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DETERMINATION OF THE OPTICAL PURITY OF A SMALL AMOUNT OF AMINO ACIDS (1 - 5 mg) EMPLOYING STEREOSELECTIVE COMPLEX FORMATION

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A new method has been established to determine the optical purity of about 1 - 5 mg of sample amino acids within a short time. The method utilizes the fact that [CH_3COCH_2-Co(acac$_2$en)(H_2O)] reacts with L-(or D-) amino acids rapidly to form Λ_L-(or Δ_D-)cis-β_1(fac)-[Co(acac$_2$en)(aa)] stereoselectively and thus, the reaction solution shows much larger optical rotation than the free amino acids.

Previously,[1,2] we reported a determination of the optical purity of a small amount of amino acids using complex formation, however, the method needed about 50 - 100 mg of sample amino acids and it took a fairly long reaction time (6 - 8 hrs). In this connection, recently, we found a stereoselective complex formation of amino acid with a cobalt(III) complex containing a tetradentate Schiff-base ligand in methanol.[3] In the reaction, amino acid coordinates to the cobalt(III)-Schiff-base complex as a bidentate ligand and the formed complex takes stereoselectively the Λ_L-(or Δ_D-)cis-β_1(fac)-structure to show much larger optical rotation than the free amino acid, if the used amino acid is L-form (or D-form) except for proline. In the case of proline, Δ_L- or Λ_D-isomer is formed. Employing the stereoselective reaction, we established a new method which provides the determination of the optical purity of about 1 - 5 mg of sample amino acids within a short time.

Although various Schiff-base complexes and at least eleven amino acids are available, we mention here only the representative reaction of an organometallic complex, [CH_3COCH_2-Co(acac$_2$en)(H_2O)][4] (acac$_2$en = dianion of N,N'-ethylenebis(acetylacetonimine)), with several amino acids in methanol.

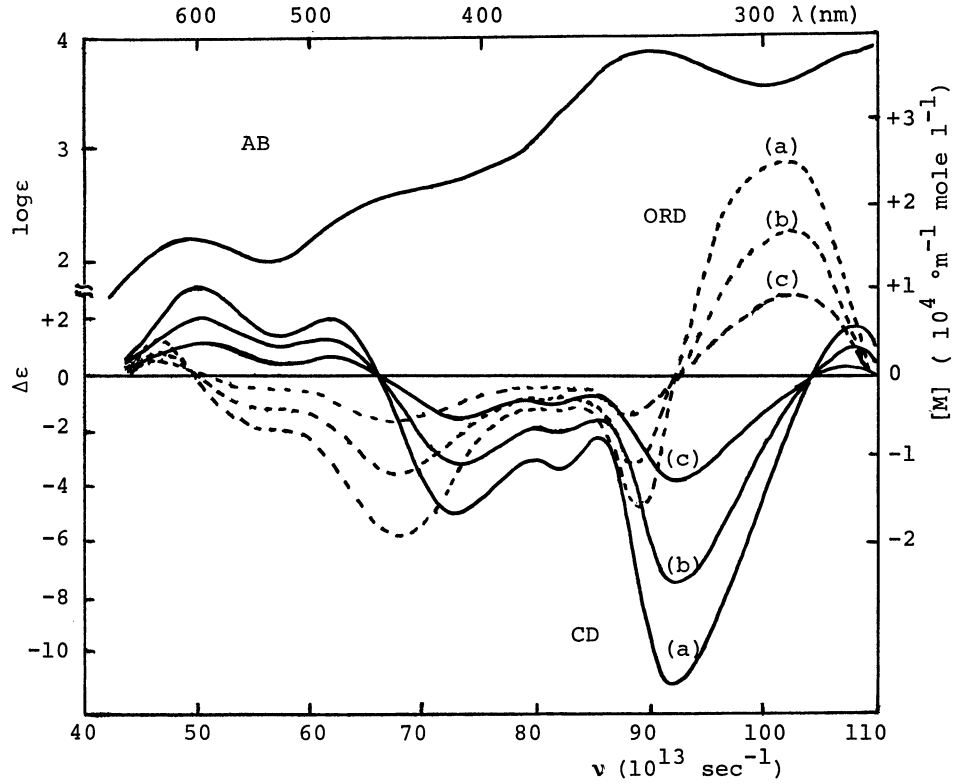

Fig. 1. AB, CD, and ORD spectra of the reaction solutions of [CH_3COCH_2-Co-($acac_2$en)(H_2O)] and L-phenylalanines having various optical purities.
(a) : O.P. = 100%,
(b) : O.P. = 66.6%,
(c) : O.P. = 33.3%.
AB spectra are the same for (a), (b), and (c).

Figure 1 shows the representative AB, CD, and ORD spectra of the reaction solutions of the organometallic complex with L-amino acids having various optical purities. This figure indicates that the reaction solution exhibits quite strong CD and ORD intensities at various wavelengths and that both CD and ORD intensities are proportional to the optical purity of the amino acid. Although not only ORD intensity but also CD intensity is applicable in this method, we used ORD intensity at 435 nm for the detailed investigation of the complex formation reaction. The reaction between the organometallic complex and amino acids (aaH) is written as follows:

$$\text{trans-[}CH_3COCH_2\text{-Co(}acac_2en\text{)(}H_2O\text{)]} + \text{L-aaH} \longrightarrow \Lambda_L\text{-cis-}\beta_1\text{-[Co(}acac_2en\text{)(aa)]} + CH_3COCH_3 + H_2O \quad (1)$$

$$\text{trans-[}CH_3COCH_2\text{-Co(}acac_2en\text{)(}H_2O\text{)]} + \text{D-aaH} \longrightarrow \Delta_D\text{-cis-}\beta_1\text{-[Co(}acac_2en\text{)(aa)]} + CH_3COCH_3 + H_2O \quad (2)$$

Here, Eqs. (1) and (2) mean that in the case of L-amino acids, Λ_L-cis-β_1-diastereoisomer is formed more than Δ_L-isomer and in the case of D-amino acids, Δ_D-cis-β_1-diastereoisomer is formed more than the corresponding Λ_D-isomer. Of cource, the ratios, Λ_L/Δ_L and Δ_D/Λ_D, are the same with each other. As the reaction is stoichiometric, if the amino acid which contains L-enantiomer more than D is used, Λ_L-isomer is formed more over than Δ_D-isomer. Therefore, the CD and ORD intensities observed in this method come from the optically active diastereoisomer. This is the reason why the CD and ORD intensities are extremely large.

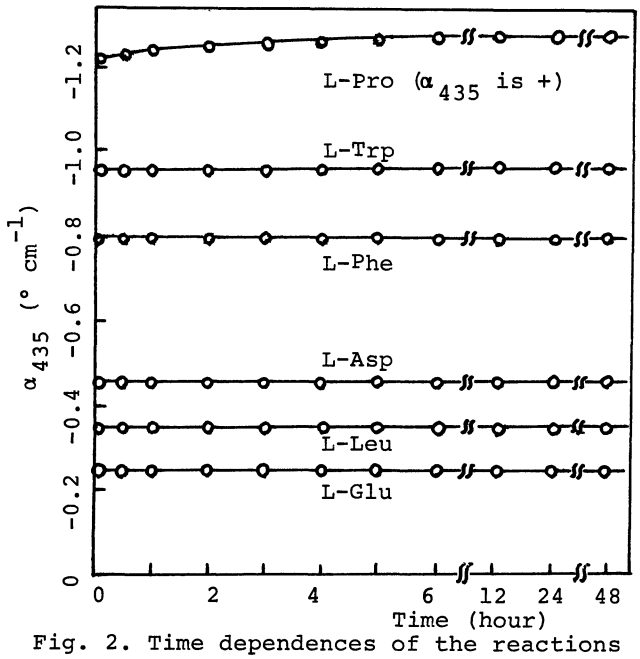

Fig. 2. Time dependences of the reactions of [CH_3COCH_2-Co(acac$_2$en)(H_2O)] (C = 4.0 x 10^{-3}M) and optically pure L-amino acids (C = 4.4 x 10^{-3}M) in methanol at 25 °C.

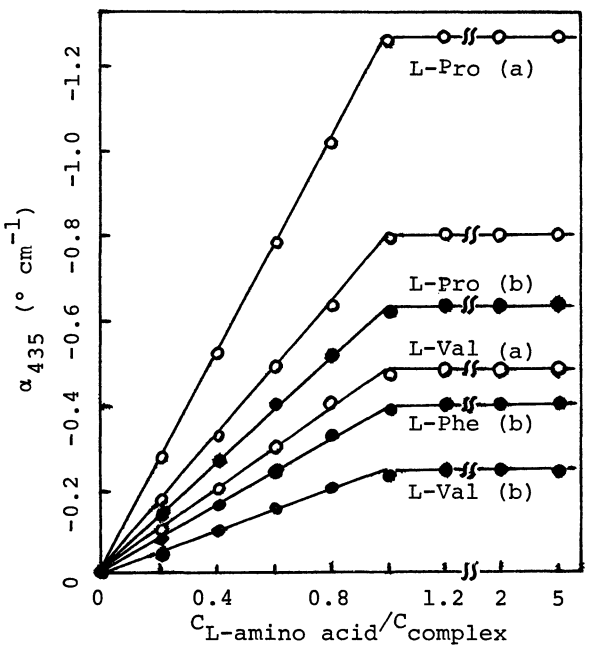

Fig. 3. Concentrational dependence.
(a) : $C_{complex}$ = 4.0 x 10^{-3}M,
(b) : $C_{complex}$ = 2.0 x 10^{-3}M.

Figure 2 shows the time dependences for the reactions. All the reactions for twelve amino acids complete within a mixing time except for proline (~ 6 hrs). Therefore, this method is applicable to measureing the optical rotation without waiting after the preparation of the reaction solution.

Figure 3 shows the concentrational dependences of the observed rotations. These experiments reveal that the molar ratios of the reactions between the organometallic complex and amino acids are all just 1 : 1 and that the observed rotations are proportional to the concentrations of the formed complexes. Thus, the higher the concentrations, the larger the optical rotations. However, since the complexes absorp light strongly, as is supposed from the AB spectrum in Fig. 1, one can not make the concentration of the complex higher than about 4.5 x 10^{-3} M, in the case of the measurement of ORD intensity at 435 nm with 1 cm cell.

Figure 4 shows the calibration curves for the optical purities of amino acids and the observed rotations for optically pure L-amino acids. All the calibration curves have the linear relations between the observed rotations and the optical purities. Therefore, one can determine the optical purity of sample amino acid by this method, if the rotation for optically pure amino acid which was measured under the same condition is known. Here, as seen in Fig. 4, the optical rotations for proline, tyrosine,

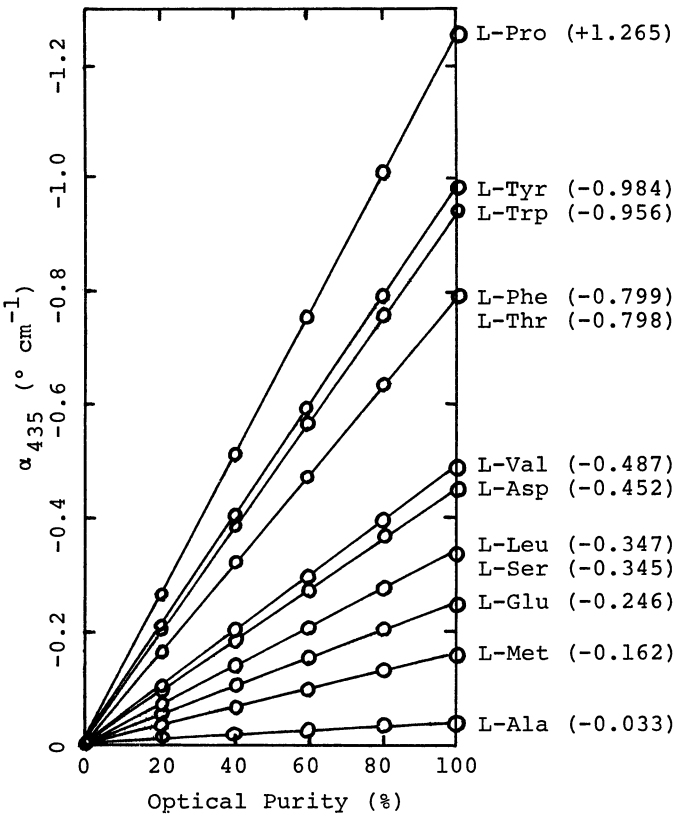

Fig. 4. The calibration curves and observed rotations, α_{435}, for optically pure L-amino acids. $C_{complex} = 4.0 \times 10^{-3}$M, $C_{amino\ acid} = 4.4 \times 10^{-3}$M.

tryptophane, phenylalanine, and threonine are quite large. Thus, it is possible for these amino acids to determine the optical purity of about 1 mg of sample.

The methods for the preparation of the solution for the measurement of optical rotation are as follows; amino acid (4.4×10^{-2} mmole, about 5 mg) is dissolved in 2.0 ml of water and it is added to a methanol solution (5.0 ml) of [CH_3COCH_2-Co(acac$_2$en)(H_2O)] (C = 8.0 mM). The mixed solution is made up to 10 ml with methanol. The rotation is measurable with 1 cm cell soon after the preparation of the solution except for proline. For proline, the measurement should be done 6 hrs after the preparation. In the cases of aspartic and glutamic acids, neutralized solutions of the amino acids with an equimolar amount of KOH are used. For tyrosine, its suspension in 2.0 ml of water is added to the complex solution and the mixture is stirred for 4 - 5 hrs to dissolve it.

This method needs no fine pH-adjustment except for aspartic and glutamic acids, and thus, the preparation of the solution is very simple. However, it should be noted that one should carry out the preparation of the solution for the measurement of rotation as soon as possible after dissolution of the organometallic complex in methanol. Because, the complex is not so stable that it gradually decomposes to an undesirable brown complex.

References

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