CHAPTER SIX

ALTERNATIVE METHOD FOR DETERMINATION OF ABSOLUTE CONFIGURATION OF CHIRAL COMPOUNDS BY CO-CRYSTAL FORMATION.

6.1 Introduction

Determination of absolute configuration of a chiral compound is very important especially for pharmaceutical compounds as they are implicated in enzyme reactions, drug action and structure-function relationships.\(^1\) There are many methods for determination of absolute configuration like circular dichroism (CD), NMR with chiral shift reagents, chemical correlation and X-ray crystallography.\(^2\) Every method has its own set of advantages and limitations. Single crystal X-ray diffraction method is the most reliable and definitive method for determination of absolute configuration but it requires good quality single crystals and the compound should contain at least one atom heavier than Na for observing an appreciable amount of anomalous scattering of X-rays.\(^3\)

When incident X-rays of moderately high energy are either scattered normally by an atom or absorbed and re-emitted at slightly different phase and intensity, this effect is called anomalous scattering. Since the anomalous photons are out of phase with the normal photons they will change both the intensity and phase of the scattered radiation. In a centrosymmetric crystal, the addition and subtraction of the anomalous photons will cancel out and they have no effect. However, for chiral crystals they will not cancel out hence Friedel’s law would not be obeyed. In this case \(|F_{\lambda,-}\neq|F_{\lambda,+}|\). Therefore one enantiomer will give a slightly better fit to the data because it will apply the correct sign to the interaction of the normal and anomalous radiation.\(^4\) The Flack parameter is the best method to get correct absolute configuration.\(^5\) This basically refines both forms at the same time and applies an occupancy of (1-x) to the form which is present and x to the other hand. Obviously x=0 suggests that assigned absolute configuration is correct and x=1 suggests that it is incorrect.

For reliable determination of absolute configuration it is necessary that the compound contains a heavy atom with a large anomalous component although there are
few isolated cases where the absolute configuration was determined by anomalous scattering from oxygen. Because many organic compounds do not contain heavy atoms, such an atom can be introduced in the compound by salt formation. This is possible where the compound is capable of forming a salt. However, introduction of a heavy atom by chemical reaction is not always facile. The present chapter suggests an alternative method for the introduction of a heavy atom in the crystal structure by co-crystal formation. There are around 40 instances in the Cambridge Structural Database (CSD) where the absolute configuration was determined by the help of a heavy atom present in the solvent included in the crystal structure. However solvent inclusion cannot be preplanned and only a small number of compounds form solvates. There are around seven co-crystals reported in the CSD where the absolute configuration was determined with the help of heavy atom present in co-crystal former. Refcodes for these co-crystals are BAHLAX10, BUMPEE10, CONPAW, FEHMOU, NUYBOY01, SURLAS and ZUSHEA. However a closer look into the literature suggests that these co-crystals were not made to determine the absolute configuration but for other purposes like resolution and subsequently the absolute configuration was determined.

To establish this methodology, some steroid molecules were selected and cocrystallisation was attempted with compounds available in the laboratory. As a result, three co-crystals of pregnenolone and cholesterol were obtained.

![Scheme 1. Co-crystals of pregnenolone 1 and cholesterol 2 with 4-iodophenol 3 and 2,4,6 trichlorophenol 4.](image-url)
6.2 Crystal structure of co-crystal 1 (pregnenolone+4-iodophenol)

Co-crystal 1 crystallises in space group $P2_12_12_1$ with one molecule of pregnenolone and one molecule of 4-iodophenol in the asymmetric unit (Figure 1). Pregnenolone molecules form infinite 1D chains by head to tail recognition via O–H…O interactions between the –OH group and the $\text{C}=\text{O}$ group (Figure 2). 4-Iodophenol forms additional O–H…O hydrogen bonds with the pregnenolone molecules and close packs itself between the two adjacent chains of pregnenolone molecules as shown in Figure 3.

The determined absolute configuration of pregnenolone in co-crystal 1 is (3S, 8S, 9S, 10R, 13S, 14S, 17S) same to that of reported for pregnenolone. The Flack parameter value 0.000(14) for assigned absolute configuration suggests that it is correct with high accuracy.

![Figure 1](image1.png)

**Figure 1.** Absolute configuration of pregnenolone in co-crystal 1, shown as (a) 50% thermal ellipsoids and (b) ball and stick model.

![Figure 2](image2.png)

**Figure 2.** Infinite linear arrangement of pregnenolone molecules and their interaction with 4-iodophenol in the crystal structure of 1.
6.3 Crystal structure of co-crystal II (pregnenolone+2,4,6-trichlorophenol)

Co-crystal II crystallises in space group $P2_1$ with one molecule of pregnenolone and one molecule of 2,4,6-trichlorophenol in the asymmetric unit (Figure 4). The primary hydrogen bonding pattern in II is similar to that of I with pregnenolone molecules forming infinite 1D chains with O–H…O interactions and 2,4,6-trichlorophenol forming additional O–H…O hydrogen bonds with pregnenolone molecules and close packed itself between the two adjacent chains of pregnenolone molecules as shown in Figures 5 and 6. However the crystal structure of II differs with I in the shape of 1D chain and packing of the molecules.

The determined absolute configuration of pregnenolone in co-crystal II is (3S, 8S, 9S, 10R, 13S, 14S, 17S), which is the same as that reported for pregnenolone.\textsuperscript{14} The Flack parameter value 0.0(2) for the assigned absolute configuration suggests that it is correct. This is further confirmed by the Flack parameter value 1.3(2) for the inverted structure.
Figure 4. Absolute configuration of pregnenolone in co-crystal II, shown as (a) 50% thermal ellipsoids and (b) ball and stick model.

Figure 5. Infinite linear arrangement of pregnenolone molecules and their interactions with 2,4,6-trichlorophenol in the crystal structure of II.

Figure 6. Packing of molecules in the crystal structure of II.
6.4 Crystal structure of co-crystal III (cholesterol+4-iodophenol 2:1)

Co-crystal III crystallises in space group $P2_1$ with two molecules of cholesterol and one molecule of 4-iodophenol in the asymmetric unit (Figure 7). Hydroxy groups of cholesterol and 4-iodophenol form infinite cooperative chains of O–H…O hydrogen bonds as shown in Figure 8. These 1D chains are further connected by I…O interactions between 4-iodophenol and cholesterol molecules respectively to give rise to a 2D network as shown in Figures 9 and 10.

The determined absolute configuration of cholesterol in co-crystal III is (3S, 8S, 9S, 10R, 13R, 14S, 17R), which is the same as that reported for cholesterol. The Flack parameter value 0.008(13) for the assigned absolute configuration suggests that it is correct.

(a)

Figure 7. Absolute configuration of cholesterol in co-crystal III, shown as (a) 50% thermal ellipsoids and (b) ball and stick model.
Figure 8. Infinite O–H…O hydrogen bond chain made up by hydroxyl groups of cholesterol and 4-iodophenol in the crystal structure of III.

Figure 9. I…O interaction between 4-iodophenol and cholesterol molecules in the crystal structure of III.

Figure 10. Packing arrangement of molecules in the crystal structure of III.
Table 1. Hydrogen bond metrics for the co-crystals in this study.

<table>
<thead>
<tr>
<th>Co-crystal</th>
<th>Interaction</th>
<th>$d/\text{Å}$ (H…A)</th>
<th>$D/\text{Å}$ (X…A)</th>
<th>$\theta/\text{deg}$ $\angle X$–H…A</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>O–H…O</td>
<td>1.76</td>
<td>2.731(3)</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>O–H…O</td>
<td>1.68</td>
<td>2.663(3)</td>
<td>176</td>
</tr>
<tr>
<td>II</td>
<td>O–H…O</td>
<td>1.82</td>
<td>2.851(4)</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>O–H…O</td>
<td>1.66</td>
<td>2.613(4)</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>C–H…Cl</td>
<td>2.74</td>
<td>3.700(9)</td>
<td>147</td>
</tr>
<tr>
<td>III</td>
<td>O–H…O</td>
<td>1.94</td>
<td>2.704(5)</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>O–H…O</td>
<td>1.66</td>
<td>2.620(4)</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>O–H…O</td>
<td>1.69</td>
<td>2.652(4)</td>
<td>164</td>
</tr>
</tbody>
</table>

6.5 Conclusion

Co-crystals I, II and III provide examples where the absolute configurations of target compounds were determined using heavy atoms of a co-crystal former and confirmed with the reported absolute configuration of the respective compounds. This method can be used for compounds with unknown absolute configuration which do not contain heavy atoms. In compounds containing acidic or basic groups, the heavy atom can be introduced by salt formation but in cases where salts do not form good single crystals or where the compounds are sensitive to pH, co-crystal formation can be tried. Compounds which do not contain heavy atoms and salt forming groups have to rely on chemical reactions for this purpose, which is not always feasible and in some cases reaction condition may lead to inversion or racemisation. Liquid compounds without salt forming groups can be also crystallised by co-crystal formation and absolute configuration can be determined.
However, co-crystals cannot always be designed. The synthon based strategy although it suggests possible co-crystal formers, cannot suggest whether a particular co-crystal will be formed or not.\textsuperscript{16} Hence co-crystals are generally obtained by trial and error methods, and these may be time-consuming and tedious. However, with the advent of high-throughput technology it should not be very difficult to get co-crystals for a particular compound as large number of experiments can be carried out and the results analysed simultaneously.\textsuperscript{17}

In short, an alternative methodology for the determination of absolute configuration by co-crystal formation is proposed in the present chapter. It is not always superior to existing methodologies for the determination of absolute configuration but in some cases it could be advantageous to choose this method as it can complement some of the drawbacks of existing methods.

\textbf{6.6 Experimental section}

\textbf{Sample preparation and crystallisation}

Co-crystals \textbf{I}, \textbf{II} and \textbf{III} were obtained by cocrystallisation of equimolar ratios of both components from EtOAc.

\textbf{X-ray Crystallography}

X-ray diffraction intensities for the co-crystals \textbf{I} and \textbf{III} were collected at 100K (Bruker Cryosystems cooler) and co-crystal \textbf{II} at 298K on a Bruker SMART 4K CCD diffractometer (Bruker Systems Inc.) using Mo $K_a$ X-radiation.\textsuperscript{18} Data were processed using the Bruker \textit{SAINT} package\textsuperscript{19} with structure solution and refinement using \textit{SHELX97} (Sheldrick, 1997).\textsuperscript{20} The structures of all the co-crystals were solved by direct methods and refined by full-matrix least-squares on $F^2$. Crystal data and details of data collections, structure solutions and refinements are summarized in appendix.
6.7 References and notes


