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Studies on the Mixed Ligand Complexes. I.[1] Synthesis of Glycine Oxine Cobalt (III) Complex

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Some of the oxine-like compounds are considered to show antibacterial properties, in the presence and absence of certain metal ions.[3-5] Albert[6-10] gave an interpretation on the increasing antibacterial activities in the presence of metal ions as a formation of cation complexes,[12] instead of neutral complexes.

It may be assumed that oxine-like compounds give some influences upon the enzyme system of bacteria. Therefore the mode of action of metal chelating agents may be considered to inhibit enzymatic activities either (1) by forming mixed ligand complexes or (2) by removing essential metals from bacterial enzymes.[12] The authors attempted synthesis of a mixed ligand complex betwecn oxine and glycine with cobalt (II) ion which is described in this paper.

Experimental

Materials——All the reagents were of analytical grade, and used without further purification. The column chromatography was carried out through Toyo Roshi No. 51 Cellulose Powder A (100—200 mesh).

Apparatus——Electronic absorption spectra were measured with a Hitachi automatic recording spectrophotometer, model-124, and for infrared spectra measurements, a Nihon Bunko JASCO, IR-E was used.

Synthesis of Glycine Oxine Cobalt(III) Complex——One gram of $Gly_3Co \cdot 2H_2O$[13] was dissolved in 50 ml of water and to this 50 ml of 1% oxine MeOH solution was added. The solution was refluxed in the presence of 1 g of active charcoal[14] for two hours on a water bath. After being cooled, the solution was separated from charcoal by filtration and the filtrate was evaporated to about 10 ml (1/10 volume) and this filtrate was separated chromatographically on a cellulose powder A (30×300 mm). The mixtures were separated with a solvent mixture of *n*-BuOH:AcOH:H_2O (4:1:2)[15] into five fractions. Each fraction was concentrated to 1/10 volume and to this either water or acetone was added. Crystals deposited were collected. Each component was rechromatographed. The properties of each fraction are listed in Table I.

Melting points of all the compounds isolated were found to be 300° and higher. From paper chromatographic properties it was indicated that fraction-1 was considered to be Co(III)-oxinate and fraction-5 was found to be Co(III)-glycinate. The brownish orange substance obtained from fraction-4 is considered to be

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Table I. The Properties of Each Fraction

Fraction	Color	Solubility	
		Soluble in	Insoluble in
F-1	yellowish green crystals	EtOH	H_2O
F-2	yellow powder	EtOH	H_2O
F-3	yellowish orange powder	H_2O	EtOH
F-4	brownish orange powder	H_2O	EtOH
F-5	violet red powder	H_2O	EtOH

mixed ligand complex expected. *Anal.* Calcd. for Glycine$_2$Co(Oxine)·2H_2O: C, 40.32; H, 4.69; N, 10.85. Found: C, 40.41; H, 4.69; N, 10.83.

Detection of the Spots——Glycine is detected with ninhydrine and Co(III) is shown in blue when placed over a flame. Other components are identified from their own characteristic color. As to the detection of the component in the decomposition products of the chelate, sample of fraction-4 was dissolved in conc. HCl, boiled for about 10 minutes and then the mixture was separated by the ascending paper chromatography using a solvent mixture of *n*-BuOH:AcOH:H_2O (4:1:2). *Rf* values obtained are shown in Table II.

Table II. *Rf* Values

		HCl decomposition substances		
Fraction-1	0.98			
Fraction-2	0.87			
Fraction-3	0.67			
Fraction-4	0.60	0.24	0.33	0.66$^{a)}$
Fraction-5	0.47			
Gly$_3$Co	0.47	0.25	0.34	
Ox$_2$Co	0.98	0.24		0.68$^{a)}$
Oxine	1.00			0.64$^{a)}$
Glycine	0.34		0.33	
Cobalt(III)	0.25	0.24		

a) *Rf* of oxine·HCl

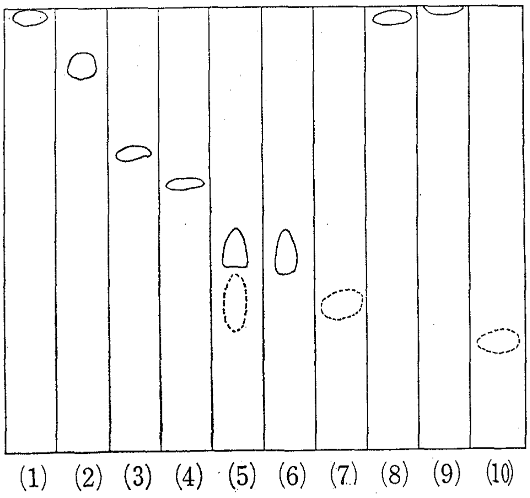

Fig. 1. Paper Chromatogram

A solvent mixture: *n*-BuOH:AcOH:H_2O (4:1:2)
temp. 20°

(1)	fraction-1	(2)	fraction-2
(3)	fraction-3	(4)	fraction-4
(5)	fraction-5	(6)	Gly$_3$Co
(7)	glycine	(8)	Ox$_2$Co
(9)	oxine	(10)	cobalt

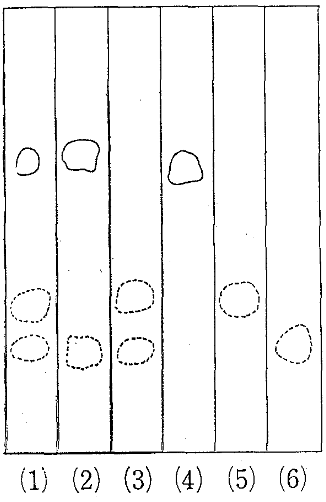

Fig. 2. Paper Chromatogram of the Decomposition Products with HCl

A solvent mixture: *n*-BuOH:AcOH:H_2O (4:1:2)
temp. 20°

(1)	fraction-4	(2)	Ox$_2$Co
(3)	Gly$_3$Co	(4)	oxine·HCl
(5)	glycine	(6)	cobalt(III)

Spectrophotometric Determination of Oxine——A certain amount of fraction-4 is weighed accurately, and it was dissolved in HCl and boiled for 10 min. The solution was used for the determination of each component. The solution was diluted with 10% HCl and its absorbancy was measured at 360 mμ. Fraction-4 was found to contain 1 mole of oxine per mole of the complex.

Colorimetric Determination of Glycine——Glycine content of fraction-4 was determined colorimetrically with ninhydrine[16] and the complex contained two moles of glycine per mole of the complex.

Determination of Cobalt[17]——The rest solution was neutrallized and to it oxine was added. Absorbancy of the chloroform extract was measured at 420 mμ. It was found that 1 mole of cobalt was contained in 1 mole of fraction-4.

Electronic Absorption Spectra[18]——Electronic absorption spectra of glycine and its chelate were measured in H_2O and those of oxine and its chelate were measured in MeOH. Spectra of the respective compounds are shown in Fig. 3 and 4, and their λ_{max} and ε are summarized in the Table III.

Infrared Spectra Analysis[19-23]——Infrared spectra were measured by KBr disc method (See Table IV).

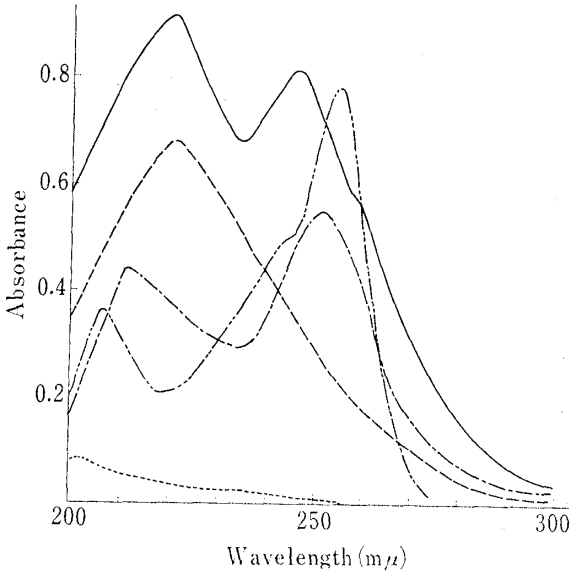

Fig. 3. Electronic Absorption Spectra in the Ultraviolet Region

	concentration	solvent
———: fraction-4	3×10^{-5}M	H_2O
– – – –: Gly_3Co	3×10^{-5}M	H_2O
- - - - - - -: glycine	1×10^{-4}M	H_2O
—•—•—: Ox_2Co	1×10^{-5}M	MeOH
—•—••: oxine	3×10^{-5}M	MeOH

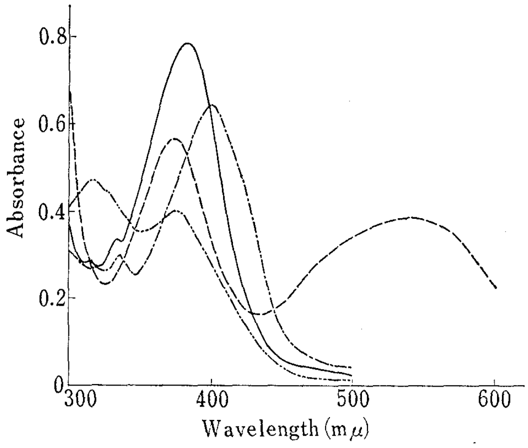

Fig. 4. Electronic Absorption Spectra in the Visible Region

	concentration	solvent
———: fraction-4	3×10^{-4}M	H_2O
– – – –: Gly_3Co	3×10^{-4}M	H_2O
—•—•—: Ox_2Co	1×10^{-4}M	MeOH
—••—••: oxine	3×10^{-4}M	MeOH

TABLE III. λ_{max} (ε) in the Electronic Absorption Spectra

Compound	Solvent	λ_{max} (ε)			
Fraction-4	H_2O	221 (30600)	246 (27500)	382 (2600)	
Gly_3Co	H_2O	221 (22800)		373 (187)	542 (130)
Glycine	H_2O				
Oxine	MeOH	207 (11900)	255 (22600)	374 (1350)	
Ox_2Co	MeOH	211 (44000)	251 (55000)		400 (6400)

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TABLE IV. Infrared Frequencies of Fraction-4, Glycine, Co(III)-Glycinate, Oxine and Co(III)-Oxinate (cm^{-1})

Fraction-4	Glycine	Gly_3Co	Oxine	Ox_2Co	Assignment
3460 broad	3400 broad	3350 s			
3240 s					
3140 s	3100 s	3160 s			NH_3^+ st. freq.
3040 s	3040 broad	3040 s	3040 s	3040 s	OH st. freq.
	2120 m				NH_3^+ st. f.
1665 sh					
1625 s	1585 broad	1570 sh			COO^- st. assym. NH_3^+ scissors
				1585 sh	
1575 sh			1578 m	1568 s	C=C and C–N st. vib.
1500 s	1495 s	1500 sh			NH_3^+ def. sym.
1420 sh	1410 s	1418 w			COO^- st. sym.
1370 s			1385 s	1370 s	OH def. C–O st.
1320 m				1315 s	
1285 sh			1285 s		
1272 m			1275 s	1275 m	
1215 sh			1212 s	1215 m	OH def. C–O st.
1175 broad			1173 s	1170 m	
1032 sh	1032 m	1040 broad			CCN assym. st.
908 m	911 s	921 m			CH_2 bending
832 m			830 s	820 s	ring vibration hydrogen def.
760 m			750 s	750 s	*ortho*-substitution

Result

When 50% MeOH solution containg Gly_3Co and Oxine was refluxed for 2 hours on a water bath, violet-red needle crystals which was soluble in water was precipitated. From infrared absorption spectrum and paper chromatographic property, it was identified to be a mixture. Therefore, nextly active charcoal was added and the solution was treated under the same condition. Paper chromatographic analysis gave a new spot, *Rf* 0.60, which is due a new mixed ligand complex $Gly_2Co(Ox)$. A yield of the mixed ligand complex is not high enough and further effort will be made to increase yield.

As is shown in Fig. 1, fraction-1 is found to be oxinate, while fraction-5 is Co(III)-glycinate. When fraction-4 is decomposed with conc. HCl for 10 min, it shows three spots, in Fig. 2, corresponding those of the components.

Elemental analysis of fraction-4 was found to be quite in good agreement with the proposed molecular formulae, $Gly_2Co(Ox) \cdot 2H_2O$.

Fraction-2 and -3 are still under investigation.

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