CHAPTER 6

CHIRAL SEPARATION OF RACEMIC CARBOXYLIC ACIDS USING CHIRAL 1,2-DIAMINES

6.1 BACKGROUND

The investigation presented in the first three chapters pertained to the synthesis of chiral molecules, which are essential for therapeutic value. In all the cases the other optical antipodes of active pharmaceutical ingredients (APIs) did not show the same therapeutic values but in some cases were also proved to be harmful. The chiral APIs with a particular configuration at chiral carbon can be prepared either by chiral synthesis or synthesize the racemic mixture of the API or their intermediates, followed by chiral separation. This chiral separation, unlike chiral synthesis involves simple reaction conditions and can achieve higher enantiomeric purity.

In this chapter, the chiral diamines synthesized in our research are employed to resolve non-conventional racemic carboxylic acids such as mandelic acid and malic acid to establish the procedures. These procedures have been employed to resolve the required drug intermediates from the corresponding racemic mixtures. The vicinal diamines employed in this work are given in Table 6.1.
Table 6.1 Chiral 1,2-diamines employed for the separation of racemic carboxylic acids

<table>
<thead>
<tr>
<th>Compound No</th>
<th>Structure of the diamine</th>
<th>Name of the diamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Structure 1" /></td>
<td>(1R,2R)-(−)-1-Phenyl-1-(1,1-dimethylethylamino)-2-(1-pyrrolidinyl)propane</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Structure 2" /></td>
<td>(1R,2R)-(−)-1-Phenyl-1-ethylamino-2-(1-pyrrolidinyl)propane</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Structure 3" /></td>
<td>(1R,2R)+(−)-1-Phenyl-1-ethylamino-2-(1-piperidinyl)propane</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Structure 4" /></td>
<td>(1R,2R)-(−)-1-phenyl-1-(2-propylamino)-2-(1-piperidinyl)propane</td>
</tr>
</tbody>
</table>

6.2 SEPARATION PROCEDURE

The diamine, used in the chiral separation of carboxylic acid, was taken as a hydrochloride salt. The base was liberated after treating the aqueous solution of the diamine hydrochloride with dilute sodium hydroxide solution. The liberated diamine was extracted in an organic solvent like
diethyl ether or dichloromethane. The organic solvent was evaporated to obtain the diamine base as viscous liquid. The diamine base was dissolved in a solvent (the solvent in specific cases is given in the experimental section) and the racemic carboxylic acid was also taken in the same solvent. The mixture was refluxed for an hour and cooled while stirring to a temperature between 25 and 30 °C. The mixture was stirred at this temperature for 10 to 24 h as specified, when the solid of the salt formed was thrown out as a precipitate from the reaction mixture. The salt formed was filtered and recrystallized from the solvent used for the reaction. The solid thus obtained was the pure version of the preferred diastereomeric salt of the carboxylic acid with the diamine used.

The principle of enantiomeric separation of racemic carboxylic acid using diamine was the formation of diastereomeric salts with each enantiomers of the racemic mixture of the carboxylic acid. In all the diamines used in this work, the nitrogens are present as tertiary amine and secondary amine in each case. It is known that secondary amine is more basic than tertiary amine. Hence it is postulated that the nitrogen of the secondary amine involved in the salt formation with both the enantiomers of the carboxylic acids resulting in the formation of diastereomeric salts (Scheme 6.1).

![Scheme 6.1](attachment:image.png)
The relative solubilities of the two-diastereomeric salts are employed to separate the two diastereomeric salts. The choice of the solvent in each case is very critical in the separation of diastereomeric salts, based on the differences in their solubilities. The correct choice of the solvent was arrived after experimentation with various solvents, where better yield of the salt along with higher enantiomeric purity was obtained. The separated diastereomeric salt is further purified by recrystallization to obtain an enantiomeric purity > 99%. The pure enantiomer of the carboxylic acid was isolated on treatment of the pure diastereomeric salt with dilute mineral acid or by treating with base. The liberated enantiomer of the carboxylic acid was either filtered as such or extracted with a solvent from aqueous phase and the pure enantiomer of the carboxylic acid was obtained on evaporation of the solvent. Making use of reported specific optical rotation in each case, the absolute configuration at the chiral center of the carboxylic acid identified.

6.2.1 (R)-(+-) Malic Acid

6.2.1.1 Chiral separation

Racemic malic acid was made to react with (1R,2R)-(+-)-1-phenyl-1-(1,1-dimethylethylamino)-2-(1-pyrrolidinyl)propane in isopropyl alcohol medium. During this reaction two diastereomeric salts were formed corresponding to (R,R,R)-diastereomer and (R,R,S)-diastereomer (Scheme 6.2). The (R,R,S)-diastereomer salt was more soluble in the solvent used and the less soluble (R,R,R)-diastereomeric salt was filtered and isolated. The absolute configuration of diastereomeric salt was derived based on the specific optical rotation of the enantiomer obtained after hydrolysis. The physical and optical data of the isolated diastereomeric salt are presented in Table 6.2. The salt corresponding to the S-isomer of malic acid remained in the filtrate along with other impurities. The required isomer of the acid was
obtained from the diastereomeric salt on treating with sodium hydroxide solution and passing through an ion-exchange cationic resin. The physical constants of the separated (R)-malic acid were determined and the structure of malic acid was identified by spectroscopic data. The absolute configuration of the enantiomeric malic acid isolated was identified to be ‘R’ based on its specific optical rotation (Table 6.3).

Scheme 6.2
Table 6.2 Details of racemic acids, 1,2-diamines used, less soluble diastereomeric salts and their physical constants

<table>
<thead>
<tr>
<th>Racemic carboxylic acid</th>
<th>Chiral 1,2-diamine used</th>
<th>Structure of less soluble diastereomeric salt</th>
<th>Specific rotation</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(RS)-Malic acid</td>
<td>(1R,2R)-(-)-1-Phenyl-1-(1,1-dimethylethylamino)-2-(1-pyrrolidinyl)propane</td>
<td><img src="image" alt="Structure of less soluble diastereomeric salt" /></td>
<td>-4.4° 1% in Pyridine</td>
<td>134 - 138</td>
</tr>
<tr>
<td>(RS)-Mandelic acid</td>
<td>(1R,2R)-(-)-1-Phenyl-1-(1,1-dimethylethylamino)-2-(1-pyrrolidinyl)propane</td>
<td><img src="image" alt="Structure of less soluble diastereomeric salt" /></td>
<td>-73.2° 1% in CH₃OH</td>
<td>178 - 184</td>
</tr>
<tr>
<td>(RS)-2-Phenyl-3-methylbutanoic acid</td>
<td>(1R,2R)-(-)-1-Phenyl-1-ethylamino-2-(1-pyrrolidinyl)propane</td>
<td><img src="image" alt="Structure of less soluble diastereomeric salt" /></td>
<td>+40.58° 1% in CH₃OH</td>
<td>111 - 116</td>
</tr>
<tr>
<td><strong>Racemic carboxylic acid</strong></td>
<td><strong>Chiral 1,2-diamine used</strong></td>
<td><strong>Structure of less soluble diastereomeric salt</strong></td>
<td><strong>Specific rotation</strong></td>
<td><strong>m.p. (°C)</strong></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>---------------------------------</td>
<td>---------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>(RS)-2-(4-Fluorophenyl)-3-methylbutanoic acid</td>
<td>(1R,2R)-(+)-1-Phenyl-1-ethylamino-2-(1-piperidinyl)propane</td>
<td><img src="image1" alt="Structure" /></td>
<td>+6.8° 1% in CH₃OH</td>
<td>144 - 148</td>
</tr>
<tr>
<td>(RS)-2-(2-oxo-1'-pyrrolidinyl)butanoic acid</td>
<td>(1R,2R)-(+)1-Phenyl-1-ethylamino-2-(1-piperidinyl)propane</td>
<td><img src="image2" alt="Structure" /></td>
<td>+28.83° 1% in CH₃OH</td>
<td>222 - 228</td>
</tr>
<tr>
<td>(RR,SS)-(++)-Phenyl-(2-piperidinyl)ethanoic acid (threo-Ritalinic acid)</td>
<td>(1R,2R)-(+)1-Phenyl-1-(2-propylamino)-2-(1-piperidinyl)propane</td>
<td><img src="image3" alt="Structure" /></td>
<td>+47.62° 1% in CH₃OH</td>
<td>248 - 256</td>
</tr>
</tbody>
</table>
Table 6.3 Physical, optical data and application of the separated enantiomeric carboxylic acids

<table>
<thead>
<tr>
<th>Enantiomer of the carboxylic acid separated</th>
<th>Specific optical rotation for the enantiomeric carboxylic acid separated</th>
<th>Melting point of the enantiomeric carboxylic acid separated</th>
<th>Yield (%)</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obtained</td>
<td>Reported (with reference)</td>
<td>Obtained °C</td>
<td>Reported (°C) (with reference)</td>
</tr>
<tr>
<td>(R)-(+)-Malic acid</td>
<td>+25.4° (2% in Pyridine)</td>
<td>+24.8° (2% in pyridine (US 4330484)</td>
<td>101 - 103</td>
<td>100-104 J. American Chem.. Soc. 1929,66,974</td>
</tr>
<tr>
<td>(R)-(−)-Mandelic cid</td>
<td>-128.4° (2% in CH₃OH)</td>
<td>-130° (2% in CH₃OH) Merck Index</td>
<td>131 - 133</td>
<td>132 – 133 J. American Chem.. Soc. 1929,51,1909</td>
</tr>
<tr>
<td>(S)-(+) 2-(4-Fluorophenyl)-3-methylbutanoic acid</td>
<td>+47.8° (1% in CH₃OH)</td>
<td>+50.4° (1% in CH₃OH) Agri.Biol. Chem 1975, 267-272</td>
<td>56 - 62</td>
<td>55 – 57 Agri.Biol. Chem 1975, 267-272</td>
</tr>
<tr>
<td>(S)-(−) 2-(2-Oxopyrrolidine-1-yl)butanoic acid</td>
<td>-16.9° (1% in CH₃OH)</td>
<td>-17.1° (1% in CH₃OH) Compared with reference compound</td>
<td>134 - 136</td>
<td>134-138 Compared with reference compound</td>
</tr>
</tbody>
</table>
6.2.1.2 Spectroscopic interpretation

1. **IR (cm\(^{-1}\)) (KBr)**

   O - H str. in the range of 3387 - 3173, C = O str. at 1719 and C - O str. at 1096 (Figure 6.1 a).

2. **\(^1\)H NMR (D\(_2\)O, 400 MHz) (\(\delta_H\))**

   2.73 (2H, m, CH\(_2\) - COOH) and 4.43 (1H, m, CH\(_2\) – CH - COOH). The OH protons are exchanged with D\(_2\)O (Figure 6.1 b).

3. **\(^{13}\)C NMR (D\(_2\)O, 100 MHz) (\(\delta_C\))**

   38.31 (CH\(_2\) - COOH), 66.68 (CH\(_2\) – CH–COOH), 174.33 (CH – COOH) and 176.33 (CH\(_2\)–COOH) (Figure 6.1 c).

4. **Mass spectrum (EI)**

   M\(^+\) m/z 134 (absent), [M - COOH]\(^+\) at m/z 89(75) and [M - COOH –H\(_2\)O]\(^+\) at m/z 71 (100) (Figure 6.1 d).

   The elemental analysis of this compound (C\(_4\)H\(_6\)O\(_5\)) gave 35.91 and 4.47 percentage of carbon and hydrogen respectively as against calculated values of 35.83 and 4.51 percentage for carbon and hydrogen respectively.

6.2.1.3 Enantiomeric purity

The separated enantiomer, (R)-(+)-malic acid, was analysed for enantiomeric purity by chiral HPLC by using chiral column (Chiralpak AD-H, 250 X 4.6 mm, 5.0 \(\mu\)m) together with corresponding racemic mixture (RS)-(±)-malic acid. The HPLC chromatograms are given in Figure 6.2 a and b.
Figure 6.1(a) IR spectrum of (R)-(+) -malic acid

Figure 6.1(b) $^1$H NMR spectrum of (R)-(+) -malic acid
Figure 6.1(c) $^{13}$C NMR spectrum of (R)-(+)-malic acid

Figure 6.1(d) Mass spectrum of (R)-(+)-malic acid
Figure 6.2 (a) Chiral HPLC chromatogram of separated (R)-(+)‐malic acid

Figure 6.2 (b) Chiral HPLC chromatogram of separated (RS)-(±)-malic acid
6.2.2 (R)-(−)-Mandelic acid

6.2.2.1 Chiral separation

The diamine used in the separation of racemic mandelic acid was (1R,2R)-(−)-1-phenyl-1-(1,1-dimethylethylamino)2-(1-pyrrolidinyl)propane and the solvent employed for the formation of salt was ethanol. The (R,R,R)-diastereomeric salt (Scheme 6.3) was less soluble in this case and hence it was filtered and purified.

Scheme 6.3

The physical and optical data of the isolated (R,R,R)-diastereomeric salt are presented in Table 6.2. The filtered solid was treated with sodium hydroxide solution and passed through an ion-exchange cationic resin to obtain R-(−)-mandelic acid as revealed by its specific optical rotation (Table 6.3). The structure of the isolated (R)-(−)-mandelic acid was confirmed by spectroscopic data.
6.2.2.2 Spectroscopic interpretation

1. IR (cm⁻¹) (KBr)

O - H str. at 3449 and 3200 to 2500, C = O str. at 1724, C – O str. at 1063 and C–H out of plane bending of mono-substituted benzene ring at 723 and 692 (Figure 6.3 a).

2. ¹H NMR (D₂O, 400 MHz) (δ_H)

5.09 (1H, m, CH - COOH) and 7.21 – 7.25 (5H, m, Harom ). The OH protons are exchanged with D₂O (Figure 6.3 b)

3. ¹³C NMR (D₂O, 100 MHz) (δ_C)

72.71 (CH - COOH), 126.95 – 137.77 (aromatic carbons) and 175.95 (COOH ) (Figure 6.3 c).

4. Mass spectrum (EI)

M⁺ m/z 152 (10) and [MH-COOH]⁺ at m/z 107 (100) (Figure 6.3d).

The elemental analysis of this compound, C₈H₈O₃, gave 63.47 and 5.29 percentage of carbon and hydrogen respectively as against the calculated values of 63.15 and 5.30 percentage for carbon and hydrogen respectively.

6.2.2.3 Enantiomeric purity

The optical purity of (R)-(-)-mandelic acid obtained through separation of racemic mandelic acid analysed by chiral HPLC column (Chiralpak AD-H, 250 X 4.6 mm, 5.0 µm) together with corresponding
racemic (RS)-(±)-mandelic acid and chromatograms are represented in Figure 6.4 a and b.

Figure 6.3 (a)  IR spectrum of (R)-(−)-mandelic acid

Figure 6.3 (b)  $^1$H NMR spectrum of (R)-(−)-mandelic acid
Figure 6.3 (c) $^{13}$C NMR spectrum of (R)-(-)-mandelic acid

Figure 6.3 (d) Mass spectrum of (R)-(-)-mandelic acid
Figure 6.4 (a) Chiral HPLC chromatogram of (R)-(−)-mandelic acid

Figure 6.4 (b) Chiral HPLC chromatogram of (RS)-(±)-mandelic acid
6.2.3 (S)-(+-)2-Phenyl-3-methylbutanoic Acid

6.2.3.1 Chiral separation

The diamine employed in this case was (1R,2R)-(--)1-phenyl-1-ethylamino-2-(1-pyrrolidinyl)propane and the solvent used was mixture of isopropyl alcohol and water in the ratio 6:4. The two diastereomeric salts formed are shown in Scheme 6.4.

The (R,R,S)-diastereomeric salt in this case was less soluble and hence it is filtered and purified to remove the trace amount of (R,R,R)-diastereomeric salt. The physical constants of (R,R,S)-diastereomer is presented in Table 6.2. The (S)-(+-)2-phenyl-3-methylbutanoic acid was obtained on treating the purified (R,R,S)-diastereomeric salt with dilute mineral acid. The absolute configuration was identified to be ‘S’ using the specific optical rotation obtained for this acid (Table 6.3).

Scheme 6.4
6.2.3.2 Spectroscopic interpretation

1. **IR (cm\(^{-1}\)) (KBr)**

   O - H str. in the range of 3200 - 2800, C = O str. at 1707, benzenoid bands at 1599 and 1470, C - O str.at 1219 and C-H out of plane bending of mono-substituted benzene ring at 727 and 696 (Figure 6.5 a).

2. **\(^{1}\text{H NMR (CDCl}_3, 400 \text{ MHz}) (\delta_{\text{H}})\)**

   0.70 (3H, d, CH\(_3\) - CH-CH\(_3\)), 1.07 (3H, d, CH\(_3\) - CH-CH\(_3\)), 2.32 (1H, m, CH\(_3\) - CH - CH\(_3\)), 3.14 (1H, d, C\(_6\)H\(_5\) - CH – COOH) and 7.23 – 7.33 (5H, m, Harom). COOH proton may have exchanged with H\(_2\)O in CDCl\(_3\) (Figure 6.5 b).

3. **\(^{13}\text{C NMR (CDCl}_3, 100 \text{ MHz}) (\delta_{\text{C}})\)**

   20.13 (CH\(_3\) – CH - C\(_3\)H\(_3\)), 21.48 (CH\(_3\) – CH - CH\(_3\)), 31.59 (CH\(_3\) – C\(_3\)H - CH\(_3\)), 60.09 (C\(_6\)H\(_5\) - CH – COOH), 127.49 – 137.75 (aromatic carbons) and 180.49 (COOH) (Figure 6.5 c).

4. **Mass spectrum (CI, MeOH)**

   [M+H]\(^{+}\) at m/z 179(2) and [MH-H\(_2\)O-CO]\(^{+}\) at m/z 133(100) (Figure 6.5 d). The ESI(-) mass spectrum shows the [M-H]\(^{+}\) ion at m/z177 as base peak.

   The elemental analysis of this compound, C\(_{11}\)H\(_{14}\)O\(_2\), gave 74.37 and 8.09 percentage of carbon and hydrogen respectively as against the calculated values of 74.13 and 7.92 percentage for carbon and hydrogen respectively.

6.2.3.3 Enantiomeric purity

The separated enantiomer, (S)-(+)2-phenyl-3-methylbutanoic acid, was analysed for enantiomeric purity by chiral HPLC by using chiral column
(Chiralpak AD-H, 250 X 4.6 mm, 5.0 μm) together with corresponding racemic mixture (RS)-(±)-2-phenyl-3-methylbutanoic acid. The HPLC chromatograms are given in Figure 6.6 a and b.

Figure 6.5 (a) IR spectrum of (S)-(+-)-2-phenyl-3-methylbutanoic acid

Figure 6.5 (b) ¹H NMR spectrum of (S)-(+-)-2-phenyl-3-methylbutanoic acid
Figure 6.5 (c) $^{13}$CNMR spectrum of (S)-(+)2-phenyl-3-methylbutanoic acid

Figure 6.5 (d) Mass spectrum of (S)-(+)2-phenyl-3-methylbutanoic acid
Figure 6.6 (a) Chiral HPLC chromatogram of (S)-(+)\(-2\)-phenyl-3-methylbutanoic acid

Figure 6.6 (b) Chiral HPLC chromatogram of (RS)-(\pm\)-2-phenyl-3-methylbutanoic acid
6.2.4  (S)-(+)2-(4-Fluorophenyl)-3-methylbutanoic Acid

6.2.4.1 Chiral separation

(1R,2R)-(−)-1-phenyl-1-ethylamino-2-(1-piperidinyl)propane as diamine and a mixture of isopropyl alcohol and water was employed as solvent in the ratio 6:4 for the chiral separation of 2-(4-fluorophenyl)-3-methylbutanoic acid. The (R,R,S)-diastereomeric salt was precipitated due to the less solubility in the solvent chosen and the (R,R,R)-diastereomeric salt was found to be more soluble (Scheme 6.5).

![Scheme 6.5](image-url)

The precipitated (R,R,S)- salt was filtered and further purified to obtain high chiral purity. The physical and optical data of the diastereomeric salt is given in Table 6.2. The required isomer of the acid was isolated on
treating the purified (R,R,S)-diastereomeric salt with mineral acid. The absolute configuration was established by specific optical rotation (Table 6.3)

6.2.4.2 Spectroscopic interpretation

1. IR (cm\(^{-1}\)) (KBr)

O- H str. in the range of 3300 to 2800, C = O str. at 1705, benzenoid bands at 1603 and 1508, C - O str.1225 and C-H out of plane bending of para-di-substituted benzene ring at 825 (Figure 6.7 a).

2. \(^1\)H NMR (CDCl\(_3\), 400 MHz) (\(\delta_H\))

0.70 (3H, d, CH\(_3\) -CH - CH\(_3\)), 1.07 (3H, d, CH\(_3\) -CH - CH\(_3\)), 2.29 (1H, m, CH\(_3\) – CH - CH\(_3\)), 3.12 (1H, d, C\(_6\)H\(_5\) - CH – COOH) and 6.96 – 7.32 (4H, m, Harom). COOH proton is exchanged with H\(_2\)O in CDCl\(_3\) (Figure 6.7 b).

3. \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) (\(\delta_C\))

20.01 (CH\(_3\) – CH - CH\(_3\)), 21.37 (CH\(_3\) – CH - CH\(_3\)), 31.74 (CH\(_3\) – CH - CH\(_3\)), 59.22 (C\(_6\)H\(_5\) - CH – COOH), 115.31 – 163.45 (aromatic carbons) and 180.32 (COOH) (Figure 6.7 c).

4. Mass spectrum (ESI, negative mode)

\([M-H]^–\) at m/z 195 (100) (Figure 6.7 d).

The elemental analysis of this compound for, C\(_{11}\)H\(_{13}\)FO\(_2\), gave 67.31 and 6.80 percentage carbon and hydrogen respectively as against the calculated values of 67.33 and 6.68 percentage for carbon and hydrogen respectively.
6.2.4.3 Enantiomeric purity

The optical purity of (S)-(+)\textendash2-(4-fluorophenyl)-3-methylbutanoic acid was analysed in chiral HPLC along with its racemic mixture and the chromatograms are given in Figure 6.8 a and 6.8 b.

![IR spectrum of (S)-(+)\textendash2-(4-fluorophenyl)-3-methylbutanoic acid](image)

**Figure 6.7 (a) IR spectrum of (S)-(+)\textendash2-(4-fluorophenyl)-3-methylbutanoic acid**

![$^1$H NMR spectrum of (S)-(+)\textendash2-(4-fluorophenyl)-3-methylbutanoic acid](image)

**Figure 6.7 (b) $^1$H NMR spectrum of (S)-(+)\textendash2-(4-fluorophenyl)-3-methylbutanoic acid**
Figure 6.7 (c) $^{13}$C NMR spectrum of (S)-(+)2-(4-fluorophenyl)-3-methylbutanoic acid

Figure 6.7 (c) Mass spectrum of (S)-(+)2-(4-fluorophenyl)-3-methylbutanoic acid
Figure 6.8 (a) Chiral HPLC chromatogram of (S)-(+)\textendash2\textendash(4-fluorophenyl)\textendash3-methylbutanoic acid

Figure 6.8 (b) Chiral HPLC chromatogram of (RS)-(\textpm)\textendash2\textendash(4-fluorophenyl)\textendash3-methylbutanoic acid
6.2.5 (S)-(-)-2-(2-Oxo-1-pyrrolidinyl)butanoic Acid

6.2.5.1 Chiral separation

The diamine chosen for the chiral separation of racemic 2-(2-oxo-1-pyrrolidinyl)butanoic acid was (1R,2R)-(−)-1-phenyl-1-ethylamino-2-(1-pyrrolidinyl)propane. Toluene was used as the solvent for selective precipitation of the required diastereomeric salt (Scheme 6.6).

![Scheme 6.6](image)

The (R,R,R)-diastereomeric salt was more soluble in the solvent chosen and hence dissolved in toluene allowing less soluble (R,R,S)-diastereomeric salt to precipitate, which was filtered and further purified in the same solvent. The optical data and the physical values of (R,R,S)-diastereomeric salt are given in Table 2. The purified salt was treated with mineral acid to isolate the (S)-(-)-2-(2-oxo-1-pyrrolidinyl)butanoic acid. The physical constants were determined and presented in Table 6.2. The absolute
configuration of (S)-2-(2-oxo-1-pyrrolidinyl)butanoic acid was established by the reported specific rotation (Table 6.3).

6.2.5.2 Spectroscopic interpretation

1. IR (cm\(^{-1}\)) (KBr)

   O - H str. at 3200 and 2800, C = O str. of acid at 1719 and C = O str. of amide at 1636 and C - O str. at 1206 (Figure 6.9 a).

2. \(^1\text{H}\) NMR (CDCl\(_3\), 400 MHz) (\(\delta_H\))

   0.93 (3H, t, CH\(_2\) - CH\(_3\)), 1.71 (1H, m, N – CH\(_2\) – CH\(^{1}\)H\(^2\) – CH\(_2\)), 2.07 (3H, m, N – CH\(_2\) – CH\(^{1}\)H\(^2\) – CH\(_2\) & CH\(_3\)), 2.51 (2H, t, CH\(_2\) – C = O), 3.52 (2H, m, N – CH\(_2\) – CH\(_2\) – CH\(_2\)) and 4.66 (1H, m, CH - COOH). COOH proton may have exchanged with H\(_2\)O in CDCl\(_3\) (Figure 6.9 b).

3. \(^{13}\text{C}\) NMR (CDCl\(_3\), 100 MHz) (\(\delta_C\))

   10.84 (3H, t, CH\(_2\) - CH\(_3\)), 18.17 (CH\(_2\) - CH\(_3\)), 21.94 (N – CH\(_2\) – CH\(_2\) – CH\(_2\)), 30.85 (N – CH\(_2\) – CH\(_2\) – CH\(_2\)), 43.93 (2H, m, N – CH\(_2\) – CH\(_2\) – CH\(_2\)), 55.46 (CH - COOH), 173.83 (C = O) and 177.31 (COOH) (Figure 6.9 c).

4. Mass spectrum (ESI)

   [M+H]\(^+\) at m/z 172(100) and [M+Na] at m/z 194(84) (Figure 6.9 d).

   The elemental analysis of this compound, C\(_8\)H\(_{13}\)NO\(_3\), gave 56.02, 7.57 and 8.00 percentage of carbon, hydrogen and nitrogen respectively as against the calculated values of 56.13, 7.65 and 8.18 percentage for carbon, hydrogen and for nitrogen respectively.
6.2.5.3 Enantiomeric purity

The separated enantiomer (S)-(−)-2-(2-oxo-1-pyrrolidinyl)butanoic acid was analysed for enantiomeric purity by chiral HPLC using chiral column (Chiralpak AD-H, 250 X 4.6 mm) together with corresponding racemic mixture. The HPLC chromatograms are given in Figure 6.10 a and b.

![IR spectrum](image1.png)

**Figure 6.9 (a)** IR spectrum of (S)-(−)-2-(2-oxo-1-pyrrolidinyl)butanoic acid

![NMR spectrum](image2.png)

**Figure 6.9 (b)** $^1$H NMR spectrum of (S)-(−)-2-(2-oxo-1-pyrrolidinyl) butanoic acid
Figure 6.9 (c) $^{13}$C NMR spectrum of (S)-(−)-2-(2-oxo-1-pyrrolidinyl)butanoic acid

Figure 6.9 (d) Mass spectrum of (S)-(−)-2-(2-oxo-1-pyrrolidinyl)butanoic acid
Figure 6.10 (a) Chiral HPLC chromatogram of (S)-(-)-2-(2-oxo-1-pyrrolidinyl)butanoic acid

Figure 6.10 (b) Chiral HPLC chromatogram of (RS)-(±)-2-(2-oxo-1-pyrrolidinyl)butanoic acid
6.2.6 (R,R)-(+)-Phenyl-(2-piperidinyl)ethanoic Acid

6.2.6.1 Chiral separation

The diamine employed in the chiral separation of phenyl-(2-piperidinyl)ethanoic acid (threo-ritalinic acid) was (1R,2R)-(−)-1-phenyl-1-(2-propylamino)-2-(1-piperidinyl)propane. Ethanol was employed as solvent for the formation of diastereomeric salts of (R,R,R,R) and (R,R,S,S). The solubility of (R,R,S,S)-diastereomeric salt was more than the corresponding (R,R,R,R)-diastereomer (Scheme 6.7) and hence it was separated by filtration and purified to achieve high enantiomeric purity. The physical constants of (R,R,R,R) -diasteromeric salt are presented in Table 6.2.

![Scheme 6.7](image)

The (R,R)-threo-phenyl-(2-piperidinyl)ethanoic acid was isolated as hydrochloride salt by neutralizing the diastereomeric salt with ammonium hydroxide followed by acidification with dil. hydrochloric acid. The physical constants were determined and reported in Table 6.2. The (R,R)-threo-phenyl-
(2-piperidinyl)ethanoic acid obtained by this chiral separation method has (R,R)-configuration which was confirmed with the help of specific optical rotation of this compound and that of the reference compound (Table 6.3).

6.2.6.2 Spectroscopic interpretation

1. **IR (cm\(^{-1}\)) (KBr)**

   O - H str. in the range of 3250 to 2950, N – H stretch 2579, C = O str. 1730, benzenoid bands 1591 and 1495, C – N str. 1298, C – O str. 1171 and C-H out of plane bending of mono-substituted benzene ring 727 and 704 (Figure 6.11 a).

2. **\(^1\)H NMR (DMSOd\(_6\), 400 MHz) (\(\delta_H\))**

   1.23 – 1.65 (6H, 3, N - CH - CH\(_2\) – CH\(_2\) - CH\(_2\) – CH\(_2\)), 2.93 (1H, m, N – CH\(^1\)H\(^2\)), 3.25 (1H, m, N – CH\(^1\)H\(^2\)), 3.70 (1H, m, N – CH), 4.01 (1H, m, C\(_6\)H\(_5\) - CH – CH\(_2\)), 7.25 – 7.40 (5H, m, Harom), 8.58 (1H, bs, CH-COOH) and 9.52 (1H, bs, CH – NH) (Figure 6.7 b).

3. **\(^13\)C NMR (DMSOd\(_6\), 100 MHz) (\(\delta_C\))**

   21.30 (N - CH - CH\(_2\) – CH\(_2\) - CH\(_2\) – CH\(_2\)), 21.53 (N - CH - CH\(_2\) – CH\(_2\) - CH\(_2\) – CH\(_2\)), 25.62 (N - CH - CH\(_2\) – CH\(_2\) - CH\(_2\) – CH\(_2\)), 44.62 (N - CH\(_2\)), 53.30 (N - CH), 56.73 (C\(_6\)H\(_5\) - CH – CH), 127.93 – 134.88 (aromatic carbons) and 172.43 (COOH) (Figure 6.11 c).

4. **Mass spectrum (ESI)**

   \([M+H]^+\) at m/z 220(100) (Figure 6.11 d).

   The elemental analysis of this compound, C\(_{13}\)H\(_{16}\)NO\(_2\), gave 61.70, 7.32 and 5.74 percentage of carbon, hydrogen and nitrogen respectively as against the calculated values of 61.05, 7.09 and 5.48 percentage for carbon, hydrogen and nitrogen respectively.
6.2.6.3 Enantiomeric purity

The separated enantiomer (1R,2R)-(+)-phenyl-(2-piperidinyl)ethanoic acid was analysed for enantiomeric purity by chiral HPLC by using chiral column (Chiralpak AD-H, 250 X 4.6 mm) together with corresponding racemic mixture. The HPLC chromatograms are given in Figure 6.12 a and b.

![Figure 6.11 (a) IR spectrum of (R,R)-(+)-phenyl-(2-piperidinyl)ethanoic acid](image1)

![Figure 6.11 (b) 1H NMR spectrum of (R,R)-(+)-phenyl-(2-piperidinyl)ethanoic acid](image2)
Figure 6.11 (c) $^{13}$C NMR spectrum of (R,R)-(+)-phenyl-(2-piperidinyl)ethanoic acid

Figure 6.11 (d) Mass spectrum of (R,R)-(+)-phenyl-(2-piperidinyl)ethanoic acid
Figure 6.12 (a) Chiral HPLC chromatogram of (R,R)-(+) -phenyl-(2-piperidinyl)ethanoic acid

Figure 6.12 (b) Chiral HPLC chromatogram of (RS,SR)-(±) -phenyl-(2-piperidinyl)ethanoic acid
The methodology used in this chiral separation is simple. It is revolved based on the solubility differences between the diastereomeric salts formed by the reaction of racemic carboxylic acid with suitable chiral diamines. The chiral purity obtained for the separated carboxylic acid is found to be high in all the cases. Hence the method of chiral separation employed in this work has general applicability.