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DETERMINATION OF THE OPTICAL PURITY OF AMINO ACIDS BY COMPLEX FORMATION

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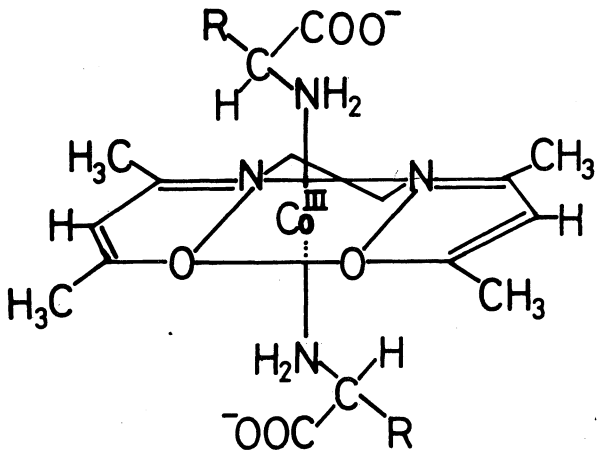
A new method has been established to determine the optical purity of small amount of amino acids (alanine, valine, and leucine). The method utilizes the fact that when the optically active amino acid is dissolved in an alkaline solution of $K[Co(baen)(gly)_2]$ complex, the mixed solution shows much larger optical rotation than that of the free amino acid in the visible region.

As has been reported previously,[1] cobalt(III)-baen (baen = N,N'-ethylenebisacetylacetonimine dianion) complex shows following two features; (1) the amino acidato ligand coordinated as a unidentate ligand at the apical site of the complex is labile and easily replaced by other amino acidato ligands, (2) when the amino acidato ligand at the apical site is optically active, the complex shows a very large optical rotation in the visible region. In this letter, we report a new method to determine the optical purity of small amount of amino acids by using these two features.



The optical purity of amino acids is usually determined by their optical rotations at Na-D line, which are generally so small that very concentrated solutions (about one mol l^{-1}) of amino acids are used for their optical rotation measurements. For example, for a one mol l^{-1} solution (6N-HCl) of optically pure L-alanine, the α-value of $+0.13^{\circ}\,cm^{-1}$ is observed at Na-D line.[2 - 4] By using the method mentioned here, nearly the same optical rotation can be attained for 1/50 - 1/100 mol l^{-1} solution of pure L-alanine at 500 nm.

Therefore, even 1/50 to 1/100 times smaller quantities of samples, as compared with those needed in the usual method, suffice to determine the optical purity of amino acids in this method.

The outline of this method is as follows: amino acid, the optical purity of which is to be determined is dissolved in an alkaline solution (pH = 10.00) of K[Co(baen)-$(gly)_2$] complex which has no optical activity. The mixed solution is left for about 10 hours to achieve ligand substitution equilibrium. Then the optical rotation of the solution, which arises mainly from the complex formation with the optically active amino acidato ligand through so-called vicinal effect, is measured and compared with that of the solution made up by mixing the complex solution with optically pure amino acid under the same experimental condition. The ratio of the two values of the optical rotation gives the optical purity of the amino acid in question. Although both CD and ORD intensities were available and gave the same result, we mention only the result obtained from the ORD intensity measurement. L-Alanine, L-valine, and L-leucine were investigated, but we describe here the case for L-alanine as an example.

Equilibria occurring in the mixed solution mentioned above can be describe as

$$[Co(baen)(gly)_2]^- \underset{gly^-}{\overset{ala^-}{\rightleftharpoons}} [Co(baen)(gly)(ala)]^- \underset{gly^-}{\overset{ala^-}{\rightleftharpoons}} [Co(baen)(ala)_2]^-$$

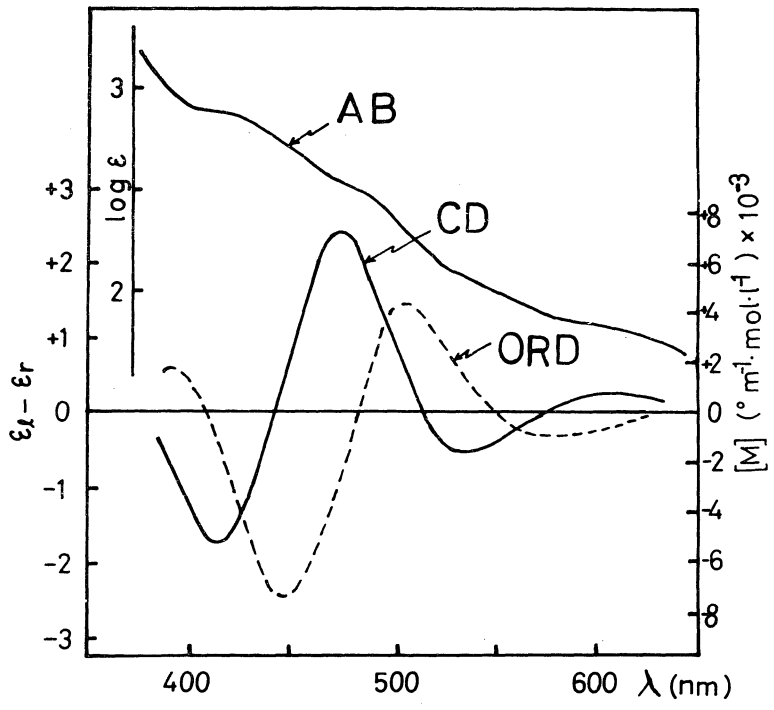


Fig. 1. AB, CD, and ORD spectra of the solution containing K[Co(baen)$(gly)_2$] and a large excess of L-alanine in H_2O. The solution pH is 10.00.

Thus, if a large excess of alanine is added to the glycinato complex solution, the complex is thought to exist mainly as $[Co(baen)(ala)_2]^-$ under equilibrium condition. This was confirmed by the observation that the AB, CD, and ORD spectra of the mixed solution containing the glycinato complex and a large excess of L-alanine were nearly the same as those of K[Co(baen)$(L\text{-}ala)_2$][1] complex. These spectral appearances were shown in Fig. 1.

However, when a small excess of L-alanine was added to 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 L-alanine. This is due to the incomplete replacement reaction. Therefore, in this study, we measured the ORD intensity under the concentrational condition suitable for the observation of the ORD intensity, $C_{complex} = 4.0 \times 10^{-3}$ mol l^{-1} and $C_{amino\ acid} = 1.6 \times 10^{-2}$ mol l^{-1} (the pH of the mixed solution was adjusted to 10.00). The ORD intensity measurement was carried out at 500 nm at 25 °C. Under these experimental conditions, the optically pure L-alanine showed the rotation of +0.122 ° cm^{-1} at 500 nm: which corresponds to that of about a one mol l^{-1} solution of the free L-alanine at Na-D line. L-Valine and L-leucine showed the optical rotations of +0.171 and +0.225 ° cm^{-1} respectively under the same experimental condition.

Fig. 2 shows the calibration curve for the optical purity of L-alanine. A good linear relationship was obtained between the optical purity (%) of L-alanine and the optical rotation of the mixed solution, and the error was within 1%.

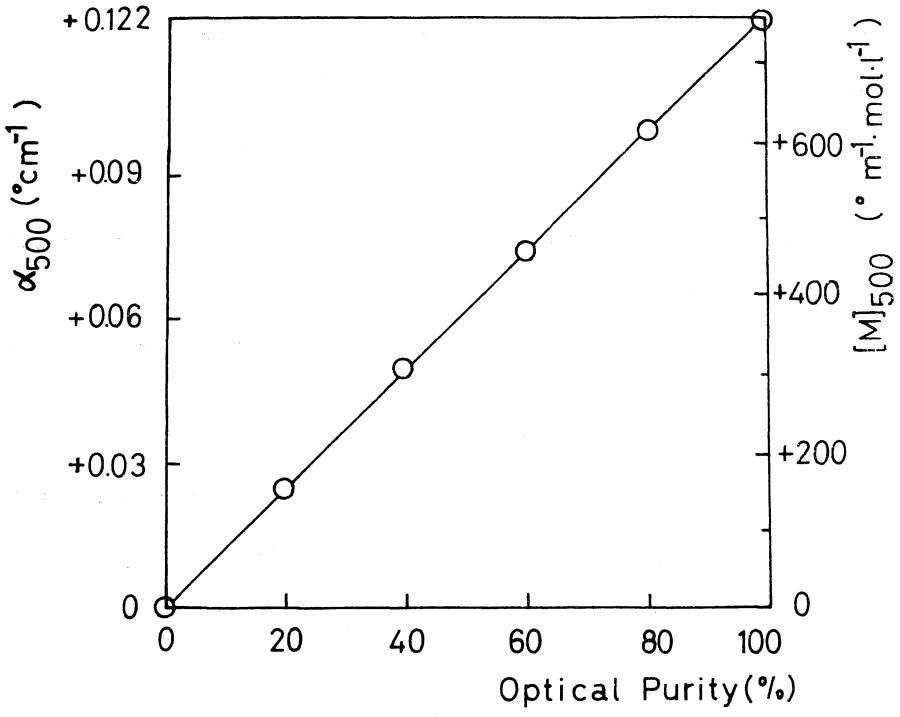


Fig. 2. The calibration curve for the optical purity of L-alanine. Concentrations: $K[Co(baen)(gly)_2] = 4.0 \times 10^{-3}$ mol l^{-1}, L-alanine = 1.6×10^{-2} mol l^{-1}. The pH of the mixed solution = 10.00. μ = 0.1 (Na_2CO_3-$NaHCO_3$). T = 25 °C.

This result implies that when the experimental condition is held constant, the equilibria between the glycinato complex and alanine anions having various optical purities are established in the same manner independently of the optical form of alanine.

The solutions for rotational measurements were prepared as follows: a 1.0×10^{-2} mol l^{-1} solution of $K[Co(baen)(gly)_2]\cdot 2H_2O$ (M.W. = 504.48), which was synthesized by the method described in reference 1), was prepared by dissolving the complex (0.5045 g) in water and the solution was made up to 100 ml with water at 25°C. Then, 20 ml of the complex solution and 5 ml of buffer solution (0.2 mol l^{-1} Na_2CO_3, 0.2 mol l^{-1} $NaHCO_3$) were mixed, and to this solution was dissolved amino acid (8.0×10^{-4} mole). The pH was adjusted to 10.00 by 0.1N-NaOH, and the solution was made up to 50 ml with water at 25°C. The rotational measurements were carried out after the solution was allowed to stand for 10 hours at 25°C.

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