Optical Resolution of Phenylalanine and Mandelic Acid by Complex Formation with Copper(II) Ion

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(Received June 17, 1992)

The optical resolutions of phenylalanine and mandelic acid were performed by complex formation with Cu^{2+}. D- and L-mandelic acids were completely resolved by forming a complex with L-phenylalanine, while the maximum optical purity of D- and L-phenylalanine by using D-mandelic acid was about 65%. The mole ratio of Cu^{2+} to phenylalanine and/or mandelic acid in the complexes was 0.5. These results were interpreted in terms of the relative stability of the complexes, i.e., the optical absorption energies in the aqueous media.

The use of chiral transition metal complexes is a subject of growing interest in such areas as biomimetic chemistry, stereoselective catalysis and racemate separation. Among them, Cu^{2+} complexes of L-amino acids are well-known examples.[1,2] L-Amino acids and their derivatives are readily available and are used as cheap building blocks in the synthesis of organic molecules as well as of open-chain and macrocyclic multidentate chiral ligands.[3–5] Gil-Av et al.[6] reported on the separation of underivatized amino acid enantiomers by reversed-phase liquid chromatography using a chiral mobile phase which can form diastereomeric complexes with D- and L-enantiomers of the amino acids. Thus, the resolution was established by the differences in the stability and the polarity of these diastereomeric species. Koga et at.[7] reported on the optical resolution of D- and L-mandelic acid (MA=Hma) by using an apparent difference in the solubility of the MA complexes with L-phenylalanine (PA=Hpa) in aqueous solution. However, the optical purity of the resolved MA was not satisfactory. We have now successfully resolved D- and L-PA and D- and L-MA by using a method that combines the chromatographic technique with the crystallization procedure.

Experimental

Apparatus. The flotation cell comprises a glass cylinder (2.5 cm diameter and 20 cm height) with a glass filter (No. 2) attached to its bottom. The pH values of the solutions were measured with a Toa (Model HM-30S) pH meter. A Toso CCPD high-performance liquid chromatograph (HPLC) attached to a Toso UV-8000 UV detector was used. The concentrations of Cu^{2+}, MA and PA were determined by a Toso TSK gel ODS-120T column (15×0.46 cm i.d.). The optical purity was determined by using a Toso TSK gel ENANTIO L1 column (25×0.46 cm i.d.) and a Toso CO-8000 column oven. The optical absorption spectra of the Cu^{2+} complexes in aqueous solutions were measured using a Hitachi U-2000 spectrophotometer. Elemental analyses of C, H, and N were carried out at the Advanced Instrumentation Center for Chemical Analysis, Ehime university.

Reagents. A stock solution of Cu^{2+} was prepared by dissolving copper(II) acetate into distilled water, whose concentration was determined by chelatometric titration with EDTA. Standard solutions of MA and PA were prepared by dissolving weighed amounts of reagents into distilled water. All of the reagents for HPLC were of GR grade.

Procedure. After adding the desired quantities of PA solution to a mixture of Cu^{2+} and MA standard solutions and adjusting the pH to the desired values, flotation of the Cu^{2+} complexes was initiated by bubbling nitrogen gas for 10 min in the flotation cell. Then, the solution was filtered out and the floatabilities of PA or MA were determined by HPLC with an ODS column. The floatabilities were calculated using the following equation:

$$F(\%) = 100 \times (a_0 - a_1)/a_0,$$

where a_0 and a_1 are the initial and final concentrations of PA or MA in the flotation cell, respectively. On the other hand, floated scums were washed with water, methanol and diethyl ether, and dried in open air; they were then offered to elemental analyses as well as analyses by HPLC with an ODS column. HPLC was carried out at room temperature. The flow rate of the mobile phase consisted of 0.05 mol dm^{-3} phosphoric acid; 30% acetonitrile was 0.8 cm^3 min^{-1}. The detection wavelength and pH were 210 nm and 3.0, respectively. The optical purity of the floated scum was also determined by HPLC with an enantiomeric resolution column at 40°C after dissolving it in 2 cm^3 of 6 mol dm^{-3} nitric acid. The flow rate of the mobile phase comprising a 1×10^{-3} mol dm^{-3} copper(II) sulfate aqueous solution was 1 cm^3 min^{-1}. The detection wavelength and pH were 254 nm and 5.0, respectively.

Results and Discussion

Optimum Conditions of Flotation. As shown in Fig. 1, the precipitate was formed only when the concentration of L-PA exceeded 2.5×10^{-3} mol dm^{-3}. The floatability of the L-PA complex reached 83% under the experimental conditions. The mole ratio of Cu^{2+} : D-MA : L-PA of the scum was determined by HPLC to be 1 : 1.07 : 1.03. Further, the analytical results for the complexes were as follows. Found: C, 53.88; H, 4.62; N, 3.83%. Calcd for $C_{17}H_{17}CuNO_5$: C, 53.89; H, 4.53; N, 3.70%. It can thus be seen that the complex was floated as [Cu^{II}(D-ma)(L-pa)]. On the other hand, precipitation for the D-PA complex required at least
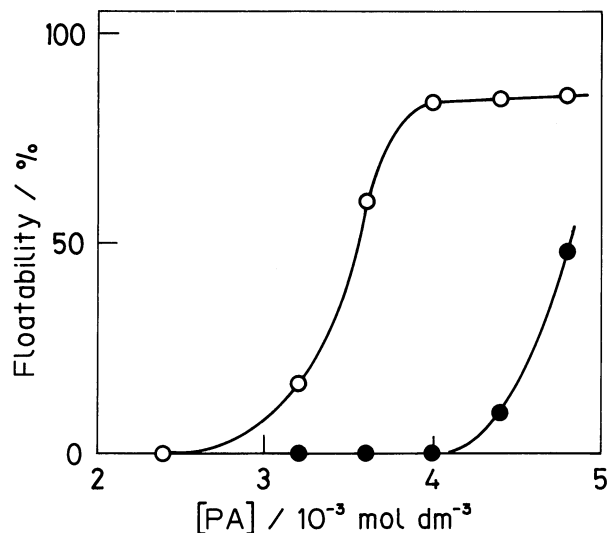
Fig. 1. Effect of the concentrations of D- and L-phenylalanine (PA=Hpa) on the flotation of their complexes. ○: L-PA, ●: D-PA. [Cu²⁺]=4.0×10⁻³ mol dm⁻³, [D-mandelic acid (MA=Hma)]=8.0×10⁻³ mol dm⁻³, pH 5.8.
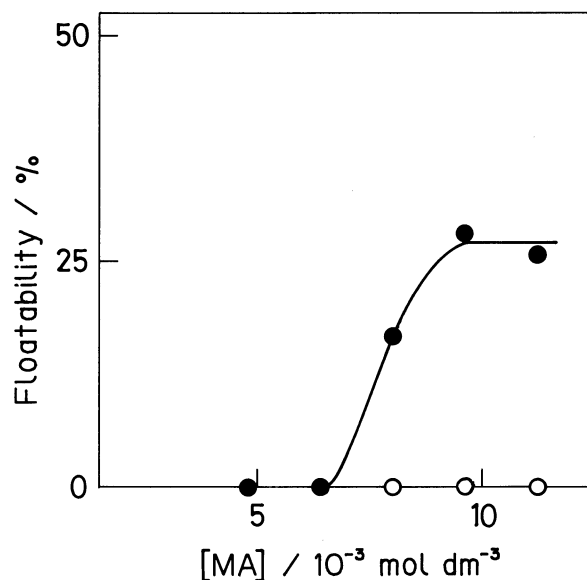

Fig. 3. Effect of the concentrations of D- and L-MA on the flotation of their complexes. ○: L-MA, ●: D-MA. [Cu²⁺]=[L-PA]=4.0×10⁻³ mol dm⁻³, pH 5.5.
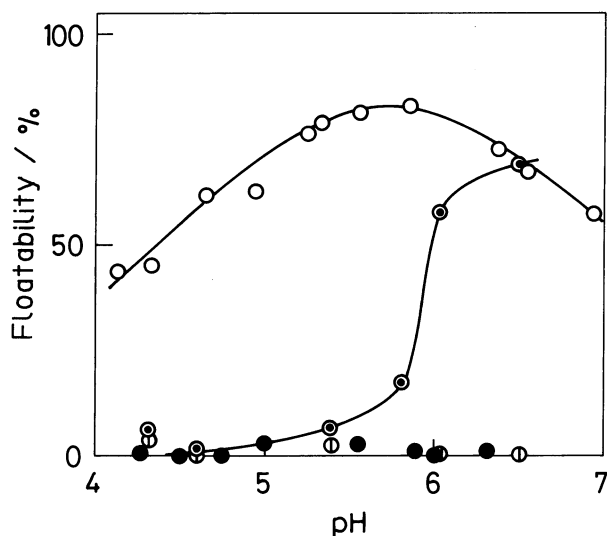
Fig. 2. pH dependence of the floatability of the D- and L-PA complexes. [Cu²⁺]=4.0×10⁻³ mol dm⁻³, [D-MA]= 8.0×10⁻³ mol dm⁻³. ○: L-PA, ●: D-PA; [PA]=4.0× 10⁻³ mol dm⁻³. ◉: D-PA, (◎: D-MA); [D-PA]=4.5× 10⁻³ mol dm⁻³.

Fig. 4. pH dependence of the floatability of the D- and L-MA complexes. ○: L-MA, ●: D-MA. [Cu²⁺]=[L-PA]= 4.0×10⁻³ mol dm⁻³, [MA]=8.0×10⁻³ mol dm⁻³.

4.0×10⁻³ mol dm⁻³ of D-PA. As shown in Fig. 2, the maximum floatability of the L-PA complex was attained at a pH value of 5.8. A decrease in the floatabilities beyond this pH may be due to hydrolysis of the complex. The D-PA complex could not be floated in the pH range from 4 to 7. On the other hand, the D-PA complex was floated with increasing pH when the D-PA concentration was 4.5×10⁻³ mol dm⁻³, although D-MA was not included in the D-PA complex, as shown in Fig. 2. The analytical results of the floated scum were as follows. Found: C,

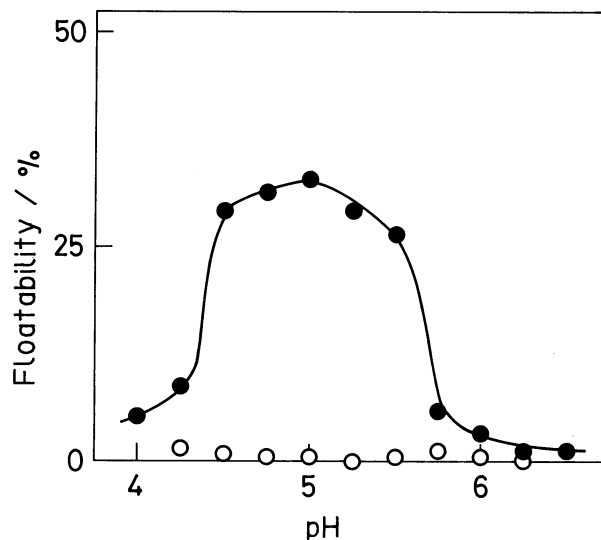53.71; H, 5.09; N, 7.12%. Calcd for C₁₈H₂₀CuN₂O₄ (Cu²⁺:D-PA=1:2): C, 55.15; H, 5.15; N, 7.15%. It is considered that the Cu²⁺ complex with only D-PA was preferentially floated when the concentration of D-PA exceeded 4.0×10⁻³ mol dm⁻³, that is, the solubility limit, as shown in Fig. 1. D- and L-PA could be resolved by forming a complex with D-MA at [D-MA]/[PA] above 2. In the case where L-MA was used, only the D-PA complex could be selectively floated.

Similarly, D- and L-MA could be resolved by using L-PA, as shown in Figs. 3 and 4. In this case, only the

Table 1.   Optical Resolution of D- and L-PA and D- and L-MA

Counter reagent	Racemic mixture ($\text{D}:\text{L}=1:1$)	pH	Yield	Optical purity
10^{-3} mol dm^{-3}	10^{-3} mol dm^{-3}		%	% ee
D-MA=16	PA = 6.0	5.8	L-PA =41.7	65.0
D-MA=20	PA = 6.0	5.8	L-PA =42.3	67.5
L-PA = 8.0	MA=16	5.8	D-MA= 9.8	100
L-PA = 5.3	MA=16	5.8	D-MA=11.4	100
L-PA =10	MA=16	5.0	D-MA=41.5	99.8
L-PA = 8.0	MA=19	5.0	D-MA=29.7	99.8

$[Cu^{2+}]=8.0\times10^{-3}$ mol dm^{-3}.
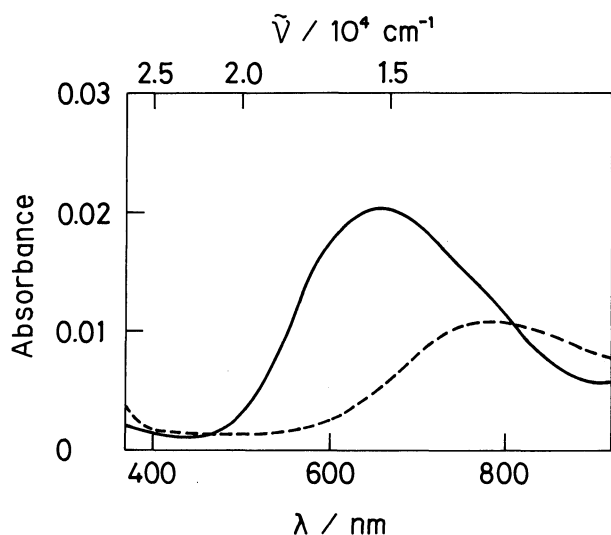
Fig. 5.   Electronic spectra of [Cu^{II}(L-pa)$_2$] and [Cu^{II}(D-ma)$_2$] complexes in aqueous solutions. ——: L-PA, ---: D-MA. [L-PA]=[D-MA]=1.6×10^{-5} mol dm^{-3}, [Cu^{2+}]=8.0×10^{-6} mol dm^{-3}, pH 5.8.
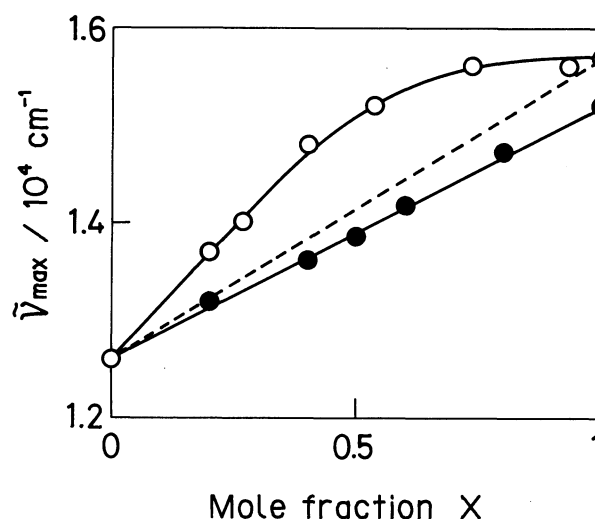

Fig. 6.   Relation between the d–d band maxima of copper(II) complexes and mole fraction (X) in $1.5(1-X)$(D-MA)–$0.5Cu^{2+}$–$1.5X$(PA) systems. O: L-PA, ●: D-PA. [Cu^{2+}]=8.0×10^{-4} mol dm^{-3}, pH 5.8.

[Cu^{II}(D-ma)(L-pa)] complex could be floated.

The Optical Resolution of Racemic Mixture. In Table 1 the optical purities of MA and PA resolved from the racemic mixture are listed under the optimum conditions of flotation. The optical purity of MA was perfectly established, while that of PA was not satisfactory. This result was due to a difference in the stability of the Cu^{2+} complexes formed in the solution, as described below.

Stability of the Complexes. The electronic spectra of the Cu^{2+} complexes in aqueous solutions are shown in Fig. 5. This absorption band is due to the ligand field d–d transition of the Cu^{2+} ion; the absorption maxima ($\bar{\nu}_{max}$) should thus depend on the stability of the complexes. The absorption bands of [Cu^{II}(L-pa)$_2$] and [Cu^{II}(D-ma)$_2$] were 640 and 787 nm, respectively. This shows that the former complex is more stable compared to the latter. This fact coincides with the thermodynamic stability constants of these Cu^{2+} complexes; thus, log K_1 values of [Cu^{II}(ma)$_2$] and [Cu^{II}(pa)$_2$] are 2.90 (I=0.058 mol dm^{-3}, at 25°C)[8] and 7.86 (I=0.05 mol

dm^{-3}, at 25°C),[9] respectively.

Figure 6 illustrates the relation between the d–d band maxima of the Cu^{2+} complexes in aqueous solution and the mole fraction of [Cu^{II}(D-pa)$_2$] and [Cu^{II}(L-pa)$_2$] (X); here, the counter complex is [Cu^{II}(D-ma)$_2$]. The absorption energy of the D-MA and D-PA system changed lineally with X, whereas it gave a convex curve for the D-MA and L-PA system. Some compositions of a more stable complex should be present in the latter system than in the former system. Figure 7 illustrates the differences of $\bar{\nu}_{max}$ values from the dashed line (excess stability, $\Delta\bar{\nu}$) in the D-MA and L-PA system, as shown in Fig. 6. The plots give a parabolic curve and exhibit the characteristic peaks at a mole fraction of 0.5. Therefore, a complex whose Cu^{2+}: D-MA: L-PA is 1:1:1 is preferable in the mixture. These results are consistent with the results of HPLC and elemental analyses. It is thus considered that the [Cu^{II}(D-pa)(D-ma)] complex was dissolved in water by an electrostatic interaction and the π–π bond between D-PA in the complex and excess D-MA. This fact has also been suggested by Koga et al.,[7]

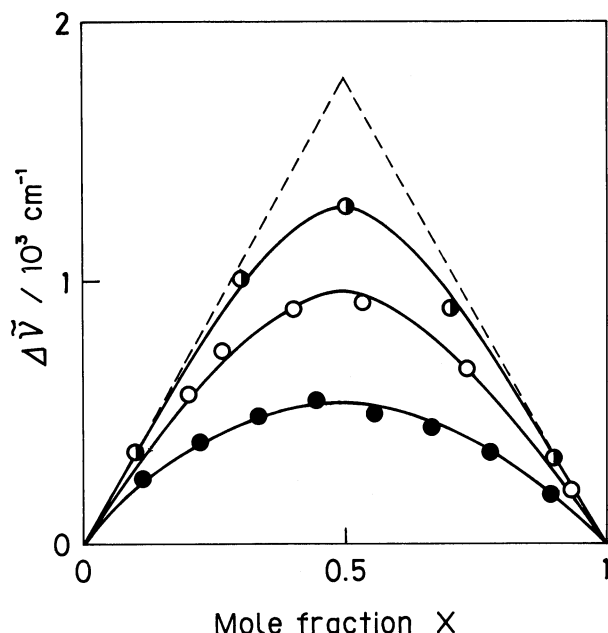Fig. 7. Relation between $\Delta\tilde{\nu}$ of copper(II) complexes and the mole fraction (X) in $a\times(1-X)$(D-MA)— $0.5Cu^{2+}-a\times X$(L-PA) systems. ◐: a=2, ○: a=1.5, ●: a=1. [Cu^{2+}]=8.0×10^{-4} mol dm^{-3}, pH 5.8.

although the Cu^{2+} ion was absent in their system.

Optical Purity of the Complexes. It is now considered to resolve into D- and L-MA by using L-PA under the following conditions: mole ratio of Cu^{2+}:D-MA:L-MA:L-PA is 1:1:1:1. Table 2 shows that the complex which is floated should be [Cu^{II}(D-ma)(L-pa)]. The other complexes comprising L-MA could not be floated due to their low stabilities, as shown in Table 2 ([Cu^{II}(L-ma)$_2$] and [Cu^{II}(L-ma)(D-ma)]), or the high solubility in an aqueous media, as described above ([Cu^{II}(L-ma)(L-pa)]); a high optical purity of D-MA was thus established. On the other hand, the stabilities of the complexes comprising D-PA and/or L-PA are large and have no difference. In order to separate D- and L-PA successfully, it is necessary to use another reagent which can form Cu^{2+} complexes with high stability.

Table 2. d–d Band Maxima of Cu^{2+}–MA–PA Complexes in Aqueous Solution

	$\tilde{\nu}_{max}/10^4$ cm^{-1}
L-PA–L-PA	1.56
L-PA–D-PA	1.56
D-PA–D-PA	1.55
D-MA–L-PA	1.45
L-MA–L-PA	1.42
L-MA–D-PA	1.41
D-MA–D-PA	1.39
L-MA–L-MA	1.28
L-MA–D-MA	1.27
D-MA–D-MA	1.27

[Cu^{2+}]:([PA]+[MA])=1:2, [Cu^{2+}]=8.0×10^{-4} mol dm^{-3}, pH 5.8.

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