Optical Resolution of DL-Proline by Forming a New Diastereoisomeric Solid Complex with L-Tartaric Acid

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DL-Proline was found to form new diastereoisomeric solid complexes with L-tartaric acid in a molar ratio of 1:1 from an aqueous ethanol. The complex of L-proline was less soluble than that of D-proline. On the basis of these properties, DL-proline was easily resolved by the usual chemical resolution technique without the necessity of converting to the derivative. The optically impure D-proline was racemized by heating at 170°C for 4 hr in water containing an equimolar amount of sodium hydroxide and was reused for resolution step.

Optically active proline is an important substance in the pharmaceutical and nutritional field and its market has been expanding rapidly in recent years. Namely, L-proline, as well as the essential amino acids, has been proven to be necessary in parenteral nutrition and is widely used as a component of amino acid infusion.

Up to now, L-proline has been produced by hydrolysis of natural protein or by fermentation methods. To find a more economical method for the production of optically active proline, we have investigated the optical resolution of synthesized DL-proline. In our previous report, some N-aryl-DL-prolines were found to be resolvable by preferential crystallization procedure. Besides this preferential crystallization procedure, if suitable diastereoisomers of DL-amino acids with readily available and cheap resolving agents can be found, the chemical resolution method could become also one of the simplest and most advantageous method. However, generally speaking, neutral amino acids themselves cannot form diastereoisomeric salts directly with ordinary weak basic or weak acidic resolving agents, such as basic amino acids, amino acid amides, L-tartaric acid and N-acylamino acids. To form diastereoisomeric salts, neutral DL-amino acids should be converted to an acidic derivative (e.g. an acyl derivative) or to a basic derivative (e.g. an ester or amide). Therefore, DL-proline has been resolved through the diastereoisomeric salts of m-nitrobenzoyl-DL-proline with cinchonine, 3,5-dinitrobenzoyl-DL-proline with brucine, or benzoxycarbonyl-DL-proline with L-tyrosinehydrazide. These conventional method are obviously laborious and unsatisfactory for the industrial production.

During the investigation of the behavior of DL-amino acids in the solutions containing a chiral compound or in the solvents having chirality, we unexpectedly found that DL-proline itself is capable of forming new diastereoisomeric solid complexes with L-tartaric acid in a molar ratio of 1:1 on crystallization from an aqueous ethanol solution. The complex of L-proline was less soluble than that of D-proline, and the difference of solubility between the two diastereoisomeric complexes was sufficient to perform the chemical resolution. In fact, DL-proline could be resolved in a good yield by separating the less soluble complex from the mixture of DL-proline and an equimolar amount of L-tartaric acid. The resolution was also achieved by the use of 0.5 equimolar amount of the resolving agent and this was advantageous for the practical production of L-proline. The optically impure D-proline recovered from mother liquor was racemized by heating and was reused for optical resolution.

Although a few examples of solid complex
formation of neutral amino acids with organic acids were reported, there has been no report on the formation of the solid complex of proline with L-tartaric acid. Also, no complex formation was observed between L-tartaric acid and other amino acids, such as DL-alanine, DL-isoleucine, DL-leucine, DL-methionine, DL-phenylalanine, DL-serine, DL-threonine, DL-tryptophan, DL-valine, except DL-histidine, DL-pipecolic acid, and DL-3, 4-methylenedioxy-α-methylphenylalanine. Proline appears to be peculiar in its ability to form the solid complex with L-tartaric acid. The isoelectric point of proline (pI=6.30) is slightly higher than those of other amino acids. Probably, this has no bearing on the salt formation ability of proline with L-tartaric acid. Since the dissociation constants of proline at 25°C are pK1' (-COOH)=1.99 and pK2' (>NH3+) =10.60, on the other hand, those of L-tartaric acid are pK1'=2.93 and pK2'=4.32, it is not conceivable that proline is able to form a typical salt with L-tartaric acid. The mechanism of the solid complex formation needs to be investigated further.

The optical resolution method now presented is very advantageous because DL-proline itself is resolved without being converted into a complicated derivative. Also the operation is very simple, and L-tartaric acid used as resolving agent is commercially available at low cost and in large quantities. If this optical resolution method is combined with an efficient synthetic method for DL-proline, it is expected to become a promising process for the industrial production of L-proline.

EXPERIMENTAL

Analyses. All samples were dried overnight in vacuo at 45–50°C. Melting points were measured with a Yamato MP-21 melting point apparatus in an unsealed capillary tube and were uncorrected. Infrared absorption spectra of samples were determined in nujol using a Shimazu infrared spectrophotometer, Model IR-27G. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter. Elemental analyses were performed with a Perkin-Elmer 240 elemental analyzer. Solubility was determined by approaching saturation equilibrium from the side of undersaturation. Solute concentration was measured with a Karl Zeiss immersion refractometer.

Solid complex of L-proline with L-tartaric acid. A mixture of L-proline (5.76 g) and L-tartaric acid (7.50 g) was dissolved in water (20 ml). To the solution, ethanol (300 ml) was gradually added and then the solution was allowed to stand overnight at room temperature. The precipitated crystals were collected, washed with ethanol, and dried to give 11.10 g (83.7%) of the solid complex of L-proline with L-tartaric acid (L-L complex). The products were almost pure, mp 153.5–154.0°C, [α]D25 = -24.2° (c=1, water). Further purification was performed by dissolving the complex (11.00 g) in water (20 ml) and by precipitating it with the addition of ethanol (300 ml) to the solution. The yield was 9.50 g (86.4%). The specific rotation of the pure L-L complex was [α]D25 = -24.2° (c=1, water). The infrared absorption spectrum and the physical properties of L-L complex are shown respectively in Fig. 1 and Table I.

Solid complex of D-proline with L-tartaric acid. Since the solid complex of D-proline with L-tartaric acid (D-L complex) was more soluble than L-L complex, the former was prepared by the following manner. A mixture of D-proline (5.76 g) and L-tartaric acid (7.50 g) was dissolved in water (5 ml). Ethanol (35 ml) was gradually added to the solution and then cooled in an ice bath. The precipitated crystals were collected, washed with ethanol, and dried to give 8.17 g (61.6%) of D-L complex, mp 138.5–139.0°C, [α]D25 = +44.4° (c=1, water). This complex (8.00 g) was recrystallized by dissolving it in water (5 ml) and by precipitating it with the addition of ethanol (35 ml) to the solution. The yield was 4.70 g (58.8%). The specific rotation of the pure D-L complex was [α]D25 = +44.4° (c=1, water). The infrared absorption spec-
TABLE I. PROPERTIES OF L-L AND D-L COMPLEX

<table>
<thead>
<tr>
<th>Solid complex (elemental composition)</th>
<th>Elemental anal., %</th>
<th>Mp, °C</th>
<th>[\alpha]_D^{25}, deg (c=1, water)</th>
<th>Solubility, g/100 ml[^a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-L Complex (C_6H_{12}NO_3)</td>
<td></td>
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</tr>
<tr>
<td>C 40.75</td>
<td>40.61</td>
<td>154.0~154.5</td>
<td>-24.2</td>
<td>0.6 0.9</td>
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<tr>
<td>H 5.70</td>
<td>5.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N 5.28</td>
<td>5.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-L Complex (C_6H_{12}NO_3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 40.75</td>
<td>40.86</td>
<td>138.5~139.0</td>
<td>+44.4</td>
<td>1.7 2.4</td>
</tr>
<tr>
<td>H 5.75</td>
<td>5.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N 5.28</td>
<td>5.23</td>
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</tbody>
</table>

[^a]: Solubility was determined in aqueous ethanol (water: ethanol; 1:15, v/v).

Optical resolution of dl-proline by forming a new diastereoisomeric solid complex

**Procedure A.** DL-Proline was resolved by using an equimolar amount of L-tartaric acid. A mixture of DL-proline (11.51 g) and L-tartaric acid (15.01 g) was dissolved in water (20 ml). Ethanol (70 ml) was gradually added to the solution under stirring and then the solution was seeded with a small amount of L-L complex. After the beginning of crystallization, ethanol (230 ml) was further added, and the mixture was stirred for 2 hr at room temperature. The precipitated crystals were collected, washed with ethanol, and dried to give 9.91 g (74.7%) of L-L complex, [\alpha]_{D}^{25} -22.8° (c=1, water), optical purity 95.9%. The resolved L-L complex (9.80 g) was recrystallized by dissolving it in water (15 ml) and by precipitating it with addition of ethanol (230 ml) to give 8.31 g (84.8%) of pure L-L complex, [\alpha]_{D}^{25} -24.2° (c=1, water).

**Procedure B.** The effect of the amount of resolving agent was investigated by changing its ratio to DL-proline. As a result, the use of 0.5 equimolar amount of the resolving agent was found to be suitable for practical resolution. A mixture of DL-proline (11.51 g) and L-tartaric acid (7.50 g) was dissolved in water (20 ml). Ethanol (120 ml) was gradually added to the warm solution and the solution was stirred overnight at room temperature. The precipitated crystals were collected, washed with ethanol, and dried to give 6.70 g (60.7%) of optically impure D-L complex, [\alpha]_{D}^{25} +31.4° (c=1, water), optical purity 62.1%.

**Preparation of L-proline.** Optically pure L-L complex (7.00 g) obtained by the above procedures was dissolved in water (30 ml). The solution was passed through a column of Amberlite IR-120 (40 ml, H⁺ form). The column was washed with water and L-proline was eluted from the column with 5% NH₄OH (70 ml). The eluates were concentrated, treated with charcoal, and concentrated again to dryness. The residue was dissolved in water (5 ml) and precipitated with addition of ethanol (230 ml) to give 9.40 g (70.9%) of L-L complex, [\alpha]_{D}^{25} -23.3° (c=1, water), optical purity 97.4%. The resolved L-L complex (9.00 g) was recrystallized by dissolving it in water (13 ml) and by precipitating it with addition of ethanol (200 ml) to the solution to give 7.70 g (85.6%) of pure L-L complex, [\alpha]_{D}^{25} -24.2° (c=1, water).

**Preparation of L-proline.** After the separation of less soluble complex in the above resolution process, the mother liquor was evaporated in vacuo to dryness. The resulting residue was dissolved in water (5 ml). Ethanol (120 ml) was gradually added to the warm solution and the solution was seeded overnight at room temperature. The precipitated crystals were collected, washed with ethanol, and dried to give 6.70 g (70.9%) of optically impure D-L complex, [\alpha]_{D}^{25} +31.4° (c=1, water), optical purity 62.1%.

**Racemization of optically impure D-proline.** Optically impure D-proline (6.00 g, optical purity 53.5%) obtained by the above procedure was dissolved in water (120 ml) containing an equimolar amount of sodium hydroxide (2.09 g). The mixture was heated in an autoclave at 170°C for 4 hr. The reaction mix-
tature was passed through a column of Amberlite IRC-50 (50 ml, H⁺ form). The column was washed with water. The effluent was concentrated, treated with charcoal, and concentrated again to dryness to give 5.82 g of DL-proline, [α]$_D^{25}$ +0.2° (c=1, water). Anal. Found: C, 51.79; H, 7.90; N, 12.08.

REFERENCES AND NOTES

9) The optical purity was calculated with the assumption that the specific rotation of the pure sample is [α]$_D^{25}$ +85.5° (c=1, water). [Lit. for α-proline: [α]$_D^{23}$ +85.2° (c=3.5, water).]