. *Qu–Qv* Plot of Dopamine

a: diphenolic species
b: phenol phenolate species
c: diphenolate species
a: pH 3.73, 1: pH 8.22, 2: pH 8.64, 3: pH 8.90, 4: pH 9.13, 5: pH 9.32, 6: pH 9.92, 7: pH 10.69, 8: pH 11.58, 9: pH 11.99, 10: pH 12.21, c: pH 12.44.
Range
U: 265—280 nm
V: 282.5—297.5 nm
W: 300—315 nm

Table II. Thermodynamic Parameters for Ionizations at 25°

Compound	ΔG_1	ΔG_2	ΔG_3	ΔH_1	ΔH_2	ΔH_3	ΔS_1	ΔS_2	ΔS_3	Ref.
	(kcal/mol)			(kcal/mol)			(e.u.)			
	12.34	14.35	16.34	6.66	9.15	10.82	−19.09	−17.44	−18.54	
	13.04	14.58		5.14	9.03		−26.51	−18.63		
	12.89	14.43		5.92	8.74		−23.39	−19.09		
		13.63			8.94			−15.71		
	13.05		16.57	6.19		9.57	−23.02		−23.49	5)
	13.22		16.52	6.66		9.34	−22.01		−24.09	5)
	13.56	11.95	16.56	7.45	9.15	9.99	−20.50	−9.40	−22.05	5)

15) T. Okamoto, "San to Enki," Tokyo Kagaku Dozine, 1967, p. 55.
16) H. Sakurai and T. Ishimitsu, *Yakugaku Zasshi*, **95**, 1384 (1975).

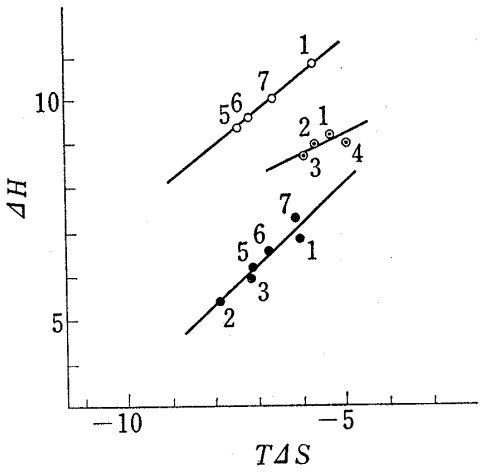


Fig. 2. Relationship between ΔH and $T\Delta S$ of Dopamine and Its Related Compounds

—⊙—: amino group dissociation.
—●—: first phenol group dissociation.
—○—: second phenol group dissociation.

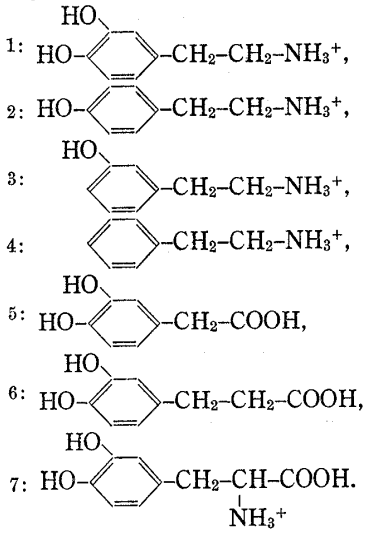


Table III. Microscopic Acid Dissociation Constants of Dopamine

k_1: 9.89,[a]	k_{12}: 9.94,	k_{23}: 11.07,	k_{123}: 11.72,
k_2: 9.48,	k_{13}: 10.14,	k_{31}: 10.38,	k_{132}: 11.52,
k_3: 9.65,	k_{21}: 10.35,	k_{32}: 10.91,	k_{231}: 11.00,

μ=0.1 ($NaClO_4$), 25°.
a) Dissociation constant of 3,4-dimethoxyphenethyiamine (9.89±0.01).

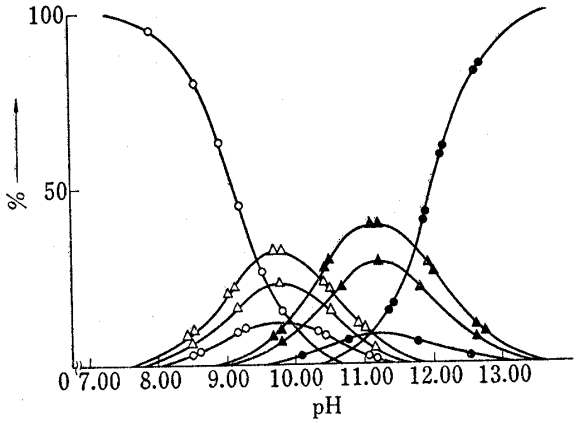


Fig. 3. Relative Concentration of Various Ionic Forms of Dopamine

—○— (I), -▲▲- (V),
-○○- (II), —▲— (VI),
-△△- (III), —●— (VII),
—△— (IV), -●●- (VIII).

ably due to formation of intramolecular hydrogen bond after the dissociation of the first phenol group.

Micro-constants of Dopamine——The scheme for the ionization of all eight different microscopic species of dopamine may be represented as shown in Chart 1, and the twelve micro-constants among those species are defined with the interrelationships as given in equations (1), (2), and (3). On the basis of the above mentioned conclusion in acid dissociation process, the micro-constants of dopamine were calculated by the use of the substitution method of Edsall *et al.*[10] and the results are shown in Table III. These values are significantly different from these of DOPA.[5] Because, the pK_1 and pK_2 are in the reverse order with dopamine and DOPA, as is above mentioned.

From the twelve micro-constants, the relative concentrations of each species of dopamine are calculated as a function of pH values (Fig. 3). It should be noted that dopamine possesses significant concentrations of all species in the pH range from 8.5 to 12.5. At physiological pH region (pH 7.2—7.4), dopamine is present as an ammonium species (I). At pH 9.75, it is present as zwitter-ionic species (III) and (IV) (about 30% and 20% respectively).

The concentration of mono-phenolate amine species, (V) and (VI), are about 40% and 30%, respectively, at pH 11.0.