Tetrahedron: *Asymmetry* 28 (2017) 1304–1313

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Determination of absolute configuration using X-ray diffraction

Simon Parsons

School of Chemistry and Centre for Science at Extreme Conditions, The University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3FJ, UK

ARTICLE INFO

Article history:
Received 7 August 2017
Accepted 21 August 2017
Available online 22 September 2017

Dedicated to the memory of Dr. Howard Flack

ABSTRACT

Methods for determination of absolute structure using X-ray crystallography are described, with an emphasis on applications for absolute configuration assignment of enantiopure light-atom organic compounds. The ability to distinguish between alternative absolute structures by X-ray crystallography is the result of a physical phenomenon called resonant scattering, which introduces small deviations from the inherent inversion symmetry of single-crystal X-ray diffraction patterns. The magnitude of the effect depends on the elements present in the crystal and the wavelength of the X-rays used to collect the diffraction data, but it is always very weak for crystals of compounds containing no element heavier than oxygen. The precision of absolute structure determination by conventional least squares refinement appears to be unduly pessimistic for light-atom materials. Recent developments based on Bijvoet differences, quotients and Bayesian statistics enable better and more realistic precision to be obtained. The new methods are sensitive to statistical outliers, and techniques for identifying these are summarised.

1. Introduction

The space group, which defines the symmetry relationships that exist between the atoms composing a crystal, is one of the most fundamental features of a crystal structure. The translational symmetry that relates one unit cell to another is part of the space group symmetry. If two molecules within the unit cell are related by a symmetry operation such as a screw axis or reflection, those operations are also part of the space group.

The inversion centre is a very common symmetry operation in crystal structures. Those structures with space groups which possess an inversion centre are called *centrosymmetric*, while those which lack an inversion centre are *non-centrosymmetric*. The same terms can be applied to the space groups themselves. Inversion centres are common because they often form a low-energy way for molecules to interact. However, an inversion centre would relate a chiral molecule to its enantiomer, and so an enantiopure compound must always crystallise in a non-centrosymmetric space group.

A non-centrosymmetric crystal structure cannot be superimposed on its inverted image, and determination of *absolute structure* amounts to assigning a particular non-centrosymmetric crystal structure to one of two possible structures which are related by inversion. The issue of absolute structure is relevant only to non-centrosymmetric crystal structures.

The inverted form of a crystal structure containing one enantiomer is a structure containing the opposite enantiomer. Therefore determination of the absolute structure of an enantiopure molecular crystal can be used to establish the absolute configuration of the molecules that comprise it. Notice that the word 'absolute' is being used here in two different contexts. To quote Howard Flack:[1] '*Absolute structure* is a crystallographer's term and applies to non-centrosymmetric crystal structures. *Absolute configuration* is a chemist's term and refers to chiral molecules.'

Inversion of a non-centrosymmetric crystal structure also leads to inversion of its diffraction pattern. In principle, therefore, the distinction between two possible absolute structures can be made by comparing the original and inverted diffraction patterns calculated from the original and inverted structural models with the one that is measured experimentally. The calculated pattern that agrees better with the experimental data defines which absolute structure is the correct one.

This would be simple were it not for the fact that X-ray diffraction patterns themselves are, at least approximately, centrosymmetric, *i.e.* the intensities of the reflections with Miller indices hkl and $\bar{h}\bar{k}\bar{l}$ are the same ($\bar{h}$ means $-h$). This is called Friedel's Law, and it arises because the sets of Miller planes hkl and $\bar{h}\bar{k}\bar{l}$ are identical. Inverted images of a centrosymmetric diffraction pattern are, of course, the same, and if Friedel's law held exactly it would be impossible to draw the distinction described in the previous paragraph.

Fortunately, an effect called resonant scattering (or anomalous scattering or dispersion) introduces deviations from Friedel's law.

E-mail address: S.Parsons@ed.ac.uk

https://doi.org/10.1016/j.tetasy.2017.08.018
0957-4166/

The source of the effect is absorption of X-ray photons by excitation of the core electrons of the atoms of the crystal. An excellent website giving further details of the physical origin of the effect and its applications in macromolecular crystallography is available.[2]

Two features of resonant scattering are relevant to absolute structure determination. First, the effect is small compared to the contribution of non-resonant scattering. Some illustrative figures are given in Table 1. The contribution to the atomic scattering factor which introduces deviations from Friedel's law is given the symbol f'' and this should be compared to the value of the non-resonant contribution (f). Secondly, its magnitude depends on the wavelength of the X-rays used to measure the diffraction pattern and on the elements present in the crystal. Data for the two most common radiations used for X-ray diffraction (Mo Kα and Cu Kα) are given in Table 1.

Resonant scattering effects are smallest for the 'light atoms' of the first two periods of the periodic table, and absolute structure determination therefore presents a particular challenge in exactly the area where it is most important, in organic chemistry. The problem is especially critical for compounds used in pharmaceutical applications, where enantiomers may show very different biological activities (*e.g.* a comparison of biological activities and odours of selected enantiomers are available in ref. 4). In the past, absolute structure determination for purely light-atom compounds has usually only been possible by preparing a derivative containing either a heavy atom such as chlorine, or a group of known chirality. This is no longer the case, and one of the aims of this paper is to summarise recent progress that enables confident assignment of absolute structure even for hydrocarbons.

2. Further space group considerations for enantiopure crystal structures

Symmetry operations may be classified as *proper* or *improper*. Proper operations include pure rotations, pure translations and screw axes, which are a combination of translation and rotation. The other operations which occur in crystal structures are inversion centres, mirror planes and other rotoinversions ($\bar{3}$, $\bar{4}$ and $\bar{6}$, equivalent to S_6, S_4 and S_3 operations in Schönflies notation) and glide planes, which are a combination of translation and reflection. These are all improper operations.

A chiral molecule is transformed into its enantiomer by an improper operation. Improper operations can therefore not occur in the space group of an enantiopure crystal. The restriction on inversion symmetry was referred to in Section 1, but the same applies to mirror planes, rotoinversions and glide planes. The most common space groups for molecular compounds, $P2_1/c$, $P\bar{1}$ and $C2/c$ are thus not possible for enantiopure crystal structures.

Many non-centrosymmetric space groups, such as $Pna2_1$ and Cc also contain improper operations, and these too are impossible for enantiopure crystals. These space groups are, nevertheless, non-centrosymmetric and the absolute structure of any crystal structure forming in them still needs to be established. In space groups $Pna2_1$ and Cc the absolute structure would define the polarity of the crystal structure with respect to the crystal morphology rather the chirality or absolute configuration of the component molecules.

Chirally-pure compounds may crystallise in the space groups containing proper operations only, the so-called Söhncke space groups. 65 of the 230 space groups fall into this category, and the most common are $P2_12_12_1$, $P2_1$ and $P1$. It is straightforward to recognise a Söhncke group: if after removal the first letter of the space group symbol all remaining characters are positive numbers then the space group is a Söhncke group and able to accommodate an enantiopure crystal structure. For example:

> $P2_12_12_1$: Removing the P gives $2_12_12_1$. These characters are only positive numbers so this is a Söhncke group.
> $P2_1/c$: Removing the P gives $2_1/c$. The '/' and 'c' are not numbers, so this is not a Söhncke group, and a compound with asymmetric centres crystallising in this space group would be a racemate.
> $I\bar{4}$: Removing the I gives $\bar{4}$. The '$\bar{4}$ ', which is also sometimes written '−4', is not a positive number, so this is not a Söhncke group either. $I4$ and $I4_1$, on the other hand, are.

The term 'chiral space group' is sometimes used in the literature to mean a space group capable of accommodating a chirally pure crystal structure, *i.e.* a Söhncke group. This usage has been criticised by Howard Flack because the term literally implies that the space group itself is chiral.[5] That is, if the symmetry operations are considered as objects then the space group is not superimposable on its mirror image. While this is true of a space group like $P3_1$, because the mirror image of a 3_1 operation is a 3_2 operation, it is not true of $P2_1$ (the mirror image of 2_1 is 2_1) even though both are Söhncke groups. There are 22 space groups, forming 11 'enantiomorphic pairs' for which the term 'chiral space group' is appropriate. $P3_1$ and $P3_2$ is one example of such a pair; $P4_12_12$ and $P4_32_12$ is another. A full list is available in most crystallographic text books and Ref. 1.

A non-centrosymmetric crystal structure can usually be inverted by simply multiplying all the fractional coordinates by −1. There are two cases where additional steps are required:

i. If the space group belongs to one of the 11 enantiomorphic pairs. In this case the space group also needs to be changed to its enantiomorphic partner, so that if a structure in $P3_1$ is to be inverted the coordinates need to be multiplied by −1 *and* the space group should be changed to $P3_2$.
ii. If the space group is one of $Fdd2$, $I4_1$, $I4_122$, $I4_1md$, $I4_1cd$, $I\bar{4}2d$ or $F4_132$. In these cases, in addition to multiplication of the coordinates by −1, an additional origin shift is required as listed in Ref. 6. Note that of these only $I4_1$, $I4_122$ and $F4_132$ are Söhncke groups; all three are relatively rare for non-macromolecular materials.

3. Friedel pairs and Bijvoet pairs

If resonant scattering effects are large enough to be observable, the symmetry of the X-ray diffraction pattern of a crystal is directly related to the space group of the crystal structure. For a crystal structure in the centrosymmetric space group $P2_1/c$, reflections

Table 1
Non-resonant (f) and resonant (f' and f'') scattering factors for C and Cl. Note that the non-resonant scattering factors (f) are not dependent on wavelength, but do depend on resolution, while the opposite is the case for f' and f''. Data from Ref. 3.

Atom	Radiation	$f(d = 5$ Å$)$/e	$f(d = 1$ Å$)$/e	f'/e	f''/e
Carbon	Mo *K*α	5.107	1.114	0.0033	0.0016
	Cu *K*α			0.0181	0.0091
Chlorine	Mo *K*α	15.234	4.023	0.1484	0.1585
	Cu *K*α			0.3639	0.7018