On wine, chirality and crystallography

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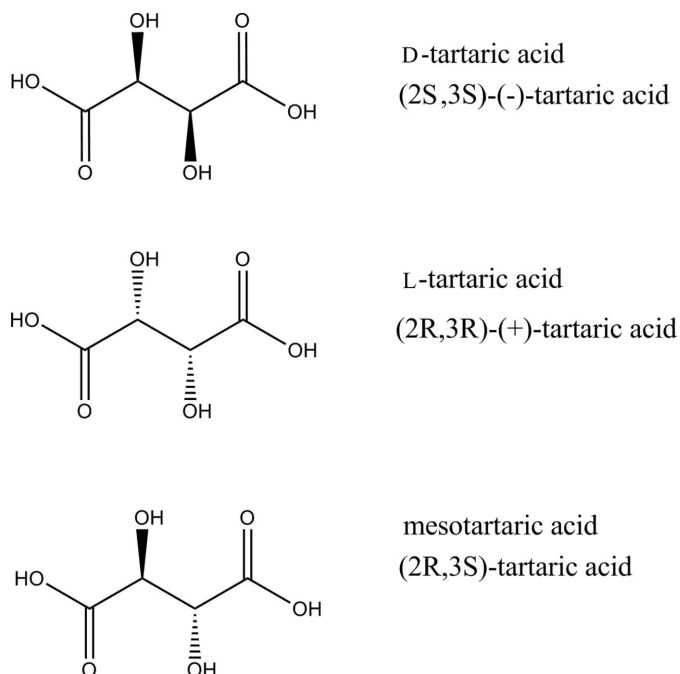
As the first centennial of X-ray diffraction is inevitably drawing closer, it is tempting to reflect on the impact that this fascinating discipline has had on natural sciences and how it has changed the world we live in. Also, next year is the 160th anniversary of the fateful April afternoon when Louis Pasteur separated D- from L-tartrate crystals, an event that many science historians recognize as the birth of stereochemistry, and the first step that the barely nascent field of crystallography took on the road to elucidate a fundamental phenomenon of chemistry and biology – chirality. Many great minds – Pasteur, Van 't Hoff, Fischer, Lord Kelvin, the Braggs, Astbury and Bijvoet, to mention just a few – contributed along the way. But one central inanimate character was there at all times – an inconspicuous somewhat obscure organic compound found in wine: tartaric acid. This is the story of its contribution to science.

1. Early history

Our story begins with a glass of wine. Tartaric acid, or according to the IUPAC convention 2,3-dihydroxybutanedioic acid, is one of the main acids in wine, and aside from grapes it is found in bananas and tamarinds, which are used as spice in Asian and Latin American cuisines and ingredients of Worcestershire sauce. Unpurified tartaric acid from grapes takes on their color, but when pure it is a white crystalline

Figure 1
Laevotartaric acid [D-(−)-tartaric acid], dextrotartaric acid [L-(+)-tartaric acid] and mesotartaric acid.

powder. The most common salt is tartar, also known as 'wine diamonds' or *Weinstein*, *i.e.* small potassium bitartrate (*i.e.* $KHC_4H_4O_6$) crystals that form on the bottom of the cork in older good-quality wines. Tartar forms spontaneously in wine casks, providing a commercial source for kitchen uses, such as stabilizing egg whites, reducing discoloration of boiled vegetables *etc.* Tartaric acid has been known to man probably as long as wine, *i.e.* for 6000–8000 years. Wine residue was found in Neolithic ceramic jars in present day Georgia, Iran and Iraq and – notably – this discovery was based on the detection of tartrate by Fourier transform infrared spectroscopy. Tartaric acid has two asymmetric carbon atoms, which allow for four possible forms (Fig. 1). The naturally occurring tartaric acid is L-(R,R)-(+)-tartaric acid or dextrotartaric acid.[1] The mirror-image, enantiomeric form [also referred to as laevotartaric acid or D-(S,S)-(−)-tartaric acid] and the optically inactive form, which contains both an R and an S carbon atom, known as mesotartaric acid, can be made synthetically. We will discuss this later. Another optically inactive form of tartaric acid, DL-$(S,S/R,R)$-(±)-tartaric acid, is a 1:1 mixture of the laevo and dextro forms, known as racemic acid or paratartrate. The word *racemic*, originally restricted to this very compound, was subsequently adopted as a general term for 1:1 enantiomeric mixtures – *i.e.* racemates.

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[1] The (+) and (−) symbols denote the direction in which polarized light is rotated by the compound and historically constitute the oldest convention used to differentiate between optically active stereoisomers. The dextro- and laevo- prefixes identify the (+) and (−) forms, respectively. The D and L symbols denote chirality as defined by Emil Fischer, *i.e.* in reference to D- and L-glucose, so that the D series is derived from (+)-glucose and the L series from (−)-glucose. However, not all D compounds are dextro- and not all L need be laevo-. Finally, the modern Cahn–Ingold–Prelog convention identified absolute configuration on carbon as either R or S, and absolute configuration need not correspond to the sense of optical rotation. This essay tracks the evolution of these conventions.

Tartaric acid was probably first isolated from tartar by the great Arabic alchemist, philosopher, astronomer and physicist, Abu Musa Jabir ibn Hayyan (*ca* 721–*ca* 815), known to science historians by his Latin name, Geber. Considered by many to be the father of chemistry, Geber is credited with the discovery of crystallization as a purification process. Aside from tartaric acid, he discovered citric acid and acetic acid, as well as hydrochloric acid and nitric acid which he mixed to obtain *aqua regia*, king's water. Geber was a prolific writer and many of his books were translated into Latin and became standard texts for alchemists in the Middle Ages; he introduced a number of chemical terms, such as 'alkali', that are still in use today.

The more contemporary recorded history of tartaric acid and its salts begins *ca* 1675, when sodium potassium tartrate, one of several double salts of tartaric acid, was first prepared by a French apothecary, Pierre Seignette, in La Rochelle, on the Atlantic coast of France. Not surprisingly, the salt is now known as the Seignette or Rochelle salt. It forms colorless to blue–white orthorhombic crystals with four water molecules per one tartrate. They grow easily to large size from aqueous solution upon cooling because of steep temperature dependence of solubility. The Seignette salt is known for its medicinal use as a purgative, but it also has other less disturbing applications.

Nearly one hundred years later, the German–Swedish chemist Carl Wilhelm Scheele (1742–1786) purified the naturally occurring tartaric acid (*i.e.* the dextro form) along with citric, oxalic, malic, gallic, lactic and uric acids. Scheele was an apothecary working in a pharmacy in Stockholm and, in 1769, together with Anders Johan Retzius (1742–1821), prepared pure tartaric acid from cream of tartar by boiling tartar with chalk and decomposing the product with sulfuric acid. Retzius went on to crystallize it. Scheele also discovered oxygen two years before Priestley, but procrastinated with the publication until 1777 and was 'scooped' by Priestley, who announced his discovery in 1774.

A second form of tartaric acid, thannic acid (later identified as paratartaric acid or racemic acid), was obtained from crude tartar around 1819 by Paul Kestner, a French manufacturer from Thann (Alsace), by overheating tartrate. Kestner mistook paratartrate for oxalic acid, but a year later J. F. John recognized it as a distinct compound. In 1826, the French chemist Joseph Louis Gay-Lussac (1778–1850) coined the name racemic acid (*acide racémique*), from the Latin *racemus*, for a bunch of grapes, and demonstrated that it had the same chemical composition as tartaric acid. This was also confirmed by Jöns Jakob Berzelius (1779–1848), the great Swedish chemist, who introduced the concept of isomerism and defined the two forms of tartaric acid as isomers. The notion was not entirely novel: in 1825, two other contemporary chemists, Justus von Liebig (1803–1873) and Friedrich Wöhler (1800–1882), resolved their dispute over two substances with apparently identical composition but different properties, *i.e.* silver cyanate and silver fulminate. The same year, Michael Faraday (1791–1867) found a liquid hydrocarbon in compressed gas with the same composition as gaseous ethylene. Finally, in 1828, in an accidental experiment that marks the birth of organic chemistry, Wöhler converted ammonium cyanate to urea. Two years later, he published his studies on cyanuric acid and urea jointly with Liebig. Thus, the idea that identical chemical compounds (as judged by their composition) can occur in different physical forms had been around for some time. In 1830, Berzelius brought the isomerism of tartaric acid to the attention of Eilhard Mitscherlich (1794–1863), who had by then already established the premise of isomorphism and polymorphism in crystallography, and shortly afterwards Mitscherlich began careful crystallographic investigations of both tartaric acid and paratartaric acid.

2. Crystals and optical activity

At this point, we should consider a parallel development in physics, *i.e.* the discovery of optical activity, which was of crucial importance to subsequent events.

Polarization of reflected light was discovered by Sir Isaac Newton (1643–1727). In 1808, Etienne-Louis Malus (1775–1812), a French officer, engineer, physicist mathematician, and a veteran of Napoleon's expedition to Egypt, discovered polarization of light by a crystalline calcium carbonate – calcite. Two years later, Malus formulated a theory of double-refraction, or birefringence, in crystals. His work was extended by Sir David Brewster (1781–1868), who in 1815 formulated a law that allows for correlating the polarization angle (*i.e.* Brewster's angle) with the refractive index. The same year, Jean-Baptiste Biot (1774–1862) discovered that if a plate of quartz or liquids such as turpentine and solutions of sugar, camphor or tartaric acid are placed between crossed Nicol prisms (so named later after William Nicol of Edinburgh), which isolate a polarized light beam, one of them must be adjusted for rotation to extinguish the light, indicating that the tested samples were rotating the polarization plane. The first mention of this fact occurs in the bulletin of the Société Philomatique, December 1815. It is important to note that Biot correctly concluded that quartz's optical activity is a property of the crystal, while that of the organic solutes is a property of individual molecules. Much later, in 1832, Biot also demonstrated the optical activity of tartaric acid solution, but it took him until 1838 to discover – to his surprise – that paratartaric acid was optically inactive. He wondered about this seemingly inexplicable phenomenon, and in 1844 received a note from Mitcherlich who was equally perplexed because the '*nature and the number of atoms, their arrangement and their distances are the same in the two substances compared*.'

3. René Just Haüy and the dawn of crystallography (and civilization as we know it)

The same year that Biot discovered the phenomenon of optical activity, in 1815, the abbot René Just Haüy (1743–1822) – who is regarded by science historians as the father of

crystallography – discovered the existence of two hemihedral forms of quartz crystals. Hemihedral crystals have only half the number of faces required by the symmetry of the crystal system to which they belong: some show faces oriented to the left and some to the right and the two are non-superimposable mirror images of each other. We now know that quartz typically crystallizes in two forms, *i.e.* $P3_121$ and $P3_221$, and, even though the SiO_4 tetrahedra that make up the crystal are achiral, the two crystals are perfect mirror images because the arrangement of molecules in the unit cell, either a left- or a right-handed helix, is chiral. This is not a unique situation among minerals but quartz is so ubiquitous that it naturally caught Haüy's eye.

It was Sir John Frederick William Herschel, 1st Baronet (1792–1871), who in 1820 communicated to the Royal Society of London a paper in which he connected Haüy's crystallographic discovery with Biot's physical one and showed that the two forms of quartz rotate the plane of polarized light in the opposite sense (Herschel was born in Germany as Friedrich Wilhelm Herschel, and is best known for his contributions to astronomy and particularly for the construction of more than four hundred telescopes, and for the discovery of Uranus, its moons, and Saturn's moons). Meanwhile, René Just Haüy became convinced that every distinct chemical substance has a unique fixed crystal form. In contrast, Mitscherlich introduced the concepts of 'isomorphism' (identity of crystalline form in spite of different chemical composition) and 'dimorphism' (*i.e.* existence of different crystal forms of the same compound). Against the backdrop of these two opposing views, Auguste Laurent (1807–1853) developed new ideas that foreshadowed contemporary structural chemistry. The son of a mining engineer, Laurent became one himself before taking a position of an assistant to Jean Baptiste André Dumas (1800–1884), the French chemist best known for his work on organic analysis and synthesis, as well as the determination of atomic weights by measuring vapor densities. In 1836, Laurent was appointed a Professor of Chemistry in Bordeaux, and moved back to Paris to Ecole Normale in late 1846. Laurent proposed that the structural grouping of atoms within molecules determined how the molecules combined in organic reactions. This theory conflicted with the then accepted view credited to Berzelius that the product of organic reactions depended solely on the electrical charge of the atoms involved. Laurent wanted to develop a theory that would reconcile the Haüy–Mitscherlich controversy and went on to postulate that substances with similar crystalline forms have a common chemical moiety, a 'fundamental radical', which could then be modified by the presence of water molecules, acids *etc.*, leading to polymorphism.

Thus, the questions of isomorphism, presence of crystallization water, crystal morphology *etc.* were central to chemistry and crystallography by 1848. They were also emphasized by Gabriel Delafosse (1795–1878), a former student of Haüy, who taught at the prestigious Ecole Normale Supérieure in Paris. One of the students in the audience was a young man by the name of Louis Pasteur.

4. Louis Pasteur, the crystallographer

Louis Pasteur (1822–1895) was recognized early for his scholastic aptitude and on that strength was accepted by Ecole Normale Supérieure. Pasteur undertook two theses to obtain his doctorate in sciences, one in chemistry and one in physics. The subjects originally had to do with pressure, chemical reactions and atomic volumes, but at the suggestion of Laurent, during the latter's short stay in Paris, Pasteur switched his interests. For chemistry, he studied the saturation capacities of arsenious acid and its salts. Among the results, he found two slightly different crystalline forms corresponding to monobasic and dibasic forms of arsenious acid. This observation supported the notion that two compounds could be nearly isomorphous and yet dimorphous (*i.e.* occurring in two distinct forms) at the same time, supporting Laurent's views. In physics, Pasteur focused on '*phenomena relating to the rotatory polarization of liquids*', as the title stated. He emphasized that optical activity could be used as a guide to chemical structure and expanded on the role of crystallography in chemistry. The main theme was the relationship between optical activity and crystal form, with the ultimate conclusion that identity of the crystalline form leads to identical optical activity. In general terms, this was not original because Laurent had already published observations to that effect but it shows how Pasteur was influenced by Laurent and how deeply he was involved in crystallographic research prior to his study of tartrates.

Both doctoral theses were presented in August 1847. Pasteur then continued to work on the issue of dimorphism because he was concerned that this phenomenon reduced the value of crystallography as an identifier of chemical composition. For example, calcite and aragonite are both crystal forms of calcium carbonate, but the former is trigonal (which we now know is $R\bar{3}c$) while the latter, which forms in mollusc shells and corals, is orthorhombic (*Pmcn*). This, of course, was in sharp contrast to Haüy's views. Pasteur's hope was to show that dimorphism can be shown to be a special case of isomorphism, blurring Mitscherlich's distinction, and in line with Laurent's views. Pasteur was already familiar with tartrates from the work published in 1841 by M. F. de la Provostaye, and tartrates were the natural system on which to test his ideas. His first paper on this subject was read on 20 April 1848. In it, he discussed the case of eight different tartrates, which were '*broadly isomorphous*' and which he argued could be co-crystallized together in any proportion. However, as a meticulous investigator he was not completely convinced by his results and conclusions. He went back to the issue of the number of water molecules in each crystal form. He focused particularly on sodium ammonium tartrate, which was for a number of reasons a very good model system to use, particularly because the enantiopure form yields crystals with well developed hemihedral faces. One problem that Pasteur encountered was that he could not find the formula for sodium ammonium paratartrate, although he presumed that it must be similar to sodium potassium tartrate (the Seignette salt), with which it was isomorphous. It is at this point that he recalled

Mitscherlich's conundrum that asked why sodium ammonium paratartrate and sodium ammonium tartrate are isomorphous, yet the paratartrate does not rotate polarized light. As Pasteur described in one of his two famous lectures given in early 1860 before the Council of the Societé Chimique de Paris, he was increasingly troubled by the problem, and eventually sorted through crystals obtained from sodium ammonium para-tartrate using only a hand lens and a pair of tweezers. He realized that Mitscherlich had made a mistake: the crystals were not all the same. They were hemihedral, with about half showing right-handed faces, and the others being mirror images (Fig. 2). He separated left-handed crystals from right-handed ones, dissolved each set and discovered that the solution of the first group is optically active to the left, and the other to the right. He had it: in Laurentian spirit he was now able to reconcile chemical structure with the crystalline polymorphism.

When Biot, who was 74 years old that year, learned about the discovery, he asked that the experiment be repeated in his presence. He watched as Pasteur separated a new batch of crystals into two piles, predicting which ones would rotate polarized light to the left and which ones to the right. At this point Biot took over and finished the experiment, starting with the more interesting left-handed crystals which were never before described. When he confirmed all predictions with his polarimeter, he said holding Pasteur's hand: '*My dear child, I have all my life so loved the sciences that this makes my heart throb with joy*.'

Years later, Pasteur made the famous comment that chance favors the prepared mind. No discovery in the history of science illustrates this premise more than the observation made by Pasteur on 29 April 1848. As it happens, paratartrate is unique in that it undergoes spontaneous separation into hemihedral crystals, but only below 27°C. Had Pasteur worked on a hot summer day, he might have never made the discovery.

5. The dawn of structural chemistry

Pasteur's life coincided with a period when the atomic theory and the foundations of structural chemistry were only begin-
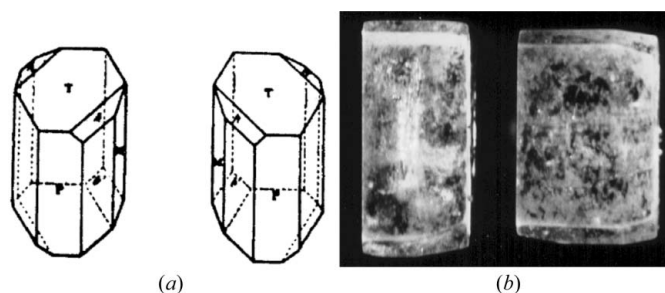
Figure 2
(*a*) Hemihedral crystals of tartaric acid as drawn by Pasteur and (*b*) actual crystals of D and L forms. From Kauffman & Myers (1998), *Chem. Educ.* **3**, doi: 10.1333/s0089780257a. With kind permission of Springer Science and Business Media.

ning to take hold. Although Robert Boyle (1627–1691) introduced the concept of an element in 1661, the idea of specific atomic weight was conceived only in 1808 by John Dalton (1766–1844) in the *New System of Chemical Philosophy*. It took another 46 years – after most of Pasteur's work on tartrates was completed – that the notion of valence was coined in 1852 by Sir Edward Frankland (1825–1899). Within the next six years, these ideas gave rise to the concept of chains of interconnected tetravalent carbon atoms and – in essence – to structural chemistry, with its graphic formulas and their implications.

Tartaric acid was again at the center of attention. Its 'correct' formula, one of only few compounds thought of in this way, was given in 1858 by Archibald Scott Couper (1831–1892) in *Annales de Chemie et de Physique*. Couper was not completely correct: he accepted 8 instead of 16 as oxygen's atomic weight, and so he has twice the correct number of oxygen atoms in his formula. Couper introduced straight lines to indicate bonds and described how carbon atoms bind to each other to make chains. His paper, however, was entrusted to Charles-Adolphe Würtz (1817–1884), a highly regarded French organic chemist, who delayed passing it on to the Academy, giving Friedrich August Kekulé (1829–1896) a chance to publish his work several months earlier. When Couper protested, Würtz expelled him from the laboratory. As a consequence, Couper suffered an emotional collapse and retreated to Scotland, never to publish anything in his remaining 30 years. His entire bibliography consists of four papers written within a single year when he was 27.

It was Alexander Crum Brown (1838–1922), a Scottish organic chemist at the University of Edinburgh, who developed structural chemical formulas. In 1864, he started drawing diagrams with atoms as circles joined by lines signifying covalent bonds. And then, in January 1865, Kekulé published his structure of the benzene ring with six carbon atoms and alternating double and single bonds to satisfy carbon's tetravalence; structural organic chemistry was born.

6. The tetrahedron

On 30 August 1852, the year that Frankland introduced the concept of valence, Jacobus Henricus Van 't Hoff was born in Rotterdam, Holland. In 1869, Van 't Hoff enrolled at the Polytechnikum in Delft to study chemistry – against the advice of his father, a medical doctor – and completed a three year program in two years. He then went for a year to the University of Leiden, known for its strength in mathematics, less so in chemistry, and then moved on to Bonn to work with August Kekulé. He was initially thrilled by the prospect of working with one of the founders of structural chemistry, but soon discovered that Kekulé was a selfish man and that it was his practice to '*convert some paying laboratory assistants into unpaid private assistants*.'

Having earned a lukewarm recommendation from Kekulé, Van 't Hoff matriculated at the University of Utrecht, passed his exams in December 1873 and went on to Paris to work with Adolphe Würtz. There he met a fellow student, Joseph Achille

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Le Bel (1857–1930). Shortly after his return to Utrecht in 1874, Van 't Hoff presented a dissertation in organic chemistry which earned him a doctorate: the thesis was entitled *Contributions to the Knowledge of Cyanoacetic and Malonic Acid* and would have been easily forgotten had it not been for Van 't Hoff's other contributions to chemistry.

While still technically a student working with Eduard Mulder, Van 't Hoff published a 15-page-long pamphlet in Dutch, which – with the benefit of hindsight – introduced one of the most critical concepts in modern chemistry, *i.e.* that of a tetrahedral carbon atom. Van 't Hoff knew from previously published work that methane is a symmetrical compound. He argued that this is sufficient to conclude that the structure of methane can be represented by a regular tetrahedron with the carbon atom at its center. With the knowledge of Pasteur's work on the tartrates and the speculation of the underlying asymmetry of the molecules, Van 't Hoff examined the composition of known optically active organic compounds and discovered that all contain at least one carbon atom in combination with four different atoms or groups. He then logically extended his idea of a tetrahedron to an asymmetric tetrahedral carbon atom.

A French translation was published the same year, but the work went virtually unnoticed. Van 't Hoff decided to expand the French version and published it in 1875 in Rotterdam under the title *La Chimie dans l'Espace* (chemistry in space): it now had 43 pages and three plates with a total of 40 figures. But even these more detailed figures, about half of which were perspective drawings, did not convincingly convey a sense of three dimensionality. So Van 't Hoff went further: he explicitly suggested that readers cut out models from cardboard, and then volunteered to mail complete collections of the models to anyone who requested them. He added that such sets were already in the hands of Baeyer, Butlerov, Henry, Hofmann, Kekulé, Frankland, Wislicenus, Würtz and Berthelot.

Three sets of these models survived to date. One set of 22 items is in the Deutsches Museum in Munich (Fig. 3) while two sets are in Museum Boerhaave in Leiden: a comprehensive set of 69 models and a recently discovered small set consisting of 10 models. The models represent hypothetical organic molecules, with bound substituents symbolized by $R1$, $R2$, $R3$ and $R4$ and, in the case of the Leiden 1 set, actual molecules. The basic shape of the models is a tetrahedron, the models are all made of cardboard, typically with 1.5−2 cm edge length.

The Munich set of models was donated to the museum in 1905 or early 1906 by Van 't Hoff himself, with a note that explained their use and significance. It is thought to comprise the original set of models that Van 't Hoff later copied for others. The second Leiden set originally belonged to Gustav Bremer, Van 't Hoff's fellow student in Utrecht, whose widow donated the set after his death in 1909 to the Chemistry Department of the University of Leiden, where it was kept until recently.

Van 't Hoff's models were not totally original in concept. August Wilhelm Hofmann, another student of Liebig, was the first to use ball-and-stick models and displayed them on 7 April 1865, during a Friday Evening discourse at the Royal Institution. Hofmann's discourse was presided over by the Prince of Wales, later Edward VII. Hoffman's example was followed by Adolph von Baeyer, Edward Frankland and, importantly, Kekulé, who used models with brass rods pointing towards the corners of a tetrahedron to visualize single, double and triple bonds by matching the tips of one, two or three pairs of rods. We already noted that Van 't Hoff worked with Kekulé after leaving Leiden, and it is very likely that he saw his mentor's models even though he later claimed that he conceived of a tetrahedron completely independently while considering the molecular basis of the optical activity of lactic acid. What is undoubtedly true is that, by explaining optical activity in the context of a tetrahedral carbon, Van 't Hoff in essence assigned physical significance to the tetrahedral atom, while Kekulé used it as an intellectual device to illustrate valencies.

It is important here that we recognize the essentially identical concept of a tetrahedral carbon published by Joseph Le Bel within weeks of Van 't Hoff's pamphlet. Although they met in Würtz's laboratory in 1873, Van 't Hoff later recalled that '. . . *we have never exchanged a word about the tetrahedron there, though perhaps both of us had cherished the idea in secret*.' Le Bel's paper is a direct continuation of the French school of the relationship between crystalline form and optical activity and suggests a general principle underlying the

Figure 3
Van 't Hoff's models. Photo: Deutches Museum, Munich; with permission.

presence of optical activity. However, it has no accompanying diagrams or perspective drawings and the tetrahedral atom is explicitly mentioned in only one paragraph.

Both Van 't Hoff and Le Bel made specific references to the four forms of tartaric acid as a result of the presence of two asymmetric, *i.e.* tetrahedral, carbon atoms.

In 1875, a German chemist Viktor Meyer (1848–1897) wrote to Van 't Hoff requesting a copy of *La Chimie dans l'Espace.* A year later, Meyer wrote to Adolf von Baeyer with a suggestion of an experimental proof of Van 't Hoff's proposal, *i.e.* synthesis of asymmetric bromochloronitroethanes. Meyer was one of the first chemists to include the concept of the tetrahedral atom in his lectures and *ca* 1878 he introduced the term 'stereochemistry'. Unfortunately, at the age of 49, Meyer succumbed to depression and on 7 August 1897 took cyanide pills.

In 1877, after three years dedicated to structural organic chemistry, Van 't Hoff abandoned the field. In 1878, he was appointed Professor of Chemistry, Mineralogy and Geology at the University of Amsterdam. He occupied this Chair for 18 years before accepting an Honorary Professorship in Berlin as a member of the Royal Prussian Academy of Sciences. He remained there until the end of his life and died on 1 March 1911 at Steglitz near Berlin. He was the recipient of the first Nobel Prize in Chemistry (in 1901). In 1885, he was appointed member of the Royal Netherlands Academy of Sciences and was awarded honorary doctorates of Harvard and Yale (1901), Victoria University, Manchester (1903), Heidelberg (1908); he received the Davy Medal of the Royal Society (1893), Helmholtz Medal of the Prussian Academy of Sciences (1911); he was also appointed Chevalier de la Legion d'Honneur (1894), Senator der Kaiser Wilhelm Gesellschaft (1911), a member or honorary member of the Chemical Society, London (1898), Royal Academy of Sciences, Göttingen (1892), American Chemical Society (1898), and the Académie des Sciences, Paris (1905). Virtually all of these distinctions were a result of his work in the area of physical chemistry. The Nobel Prize was awarded '*in recognition of the extraordinary services he has rendered by the discovery of the laws of chemical dynamics and osmotic pressure in solutions*.'

7. Making sense of it all

During the winter of 1890, Hermann Emil Fischer (1852–1919) was deeply immersed in his investigations of sugars. He was considering the relationship between pentoses and trihydroglutaric acids but it was not clear to him how many such acids were possible, probably because Van 't Hoff had mistakenly written in his *Lagerung der Atome im Raume* (1877) that there were three isomers. Fortuitously, he was at the time in Bordighera, a resort on the Italian Riveria near Nice, in the company of Adolf von Baeyer. The German chemist, all too familiar with Van 't Hoff's tetrahedral carbon atom, when asked if he knew the answer, tried to solve the problem using bread crumbs to make models of carbon atoms and toothpicks for bonds, but gave up. Upon his return to Würzburg, Fischer probably contacted Van 't Hoff, and resolved the issue. Then,

between 1891 and 1894, Fischer established the stereochemical configuration of all the known sugars and correctly predicted the possible isomers. He also realized that one could correlate the configurations of asymmetric carbon atoms in different molecules if one knew at least one of the series but that it was not possible to infer from macroscopic models what the absolute configuration is at the microscopic level. So to be able to formulate all stereochemical relationships, he decided to assign one of the two possible absolute configurations to a reference compound based on optical activity, with the calculated risk of being 50% wrong.

Initially, Fischer adopted Van 't Hoff's notation using (+) and (−) signs, but in the second of his papers on sugar stereochemistry in 1891 he introduced his *projection*. He used tartaric acid for this purpose and he described it later in his autobiography as follows:

'*With the help of Friedländer's convenient rubber models, one can construct molecules of right-handed tartaric acid, left-handed tartaric acid, and inactive tartaric acid and lay them in the plane of the paper so that the four carbon atoms are in a straight line and the attached hydrogens and hydroxyls lie above the plane of the paper.*'

Fischer's projections did not show tetrahedral carbon atoms, but two-dimensional projections that were obtained when rubber models were literally squashed onto the paper. Fischer then defined *d*- and *l*-saccharic acid, choosing arbitrarily one of the two possible projections for the *d* isomer. All compounds that were correlated by chemical transformations to dextrorotatory glucose, related to *d*-saccharic acid, were classified as *d*, and all enantiomers as *l*. However, lower and uppercase *d* and *l* had been used before to specify the sign of rotation, *i.e.* in place of (+) and (−), and later a new convention was reached which introduced small capital letters D and L.

Emil Fischer received the second Nobel Prize in Chemistry in 1902, the first to be awarded for research in organic chemistry, for work on purines and sugars. His stereochemical correlations were not flawless, and in 1906 Martin Andre Rosanoff (1874–1951), a Russian born American chemist, took the idea further to remove some erroneous logic and correlated sugars with (+) and (−) glyceraldehydes. He also tried to replace Fischers D and L notation with δ and λ, but that was not accepted. The system based on Rosanoff's principles but using D and L notation is referred to as the Fischer–Rosanoff notation.

Even Rosanoff's work did not clarify completely the stereochemistry of tartrates. The problem was that (+)-tartaric acid could be correlated with either (+)- or (−)-glyceraldehydes depending on the reaction pathway. The quest for the elucidation of absolute configuration seemed endless.

8. Seeing is not always believing: the structure of diamond

In 1912, at the suggestion of Max von Laue (1879–1960), Paul Friedrich and Walter Knipping exposed zincblende (a cubic form of zinc sulfide) to X-rays, discovered by Wilhelm Conrad
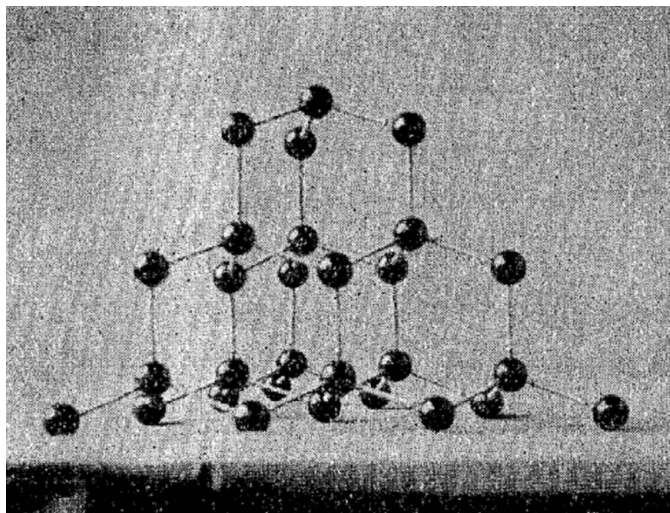
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Roentgen (1845–1923) in 1895, and recorded the first X-ray diffraction pattern. In June 1913, William Lawrence Bragg (1890–1971) published his investigation of alkaline halides, including sodium chloride, starting with a new interpretation of the diffraction phenomenon involving the relationship $n\lambda = 2d \sin \theta$. The paper appeared in the *Proceedings of the Royal Society of London* and was communicated by the author's father, William Henry Bragg, F.R.S. (1862–1942). By strange coincidence, at the age of five William Lawrence became the first recorded person ever to be X-rayed in his native Australia: he fell off his bike, broke his arm, and was X-rayed by his father, then a Professor at the University of Adelaide.

On 30 July 1913, the Braggs submitted another paper entitled *The Structure of Diamond*. In this paper, they expanded on the difference between the Laue method which utilizes a broad spectrum of X-rays, or *Bremsstrahlung* (referred to as heterogeneous X-rays in the paper) and the 'reflection method', which utilizes the famous Bragg relationship and monochromatic (or homogeneous) X-rays. Since filters were not yet in use, the Braggs searched for anticathode material that would give strong characteristic lines. They note that platinum, used by Friedrich and Knipping, gives intense background radiation, while rhodium gives very strong characteristic radiation at $\lambda = 0.607$ and 0.533 Å. Accordingly, they used rhodium (Rh) radiation to record spectra from diamonds and arrived at the correct structure (Fig. 4):

'*The union of every carbon atom to four neighbors in a perfectly symmetrical way might be expected in view of the persistent tetravalency of carbon. The linking of six carbon atoms into a ring is also an obvious feature of the structure.*'

Interestingly, nowhere do the Braggs allude to Van 't Hoff's model, nor do they hint at the obvious stereochemical implications. This is perhaps because it was not necessarily obvious to them whether the packing of carbon atoms in the cubic crystals of diamond actually reflected covalent structure. They followed the previous conclusion with a cautious statement:
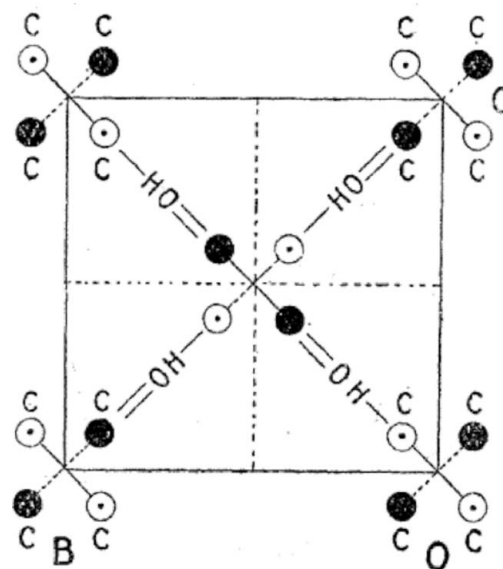
'*But it would not be right to lay much on these facts at present, since other crystals which do not contain carbon atoms possess, apparently, a similar structure.*'

Thus, the spectacular validation of Van 't Hoff's proposal seems to have come and gone without much fanfare. Both Braggs were awarded a shared Nobel Prize in Physics in 1915 but due to the outbreak of World War I it was not until 1922 that William Lawrence Bragg delivered his Nobel Lecture. Although he showed the structure of diamond, he barely mentions it in the lecture and never expands on the tetrahedral carbon atom. William Henry Bragg was knighted in 1920 and his son in 1941.

9. William T. Astbury

While the structure of inorganic salts and diamond could be inferred from relatively simple considerations of unit-cell dimensions, packing and atomic volumes, organic structures presented a much greater challenge. At the top of everyone's list – naturally – was tartaric acid. The first to take up the challenge was William T. Astbury (1889–1961).

Born in Longton (in the Stoke district) to a potter's turner, Astbury had to support his entire education through scholarships. In Cambridge, he won scholarships in chemistry, physics and mathematics, but his studies at Jesus College were interrupted by the outbreak of World War I. After his return to Cambridge in 1919, Astbury became interested in crystallography through a course in mineralogy taught by Arthur Hutchinson (1866–1937). Hutchinson was a fabulous teacher and John Desmond Bernal (1901–1971) recorded that his course was '*by far the best in crystallography to be given in the*

Figure 4
A model of diamond based on the crystal structure determined by the Braggs. Reprinted with permission from Bragg & Bragg (1913). *Proc. R. Soc. London Ser. A*, **89**, 277–291. Copyright (1913) The Royal Society of London.

Figure 5
William Astbury's failed attempt at the elucidation of the structure of tartaric acid. Reprinted with permission from Astbury (1923). *Proc. R. Soc. London Ser. A*, **102**, 506–528. Copyright (1923) The Royal Society of London.

United Kingdom', illustrated with models and apparatus of his own design. Prior to his appointment in Cambridge in 1895, Hutchinson studied chemistry with Emil Fisher at Wüzburg and attended lectures in physics by Roentgen. After World War I, Hutchinson followed closely the developments in the new area of X-ray studies of crystal structures and in 1921 recommended Astbury to Sir William Henry Bragg at University College, London.

According to Bernal, Astbury '*knew more crystallography than either Sir William or Sir Lawrence Bragg.*' He is remembered, of course, for his contributions to the early studies of protein and DNA structure, but what is less well known is that Astbury's first two scientific papers dealt with the question of the relationship between optical rotation and molecular structure of tartaric acid. The first paper, *The Crystalline Structure and Properties of Tartaric Acid*, was published in the *Proceedings of the Royal Society* on 1 February 1923. This was probably the first diffraction study of a monoclinic crystal, and only a handful of reflections were measured using Bragg's ionization spectrometer, using a Coolidge X-ray tube with a molybdenum anticathode. Needless to say, Astbury did not really find the correct structure (Fig. 5) because his structural arguments were based on unit-cell dimensions and packing inferred from Bragg's atomic radii. Perhaps the most notable is Astbury's conclusion that '*It is shown that it is impossible by the diffraction of X-rays to distinguish between the dextro- and laevo- forms of an optically active body.*' His second paper, also communicated by Sir William, appeared on 1 September 1923 and dealt with the structure of the anhydrous racemic acid. As before, the details were not quite right, but he concluded that '*The fundamental cell is associated with one molecule of the right-handed tartaric acid and one molecule of the left-handed tartaric acid. There is no evidence from X-ray examination that racemic acid exists as an independent doublet of molecular weight 300.*'

Astbury's ground-breaking work on protein and DNA structure belongs to another story. He received many honors but did not earn a Nobel Prize.

10. Structure at last

With the introduction of the Fourier and Patterson techniques in the 1920s and 1930s, organic structures finally moved within reach of rigorous X-ray diffraction analysis. The time had arrived for a detailed structure determination of a tartrate.

The first was that of the Seignette salt ($NaKC_4H_4O_6\cdot4H_2O$), published in August 1940 in the *Proceedings of the Royal Society* by C. Arnold Beevers & W. Hughes. By the standards of the day, the task was formidable: 47 parameters had to be determined and the difficulties were compounded by lack of a center of symmetry ($P2_12_12$) and the need to determine phases rather than signs of reflections. The structure was solved by the canonical isomorphous replacement method, with ammonium, potassium, rubidium and thallium salts used as derivatives. Data were obtained from Weissenberg photographs and the structure was determined using Patterson synthesis and Fourier analysis. The tartrate molecule was found to have its four backbone carbon atoms almost exactly coplanar (Fig. 6). Tetrahedral coordination of water is observed and found to be in agreement with the Bernal–Fowler structure. Interestingly, there is no discussion in the paper of the absolute configuration.

It took another eight years before C. A. Beevers and F. Stern published in 1948 a short note in *Nature* on the structure of D-tartaric acid. This was a more difficult case because, unlike in the case of the Seignette salt, there were no candidate atoms for isomorphous replacement. The structure was solved from Patterson maps and Fourier projections were used to evaluate the final model, which took another two years before it was published in *Acta Crystallographica* in 1950. Crystallographic R factors for the three zones $F(0kl)$, $F(h0l)$ and $F(hk0)$ were 0.26, 0.29 and 0.30, leaving clearly a bit to the imagination by today's standards. Nonetheless, the authors proclaimed the coordinates to be within ±0.05 Å and concluded that the molecule of tartaric acid had a general configuration identical with that found in the Seignette salt. In the last paragraph it is stressed that:

'... *the methods described in this paper cannot distinguish between the* D- *and* L-*forms of an optically active compound, since parameters derived from those given by the operation of a center of symmetry would give the same intensities.*'

Shortly after this publication, G. S. Parry published his structural investigation of the triclinic ($P\bar{1}$) monohydrate of racemic tartaric acid, easier to crystallize than Astbury's anhydrous species. In this case, in addition to Patterson and Fourier methods, the author also used Harker–Kasper inequalities to obtain some phase relationships for $h0l$ reflections. The crystallographic R factors for the three zones were 0.19, 0.23 and 0.26. Yet again, the structure turned out to be essentially the same as in the Seignette salt and D-tartaric
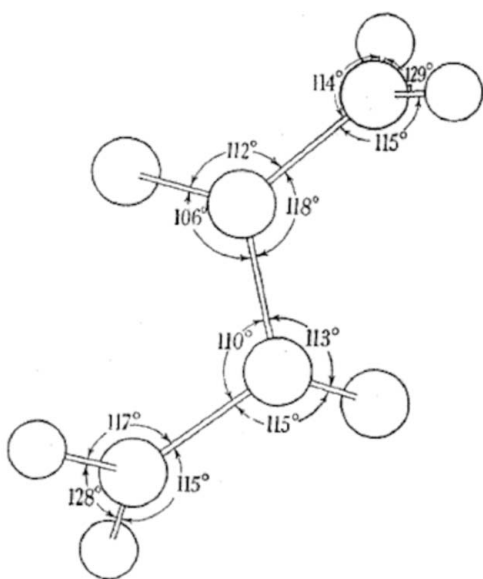
Figure 6
The structure of the tartrate moiety in the Seignette (Rochelle) salt as determined by Beevers and Hughes. Reprinted with permission from Beevers & Hughes (1941). *Proc. R. Soc. London Ser. A*, **177**, 251–259. Copyright (1941) The Royal Society of London.

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acid except that, as expected, an enantiomeric pair of molecules was observed.

Only one enigma remained to be solved: the absolute configuration.

11. Bijvoet makes a difference

In spite of the phenomenal advances in crystallography, which by 1950 had boldly focused on proteins and viruses, X-ray diffraction suffered from the serious limitation, lucidly explained by Astbury and then by Beevers and Stern, that the absolute configuration seemed unattainable. The problem was originally defined in 1913 by the French crystallographer Georges Friedel (1865–1933): the diffraction pattern is centrosymmetric and therefore would forever obscure the absolute configuration of an asymmetric molecule.

As early as 1925, Hermann Francis Mark (1895−1992) and his postdoctoral fellow Leo Szilard (1898–1964), working at the Kaiser Wilhelm Institut in Berlin demonstrated using rubidium bromide crystals, and strontium and bromide radiation, that when the scattered frequency is in the neighborhood of one of the '*characteristic frequencies of the scattering element*', *i.e.* near an absorption edge, the phenomenon is no longer independent of wavelength. (It is perhaps worth recalling as an aside that Mark was a pioneer of crystallography and structural chemistry, considered to be one of the fathers of polymer chemistry, and it was he who recommended one of his students, Max Ferdinand Perutz, to John Desmond Bernal in Cambridge, and suggested that Perutz study protein structure. The rest is history. Leo Szilard went on to demonstrate the chain reaction.) The 'anomalous dispersion' observed by Mark and Szilard was then taken up in 1930 by Coster, Knol and Prins who showed that the 111 and $\overline{1}\overline{1}\overline{1}$ reflections of zincblende have unequal intensities when the wavelength is close to the zinc absorption edge. A. J. Bradley and R. A. H. Hope followed in 1932 with studies of FeAl

Figure 7
X-ray powder photographs of FeAl using different radiations: (*a*) Mo *Kα* radiation; (*b*) Co *Kα* and *Kβ* radiations; (*c*) Co *Kα* and *Kβ* radiations; (*d*) Fe *Kα* and *Kβ* radiations; (*e*) Cr *Kα* and *Kβ* radiations. Reprinted with permission from Bradley & Hope (1932). *Proc. Roc. Soc. London Ser. A*, **136**, 272–288. Copyright (1932) The Royal Society of London.

powder where, due to iron atoms occupying the corners and aluminium atoms the centers of the unit cells, the structure factors are either $f_{Fe} + f_{Al}$ or $f_{Fe} - f_{Al}$ (Fig. 7). A method for calculation of the atomic form factor for X-rays in the region of anomalous dispersion was then proposed by D. Coster and A. J. Knol who in their 1933 paper noted that '*mathematically speaking the atomic factor is in general ... a complex quantity of which not only the modulus but also the argument (i.e. the phase difference between the primary and the scattered beam) is a function of the wavelength and the angle θ.*' It took the brilliant mind of Johannes Martin Bijvoet (1892–1980) to realize what the consequences are. Bijvoet became interested in crystals at a young age in high school, when a visiting physicist demonstrated birefringence. After two years of studies in Greek and Latin, Bijvoet entered the University of Amsterdam to study chemistry, physics, astronomy and mathematics. He served in the army in World War I, although The Netherlands stayed out of the war and Bijvoet never had to fight. He resumed his studies as a doctoral student in chemistry and physics, and was particularly impressed with the lectures of J. D. van der Waals Jr, the son of the Nobel Prize winner for physics in 1910. In 1919, after graduating *cum laude*, Bijvoet became an assistant to Professor A. Smits in the Department of Inorganic Chemistry. The structure of sodium chloride had been published by the Braggs a few years earlier and Smits was highly skeptical: like other traditional chemists, he would not accept the notion that there are no identifiable NaCl molecules. Bijvoet and his friend A. Karssen decided to pursue X-ray diffraction and to investigate the properties of salts for themselves. This endeavor resulted in the determination of the structures of lithium and lithium hydride, which Bijvoet wrote up in his doctoral dissertation in 1923, as well as sodium chlorate and sodium bromate. Crystallography became a lifetime passion for Bijvoet. He served as a Reader at the University of Amsterdam until 1939, and then moved to Utrecht to become the Chair of General and Inorganic Chemistry at the State University. By a strange turn of events, for the first 13 years he worked in the Van 't Hoff Laboratory.

In Utrecht, Bijvoet's attention moved to the complex problems of organic chemistry and structure of non-centrosymmetric compounds. He set out to determine the structure of strychnine, which could be fortuitously crystallized with *C*2 symmetry as isomorphous selenate or sulfate salt. Bijvoet realized that a single-isomorphous-replacement procedure might be used to determine the structure. The signs of structure factors for both salts could be easily determined for the centric [0,1,0] projection from isomorphous differences, as was first done for phthalocyanine. The non-centrosymmetric [0,0,1] projection presented a problem because phase angles and not signs had to be determined, and a pair of isomorphous crystals yielded the obvious ambiguity. Bijvoet suggested that a symmetrical Fourier synthesis can be used with every term included twice, using both phase angles, leading to an electron-density map with a superposition of two mirror images of the molecule. The [0,1,0] projection along with stereochemical considerations was used to resolve the image and provide the structural formula. Bijvoet became immediately aware that
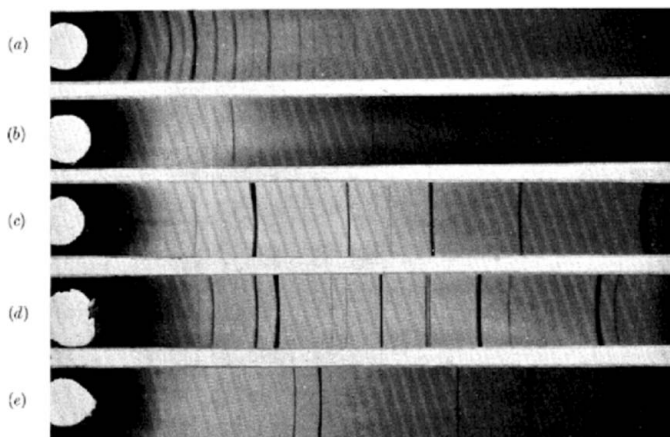
two derivatives would yield unambiguous phases. A bromide salt of strychnine was under investigation in Glasgow by J. H. Robertson and C. A. Beevers and Bijvoet noted in his 1951 paper in *Acta Crystallographica*:

'*In order to avoid the double model the replacement method could be extended as follows: Consider three substances, namely, (I) a-chlorine strychnine sulphate, (II) a-chlorine strychnine selenate and (III) a-bromine strychnine sulphate which are isomorphous. Now comparison between (I) and (II) will give two possibilities for the phase angle of every reflexion of substance (I). Comparison between (I) and (III) also will give two possibilities for the α values of (I). If both DF vectors are different – and mostly they are – both pairs of values must have one in common. So for nearly every reflexion the α value can be determined and one model will result. As the relative positions of chlorine and sulphur will result from Patterson syntheses, there are two possibilities which would give two models, the separate image or mirror image.*'

While this is the first explicit formulation of the double-isomorphous-replacement approach, it failed to address the choice of origin and the identification of the correct enantiomorph. These questions were raised in the famous paper by David Harker in 1956, who elaborated on Bijvoet's arguments and extended its application to proteins.

As early as in 1949, Bijvoet also realized that an anomalous signal can be used to identify the correct enantiomorph. He wrote:

'*There is in principle a general way of determining the sign [of a phase angle]. ... We can use the abnormal scattering of an atom for a wavelength just beyond its absorption limit. ... It also becomes possible to attribute the d or l structure to an optically active compound on actual grounds and not merely by a basic convention.*'

Together with colleagues A. F. Peerdeman and A. J. van Bommel, Bijvoet went on to prove the point experimentally. What better model system than tartaric acid? The Rb salt was a perfect choice and for the first time Fischer's convention was put to an experimental test. Rubidium's absorption edge is at 0.8157 Å, and Bijvoet used Zr $K\alpha$ radiation (0.7859 Å) to

obtain the anomalous signal. The results were published in a short note to *Nature* on 18 August 1951 (essentially the same work was published a few months earlier in the *Proceedings of the Royal Netherlands Academy of Sciences*, although notably the order of authors is altered with Peerdeman being first). It turned out that Fischer's convention was correct (Fig. 8).

The impact of this work was immediate. Only months prior to Bijvoet's papers, the configurational relationships between carbohydrates, hydroxyl acids and amino acids had been worked out systematically and described by P. Brewster, E. D. Hughes, C. K. Ingold and P. A. D. S. Rao in their lucid 1950 *Nature* article. Once the absolute configuration of tartaric was established, nearly all of these compounds could have their absolute configurations inferred systematically from that reference point. However, Bijoet additionally went on to confirm the absolute configuration of amino acids with his work on the D-(−)-isoleucine. At the time when this work was undertaken, already 15 amino acids had been characterized by crystallography, but their absolute configuration had not been directly validated. Bijvoet used the hydrobromide salt crystals and to obtain useful anomalous data for the Br K edge (λ = 0.918 Å) he chose to use U $L\alpha_1$ radiation (λ = 0.9087 Å), monochromated with a curved quartz crystal. His X-ray tube could withstand a maximum of 100 W and 400 h exposure was required to obtain one first-layer Weissenberg photograph. Intensities for Friedel pairs were estimated visually '*by a few people independently*' and the D configuration was confirmed as expected. The results were published in 1954 with J. Trommel in *Acta Crystallographica*.

12. What's in a name?

Interestingly, while the term 'stereochemistry' was already in wide use, you will look in vain for any reference to 'chirality' in Bijvoet's paper on tartaric acid absolute configuration. The term chirality was originally introduced to chemistry by William Thomson (1824−1907), later in life known as Lord Kelvin, or more precisely First Baron Kelvin of Largs, OM, GCVO, PC, PRS, FRSE. Kelvin was one of the most accomplished, most revered and most famous scientists in history. He published more than 600 scientific papers, filed over 70 patents and after he died his ashes were buried at the request of the Royal Society in the nave of Westminster Abbey, near the grave of Sir Isaac Newton. His contributions to science and engineering were many, including a key role in laying down the transatlantic cable in 1866, a venture which brought him fortune and a knighthood that year. His name (or more accurately the name of a somewhat obscure river which flows past the University of Glasgow in Scotland) has been immortalized in the scale of absolute temperature which he developed. As an aside, it is also worth mentioning that Lord Kelvin made many assertions during his lifetime that – fortunately for him – he is not remembered for. He is on record stating that '*X-rays will prove to be a hoax*', and in 1900 declared that '*There is nothing new to be discovered in physics now. All that remains is more and more precise measurement.*'
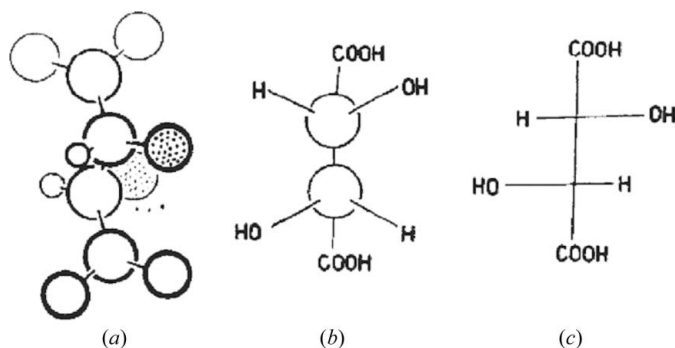
Figure 8
Absolute configuration of dextrorotatory tartaric acid as determined by Bijvoet: (*a*) crystal structure; (*b*) normalized configuration; (*c*) Fischer's projection. Reprinted by permission from Macmillan Publishers Ltd: Bijvoet, Peerdeman & van Bommel (1951). *Nature (London)*, **168**, 271–272. Copyright (1951).

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In 1884, Kelvin gave a series of 20 lectures at Johns Hopkins University in Baltimore. The Baltimore lectures were interactive and spontaneous, with an eclectic choice of subjects. It was not until 1904 that lecture notes were published, and in the Appendix they included the following statement:

'*I call any geometrical figure, or group of points, chiral, and say that it has chirality, if its image in a plane mirror, ideally realized, cannot be brought to coincide with itself.*'

Apparently the term chiral (from the Greek word *kheir*, hand) was never used in the Baltimore lectures but added at the time of publication of the notes, presumably by Kelvin himself. Greek-based terminology was coined by Kelvin at least as early as 1873 (*i.e.* a year *before* Van 't Hoff and Le Bel published their concept of a tetrahedral carbon atom) when he gave a lecture – whose text was never published – at the Royal Society of Edinburgh: *Note on Homocheiral and Heterocheiral Similarity*. With cheirality (and later chirality) confined to algebraic and topological problems, it is not difficult to understand why chemists ignored the term for nearly one century. It was re-introduced by Lancelot Law Whyte (1896–1972), a Scottish financier, engineer and author, in two letters that appeared in *Nature* both entitled *Chirality* in 1957 and 1958. It was permanently engraved into the chemical vocabulary by Kurt M. Mislow (1923–), a student of Linus Pauling (1901–1994), in his *Introduction to Stereochemistry* published in 1965, and by Robert S. Cahn (1899–1981), Sir Christopher K. Ingold (1893–1970) and Vladimir Prelog (1906–1998), in their classic 1966 paper *Specification of Molecular Chirality*.

Bijvoet's discovery of the absolute configuration of tartaric acid led directly to the revision of the existing unsatisfactory configurational notation. In November 1950, Robert S. Cahn and Sir Christopher K. Ingold had already established new principles for notation for tetrahedral carbon atoms and introduced the so-called sequence rules, according to which the ligands around the tetrahedral atom were first ordered in a sequence by atomic numbers and then used to establish clockwise or anticlockwise order (looking from the side opposite the ligand of lowest priority) to determine the D or L configuration, respectively. They still used D-glyceraldehyde as a reference point, but they added with prescience that "*The time will come when absolute configuration can be determined with certainty; and the 'standard substance' will be a redundant concept.*"

Six years later, in 1956, with the problem of absolute configuration elegantly resolved by Bijvoet, Vladimir Prelog joined Cahn and Ingold in an effort to further improve a notation that would establish a general and absolute system. To free the new paradigm from the shackles of earlier conventions, the authors abandoned the old D and L descriptors in favor of new ones, *i.e.* R and S, derived from Latin words *rectus* (right) and *sinister* (left). The system was further improved in the aforementioned 1966 paper by the same authors, which appeared in *Angewandte Chemie International Edition in English*.

Thus, the different forms of tartaric acid have finally earned accurate and precise names: the naturally occurring dextro-tartaric acid is (2R,3R)-2,3-dihydroxysuccinic acid, its enantiomeric laevo form became (2S,3S)-2,3-dihydroxysuccinic acid) and mesotartaric acid is (2R,3S)-2,3-dihydroxysuccinic acid.

13. From small to large: implications for biomolecular crystallography

Bijvoet's work had immediate implications for chemistry and biochemistry in general. This was well understood by Bijvoet and his colleagues. In the *Introduction* to *Anomalous Scattering*, a volume edited by Ramaseshan & Abrahams in 1975, A. F. Peerdeman remarks: '*It was about that time after the first elation over the success of our anomalous child that I had a feeling of slight disappointment because this child appeared to be anomalous in another respect. It seemed to be fully grown at its birth.*' Beyond everything that followed from the determination of absolute configuration, the work also laid foundations for the two techniques that were subsequently used to solve the phase problem in macromolecular crystallography: the multiple-isomorphous-replacement method, and single- and multiwavelength anomalous dispersion.

However, not everyone was immediately fully aware of the importance of these developments. It is interesting to note that the α-helix proposed in 1951 by Linus Pauling was actually ... left handed and made up of D-amino acids (*sic*!). This is strange, because Pauling may have actually known about Bijvoet's work – even prior to its publication – from J. G. Kirkwood (1907–1959), a colleague at Caltech, who also worked on absolute configuration and who independently confirmed Fischer's arbitrary assignment by comparing observed and calculated optical rotations. Pauling should have also been familiar with the *Nature* 1950 paper on the configurational relationships between carbohydrates and amino acids, in which Brewster and colleagues explicitly proved by stereospecific reactions that natural amino acids are the L enantiomers. However, surprising as it seems, this may not have been an important issue to Pauling. The handedness of the α-helix was of course effectively resolved by the crystal structure of myoglobin determined by John C. Kendrew (1917–1997).

In contrast to the stereochemical details of the α-helix, the double helix of the DNA was never in question. In their original model building, James D. Watson (1928–) and Francis H. C. Crick (1916–2004) used the standard configuration of β-D-deoxyribofuranose described in 1952 by Sven Furberg (1920–1983), who previously also discovered that the sugar and base rings were perpendicular to each other in DNA. Since this was based on the standard convention, by 1953 it was implicitly assumed to be correct. Nonetheless, in the lesser known expanded version of their double-helix model, as published in the *Proceedings of the Royal Society* in 1954 by Crick and Watson (note the reversed order of names compared to the *Nature* paper) inserted a footnote following the sentence on p. 87 that states: '*... we find by trial that the model can only be built in the right-handed sense*'; the footnote

asserts that: '*The Fischer convention has recently been shown to be correct (Bijvoet, Peerdeman and van Bommel, 1951)*.'

The recognition of Bijvoet's work for macromolecular crystallography took even longer. Even though Max Perutz (1914–2002) struggled for years to solve the phase problem for haemoglobin, he overlooked Bijvoet's results. In fact, both John D. Bernal and J. M. Robertson suggested in 1939 that isomorphous replacement might be the way to determine phases for insulin, but these were just 'hunches' that Max Perutz did not take seriously because he did not see how a single mercury atom (specifically suggested by Robertson) could affect diffraction of thousands of carbon, nitrogen and oxygen atoms. Neither did Perutz pay much attention to A. J. C. Wilson's paper on data statistics, since he did not think it was relevant. Even with a mercury derivative in hand, and having established that heavy atoms make a significant contribution to diffraction because their electrons scatter in phase in contrast to the protein's light atoms that largely cancel each other due to interference, Perutz was still oblivious to Bijvoet's work. It took Dorothy Hodgkin to bring Bijvoet's 1951 paper on strychnine to Perutz's attention and to suggest that a multi-isomorphous approach would solve the problem in the general non-centrosymmetric case. She told Perutz: '*when a paper is written by a person like Bijvoet, you must read each word and sentence very, very carefully.*'

While the development of phasing methods in macromolecular crystallography is a separate tale, it is important to remember that tartaric acid was a critical stepping stone on the way to structures of viruses, ribosomes and other large biological complexes.

14. Epilogue

Following the pioneering determination of the absolute configuration of rubidium sodium tartrate, Bijvoet initiated studies of other tartrate salts. In 1958, along with A. J. van Bommel, he completed the refinement of the ammonium hydrogen D-tartrate, using intensities measured by a Geiger counter and a refinement protocol that used anisotropic temperature-factor parameters. As expected, he found the absolute configuration to be identical to that of the Rb–Na salt. D-tartaric acid was revisited again in 1966 by Y. Okaya and colleagues with the use of a computer-controlled diffractometer and neutron diffraction data. This allowed for unequivocal characterization of the stereochemistry of hydrogen bonds. The structure of D-tartaric acid monohydrate was reported in *Acta Crystallographica E* only recently, in 2006, by Qing-Bao Song and collaborators from Zhejiang and Shanghai. The structure of the racemic tartaric acid monohydrate, first reported by Parry (1951), was re-determined by Jing-Jing Nie and his colleagues in China and Taiwan, and published in 2001 in *Acta Crystallographica E*.

Research into chirality is still one of the most active fields in chemistry and structural biology, largely due to the implications for chiral drug design and synthesis. According to the Institute for Scientific Information Web of Knowledge Database, during the last decade there were over 8700 papers identified using the keyword 'chirality' and these papers elicited over 100000 citations.

Traditional experimental approaches to determining chirality fail when confronted with subtle problems. In a most recent *tours de force* published in *Nature*, Jaques Haesler, Werner Hug and colleagues from the University of Fribourg in Switzerland tested the limits of an experimental approach that utilizes Raman optical activity (ROA), *i.e.* circular polarization dependence of vibrational Raman scattering of visible laser light. With a new instrument, in which simultaneous data acquisition of right- and left-polarized Raman spectra eliminates most of the noise due to subtle laser fluctuations *etc.*, they directly determined the absolute configuration of (R)-[$^2H_1$,$^2H_2$,$^2H_3$]-neopentane, which is $C(CH_3)_4$ in which the four methyl substituents differ only by the degree of deuteration, *i.e.* $-CH_3$, $-CH_2D$, $-CHD_2$ and $-CD_3$ groups. Although the basic premise of ROA had been proposed in 1973, the new instrumentation pushes the sensitivity to allow measurements of circularly polarized components down to a few parts in 10^{-5} of the total band intensity. The methodology can be used to probe small molecules as well as large biological complexes including viruses, gathering information about the fold of coat proteins and the structure of DNA.

So let us raise a toast of a smooth Australian Cabernet Sauvignon – to tartaric acid!

Incidentally: in the 6th century BC, the Greeks already had a custom of toasting the health of their friends to assure them that the wine they were given was not poisoned, an otherwise perfectly acceptable social custom of dealing with problems such as a business competitor, a troublesome wife, or perhaps a hostile grant reviewer. To demonstrate friendship, the host would drink the wine before his guests and then raise his glass to his friends inviting them to share the drink. The Romans had similar social customs (*i.e.* poisoning wives and neighbors, especially when inconveniently far from the cliffs of Capri) and the practice of toasting was continued. The term 'toast' (in case you wondered) comes from the Roman practice of dropping a piece of burnt bread into the wine. This was done to temper some of the bad wines the Romans sometimes had to drink. This custom survived and, at the beginning of Scene V, Act III in Shakespeare's *Merry Wives of Windsor*, Falstaff calls '*Go fetch me a quart of sack; put toast in't*.' The charcoal reduces the excess of tartaric acid in wines that are past their prime, making them more drinkable.

But that is an entirely different story ...

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University of Virginia. Additional reading is given in the references as well as those articles cited in the text.

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