[Chem. Pharm. Bull. 23(1) 29—34 (1975)] UDC 547.864.04 : 547.559.057

Studies on the Metal Chelate Compounds of Phenazine Derivatives. IX.[1) Formation Constants of Metal Chelates of Hydroxybenzo[*a*]-phenazine Derivatives

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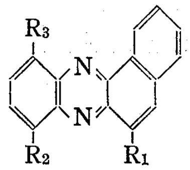
(Received May 17, 1974)

The acid dissociation constants of hydroxyphenazine derivatives having the oxine-like functional group and the formation constants of their metal chelates with cobalt [II], nickel [II], copper [II], zinc [II] and cadmium [II] were measured in a 50% (v/v) ethanol solution at 25 ± 1° by the spectrophotometry. Considering from the formation constants of Ni- and Zn-chelates, it was found that the metal chelates of hydroxyphenazine derivatives indicated the similar steric hindrance to those of the 2-substituted oxine metal chelates.

It is known that antibacterial activities of the chelating agents having the oxine-like functional group, such as 5-hydroxyquinoxaline and 1-hydroxyphenazine, are increased by complexation with metal ions.[3,4) The authors have been engaged in the fundamental studies on the metal chelate formation of phenazine derivatives of biological interest. In a previous paper,[1) the overall formation constants of the metal chelates of 1-hydroxyphenazine (1-OH-phz) were reported.

Syntheses of 6-hydroxybenzo[*a*]phenazine (6-OH-Bzphz), 8-hydroxybenzo[*a*]phenazine (8-OH-Bzphz) and 11-hydroxybenzo[*a*]phenazine (11-OH-Bzphz) were reported in the literature.[5,6) However, any study on the metal chelate formation of these compounds has not yet been reported. Therefore, a study of metal chelate compounds of hydroxyphenazine derivatives has been attempted. This paper deals with the determination of acid dissociation constants of hydroxybenzo[*a*]phenazine derivatives and the formation constants of their metal chelates (Co, Ni, Cu, Zn and Cd) by the spectrophotometric method. Since hydroxybenzo[*a*]phenazine derivatives and their metal chelates were not soluble in water, measurements of acid dissociation constants and formation constants were carried out in a 50% (v/v) ethanol solution.



	R_1	R_2	R_3
6-OH-Bzphz	OH	H	H
8-OH-Bzphz	H	OH	H
11-OH-Bzphz	H	H	OH

Chart 1

Experimental

Reagents——Hydroxyphenazine derivatives were synthesized by the methods described in the literature. 1-OH-phz,[7) mp 156°. *Anal.* Calcd. for $C_{12}H_8ON_2$: C, 73.46; H, 4.11; N, 14.28. Found: C, 73.35; H, 4.05; N, 14.20. Mass Spectrum *m/e*: 196 (M^+). 6-OH-Bzphz, mp 200°. *Anal.* Calcd. for $C_{16}H_{10}ON_2$: C, 78.05; H, 4.07; N, 11.38. Found: C, 78.01; H, 4.29; N, 11.46. Mass Spectrum *m/e*: 246 (M^+). 8-OH-

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1) Part VIII: Y. Kidani, K. Inagaki, and H. Koike, *Yakugaku Zasshi*, **93**, 1089 (1973).
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3) A. Albert, "Selective Toxity," Methuen, London, 1965.
4) H. Erlenmeyer, J. Bäumler, and W. Roth, *Helv. Chim. Acta*, **34**, 941 (1953).
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7) I. Yosioka, *Yakugaku Zasshi*, **72**, 1128 (1952).

Bzphz, mp 189°. Found: C, 77.91; H, 4.06; N, 11.21. Mass Spectrum *m/e*: 246 ($\mathrm{M}^{+\cdot}$). 11-OH-Bzphz, mp 203°. Found: C, 77.93; H, 3.93; N, 11.17. Mass Spectrum *m/e*: 246 ($\mathrm{M}^{+\cdot}$).

Reagent solutions were prepared by dissolving the ligands in 99.5% ethanol. The solutions of various metal ions were prepared by dissolving a respective metal ion in deionized water and were standardized by the atomic absorption spectrophotometric procedure. A Britton Robinson solution was used for the measurement of acid dissociation constants of the hydroxyl group. An acetate buffer was used for the measurement of the formation constants. The other reagents used in this study were of reagent grade.

Apparatus——Measurements of pH were made with a Hitachi-Horiba pH Meter Model 5. The hydrogen ion concentration was directly calculated from pH meter-reading. Spectrophotometric measurements were made with a Hitachi reacording spectrophotometer Model EPS-3T and with a Hitachi spectrophotometer Model 139. Quantitative analyses of the metal ions were made with a Hitachi atomic absorption spectrophotometer Model 207.

Measurements of Acid Dissociation Constants——pK_{OH} (pK_a value of the hydroxy group); In a 20 ml measuring flask, 5 ml of 1.2×10^{-5} M ethanol solution of a ligand and 5 ml of a Britton Robinson buffer solution were added and then was diluted to 20 ml exactly with a 50% (v/v) aqueous ethanol. The ionic strength was maintained at 0.1 with potassium chloride and absorbance of the solution was measured at 25 ± 1° using a 50 mm quartz cell. The wavelengths measured are: 6-OH-Bzphz, 330 mm; 8-OH-Bzphz, 319 nm; 11-OH-Bzphz; 320 nm. pK_{NH} (pK_a value of the ring nitrogen atom); In a 25 ml measuring flask, 2 ml of 1.0×10^{-3} M ethanol solution of a ligand and 0.1—20 ml of 2 M hydrochloric acid in a 50% (v/v) ethanol were added and then was diluted to 25 ml exactly with a 50% (v/v) ethanol. Absorbance of the solution was measured at 25 ± 1° using a 10 mm quartz cell. The wavelengths measured are: 6-OH-Bzphz, 425 nm; 8-OH-Bzphz, 438 nm; 11-OH-Bzphz, 437 nm; 1-OH-phz, 383 nm.

Measurements of Formation Constants——$\log K_1$ (first successive formation constant); In a 25 ml measuring flask, 5 ml of 2.5×10^{-4} M ethanol solution of a ligand and 5 ml of 0.02 M metal ion solution, (Co, Ni, Cu, Zn, and Cd) were added and then was adjusted at required pH by adding 0.1 M hydrochloric acid. The ionic strength was maintained at 0.1 with potassium chloride, and absorbance of the solution was measured at 25 ± 1° using a 50 mm quartz cell. $\log \beta_2$ (overall formation constant); In a 25 ml measuring flask, 5 ml of 1.0×10^{-3}—2.5×10^{-4} M ethanol solution of a ligand, 5 ml of 2.0×10^{-5} M metal acetate solution and 10 ml of acetate buffer solution were added and then was diluted to 25 ml exactly with a 50% (v/v) ethanol. Ionic strength was maintained at 0.1 with potassium chloride, and absorbance of the solution was measured at 25 ± 1° using a 50 mm quartz cell.

Results and Discussion

Determination of Acid Dissociation Constants

The electronic absorption spectra were measured at various pH in a 50% (v/v) ethanol solution in order to obtain the pK_{OH} value, shown in Fig. 1. The absorption ($\lambda_{\max}=292$ nm, $\log \varepsilon=4.56$) is considered to be due to the neutral form of 8-OH-Bzphz, while that of the anion form showed a maximum at 319 nm ($\log \varepsilon=4.53$). An isosbestic point was observed at 304 nm. Therefore, the pK_{OH} value was calculated on the basis of the Hildebrand method[8] from the relationship between pH and absorbance at 319 nm.

In the case of 6-OH-Bzphz, the neutral form shows $\lambda_{\max}$ at 305 nm ($\log \varepsilon=4.63$), the anion form shows $\lambda_{\max}$ at 330 nm ($\log \varepsilon=4.62$) and the isosbestic point is observed at 314 nm. In the case of 11-OH-Bzphz, the neutral form shows $\lambda_{\max}$ at 293 nm ($\log \varepsilon=4.84$), the anion form shows $\lambda_{\max}$ at 320 nm ($\log \varepsilon=4.49$) and the isosbestic point is observed at 305 nm.

It may be considered that the protonation takes place at the ring nitrogen atom, being attributable to either the 7- or the 10-position. The acid dissociation constant (pK_{NH} value) of the ring nitrogen atom of the ligand was less than unity. The absorption maximum of the neutral form of 8-OH-Bzphz appeared at 400 nm, while the absorption maximum of the cation form was observed at 438 nm. It means that, the absorption band shifts to the longer wavelength by the protonation at the nitrogen atom, being shown in Fig. 2. Hence, the pK_{NH} value has been determined on the basis of the following equation.[9]

$$\frac{L_{\mathrm{T}}}{A-A_{\mathrm{LH}}}=\frac{1}{\varepsilon_{\mathrm{LH_2}^+}-\varepsilon_{\mathrm{LH}}}\times\frac{K_{\mathrm{NH}}}{[\mathrm{H}^+]}+\frac{1}{\varepsilon_{\mathrm{LH_2}^+}-\varepsilon_{\mathrm{LH}}}$$

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[8] G.P. Hildebrand and C.N. Reilley, *Anal. Chem.*, **29**, 258 (1957).

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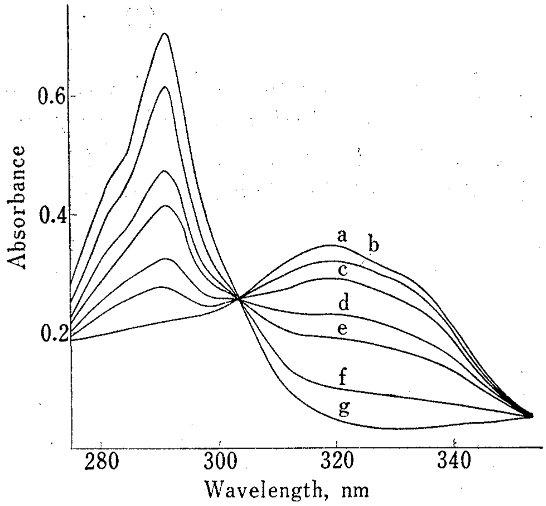

Fig. 1. Electronic Absorption Spectra of 8-OH–Bzphz at Various pH in a 50% Ethanol Solution

[8-OH-Bzphz]=3.0×10⁻⁶ M, using 50 mm quartz cell pH: a) 12.70, b) 10.87, c) 10.43, d) 9.97, e) 9.72, f) 9.13, g) 7.43

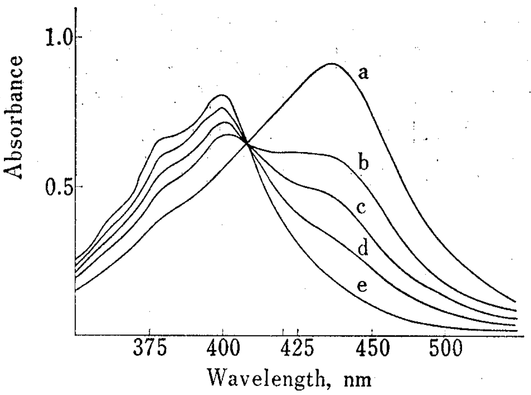

Fig. 2. Electronic Absorption Spectra of 8-OH–Bzphz at Various pH in a 50% (v/v) Ethanol Solution

[8-OH-Bzphz]=8.0×10⁻⁵M, using a 10 mm quartz cell pH: a) <0, b) 0.3, c) 0.5, d) 0.8, e) 2.8

Table I. Acid Dissociation Constants of Hydroxyphenazine Derivatives in a 50% (v/v) Ethanol Solution

Ligands	pK_{OH}	pK_{NH}
1-OH–phz	9.21[a)]	0.3
6-OH–Bzphz	10.10	−0.2
8-OH–Bzphz	9.90	−0.1
11-OH–Bzphz	9.80	0.3

a) The value was taken from ref. 1.

where $\varepsilon_{LH2}{}^{+}$ and ε_{LH} represent the molar absorption coefficient of the cation form and the neutral form, respectively, at 438 nm. L_T is the total concentration of ligand, A is the absorbance of the solution, and A_{LH} equals to $\varepsilon_{LH}L_T$.

In the case of 6-OH-Bzphz, the neutral form shows λ_{max} at 384 nm, the cation form shows λ_{max} at 425 nm and the isosbestic point is observed at 393 nm. In the case of 11-OH-Bzphz, the neutral form shows λ_{max} at 403 nm, the cation form shows λ_{max} at 437 nm and the isosbestic point is observed at 410 nm. In the case of 1-OH-phz, the neutral form shows λ_{max} at 366 nm, the cation form shows λ_{max} at 383 nm and the isosbestic point is observed at 397 nm. The pK_a values obtained are summarized in Table I. All of the pK_{OH} values of hydroxybenzo[*a*]phenazine derivatives are larger than that of 1-OH-phz. However, the pK_{NH} values of hydroxybenzo[*a*]phenazine decrease reversely, as the pK_{OH} values increases.

Determination of the Formation Constants

Change of the absorption curves at various pH was measured in the presence of a very large excess of metal ion. Therefore, the chelate is considered to be in a 1:1 ratio. Under the conditions, the metal chelate formation is represented by the following equation[10];

$$M^{2+} + LH \rightleftarrows LM^{+} + H^{+} \tag{1}$$

$$K_1' = \frac{[LM^+][H^+]}{[M^{2+}][LH]} \qquad K_1' = \frac{(A-A_{LH})[H^+]}{(A_{max}-A)[M^{2+}]} \tag{2}$$

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10) A. Corsini, Q. Fernando, and H. Freiser, *Anal. Chem.*, 35, 1424 (1963).

$$K_1 = \frac{[\mathrm{LM}^+]}{[\mathrm{L}^-][\mathrm{M}^{2+}]} \qquad K_1 = \frac{K_1'}{K_{\mathrm{OH}}} \tag{3}$$

where A is the absorbance of the solution, and $A_{\max}$ is the absorbance of the solution when the chelate formed completely. A_{LH} is the absorbance of the solution in which any chelate does not present at all. K_1' value can be obtained by equation (2). A plot of $(A_{\max}-A)/(A-A_{\mathrm{LH}})$ *vs.* $[\mathrm{H}^+]$ should be linear. If another species of metal chelate is present in the solution, a plot of $(A_{\max}-A)/(A-A_{\mathrm{LH}})$ will not be linear.

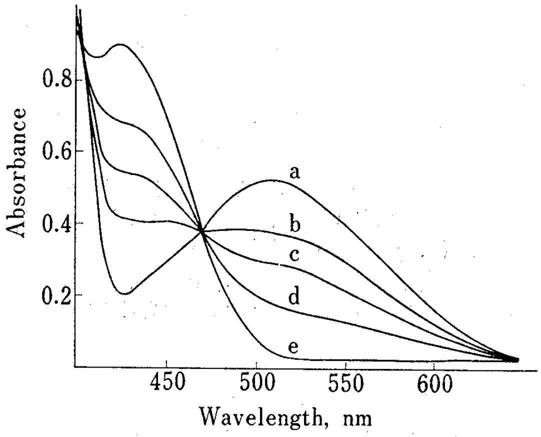

Fig. 3. Absorption Spectra of the 1:1 Metal Chelate of 6-OH–Bzphz

[6-OH-Bzphz]=5.0×10⁻⁵M, [Zn²⁺]=4.0×10⁻³M, using 50 mm quartz cell
pH: a) 6.00, b) 5.20, c) 4.82, d) 4.40, e) 2.70

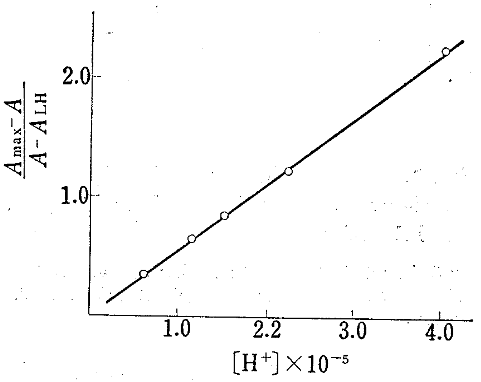

Fig. 4. Determination of K_1' for 1:1 Zinc Chelate of 6-OH–Bzphz

TABLE II. Absorption Maxima and Isosbestic Points of the 1:1 Metal Chelates

		Co	Ni	Cu	Zn	Cd
1-OH–phe	absorption maximum (nm)	524	538	532	512	529
	log ε	3.29	3.21	3.23	3.19	3.16
	isosbestic points (nm)	463	470	468	463	466
6-OH-Bzphz	absorption maximum (nm)	518	530	325[a]	512	527
	log ε	3.30	3.20	4.17[a]	3.29	3.29
	isosbestic point (nm)	466	473	302[a]	470	473
8-OH-Bzphz	absorption maximum (nm)	494	501	320[a]	480	494
	log ε	3.30	3.20	4.17[a]	3.29	3.27
	isosbestic point (nm)	442	446	302[a]	442	445

a) The values were taken from ultraviolet region, because the copper chelates of hydroxybenzo[*a*]phenazine are slightly soluble.

The determination of the formation constant of zinc chelate of 6-OH-Bzphz will be described mainly. The spectral change of zinc chelate of 6-OH-Bzphz are shown in Fig. 3. The absorbance at 512 nm, which is due to the zinc chelate, increased with an increase of pH and there was a linear relationship between $(A_{\max}-A)/(A-A_{\mathrm{LH}})$ and $[\mathrm{H}^+]$, as it is shown in Fig. 4. The absorption maximum and wavelength at the isosbestic point of the other metal chelates are tabulated in Table II. The formation constants of the 1:1 metal chelates of hydroxyphenazine derivatives except 11-OH-Bzphz are shown in Table IV.

Overall formation constants of the metal chelates of 6-OH-Bzphz and 8-OH-Bzphz were measured in a 50% (v/v) ethanol solution by the same method as described in a previous paper.[1] However, since the copper chelates of 8-OH-Bzphz and 6-OH-Bzphz are insoluble in a 50% (v/v) ethanol solution, their overall formation constants have not been determined.

The conditional formation constants of the metal chelates (Co, Ni, Zn and Cd) are summarized in Table III. Binding ratio of the metal chelates are found to be 2: 1 (L_2M) from the geraden method.[11]

TABLE III. Conditional Formation Constants of the 2: 1 Metal Chelates of Hydroxybenzo[*a*]phenazine Derivatives and Wavelength measured

Metal chelates		Conditional formation constant	pH	Wavelength (nm)
6-OH–Bzphz	Co	2.72×10^7	6.50	328
	Ni	4.75×10^7	6.50	331
	Zn	3.94×10^7	6.50	330
	Cd	5.46×10^6	6.92	330
8-OH–Bzphz	Co	3.39×10^7	6.40	320
	Ni	4.06×10^7	6.40	324
	Zn	3.27×10^7	6.40	319
	Cd	2.54×10^7	7.15	320

The metal chelate formation of 11-OH-Bzphz could not be observed in a 50% (v/v) ethanol solution, partly because of the large interference of the benzene ring upon chelation. However, their metal chelates could be isolated from an anhydrous ethanol solution.[12]

The relationship between the formation constants (log K_1) and the acid dissociation constants of the ligand is shown in Fig. 5. Expectedly, the formation constants increase linearly with an increase of the pK_{OH} values.

TABLE IV. Formation Constants of the 1: 1 Metal Chelates of Hydroxyphenazine Derivatives

		Co	Ni	Cu	Zn	Cd
1-OH–phz	log K_1	6.9	7.0	8.9	7.1	6.1
	log β_2	13.6[a]	13.7[a]	17.5[a]	13.6[a]	12.0[a]
6-OH–Bzphz	log K_1	7.5	7.7	9.6	7.8	6.6
	log β_2	14.7	14.9	—	14.8	13.1
8-OH–Bzphz	log K_1	7.4	7.5	9.5	7.6	6.6
	log β_2	14.5	14.6	—	14.5	12.9

a) The value were taken from ref. 1.

The order of the log K_1 values is Cu>Zn≥Ni>Co>Cd, and that of log β_2 values is Ni≥Zn>Co>Cd which is the same as the Irving-Williams series.[13] Generally, both the log K_1 and log β_2 values of Ni-chelates of chelating agents having the nitrogen and oxygen atom as the donor ligand are larger than those of Zn-chelates. In the case of metal chelates of 1-OH-phz, 6-OH-Bzphz and 8-OH-Bzphz, log K_1 values of Zn-chelates are larger than those of Ni-chelates, while the log β_2 values of Zn-chelates are smaller than those of Ni-chelates. The difference (Δlog K_1 and Δlog β_2) between Zn- and Ni-chelates is 0.1. Generally, the relation of formation constants between Ni- and Zn-chelates gives a useful information about the structural effect such as steric hindrance.[14,15] In this paper, the discussion will be centered mainly in the formation constants of both Ni- and Zn-chelates of oxine-like chelating agents.

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11) E. Asmus, *Z. Anal. Chem.*, **178**, 104 (1960).
12) Unpublished data.
13) H. Irving and R.J.P. Williams, *J. Chem. Soc.*, **1953**, 3192.
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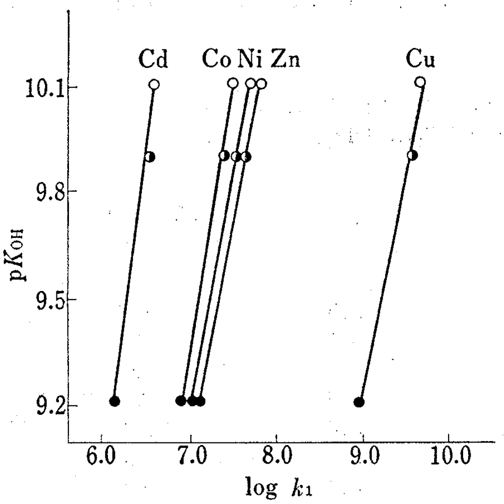

Fig. 5. Relationship between pK_{OH} and log K_1

●: 1-OH-phz, ○: 6-OH-Bzphz, ◑ :8-OH-Bzphz

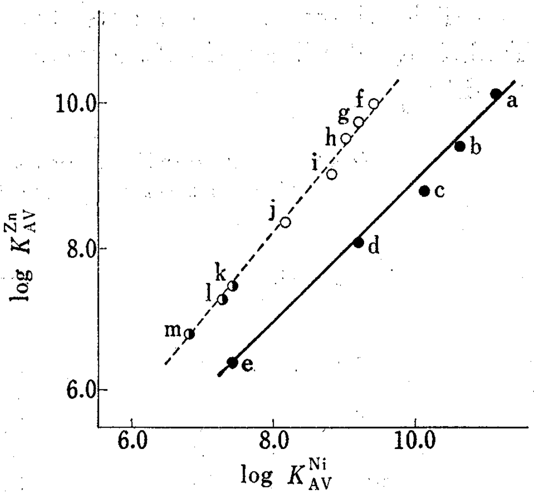

Fig. 6. Relation of Formation Constants between Zn- and Ni-Chelates

a) 4-methyloxine (ref. 19), b) oxine (ref. 18), c) oxine (ref. 17), d) oxine-5-sulfonic acid (ref. 20), e) 5-hydroxyquinoxaline (ref. 17), *f*) 2-buthyloxine (ref. 16), g) 2-propyloxine (ref. 16), h) 2-methyloxine (ref. 16), i) 2-ethyloxine (ref. 16), j) 2-methyloxine (ref. 17), k) 6-OH-Bzphz, l) 8-OH-Bzphz, m) 1-OH-phz

In the case of the 2-substituted oxine chelates,[16] Ni-chelate is more susceptible to the steric hindrance than Zn-chelate, and consequently the formation constants of the Zn-chelates are larger than those of Ni-chelates. On the other hand, the formation constant of the 5-hydroxyquinoxaline Ni-chelate is larger than that of Zn-chelate,[17] while the formation constants of Ni-chelates of hydroxyphenazine derivatives are almost equal to those of Zn-chelates. The formation constants of hydroxyphenazine derivatives will be smaller than the value expected.

If the Zn-chelate has tetrahedral structure, and the Ni-chelate has either planar or octahedral structure, the effect of the steric hindrance for the Zn-chelate will be smaller, while that of Ni-chelate will be larger. Hence, log K_{AV}^{Zn} is plotted against log K_{AV}^{Ni} to know the influence of the steric hindrance (Fig. 6), where the log K_{AV}^{Zn} and log K_{AV}^{Ni} represent the 1/2 log β_2, respectively. From Fig. 6, the behavior of the formation constants of Ni- and Zn-chelates of 5-hydroxyquinoxaline is similar to that of oxine,[18] 4-methyloxine[19] and oxine-5-sulfonic acid.[20] On the contrary, the metal chelates of hydroxyphenazine delivatives indicate the similar tendency to 2-substituted oxine chelates having the steric hindrance. The behavior of the formation constants of hydroxyphenazine derivatives is thus seem to be characteristic of steric hindrance as well as 2-alkyl-substituted oxine metal chelates.

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19) W.D. Johnston and H. Freiser, *Anal. Chem. Acta*, **11**, 201 (1954).
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