Notes

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Studies on the Metal Chelate Compounds of Phenazine Derivatives. XI.[1) Metal Chelates of 1-Phenazinethiol

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The acid dissociation constant of 1-phenazinethiol and its metal chelate formation constants have been determined spectrophotometrically in a 50% (v/v) ethanol at 25±1°. Metal chelates of 1-phenazinethiol were synthesized, and the salient feature of infrared (IR) spectra of these chelates were discussed. Considering from the formation constant of zinc chelate and its IR spectrum, it may be suggested that zinc-sulfur linkage of the zinc chelate has covalent character.

Chelating agents having the sulfur and nitrogen donor atoms are more selective than those having oxygen and nitrogen donor atoms, probably, because of the possibility of π-bonding between metal and sulfur atom. Metal chelate formation of 1-phenazinethiol (1-SH-phz) has not yet been reported, and the acid dissociation constant of 1-SH-phz and its metal chelate stability constants have been determined spectrophotometrically in a 50% (v/v) ethanol solution. The stability constants have been investigated in comparison with those of the metal chelates of the related ligands, such as 1-hydroxyphenazine(1-OH-phz),[3) oxine,[4) 2-methyloxine,[5) thiooxine,[6) and 2-methylthiooxine.[7) Further, the metal chelates of 1-SH-phz were synthesized and their infrared spectral analyses were also described.

Experimental

Reagent——Potassium salt of 1-SH-phz(1-SK-phz) was purified by the following procedure; Crude 1-SK-phz[8) was dissolved in ethanol by refluxing on a water bath and filtered under nitrogen stream, and then diethyl ether was added to the filtrate. A few minutes later, pure 1-SK-phz was precipitated out. *Anal.* Calcd. for $C_{12}H_7N_2SK\cdot 1/4H_2O$: C, 56.6; H, 3.0; N, 11.0. Found: C, 56.7; H, 3.0; N, 11.2. 1-SK-phz is oxidized gradually to disulfide when kept in open air. However, it can be stored for about 1 month in nitrogen atomosphere.

Measurements of Acid Dissociation Constants——pK_{SH}: Solution of 1-SK-phz (1.57×10^{-4}) were prepared in a 50% (v/v) ethanol at pH ranging from about 4.0 to 12.0. The absorption spectra of the solution began to change about 30 minutes. Thus the measurement was carried out within 30 minutes after adding 1-SK-phz, at ionic strength, 0.1, and 25±1°. Absorption spectra of the solution have absorption maxima at 370 and 426 nm for neutral form, and at 378 and 538 nm for anionic form. The pK_{SH} was calculated according to the Hildebrand method.[9)

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pK_{NH}: Solution of 1-SK-phz (3.02×10^{-5} M) were prepared in a 50% (v/v) ethanol whose pH was adjusted with 2.0 M hydrochloric acid ranging from 0.3 to 3.9. Ionic strength was not adjusted because of the low pK_{NH} value. Absorption spectra of the solution give the absorption maxima at 370 and 434 nm for the cationic form. The pK_{NH} value was calculated according to the method of Yamamoto, *et al.*[10]

Measurements of Stability Constants——In each of a 25 ml measuring flask, 3 ml of 4.0×10^{-4} M ethanol solution of 1-SK-phz and 3 ml of 0.1 M aqueous solution of metal ions (Zn^{2+}, Co^{2+}, Pb^{2+}) were added and then it was adjusted at a required pH by adding 0.5 M hydrochloric acid. Ionic strength was maintained at 0.1 with potassium chloride. Absorbance of the solution was measured immediately using a 50 mm quartz cell at 25 ± 1°. The wavelengths measured are: Co–chelate, 572 nm; Zn–chelate, 532 nm and Pb–chelate, 510 nm. Measurements of the stability constants are limited to those of the 1 : 1 chelates because 2 : 1 chelates are very insoluble, even in a 50% (v/v) ethanol. The stability constants were calculated on the basis of the method of Corsini, *et al.*[6] However, the spectra of Cu–, Ni– and Cd–chelates changed rapidly, so that their stability constants could not be determined.

Syntheses of Metal Chelates——Zn–chelate: Zinc acetate, 1-SK-phz and acetate buffer (pH 5.0) were added in separatory funnel and the Zn–chelate formed was extracted with chloroform. After the chloroform solution was concentrated by evaporation, *n*–hexane was added to precipitate the Zn–chelate. Bluish black precipitate, *Anal.* Calcd. for $(C_{12}H_7N_2S)_2\cdot Zn$; C, 59.1; H, 2.9; N, 11.5. Found; C, 59.0; H, 2.6; N, 11.3.

Co–chelate: The Co–chelate was prepared by mixing cobaltous acetate with 1-SK-phz in ethanol solution. The precipitates deposited were collected and were dissolved in dimethylformamide, and then methanol was added to reprecipitate the Co–chelate. Black precipitate, *Anal.* Calcd. for $(C_{12}H_7N_2S)_2\cdot Co$; C, 59.9; H, 2.9; N, 11.6. Found; C, 59.6; H, 2.9; N, 11.5.

Pb–chelate: The Pb–chelate was prepared by mixing lead nitrate with 1-SK-phz in an acetate buffer solution (pH 5.0). The precipitates deposited were collected on sintered glass and washed with an acetate buffer solution, ethanol and chloroform. Purple precipitate, *Anal.* Calcd. for $(C_{12}H_7N_2S)_2\cdot Pb$; C, 45.8; H, 2.2; N, 8.9. Found; C, 46.0; H, 1.9; N, 8.7.

Ni–chelate: Nickel acetate and 1-SK-phz were added together in a separatory funnel and it was extracted with chloroform in the presence of pyridine. The chloroform solution was concentrated by evaporation and then *n*–hexane was added to form precipitates. Black precipitate, *Anal.* Calcd. for $(C_{12}H_7N_2S)_2\cdot Ni$; C, 59.9; H, 2.9; N, 11.7. Found; C, 56.0; H, 2.8; N, 11.2. Elemental analysis of the Ni–chelate was slightly different from the calculated value, however, its infrared spectrum exhibited the same pattern with that of the Co–chelate.

Result and Discussion

The pK_{SH} of 1-SH-phz is smaller than the pK_{OH} of 1-OH-phz, as indicated in Table I. The difference (ΔpK_a) between 1-SH-phz and 1-OH-phz is 1.73 in log units. The proton affinity to oxygen atom is usually greater than that of sulfur atom and this fact is in agreement with experimental result. Although the proton affinity of 1-SH-phz is smaller than that of 1-OH-phz, the log K_1 value of Zn-chelate of 1-SH-phz is larger than that of 1-OH-phz. A similar relationship was also observed between Zn-chelate of 2-methyloxine and 2-methylthiooxine. The corresponding difference between Co-chelate of 1-SH-phz and 1-OH-phz is smaller comparatively than that of Zn-chelates. The stability of the chelate may be more enhanced in Zn-chelate by covalent character in the metal-sulfur coordination than in the Co-chelate.

TABLE I. Acid Dissociation Constants and Stability Constants

Compounds	pK_{SH}	pK_{OH}	pK_{NH}	log K_1		
				Zn (II)	Co (II)	Pb (II)
1-SH-phz	7.48	—	0.9	8.6	7.2	8.5
1-OH-phz[3]	—	9.21	0.3	7.1	6.9	—
Thiooxine[6]	9.20	—	1.74	11.0	7.9	11.9
Oxine[4]	—	11.54	3.97	10.0	10.6	10.6
2-Methylthiooxine[7]	9.76	—	1.96	11.1	9.6	—
2-Methyloxine[5]	—	11.71	4.58	9.8	9.6	—

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[10] K. Yamamoto and K. Ohashi, *Nippon Kagaku Zasshi*, **90**, 1138 (1969).

IR-Spectra

Measurements of infrared (IR) spectra (4000—500 cm^{-1}) were made by means of the KBr disk method. In the present work, the salient features of these IR spectra are mainly discussed. 1-SK-phz and its chelates showed very strong bands in 739—760 cm^{-1}, which are assignable to the C–H out of plane bending vibration,[11,12] and these bands shifted toward lower frequencies in the chelate spectra. Mido, *et al.* reported that the strong bands at around 990 and 670 cm^{-1} of metal thiooxinates were assigned to the metal-sensitive band.[13] In the case of the metal chelates of 1-SH-phz, it may be considered that the strong bands at around 960 and 600 cm^{-1} correspond to the metal-sensitive bands. The frequencies of the 960 cm^{-1} band are in the order Ni>Co>Zn>Pb, while those of the 600 cm^{-1} band are in the order Zn>Ni>Co>Pb. The relationship is in agreement with the order of metal thiooxinates. Measurements of far-IR spectra (500—220 cm^{-1}) were made by means of the nujol mull method. Metal-sulfur stretching vibration[14,15] lies over the range of 480—210 cm^{-1}. The chelates of

Table II. Infrared Bands of 1-SH-phz Metal Chelates in the Region 1000—500 cm^{-1}

Disulfide of 1-SH-phz	1-SK-phz	Co	Ni	Zn	Pb
951^{vs}	964^{vs}	968^{s}	969^{s}	966^{vs}	958^{vs}
919^{m}	920^{s}	921^{w}	921^{w}	923^{m}	918^{m}
840^{s}	840^{s}	853^{s}	853^{s}	853^{s}	848^{s}
819^{m}	813^{m}	817^{m}	817^{m}	817^{m}	817^{m}
778^{m}				769^{s}	778^{s}
754^{vs}	761^{vs}	753^{vs}	756^{vs}	761^{vs}	755^{vs}
739^{vs}	748^{vs}	739^{vs}	739^{vs}	739^{vs}	742^{vs}
		690^{w}	697^{w}		
666^{s}	675^{m}	680^{w}	682^{w}	691^{vs}	682^{vs}
599^{s}	600^{s}	603^{s}	606^{s}	608^{s}	602^{s}
576^{vw}	590^{vw}	581^{vw}	581^{vw}	578^{w}	580^{m}
551^{w}	559^{vw}	557^{w}	558^{w}	558^{w}	555^{w}
	535^{vw}	541^{vw}	542^{vw}	542^{vw}	538^{w}

abbreviation; vw, very weak; w, weak; m, medium; s, strong; vs, very strong

Table III. Infrared Bands of 1-SH-phz Metal Chelates in the 500—220 cm^{-1}

Disulfide of 1-SH-phz	1-SK-phz	Co	Ni	Zn	Pb
487^{m}	483^{m}	483^{w}	484^{w}	489^{m}	480^{m}
464^{w}	440^{m}	448^{m}	448^{s}	446^{s}	439^{m}
400^{m}	402^{m}	416^{m}	418^{m}	408^{m}	405^{w}
	386^{m}	388^{s}	388^{s}	385^{s}	380^{s}
		337^{s}	340^{s}	360^{m}	312^{s}
290^{m}	280^{m}	280^{m}	287^{s}	286^{s}	292^{m}
		261^{m}	263^{m}	273^{s}	235^{s}

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12) C. Stammer and A. Taurins, *Spectrochim. Acta*, **19**, 1625 (1963).
13) Y. Mido and E. Sekido, *Bull. Chem. Soc. Japan*, **44**, 2127, 2130 (1971).
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15) K. Nakamoto, "Infrared Spectra of Inorganic and Coordination Compounds," John Willey & Sons, Inc., New York, 1970, p. 256.

thiosalicylic acid[16] and monothioacetylacetone[17] showed M-S stretching vibration at 392—278 cm^{-1}. In the case of the metal chelates of 1-SH-phz, the bands at 360—312 cm^{-1} and 270—235 cm^{-1} are distinctly understood to be metal-sensitive, and the former may be considered to be associated with the metal-sulfur bond, because these bands can not be observed in the 1-SK-phz spectrum. Both the frequencies at 360—312 cm^{-1} and 273—235 cm^{-1} are in the order $Zn > Ni > Co > Pb$. It may be suggested that the Zn-chelate has covalent character between zinc and the sulfur atom, because the frequency of the Zn-chelate is the highest.

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Effect of Thyrocalcitonin on Calcium Efflux in Liver Slices of Rats

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The effect of thyrocalcitonin (TCT) on the calcium content of liver slices has been studied. At 30 min incubation period, TCT significantly ($p<0.01$) increased the accumulation of calcium in the concentration range from 0.1 to 8.0 mU/ml with half-maximal concentration occurring at about 0.1 mU/ml. During the 60 min incubation period, the effect of TCT on the liver calcium accumulation was additively enhanced. On the other hand, the radiocalcium ($^{45}CaCl_2$) efflux in the absence of the hormone increased linearly during the incubation period. The radiocalcium efflux in the presence of the hormone increased later with time than that in the absence of the hormone, and the inhibition of efflux was 25% of the control ($p<0.01$). These data suggest that TCT increased calcium accumulation in the liver cells by inhibiting the efflux of calcium.

It is known that thyrocalcitonin (TCT) increases calcium concentration in kidney cells. A number of studies on kidney cells[2,3] and on HeLa cells[4,5] have demonstrated that calcium entry is a passive phenomenon, while its extrusion is a metabolically dependent transport process. Accumulation of calcium in cultured kidney cells is enhanced by a decrease of calcium efflux with TCT,[3] and parathyroid hormone increases the calcium accumulation by an increase of calcium influx.[2] More recently we found that TCT markedly increased hepatic calcium concentration *in vivo*, and suggested that the action of TCT on liver calcium is not dependent on cyclic adenosine monophosphate (AMP).[6] The present study was therefore undertaken to investigate the effect of TCT on calcium transport in liver slices of rats, especially to examine whether TCT would inhibit the active extrusion of calcium from liver cells. The present results show that TCT increases the calcium concentration of the liver cells possibly by decreasing calcium efflux.

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