Determination of the Optical Purity of Amino Acids by Complex Formation.* Determination of Optimum Condition for Rotational Measurement

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A new method has been established for the determination of the optical purity of a small amount of amino acids (alanine, valine, leucine, and glutamic acid). The method utilizes the fact that when an optically active amino acid is dissolved in an alkaline solution of K[Co(acac$_2$en)(gly)$_2$], the solution shows much larger optical rotation than the free amino acid in the visible region. 1/50 to 1/100th the amount of an amino acid (about 0.1—0.05 grams) suffices for the determination of optical purity as compared with that needed in the usual method. The optimum conditions for measurement are: wavelength, 500 nm; pH, 10.00; time required to establish the substitution equilibrium, 6—8 hours; concentration of the complex, (4—5)$\times 10^{-3}$ mol l^{-1}; concentration of amino acid, (2—1)$\times 10^{-2}$ mol l^{-1}.

The optical purity of amino acids is usually determined by their optical rotations at the Na–D line.[1-4] These are generally so small that concentrated solutions (about 0.1—1.0 mol l^{-1}) are needed. For example, a 1.12 mol l^{-1} solution (6M-HCl) of optically pure L-alanine shows α_D-value only +0.14° with a 1 cm cell. Thus, several grams of sample is required for the usual method. The quantity of amino acids handled in biochemical research is so small that it is difficult to measure the optical rotation in such cases. Great improvements have been made in polarimeters and it has become possible to measure the optical rotation of active compounds with an accuracy of 10^{-3} °, but further improvement cannot be expected. Badr *et al.*[5] reported that when an amino acid in anhydrous methanol containing a base is treated with excess carbonyl compound, a considerably large optical rotation is observed in the 250 nm region. The reaction is useful for the assignment of the configuration of a small amount of amino acid, but as it is not quantitative, the method has not been used to determine the optical purity of amino acids.

It was found that cobalt(III)–acac$_2$en-complex, [Co(acac$_2$en)(X)$_2$]$^-$ (acac$_2$en^{2-}=*N*,*N*′-ethylenebis(acetylacetonimine) dianion, X$^-$=amino acid anion) has the following characteristics:[6] 1) The amino acidato ligand X$^-$, coordinated as a unidentate ligand at the axial site of the complex, is labile and easily replaced by an other amino acidato ligand Y$^-$ under alkaline conditions. The reaction between the complex [Co(acac$_2$en)(X)$_2$]$^-$ and Y$^-$ can be described by

$$[Co(acac_2en)(X)_2]^- + Y^- \rightleftharpoons [Co(acac_2en)(X)(Y)]^- + X^- \quad (1)$$

$$[Co(acac_2en)(X)(Y)]^- + Y^- \rightleftharpoons [Co(acac_2en)(Y)_2]^- + X^- \quad (2)$$

2) When the amino acidato ligand at the axial site is optically active, the complex shows a much larger optical rotation than the free amino acid in the visible region. These results suggest that if an optically active amino acid is mixed with optically inactive [Co(acac$_2$en)(X)$_2$]$^-$ under alkaline conditions, rapid exchange reactions such as (1) and (2) occur, and the mixed solution shows a much larger optical rotation than the solution containing the amino acid only. On this basis, we established a new method to determine the optical purity of a small amount of amino acid. Determination of the optimum condition for rotational measurements is discussed herewith.

Experimental

Preparation of Complexes. K[Co(acac$_2$en)(gly)$_2$]·2H$_2$O was prepared by the method reported.[6] The other complexes were prepared by a method similar to that used for [Co(acac$_2$en)(NH$_3$)$_2$]Cl·2H$_2$O.[7,8] The elemental analysis data of new complexes are as follows: *N*,*N*′-ethylenebis(benzoylacetoniminato)diamminecobalt(III) chloride dihydrate, [Co(bzac$_2$en)(NH$_3$)$_2$]Cl·2H$_2$O, Found: C, 51.89; H, 5.99; N, 10.26%. Calcd for C$_{22}$H$_{28}$N$_4$O$_2$CoCl·2H$_2$O: C, 51.72; H, 6.33; N, 10.97%. *N*,*N*′-Ethylenebis(trifluoroacetylacetoniminato)diamminecobalt(III) chloride monohydrate, [Co(tfac$_2$en)(NH$_3$)$_2$]Cl·H$_2$O, Found: C, 29.83; H, 4.16; N, 11.93%. Calcd for C$_{12}$H$_{18}$N$_4$O$_2$CoF$_6$Cl·H$_2$O: C, 30.23; H, 4.24; N, 11.76%.

Reagents. All chemicals were of analytical grade and used without further purification. Specific rotations measured by the usual method are as follows (% given in ( ) is the optical purity of amino acid). L-alanine (100%), $[\alpha]_D^{25}$=+15.0°, D-alanine (100%), $[\alpha]_D^{25}$=−14.9° (5 g/50 ml of 6M-HCl); L-valine (100%), $[\alpha]_D^{25}$=+28.5°, D-valine (100%), $[\alpha]_D^{25}$=−28.5° (4 g/50 ml of 6M-HCl); L-leucine (100%), $[\alpha]_D^{25}$=+16.3°, D-leucine (100%), $[\alpha]_D^{25}$=−16.3° (2 g/50 ml of 6M-HCl); L-glutamic acid (100%), $[\alpha]_D^{25}$=+32.4°, D-glutamic acid (100%), $[\alpha]_D^{25}$=−32.3° (5 g/50 ml of 2M-HCl). Amino acids of different optical purity were prepared by mixing optically pure amino acid with racemic amino acid (for example, L-alanine (50%) was prepared by mixing L-alanine (100%) with racemic alanine (0%) in a ratio of 1:1).

Measurements. The ORD and CD spectra were recorded at 25 °C on a JASCO Model ORD/UV-5 spectrophotometer with a CD attachment. The absorption spectra were measured at 25 °C with a Shimadzu UV-200 double beam spectro-

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photometer. The pH was measured with a Hitachi-Horiba Model F-7 pH-meter at 25 °C.

Results and Discussion

Determination of the Optimum Condition for Rotational Measurement. The molar rotations of L-alanine (100%) and its metal complexes are given in Table 1. The [M]-values of metal complexes containing amino acids are greater than those of free amino acids at special wavelengths, the [M]-values of Co(acac$_2$en)-complex being remarkably large. From a comparison of the [M]-values per one mole of amino acid, we see that the amino acid coordinated to the Co(acac$_2$en)-complex has 100—200 times as large an optical rotation as the free amino acid. The AB, CD, and ORD spectra of solutions prepared by mixing optically inactive K[Co(acac$_2$en)(gly)$_2$] with a large excess (100 times) of alanine under alkaline conditions are shown in Fig. 1. We see that ORD- and CD-intensities of the mixed solutions are in line with the optical purities of the alanine. The results suggest that the optical purity of amino acids can be determined by comparing the rotational (and also CD) value of a solution containing K[Co(acac$_2$en)(gly)$_2$] and optically pure amino acid with that of a solution containing the complex and the amino acid whose optical purity is to be determined. In this case a large excess of amino acid is used. However, when a small excess of amino acid (100%) is mixed with the glycinato complex solution, the resulting solution shows a small optical rotation as compared with that of the complex solution containing a large excess of amino acid (100%). This is due to the incomplete replacement reaction. Thus, the determination of optimum concentrations of the complex and amino acid is very important.

K[Co(acac$_2$en)(gly)$_2$] was used as an optically inactive Co(acac$_2$en)-complex, and alanine, valine, leucine and glutamic acid were examined. Since the chemical and spectral behavior of L- and D-form amino acids is the same except for the inversion of the CD- and

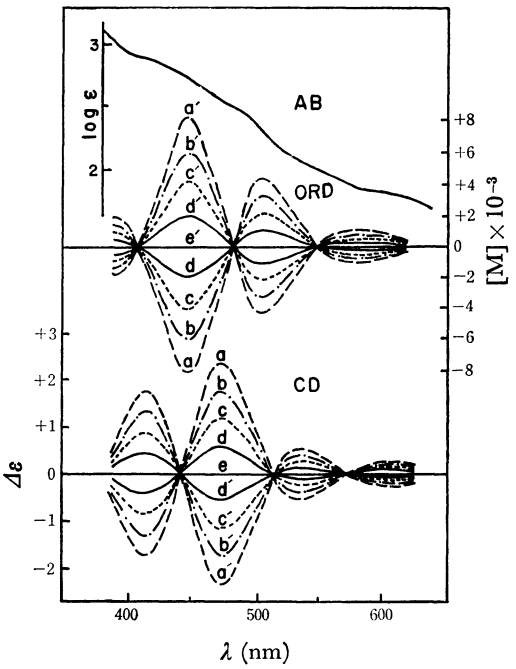

Fig. 1. AB, CD, and ORD spectra of the aqueous solution containing K[Co(acac$_2$en)(gly)$_2$] and a large excess (100 times) of alanine. The solution pH is 10.00. a and a′, L-alanine (100%) and D-alanine (100%); b and b′, L- and D-alanine (75%); c and c′, L- and D-alanine (50%); d and d′, L- and D-alanine (25%); e, racemic alanine.

ORD-signs, and the four amino acids behave similarly, L-alanine is given here with as an example. Although free amino acid is CD-inactive in the visible region, it becomes CD-active upon complex formation. Both CD- and ORD-intensities were therefore used to investigate optimum conditions.

Wavelength: The mixed solutions show ORD peaks at 380, 450, 500, and 580 nm, and CD peaks at 425, 475, 540, and 600 nm (Fig. 1). Peaks near 600 nm have low intensities, and those near 400 nm are accom-

Table 1. Molar rotation of L-alanine (100%) and its metal complexes

Compound	Molar rotation per one mole of complex* (°m^{-1} mol^{-1} l)	Molar rotation per one mole of amino acid (°m^{-1} mol^{-1} l)	Wavelength (nm)	Solvent
L-Alanine[1]		+2.41	500	Water
		+1.75	589	Water
		+20.76	500	2M-HCl
		+12.74	589	2M-HCl
		+7.06	500	2M-NaOH
		+4.14	589	2M-NaOH
K[Co(acac$_2$en) (L-ala)$_2$][6]	+4513	+2257	500	Water
	−9595	−4798	450	Water
[Co(NH$_3$)$_5$(L-alaH)](ClO$_4$)$_3$[8]	*ca.* −100	*ca.* −100	500	Water
trans-[Co(en)$_2$(L-alaH)$_2$]Cl$_3$[9]	+635	+318	500	Water
	−615	−308	*ca.* 590	Water
[Co(NH$_3$)$_4$(L-ala)]SO$_4$[8]	*ca.* −900	*ca.* −900	500	Water
[Cu(L-ala)$_2$][1]	+155	+78	522	Water
	−265	−133	654	Water

* Maximum values in the visible region.

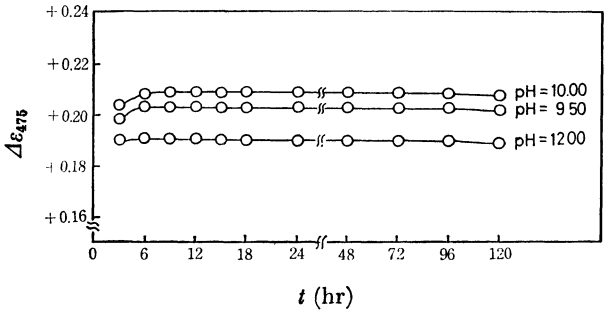

Fig. 2. The time dependence on the substitution equilibrium between K[Co(acac$_2$en)(gly)$_2$] and L-alanine (100%) at various pH. $C_{complex}=2.0\times10^{-3}$ mol l^{-1}, $C_{L-alanine}=2.0\times10^{-2}$ mol l^{-1}, $\mu=0.1$ (Na_2CO_3+$NaHCO_3$), T=25 °C.
$\Delta\varepsilon$ is given by the values for one mole of L-alanine at 475 nm.

panied by strong absorptions, and are therefore not suitable for measurements of CD- and ORD-intensities. The wavelengths of peaks at 475 nm (CD) and 500 nm (ORD) were chosen as their intensities are strong, and their CD- and ORD-signs coincide with the ORD-sign of free amino acids.

Time Dependence: In order to employ complex formation reactions such as (1) and (2) for determination of the optical purity of amino acids, it is necessary for the substitution reaction in solution to be in equilibrium. Thus the time needed to reach equilibrium was examined (Fig. 2). It is seen that 6—8 hr was required and that is less dependent on the pH of the solution. The solutions show constant CD-intensities for 3—4 days, which gradually decrease owing to the racemization of the optically active amino acid in a basic condition. The CD- and ORD-intensities of the mixed solutions were thus measured 10—24 hr after preparation of the solution.

pH-Dependence: The CD-intensity (and also ORD-intensity) depends upon the pH of the solution. The pH-dependence is shown in detail in Fig. 3. The solution shows maximum intensity at pH *ca.* 10. The intensity decreases in low pH region and in an alkaline

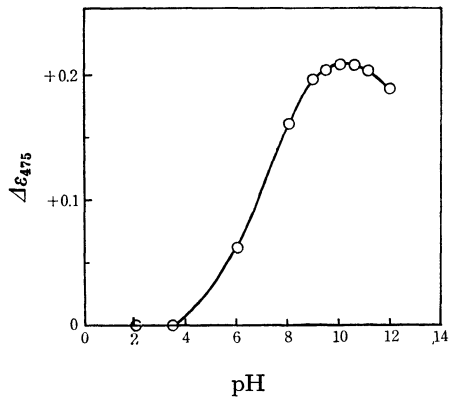

Fig. 3. The pH dependence on the CD-intensities of the mixed solutions containing L-alanine (100%). $\Delta\varepsilon$ is given by the values for one mole of L-alanine at 475 nm. $C_{complex}=2.0\times10^{-3}$ mol l^{-1}, $C_{L-alanine}=2.0\times10^{-2}$ mol l^{-1}, $\mu=0.1$ (Na_2CO_3+$NaHCO_3$), $T=25$ °C.

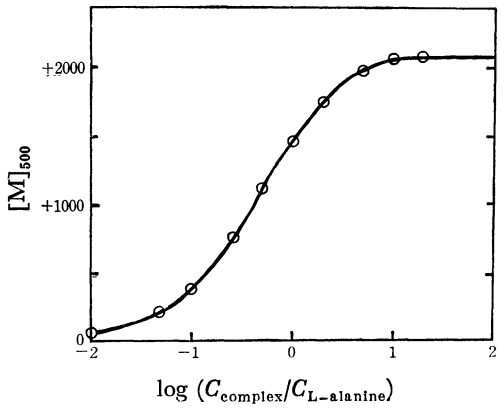

Fig. 4. Molar rotations of the mixed solutions containing K[Co(acac$_2$en)(gly)$_2$] and L-alanine (100%) in various mixing ratio. The pH of the solutions is 10.00. $[M]_{500}$ is given by the values for one mole of L-alanine. $\mu=0.1$ (Na_2CO_3+$NaHCO_3$), $T=25$ °C.

region above pH=10. This decrease seems to be due to the formation of aquo- and hydroxo-complexes in acidic and strongly basic conditions, respectively.

In order to adjust both the ionic strength μ and pH, a 1:1 mixture of Na_2CO_3 and $NaHCO_3$ was added to the solution. The coordination of CO_3^{2-} and HCO_3^- to the complex could be neglected for $\mu=(0.05—0.2)$.

Concentrational Dependence: The ORD-intensities of solutions containing K[Co(acac$_2$en)(gly)$_2$] and L-alanine (100%) in various concentrations are shown in Fig. 4. The results for CD-intensities are given in Table 2. It is observed that the larger the mixing ratio $C_{complex}/C_{L-alanine(100\%)}$, the greater the CD- and ORD-intensities per one mole of L-alanine (100%). Thus, in order to measure the CD- and ORD-intensities in high sensitivity for amino acid, a high concentration of complex and a low concentration of amino acid should be used. However, since the solution shows strong absorption in the visible region, the complex

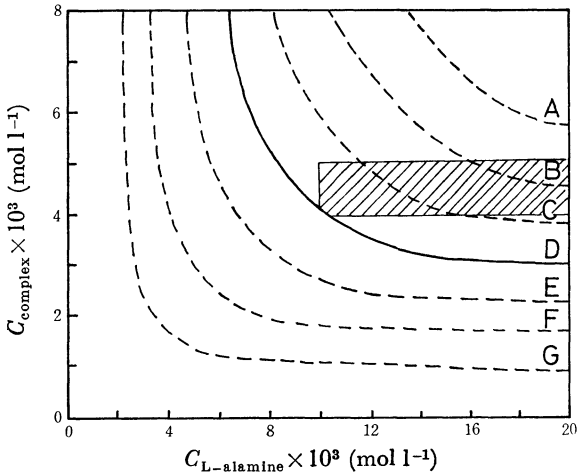

Fig. 5. The estimated rotations at 500 nm of the mixed solutions containing K[Co(acac$_2$en)(gly)$_2$] and L-alanine (100%) under various concentrational conditions. α_{500}-values are given by the contours (° cm^{-1}): A, $\alpha_{500}=1.6\times10^{-1}$; B, $\alpha_{500}=1.4\times10^{-1}$: C, $\alpha_{500}=1.2\times10^{-1}$; D, $\alpha_{500}=1.0\times10^{-1}$; E, $\alpha_{500}=0.8\times10^{-1}$; F, $\alpha_{500}=0.6\times10^{-1}$; G, $\alpha_{500}=0.4\times10^{-1}$.

TABLE 2. CD INTENSITIES OF SOLUTIONS CONTAINING K[Co(acac$_2$en)(gly)$_2$] AND L-ALANINE (100%) IN VARIOUS MIXING RATIO
pH=10.00, μ=0.1 (Na_2CO_3+$NaHCO_3$), T=25 °C, λ=475 nm.

Mixing ratio ($C_{complex}/C_{alanine}$)	Concentration (mol l^{-1})	$(\Delta\varepsilon)_{obs}$ ($cm^{-1}\times10^2$)	$\Delta\varepsilon_{obs}$ for one mole of alanine (cm^{-1} mol^{-1} l)	$\Delta\varepsilon_{calcd}$ for one mole of alanine (cm^{-1} mol^{-1} l)
10	$C_{complex}=5.0\times10^{-3}$	+0.056	+1.12	1.11
	$C_{alanine}=5.0\times10^{-4}$	+0.056	+1.12	
5	$C_{complex}=4.0\times10^{-3}$	+0.169	+1.06	1.07
	$C_{alanine}=8.0\times10^{-4}$	+0.170	+1.06	
2	$C_{complex}=4.0\times10^{-3}$	+0.378	+0.945	0.942
	$C_{alanine}=2.0\times10^{-3}$	+0.380	+0.950	
1	$C_{complex}=5.0\times10^{-3}$	+0.399	+0.798	0.795
	$C_{alanine}=5.0\times10^{-3}$	+0.398	+0.796	
1/2	$C_{complex}=5.0\times10^{-3}$	+0.593	+0.593	0.606
	$C_{alanine}=1.0\times10^{-2}$	+0.598	+0.598	
1/4	$C_{complex}=4.0\times10^{-3}$	+0.656	+0.410	0.411
	$C_{alanine}=1.6\times10^{-2}$	+0.657	+0.411	
1/5	$C_{complex}=2.0\times10^{-3}$	+0.354	+0.354	0.354
	$C_{alanine}=1.0\times10^{-2}$	+0.354	+0.354	
1/10	$C_{complex}=2.0\times10^{-3}$	+0.416	+0.208	0.209
	$C_{alanine}=2.0\times10^{-2}$	+0.414	+0.207	
1/20	$C_{complex}=2.0\times10^{-3}$	+0.458	+0.115	0.115
	$C_{alanine}=4.0\times10^{-2}$	+0.462	+0.116	
1/100	$C_{complex}=2.0\times10^{-3}$	+0.510	+2.55 ($\Delta\varepsilon_{obs}$ for one mole of complex)	
	$C_{alanine}=2.0\times10^{-1}$			

concentration cannot be made higher than *ca.* 5.0×10^{-3} mol l^{-1}. If a very low concentration of amino acid is used, the solution shows negligibly small CD- and ORD- intensities. Under these circumstances, it is difficult to determine the optimum concentrations. The solution equilibrium was therefore treated theoretically and an estimation of rotational values of the mixed solutions was examined. Results of calculation for the rotational values of the mixed solutions are shown in Fig. 5, where the estimated rotations are indicated by contours. The solid line indicates the α_{500}-value of 0.1° cm^{-1}, which is the minimum limit to obtain a rotational value to three significant figures. The optimum concentrations for rotational measurement are determined to be $C_{complex}=(5-4)\times10^{-3}$ mol l^{-1}, $C_{alanine}=(1-2)\times10^{-2}$ mol l^{-1} (shaded area, Fig. 5).

Theoretical Approach to Solution Equilibrium and Rotational Values of the Mixed Solution. When we mix K[Co(acac$_2$en)(gly)$_2$] with optically active amino acid YH in the initial concentrations of x mol l^{-1} and y mol l^{-1}, respectively, the concentrations of the complexes under equilibrium conditions can be written as follows:

$$C_{[Co(acac_2en)(gly)_2]^-} = [4x^2/(\alpha H_{gly})^2Z]x \quad mol\cdot l^{-1},$$

$$C_{[Co(acac_2en)(gly)(Y)]^-} = [4xyD/(\alpha H_{gly})(\alpha H_Y)Z]x \quad mol\cdot l^{-1},$$

$$C_{[Co(acac_2en)(Y)_2]^-} = [y^2D^2/(\alpha H_Y)^2Z]x \quad mol\cdot l^{-1},$$

where $Z=[4x^2/(\alpha H_{gly})^2+4xyD/(\alpha H_{gly})(\alpha H_Y)+y^2D^2/(\alpha H_Y)^2]$, αH_{gly} and αH_Y are αH-values of glycine and YH, respectively. D is a parameter which represents the relative coordinating ability of amino acids, taking the coordinating ability of gly^- as 1.00. Amino acids including glycine were thought to coordinate to Co(acac$_2$en)-complex in their anionic forms, and were assumed to distribute to Co(acac$_2$en)-complex statistically.[6] In the above expression, aquo- and hydroxo-complexes were neglected. The compounds which contribute to the rotation (and CD-intensity) are [Co(acac$_2$en)(gly)(Y)]$^-$, [Co(acac$_2$en)(Y)$_2$]$^-$, free Y^- and YH. Thus, the rotational value (or CD-intensity) at wavelength λ of the mixed solution is given by

$$A_\lambda = F\{B_\lambda x[2xyD/(\alpha H_{gly})(\alpha H_Y)+y^2D^2/(\alpha H_Y)^2]/Z+E_\lambda\} \quad (3)$$

where A_λ is the rotational value (or CD-intensity) at λ of the solution, B_λ the molar rotation (or molar CD-intensity) at λ of [Co(acac$_2$en)(Y)$_2$]$^-$ in which Y^- is an optically pure amino acid anion, E_λ the total rotational value (or CD-intensity) of free Y^- and YH, and F the optical purity of the amino acid YH, given by F=[concentration of optically active amino acid]/[total concentration of amino acid]. The molar rotation (or CD-intensity) of [Co(acac$_2$en)(gly)(Y)]$^-$ was assumed to be $1/2\times B_\lambda$, because the vicinal effect is generally additive.[11,12] If we mix K[Co(acac$_2$en)-(gly)$_2$] with optically pure amino acid, the rotational value (or CD-intensity) of the solution can be calculated by putting F=1. B_λ-values were estimated from the ORD- and CD-intensities of the solution containing K[Co(acac$_2$en)(gly)$_2$] and a large excess of optically pure amino acids. E_λ-values were estimated to be zero because free amino acids show negligibly small optical rotations in low concentrations and they are CD-inactive in the visible region. D-values of the solutions were estimated from the observed CD-intensities of the solutions having various mixing ratio, $C_{complex}/C_{aminoacid(100\%)}$. The values of B_λ and D thus determined are listed in Table 3.

By using these values, the CD- and ORD-intensities of the mixed solutions were calculated by Eq. (3). The results are given in Table 2 and Fig. 4. The

Table 3. Parameters B_i and D

Amino acid	pK_1	pK_2	B_{475} (CD) (cm^{-1} mol^{-1} l)	B_{500} (ORD) ($°m^{-1}$ mol^{-1} l)	D
Glycine	2.350	9.780	0.00	0	1.00
L-Alanine (100%)	2.348	9.866	+2.55	+4735	0.98
L-Valine (100%)	2.286	9.744	+4.95	+6625	0.90
L-Leucine (100%)	2.328	9.744	+4.70	+9220	0.77
L-Glutamic acid (100%)	2.10	4.07	+2.50	+6140	0.76
	9.47 (pK_3)				

Table 4. pH dependence of a mixed solution of L-alanine (100%)
$C_{K[Co(acac_2en)(gly)_2]}=5.0\times10^{-3}$ mol l^{-1}, $C_{L\text{-alanine}}=5.0\times10^{-2}$mol l^{-1}, $\mu=0.1$ ($Na_2CO_3+NaHCO_3$), T=25°C, λ =475 nm.

pH	($\Delta\varepsilon$) ($cm^{-1}\times10^2$)	$\Delta\varepsilon_{obs}$ for one mol of alanine ($cm^{-1}\cdot mol^{-1}\cdot l$)	$\Delta\varepsilon_{calcd}$ for one mol of alanine ($cm^{-1}\cdot mol^{-1}\cdot l$)
8.00	+0.750	+0.150	0.204
9.00	+0.976	+0.195	0.191
9.50	+1.008	+0.202	0.203
10.00	+1.030	+0.206	0.209
10.50	+1.036	+0.207	0.211
11.00	+1.020	+0.204	0.211
12.00	+0.944	+0.189	0.212

calculated values agree with the observed ones. The pH-dependence of Eq. (3) is given in Table 4. Agreement between the calculated and observed values is good in the pH region 9—10.5, but not in the others. The discrepancy might be due to the formation of aquo- and hydroxo-complexes. It is thus concluded that Eq. (3) holds in the pH region where the formation of aquo- and hydroxo-complexes is negligble.

The coordinating abilities (D-value) of the amino acids depend upon their basicity and steric factor. For the above system, the linear relation between the D-values and pK_2-values (pK_3 for L-glutamic acid) of the amino acids can not be found, since all the amino acids cited have nearly the same pK_2-values (pK_3 for L-glutamic acid) (the amino acid anion coordinates to Co(acac$_2$en)-complex with its amino group,[6] and therefore its pK_2-value was considered). Since the decreasing order of the D-values coincides with the increasing order of the steric crowding of the amino acids, it is assumed that the difference in coordinating ability of the amino acids to Co(acac$_2$en)-complex is mainly dependent on the steric factor.

Calibration Curve. The CD- (475 nm) and ORD- (500 nm) intensities were thus measured with solutions containing K[Co(acac$_2$en)(gly)$_2$] and amino acids of various optical purities. Experimental conditions were as follows: $C_{complex}=4.0\times10^{-3}$mol l^{-1}, $C_{aminoacid}=1.6\times10^{-2}$ mol l^{-1}, pH of the solution=10.00, $\mu=0.1$ ($Na_2CO_3+NaHCO_3$), cell length=1.0 cm, time=10—24 hr. From the results of measurements, calibration curves were obtained for four amino acids. All showed a linear relation between the ORD- (CD) intensities of the mixed solutions and the optical purities of the amino acids, the error being within 1%. It is concluded that we can determine the optical purity of a small amount of amino acid by comparing the ORD- (CD) intensity of a mixed solution containing optically pure amino acid with that of a mixed solution containing the amino acid whose optical purity is to be determined. The ORD- and CD-intensities of solutions prepared by mixing K[Co(acac$_2$en)(gly)$_2$] and optically pure amino acids are given in Table 5.

Table 5. CD- and ORD-intensities of a mixed solution containing K[Co(acac$_2$en)(gly)$_2$] and optically pure amino acid
$C_{complex}=4.0\times10^{-3}$ $mol^{-1}\cdot l$, $C_{amino\ acid}=1.6\times10^{-2}$ $mol\cdot l^{-1}$, pH of the solution=10.00, μ =0.1 ($Na_2CO_3+NaHCO_3$), T=25 °C, Time=(10—24) hr.

Amino acid	α_{500} ($°cm^{-1}$)	$\Delta\varepsilon_{475}$ (cm^{-1})
L-Alanine (100%)	+0.122	$+6.56\times10^{-3}$
D-Alanine (100%)	−0.122	-6.56×10^{-3}
L-Valine (100%)	+0.171	$+12.85\times10^{-3}$
D-Valine (100%)	−0.171	-12.85×10^{-3}
L-Leucine (100%)	+0.225	$+11.41\times10^{-3}$
D-Leucine (100%)	−0.225	-11.41×10^{-3}
L-Glutamic acid (100%)	+0.151	$+6.13\times10^{-3}$
D-Glutamic acid (100%)	−0.151	-6.13×10^{-3}

The solution for rotational measurement is prepared as follows: 20 ml of a 1.0×10^{-2} mol l^{-1} solution of K[Co(acac$_2$en)(gly)$_2$]·2H$_2$O (M.W.=504.48) and 5 ml of a buffer solution (0.2 mol l^{-1} Na_2CO_3+0.2 mol l^{-1} $NaHCO_3$) are mixed together. Then an amino acid (8.0×10^{-4} mol) is dissolved in this solution. The pH of the solution is adjusted to 10.00 by 0.1N-NaOH, the solution being made up to 50 ml with water. The rotational measurement is carried out after the solution is allowed to stand for about 10 hr at 25 °C.

Comparison with the Usual Method. The usual method requires 5—10 grams of amino acids to determine the optical purity. The method established herewith requires only *ca.* 0.1 g (8.0×10^{-4} mol). As small a quantity as 1/50 to 1/100 th of the sample suffices for the determination of the optical purity as compared with that needed in the usual method.

Other Schiff-Base Complexes. Several other cobalt(III)-Schiff-base complexes were examined in order to find other complexes which show larger a optical rotation than K[Co(acac$_2$en)(gly)$_2$] when they react with optically active amino acids, and which cause a more rapid substitution reaction of the axial ligand. The results are shown in Fig. 6. The Co(acac$_2$en)-

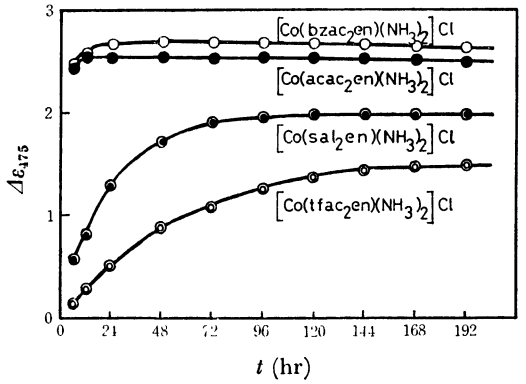

Fig. 6. The time dependence on the CD-intensity (475 nm) of the mixed solution containing Co(III)-Schiff-base complex and L-alanine (100%). $\Delta\varepsilon$ is given by the values for one mole of complex.
$C_{complex}=4.0\times10^{-3}$ mol l^{-1}, $C_{L\text{-alanine}}=8.0\times10^{-2}$ mol l^{-1}, the pH of the solution=9.50, $\mu=0.2$ (Na_2CO_3+$NaHCO_3$), $T=25$ °C.

complex is the most suitable for rotational measurement. All the complexes investigated, [Co(acac$_2$en)(NH$_3$)$_2$]Cl, [Co(bzac$_2$en)(NH$_3$)$_2$]Cl, [Co(tfac$_2$en)(NH$_3$)$_2$]Cl, and [Co(sal$_2$en)(NH$_3$)$_2$]Cl, show AB, CD, and ORD spectra similar to those for K[Co(acac$_2$en)(gly)$_2$] when mixed with L-alanine (100%). A long time is needed to establish the substitution equilibrium except for the Co(acac$_2$en)-complex. Since the chemical and spectral behavior of [Co(acac$_2$en)(NH$_3$)$_2$]Cl is very similar to that of K[Co(acac$_2$en)(gly)$_2$] and the preparation of the amine-complex is easier than that of the glycinato-complex, [Co(acac$_2$en)(NH$_3$)$_2$]Cl might be substituted for K[Co(acac$_2$en)(gly)$_2$].

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