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Stereochemistry

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Optical activity and stereoisomerism

In the first half of the 19th century various aspects of geometrical optics such as diffraction, interference and the polarization of light were of interest to a number of scientists, especially in France. Although a relationship between the phenomenon of optical rotation and the structure of matter was assumed by the early investigators, the experimental studies that ultimately served as the key to an understanding of the relationship were those of Pasteur in the late 1840s and Wislicenus in the late 1860s.

The phenomenon of polarized light was suggested in 1690 in the studies of Christian Huygens (1629–1695) who, as a result of his study of the behavior of light through crystals of Iceland spar (calcite, a form of $CaCO_3$), concluded that the crystals imparted to the waves of light 'a certain form or disposition' which enabled them to give either a single or double image. (The double refraction of light by Iceland spar was first observed by Erasmus Bartholinus in 1669.) In 1809–10, E. L. Malus noted that the light reflected from a transparent surface such as water or glass '. . . has all the characteristics of one of the beams produced by the double refraction of a crystal of spar. . .'. Malus used the term 'polarization' to describe the character of the light. In 1821, Augustin Fresnel stated that the vibrations of light could not be longitudinal, but must be transverse, and 'that the vibrations of a polarized beam must be perpendicular to what is called its plane of polarization'.

The French physicist, Jean Baptiste Biot (1774–1862) (Plate 6), was the first to investigate the phenomenon of optical activity. In a series of studies undertaken from 1811 to 1817, he observed the rotation of the plane of polarization when polarized light was passed through a piece of quartz that had been cut perpendicular to its axis. The amount of rotation was proportional to the thickness of the quartz. (By 1818, he had also demonstrated that the rotation was '. . . inversely proportional to the squares of the lengths of their vibrations in the system of waves' (that is,

Plate 6. Jean Baptiste Biot (1774–1862) (Edgar Fahs Smith Collection).

$\alpha = k/\lambda^2$, where α = the rotation, k = a constant, and λ = the wavelength of the light).

One of the more interesting discoveries made in this period was that the optical activity was not restricted to crystalline substances, but was observed in certain liquids and solutions. The optical activity of some natural liquids (oil of turpentine, oil of lemon, and camphor dissolved in alcohol) was reported in 1815–17. The optical activity of sugar solutions was observed in 1818. (The 'inversion' of the optical rotation of an acidified sugar solution due to the hydrolysis of the sugar was not observed until 1832.)

Biot had also noted that some species of quartz rotated polarized light in opposite directions, an observation that he was unable to explain after he had examined the crystal shapes. A typical crystal of quartz might be considered to have a shape approximating a hexagonal prism. Early in the century, the Abbé Haüy had observed the location of small facets at the top edges of quartz crystals that reduced the crystal symmetry. He noted that the placement of these facets (originally called 'plagihedral', later 'hemihedral') was such that the mirror image of the crystal would not be superimposable on itself – that is to say, there were 'left-handed' and 'right-handed' crystals. It was not until 1820 that John F. W. Herschel correlated the sign of rotation of plane-polarized light observed by Biot with the 'handedness' of the crystal. Herschel also observed that the chemical dissolution of an optically active piece of quartz destroyed the activity. Herschel concluded that greater attention should be paid to the presence or absence of hemihedral facets, a suggestion taken up by Pasteur some 25 years later in his studies of tartrate crystals.

Tartar is a crude form of potassium acid tartrate ($KHC_4H_4O_6$) that had been isolated as a deposit formed during wine fermentation. The free acid, tartaric acid ($C_4H_6O_4$), was first isolated by Carl Wilhelm Scheele (1742–1786) in 1769 and subsequently was produced commercially from tartar. In 1821 Charles Kestner, a manufacturer of chemicals in the Vosges in Alsace, obtained several hundred kilograms of a solid from a wine fermentation. Since the solid had different solubility properties from the tartar normally isolated, Kestner thought it might be oxalic acid. The supply obtained by Kestner was the only available to chemists for analysis for another 30 years. In 1828, Joseph Louis Gay-Lussac (1778–1850) determined that the new acid had the same chemical composition as tartaric acid and he accordingly named it 'acide racémique' (from the Latin *racemus*, grape). It was known as racemic acid in English. Berzelius used the name 'paratartaric acid' in 1832 and cited the existence of the two acids as an example of isomerism. In the discussions that follow, the names 'tartaric acid' and 'racemic acid' will be used in their original meaning. Upon the conclusion of the historical discussions that led to the understanding of the relationship between the two, the modern names will then be used.

In 1838, Biot reported that racemic acid was optically inactive, in contrast to tartaric acid which he had observed was optically active. Biot had attempted to ascertain the cause of the difference in properties but without success. Berzelius thought that the isomerism in the two acids might manifest itself in differences in the crystal structures of the salts. Since his studies were not very fruitful, he encouraged Eilhardt Mitscherlich (1794–1863) to pursue the study further since Mitscherlich had made considerable contributions to crystallography. By 1831, Mitscherlich had found that only the sodium-ammonium double salt of each had the same crystal form. Mitscherlich, however, did not publish the results of his investigations. He did not take any interest in the problem for another ten years.

In 1841, Frédéric Hervé de la Provostaye published a series of papers on crystallography. One of these was concerned with the crystal forms of tartaric acid and racemic acid. He found that in general the crystals of the various tartrate and racemate salts were not isomorphic. (The law of isomorphism had been formulated in 1819 by Mitscherlich. The law stated that the crystalline form of two substances should be the same if the number of combined atoms was the same. This would be expected to be

Plate 7. Louis Pasteur (1822–1895) in 1857 (Institut Pasteur).

the case even when the constituent elements were not identical. For example, crystals of the potassium and sodium salts should be isomorphic.) He had hoped to learn whether or not isomeric compounds should produce isomorphic crystals. In 1848, Louis Pasteur (1822–1895) (Plate 7), who was then an assistant to Biot, undertook a reinvestigation of de la Provostaye's work in order to give him some experience in the study of crystallography. He confirmed de la Provostaye's observations but with one important exception. He observed that the crystals of tartaric acid and its salts exhibited hemihedral facets. Racemic acid crystals, on the other hand, did not show this hemihedrism. Pasteur was therefore surprised to read a communication, published in 1844, in which Mitscherlich reported on the crystallographic identity of the sodium-ammonium salt of tartaric acid and racemic acid:

> The double paratartrate and the double tartrate of soda and ammonia have the same chemical composition [$(Na)(NH_4)C_4H_4O_6$], the same crystalline form with the same angles, the same specific weight, the same double refraction, and consequently the same inclination in the optical axes. When dissolved in water their refraction is the same. But the dissolved tartrate deviates the plane of polarization, while the paratartrate is indifferent, as has been found by M. Biot for the whole series of those two kinds of salts. Yet, here the nature and number of the atoms, their arrangement and distances, are the same in the two substances compared.

Pasteur had some difficulty in believing this conclusion since he assumed that the inactivity of the racemate salt should manifest itself in a crystal displaying no hemihedral facets. Pasteur prepared the sodium-ammonium salt and noted that not only did the tartrate contain the hemihedral facets, but to his surprise, the racemate as well. There was, however, a subtle difference between the two that had escaped Mitscherlich:

> Only the hemihedral facets in the tartrates all lay in the same direction; in the racemates some lay toward the right and some toward the left. In spite of the unexpected character of this result, I continued to follow up my idea. I carefully separated the crystals which were hemihedral to the right from those hemihedral to the left and examined their solutions separately in the polarizing apparatus. I then saw with no less surprise than pleasure that the crystals hemihedral to the right deviated the plane of polarization to the right, and that those hemihedral to the left deviated it to the left, and when I took an equal weight of each of the two kinds of crystals, the mixed solution was indifferent towards the light in consequence of the neutralization of the two equal and opposite individual deviations.

Thus, Pasteur had discovered that the cause of the optical inactivity of the racemic acid was due to it being a mixture of 'right-handed' and 'left-handed' tartaric acid. He had discovered the phenomenon of enantiomerism (or 'enantiomorphism' as it was called earlier) in the racemate crystals. Pasteur's success is even more remarkable when you consider that above approximately 26 °C (79 °F) both the tartrate and racemate crystallize as a monohydrate having no hemihedral faces or asymmetry. When Biot was told of the result, he insisted that Pasteur repeat the experiment in his presence. After Biot had prepared the solution from the two kinds of crystals and had seen they were optically active, Pasteur related: 'The illustrious old man took me by the arm and said: "My dear child, I have loved science so much throughout my life that this makes my heart throb." '

Since the hemihedrism of the crystals is not always easily perceived, Pasteur prepared numerous crystal models to illustrate his lectures and also for Biot, whose eyesight was failing. Figure 17 shows two of Pasteur's models of the enantiomeric tartrate crystals.

The results of the studies undertaken by Pasteur in the next decade were no less remarkable. By the 1850s, he had discovered optical activity associated with a number of organic substances such as asparagine,

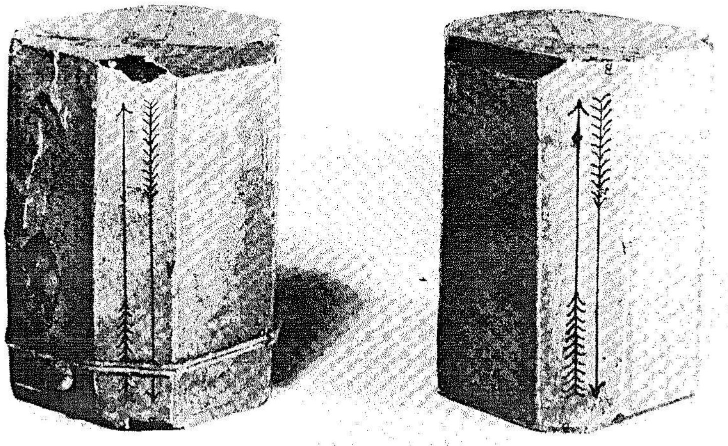


Figure 17. Pasteur's models of enantiomeric tartrate crystals (Institut Pasteur).

aspartic acid and malic acid. He also observed that the isomeric compounds, fumaric acid and maleic acid, were optically inactive. A rather interesting study was made by Pasteur of the crystals of strontium formate which were observed to be hemihedral and optically active. A solution of strontium formate, however, was optically inactive. He concluded that the hemihedrism of the crystal was

> . . . not due to the arrangement of the atoms in the chemical molecule, but to the arrangement of the physical molecules in the whole crystal, such that when the crystalline structure disappears in the act of dissolution there is no more dissymmetry.

This substance provides a second example (the other is quartz) of a substance whose optical activity is observed only in the crystalline form and on which can be observed the hemihedral facets. However, Pasteur also found that the presence of hemihedrism in the crystalline form is not always predictive of optical activity of solutions. For example, amyl alcohol (2-methyl-1-butanol) obtained from certain natural sources is optically active in solutions or as a liquid. Crystals of the barium sulphamylate of the alcohol do not display any hemihedral facets. Thus

we can see that the absence or presence of hemihedrism in crystals cannot be used to predict the optical activity of the solutions. In this regard, Pasteur's investigation of malic acid, $HOOCH_2CH(OH)COOH$, might have catalyzed the final stereochemical revolution that Wislicenus' study of lactic acid, $CH_3CH(OH)COOH$, did a decade later. For a number of reasons, it did not.

In 1850, M. Dessaignes reported that he had prepared aspartic acid, $HOOCCH_2CH(NH_2)COOH$, by the thermal decomposition of either ammonium malate or ammonium fumarate. Aspartic acid obtained from natural sources is optically active. Pasteur considered it inconceivable that a laboratory synthesis could produce an optically active substance, even a racemic mixture, from an optically inactive substance. The synthesis of aspartic acid from the optically active malic acid was acceptable to Pasteur, but it was difficult to believe that it might be prepared from fumaric acid. Pasteur obtained samples of the synthetic aspartic acid and determined that they were both optically inactive. Pasteur then converted some of the 'synthetic' aspartic acid to malic acid. On discovering that the malic acid was optically inactive, he concluded:

> Is it not evident that we have here to deal with a malic acid identical with the natural one [optically active], except for the simple suppression of its molecular dissymmetry? It is natural malic acid *untwisted*, if I may so express myself. The natural acid is a spiral stair as regards the arrangement of its atoms, this acid is the same stair made of the steps but straight in place instead of being spiral.
>
> It might be asked whether the new malic acid was not the racemic form of this group, that is, the compound of the right and left malic acids. This has very slight probability, for in this case not only would one active body be made from an inactive substance, but two active bodies would be produced, a dextro- and levorotatory substance.

Pasteur was thus convinced that it was impossible to synthesize an optically active substance – even if it was present in a racemic mixture. He therefore never attempted a chemical resolution (a procedure he developed about the same time) of the inactive product into its enantiomers. The fact that the crystals of malic acid do not exhibit hemihedral facets undoubtedly contributed to his thought that the malic acid was inactive due to its 'untwisted' symmetrical molecular form. We have seen, however, that the presence of crystal hemihedrism is not always essential to the prediction of molecular chirality.

However, Pasteur's views on the causes of the optical inactivity of malic acid led him to predict the existence of a second inactive form of tartaric acid. In 1853, Pasteur heated a solution containing tartaric acid and the alkaloid, cinchonicine. From the mixture he obtained not only racemic acid but also an optically inactive isomeric compound. From the discussion above, it is not difficult to understand Pasteur's surprise at finding racemic acid as a product – since its synthesis would involve a laboratory conversion of (+)-tartaric acid to (–)-tartaric acid. The optical inactivity of the new isomer was thought by Pasteur to arise from the 'untwisting' of ordinary tartaric acid. For some time this substance was known as 'inactive tartaric acid' in contrast to 'racemic acid'.

By the 1860s, Pasteur had classified the tartaric acid isomerism as follows:

1. *Tartaric acid*. The optically active form obtained from wine fermentation: *dextro*-tartaric acid (now designated (+)-tartaric acid).

2. *Racemic acid* (or paratartaric acid). The compound is composed of a 50:50 mixture of the enantiomeric tartaric acid: *dextro*- and *levo*-tartaric acid (now designated (±)-tartaric acid).

3. *Inactive tartaric acid.* This was Pasteur's 'untwisted' tartaric acid, now designated as *meso*-tartaric acid.

In the 1850s, Pasteur devised a more efficient method (chemical resolution) of separating the enantiomers in a racemic mixture. The method was developed as a consequence of his observation of the solubility differences of the salts of tartaric acid prepared from optically active alkaloids:

> When one prepares the racemate of cinchonicine, for example, it always occurs for a certain concentration of liquor, that the first crystallization is in major part formed of the *levo*-tartrate of cinchonicine; the *dextro*-tartrate remains in the mother liquor. Parallel results present themselves with quinicine, only, in this case, it is the *dextro*-tartrate which is first removed.

In short, Pasteur had discovered that he could take advantage of the solubility differences of diastereomeric salts as a method of separating enantiomers. In 1858, Pasteur also observed that a yeast mould growing in a solution containing the (±)-tartrate destroyed the (+)-tartrate leaving the (–)-tartrate.

The availability of these two methods (the 'chemical' and 'biochemical' methods) for obtaining an optically active substance was soon exploited by organic chemists. The reports of the isolation of increasing numbers of optically active substances in the 1860's coincides with the growing use of graphic formulas by organic chemists to illustrate the chemical structures of organic compounds. However, it was almost another twenty years before a relationship was perceived between the phenomenon of optical activity and molecular structure. Many chemists persisted in the belief that the observation of optical activity had little chemical significance. This belief had been stated sometime earlier (1841) by Charles Gerhardt (1816–1856):

> We chemists require chemical differences to distinguish between two bodies, and it therefore seems to me that those who attach such great importance to rotatory power are deluding themselves strongly if they look to it for the future of chemistry.

Although Gerhardt wrote this before Pasteur's work was published, the attitude persisted in the minds of most chemists for many decades. Even in the 1860s, some chemists described compounds that were identical in all respects except in this physical property as 'physical isomers'.

Pasteur himself was close to understanding the relationship between optical activity and molecular structure, but the final solution escaped him because he had turned to non-chemical studies in the 1850s and was therefore not in a position to appreciate the insights provided by the introduction of a theory of valency. This can be seen in a lecture given by Pasteur in 1860 in which he speculated on the reason why the dissolution of substances, that are optically active in the solid state, sometimes results in the loss of optical activity, other times in the retention of activity:

> Imagine a spiral stair whose steps are cubes, or any other objects with superposable images. Destroy the stair and the dissymmetry will have vanished. The dissymmetry of the stair was simply the result of the mode of arrangement of the component steps. Imagine, on the other hand, the same spiral stair to be constructed with irregular tetrahedra for steps. Destroy the stair and the dissymmetry will still exist, since it is a question of a collection of tetrahedra!

Pasteur then went on to consider the possible geometries of the molecular species that might produce the dissymmetry (asymmetry) that correlated with the observation of optical activity:

> Are the atoms of the right acid grouped on the spirals of a dextrogyrate helix, or placed at the summits of an irregular tetrahedron, or disposed according to some particular dissymmetric grouping or other? We cannot answer these questions.

> But it cannot be doubted that there exists an arrangement of the atoms in a dissymmetric order, having a non-superposable image, and it is no less certain that the atoms of the *levo*-acid realize precisely the inverse dissymmetric grouping to this.

Pasteur's inattention to the developments in structural organic chemistry in the 1850s prevented him from considering the possible atomic arrangements of substances like tartaric acid that might produce the required 'dissymmetric groupings'. However, even the organic chemists who were aware of these developments considered Pasteur's proposals speculative inasmuch as there were difficulties enough in unraveling more conventional problems in organic chemistry. Only gradually did it become apparent that the phenomenon of 'optical isomerism' required a different theoretical interpretation than had been proposed to explain 'chemical' or 'structural' isomerism. The new interpretation arose from the experimental studies of the properties of a single compound, lactic acid.

Lactic acid had been discovered by Carl Wilhelm Scheele (1742–1786) in 1770 as a product isolated from sour milk. Although elemental analysis of the compound had been reported early in the 19th century, little was known of the chemical nature of the compound until the 1850s as the result of a controversy between Charles Wurtz and Hermann Kolbe. The basic difficulty revolved around the issue as to whether lactic acid should be considered 'monobasic' or 'dibasic'. To further complicate the issue was the report of the isolation of an acid from muscle tissue that had properties similar to lactic acid. This acid, referred to in the literature as 'sarcolactic acid' or 'paralactic acid', had the same chemical formula as lactic acid, but differed in its chemical and physical properties. By the early 1860s, those chemists concerned with the problem had concluded that the two acids were simply isomeric compounds: lactic acid corresponding to 2-hydroxypropanoic acid; sarcolactic acid, 3-hydroxypropanoic acid. One of the chemists who had become involved in the controversy was Johannes Adolf Wislicenus (1835–1902) (Plate 8). By 1863, he was persuaded that the two acids were not positional isomers. He concluded that the difference between the two could not be understood by the formulas then in use since they were limited to pictures of the substance in two dimensions. By the late 1860s, as the result of further experimental work, it was clear that the two acids were not positional isomers. But by this time, however, Wislicenus had discovered that whereas the substance known as lactic acid (isolated from sour milk) was optically inactive, sarcolactic acid was optically active. This observation was inexplicable to Wislicenus and suggested the existence of a new kind of isomerism:

Plate 8. Johannes Adolf Wislicenus (1835–1902).

> Thus is given the first certainly proved case in which the number of isomers exceeds the number of possible structures. Facts like these compel us to explain different isomeric molecules with the same structural formula by different positions of their atoms in space and to seek for definite representations of these.

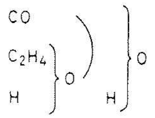
It is interesting to note that Kekulé merely summarized Wislicenus' remarks in a published account of the meeting at which Wislicenus spoke in 1869. Kekulé did not consider how his models might be used to solve the dilemma posed by Wislicenus:

> The speaker drew our attention to the fact that the existence of three hydroxypropionic acids demonstrated the limitations of the structural formulas in general use, and also of the views that are usually expressed by these formulas. Such subtler cases of isomerism might perhaps be explained by the spatial representation of the combination of atoms, that is, by models.

Wislicenus continued to work on the problem for another four years but came no closer to understanding the cause of the differences. In 1873, he again concluded:

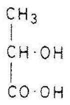
> If molecules can be structurally identical and yet possess dissimilar properties, this can be explained only on the ground that the difference is due to a different arrangement of the atoms in space.

The new kind of isomerism was described by Wislicenus as 'geometrical isomerism' – a term that took on a different meaning a few years later. That Wislicenus did not attempt to arrange the 'atoms in space' with some sort of models might seem unusual to us now. However, the 'type' formulas used earlier by Wislicenus do not lend themselves to illustrations with models as well as the Crum Brown–Kekulé formulas (Figs. 10–12, 14):



$$\left.\begin{matrix} \left.\begin{matrix} CO \\ \left.\begin{matrix} C_2H_4 \\ H \end{matrix}\right\} O \end{matrix}\right) \\ H \end{matrix}\right\} O$$

Type formula (1863)



$$\begin{matrix} CH_3 \\ | \\ CH\cdot OH \\ | \\ CO\cdot OH \end{matrix}$$

Graphic formula (1873)

By 1873, he was using graphic formulas which could have lent themselves to representations using the models developed by Kekulé. There is no evidence that he ever used these models, however. Perhaps Wislicenus did not wish to risk taking his time on such speculations when further experimental work was needed to establish for certainty that he was in fact observing a new kind of isomerism. As we have seen in the case of the paper published by Paternò, the literature contained a number of examples of reports of excessive isomers that later proved to be non-existent.

It is also of interest to point out that the cause of the optical inactivity of milk-lactic acid was never dealt with. No chemical resolution was attempted (this was also the case with Pasteur's inactive malic acid). Thus, that the enantiomer of sarcolactic acid might be present in the inactive milk-lactic acid was never revealed. One can only conclude that Pasteur's observations in 1860, and earlier, had not had the profound effect on the 'geometrical' speculations of organic chemists that we might suppose from our present perspective.

Plate 6. Jean Baptiste Biot (1774–1862) (Edgar Fahs Smith Collection).

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One of the more interesting discoveries made in this period was that the optical activity was not restricted to crystalline substances, but was observed in certain liquids and solutions. The optical activity of some natural liquids (oil of turpentine, oil of lemon, and camphor dissolved in alcohol) was reported in 1815–17. The optical activity of sugar solutions was observed in 1818. (The 'inversion' of the optical rotation of an acidified sugar solution due to the hydrolysis of the sugar was not observed until 1832.)

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Pasteur had some difficulty in believing this conclusion since he assumed that the inactivity of the racemate salt should manifest itself in a crystal displaying no hemihedral facets. Pasteur prepared the sodium-ammonium salt and noted that not only did the tartrate contain the hemihedral facets, but to his surprise, the racemate as well. There was, however, a subtle difference between the two that had escaped Mitscherlich:

> Only the hemihedral facets in the tartrates all lay in the same direction; in the racemates some lay toward the right and some toward the left. In spite of the unexpected character of this result, I continued to follow up my idea. I carefully separated the crystals which were hemihedral to the right from those hemihedral to the left and examined their solutions separately in the polarizing apparatus. I then saw with no less surprise than pleasure that the crystals hemihedral to the right deviated the plane of polarization to the right, and that those hemihedral to the left deviated it to the left, and when I took an equal weight of each of the two kinds of crystals, the mixed solution was indifferent towards the light in consequence of the neutralization of the two equal and opposite individual deviations.

Thus, Pasteur had discovered that the cause of the optical inactivity of the racemic acid was due to it being a mixture of 'right-handed' and 'left-handed' tartaric acid. He had discovered the phenomenon of enantiomerism (or 'enantiomorphism' as it was called earlier) in the racemate crystals. Pasteur's success is even more remarkable when you consider that above approximately 26 °C (79 °F) both the tartrate and racemate crystallize as a monohydrate having no hemihedral faces or asymmetry. When Biot was told of the result, he insisted that Pasteur repeat the experiment in his presence. After Biot had prepared the solution from the two kinds of crystals and had seen they were optically active, Pasteur related: 'The illustrious old man took me by the arm and said: "My dear child, I have loved science so much throughout my life that this makes my heart throb." '

Since the hemihedrism of the crystals is not always easily perceived, Pasteur prepared numerous crystal models to illustrate his lectures and also for Biot, whose eyesight was failing. Figure 17 shows two of Pasteur's models of the enantiomeric tartrate crystals.

The results of the studies undertaken by Pasteur in the next decade were no less remarkable. By the 1850s, he had discovered optical activity associated with a number of organic substances such as asparagine,

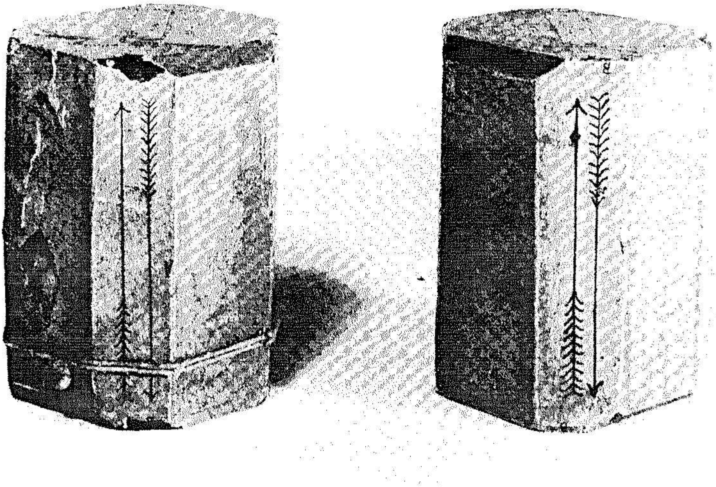


Figure 17. Pasteur's models of enantiomeric tartrate crystals (Institut Pasteur).

aspartic acid and malic acid. He also observed that the isomeric compounds, fumaric acid and maleic acid, were optically inactive. A rather interesting study was made by Pasteur of the crystals of strontium formate which were observed to be hemihedral and optically active. A solution of strontium formate, however, was optically inactive. He concluded that the hemihedrism of the crystal was

> . . . not due to the arrangement of the atoms in the chemical molecule, but to the arrangement of the physical molecules in the whole crystal, such that when the crystalline structure disappears in the act of dissolution there is no more dissymmetry.

This substance provides a second example (the other is quartz) of a substance whose optical activity is observed only in the crystalline form and on which can be observed the hemihedral facets. However, Pasteur also found that the presence of hemihedrism in the crystalline form is not always predictive of optical activity of solutions. For example, amyl alcohol (2-methyl-1-butanol) obtained from certain natural sources is optically active in solutions or as a liquid. Crystals of the barium sulphamylate of the alcohol do not display any hemihedral facets. Thus we can see that the absence or presence of hemihedrism in crystals cannot be used to predict the optical activity of the solutions. In this regard, Pasteur's investigation of malic acid, $HOOCH_2CH(OH)COOH$, might have catalyzed the final stereochemical revolution that Wislicenus' study of lactic acid, $CH_3CH(OH)COOH$, did a decade later. For a number of reasons, it did not.

In 1850, M. Dessaignes reported that he had prepared aspartic acid, $HOOCCH_2CH(NH_2)COOH$, by the thermal decomposition of either ammonium malate or ammonium fumarate. Aspartic acid obtained from natural sources is optically active. Pasteur considered it inconceivable that a laboratory synthesis could produce an optically active substance, even a racemic mixture, from an optically inactive substance. The synthesis of aspartic acid from the optically active malic acid was acceptable to Pasteur, but it was difficult to believe that it might be prepared from fumaric acid. Pasteur obtained samples of the synthetic aspartic acid and determined that they were both optically inactive. Pasteur then converted some of the 'synthetic' aspartic acid to malic acid. On discovering that the malic acid was optically inactive, he concluded:

> Is it not evident that we have here to deal with a malic acid identical with the natural one [optically active], except for the simple suppression of its molecular dissymmetry? It is natural malic acid *untwisted*, if I may so express myself. The natural acid is a spiral stair as regards the arrangement of its atoms, this acid is the same stair made of the steps but straight in place instead of being spiral.
>
> It might be asked whether the new malic acid was not the racemic form of this group, that is, the compound of the right and left malic acids. This has very slight probability, for in this case not only would one active body be made from an inactive substance, but two active bodies would be produced, a dextro- and levorotatory substance.

Pasteur was thus convinced that it was impossible to synthesize an optically active substance – even if it was present in a racemic mixture. He therefore never attempted a chemical resolution (a procedure he developed about the same time) of the inactive product into its enantiomers. The fact that the crystals of malic acid do not exhibit hemihedral facets undoubtedly contributed to his thought that the malic acid was inactive due to its 'untwisted' symmetrical molecular form. We have seen, however, that the presence of crystal hemihedrism is not always essential to the prediction of molecular chirality.

However, Pasteur's views on the causes of the optical inactivity of malic acid led him to predict the existence of a second inactive form of tartaric acid. In 1853, Pasteur heated a solution containing tartaric acid and the alkaloid, cinchonicine. From the mixture he obtained not only racemic acid but also an optically inactive isomeric compound. From the discussion above, it is not difficult to understand Pasteur's surprise at finding racemic acid as a product – since its synthesis would involve a laboratory conversion of (+)-tartaric acid to (–)-tartaric acid. The optical inactivity of the new isomer was thought by Pasteur to arise from the 'untwisting' of ordinary tartaric acid. For some time this substance was known as 'inactive tartaric acid' in contrast to 'racemic acid'.

By the 1860s, Pasteur had classified the tartaric acid isomerism as follows:

1. *Tartaric acid.* The optically active form obtained from wine fermentation: *dextro*-tartaric acid (now designated (+)-tartaric acid).

2. *Racemic acid* (or paratartaric acid). The compound is composed of a 50:50 mixture of the enantiomeric tartaric acid: *dextro-* and *levo*-tartaric acid (now designated (±)-tartaric acid).

3. *Inactive tartaric acid.* This was Pasteur's 'untwisted' tartaric acid, now designated as *meso*-tartaric acid.

In the 1850s, Pasteur devised a more efficient method (chemical resolution) of separating the enantiomers in a racemic mixture. The method was developed as a consequence of his observation of the solubility differences of the salts of tartaric acid prepared from optically active alkaloids:

> When one prepares the racemate of cinchonicine, for example, it always occurs for a certain concentration of liquor, that the first crystallization is in major part formed of the *levo*-tartrate of cinchonicine; the *dextro*-tartrate remains in the mother liquor. Parallel results present themselves with quinicine, only, in this case, it is the *dextro*-tartrate which is first removed.

In short, Pasteur had discovered that he could take advantage of the solubility differences of diastereomeric salts as a method of separating enantiomers. In 1858, Pasteur also observed that a yeast mould growing in a solution containing the (±)-tartrate destroyed the (+)-tartrate leaving the (–)-tartrate.

The availability of these two methods (the 'chemical' and 'biochemical' methods) for obtaining an optically active substance was soon exploited by organic chemists. The reports of the isolation of increasing numbers of optically active substances in the 1860's coincides with the growing use of graphic formulas by organic chemists to illustrate the chemical structures of organic compounds. However, it was almost another twenty years before a relationship was perceived between the phenomenon of optical activity and molecular structure. Many chemists persisted in the belief that the observation of optical activity had little chemical significance. This belief had been stated sometime earlier (1841) by Charles Gerhardt (1816–1856):

> We chemists require chemical differences to distinguish between two bodies, and it therefore seems to me that those who attach such great importance to rotatory power are deluding themselves strongly if they look to it for the future of chemistry.

Although Gerhardt wrote this before Pasteur's work was published, the attitude persisted in the minds of most chemists for many decades. Even in the 1860s, some chemists described compounds that were identical in all respects except in this physical property as 'physical isomers'.

Pasteur himself was close to understanding the relationship between optical activity and molecular structure, but the final solution escaped him because he had turned to non-chemical studies in the 1850s and was therefore not in a position to appreciate the insights provided by the introduction of a theory of valency. This can be seen in a lecture given by Pasteur in 1860 in which he speculated on the reason why the dissolution of substances, that are optically active in the solid state, sometimes results in the loss of optical activity, other times in the retention of activity:

> Imagine a spiral stair whose steps are cubes, or any other objects with superposable images. Destroy the stair and the dissymmetry will have vanished. The dissymmetry of the stair was simply the result of the mode of arrangement of the component steps. Imagine, on the other hand, the same spiral stair to be constructed with irregular tetrahedra for steps. Destroy the stair and the dissymmetry will still exist, since it is a question of a collection of tetrahedra!

Pasteur then went on to consider the possible geometries of the molecular species that might produce the dissymmetry (asymmetry) that correlated with the observation of optical activity:

> Are the atoms of the right acid grouped on the spirals of a dextrogyrate helix, or placed at the summits of an irregular tetrahedron, or disposed according to some particular dissymmetric grouping or other? We cannot answer these questions.

> But it cannot be doubted that there exists an arrangement of the atoms in a dissymmetric order, having a non-superposable image, and it is no less certain that the atoms of the *levo*-acid realize precisely the inverse dissymmetric grouping to this.

Pasteur's inattention to the developments in structural organic chemistry in the 1850s prevented him from considering the possible atomic arrangements of substances like tartaric acid that might produce the required 'dissymmetric groupings'. However, even the organic chemists who were aware of these developments considered Pasteur's proposals speculative inasmuch as there were difficulties enough in unraveling more conventional problems in organic chemistry. Only gradually did it become apparent that the phenomenon of 'optical isomerism' required a different theoretical interpretation than had been proposed to explain 'chemical' or 'structural' isomerism. The new interpretation arose from the experimental studies of the properties of a single compound, lactic acid.

Lactic acid had been discovered by Carl Wilhelm Scheele (1742–1786) in 1770 as a product isolated from sour milk. Although elemental analysis of the compound had been reported early in the 19th century, little was known of the chemical nature of the compound until the 1850s as the result of a controversy between Charles Wurtz and Hermann Kolbe. The basic difficulty revolved around the issue as to whether lactic acid should be considered 'monobasic' or 'dibasic'. To further complicate the issue was the report of the isolation of an acid from muscle tissue that had properties similar to lactic acid. This acid, referred to in the literature as 'sarcolactic acid' or 'paralactic acid', had the same chemical formula as lactic acid, but differed in its chemical and physical properties. By the early 1860s, those chemists concerned with the problem had concluded that the two acids were simply isomeric compounds: lactic acid corresponding to 2-hydroxypropanoic acid; sarcolactic acid, 3-hydroxypropanoic acid. One of the chemists who had become involved in the controversy was Johannes Adolf Wislicenus (1835–1902) (Plate 8). By 1863, he was persuaded that the two acids were not positional isomers. He concluded that the difference between the two could not be understood by the formulas then in use since they were limited to pictures of the substance in two dimensions. By the late 1860s, as the result of further experimental work, it was clear that the two acids were not positional isomers. But by this time, however, Wislicenus had discovered that whereas the substance known as lactic acid (isolated from sour milk) was optically inactive, sarcolactic acid was optically active. This observation was inexplicable to Wislicenus and suggested the existence of a new kind of isomerism:

Plate 8. Johannes Adolf Wislicenus (1835–1902).

> Thus is given the first certainly proved case in which the number of isomers exceeds the number of possible structures. Facts like these compel us to explain different isomeric molecules with the same structural formula by different positions of their atoms in space and to seek for definite representations of these.

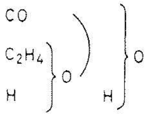
It is interesting to note that Kekulé merely summarized Wislicenus' remarks in a published account of the meeting at which Wislicenus spoke in 1869. Kekulé did not consider how his models might be used to solve the dilemma posed by Wislicenus:

> The speaker drew our attention to the fact that the existence of three hydroxypropionic acids demonstrated the limitations of the structural formulas in general use, and also of the views that are usually expressed by these formulas. Such subtler cases of isomerism might perhaps be explained by the spatial representation of the combination of atoms, that is, by models.

Wislicenus continued to work on the problem for another four years but came no closer to understanding the cause of the differences. In 1873, he again concluded:

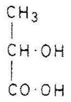
> If molecules can be structurally identical and yet possess dissimilar properties, this can be explained only on the ground that the difference is due to a different arrangement of the atoms in space.

The new kind of isomerism was described by Wislicenus as 'geometrical isomerism' – a term that took on a different meaning a few years later. That Wislicenus did not attempt to arrange the 'atoms in space' with some sort of models might seem unusual to us now. However, the 'type' formulas used earlier by Wislicenus do not lend themselves to illustrations with models as well as the Crum Brown–Kekulé formulas (Figs. 10–12, 14):



$$\left.\begin{matrix}\left.\begin{matrix}\mathrm{CO}\\ \left.\begin{matrix}\mathrm{C_2H_4}\\ \mathrm{H}\end{matrix}\right\}\mathrm{O}\end{matrix}\right) \\ \mathrm{H}\end{matrix}\right\}\mathrm{O}$$

Type formula (1863)



$$\begin{matrix}\mathrm{CH_3}\\ | \\ \mathrm{CH\cdot OH}\\ | \\ \mathrm{CO\cdot OH}\end{matrix}$$

Graphic formula (1873)

By 1873, he was using graphic formulas which could have lent themselves to representations using the models developed by Kekulé. There is no evidence that he ever used these models, however. Perhaps Wislicenus did not wish to risk taking his time on such speculations when further experimental work was needed to establish for certainty that he was in fact observing a new kind of isomerism. As we have seen in the case of the paper published by Paternò, the literature contained a number of examples of reports of excessive isomers that later proved to be non-existent.

It is also of interest to point out that the cause of the optical inactivity of milk-lactic acid was never dealt with. No chemical resolution was attempted (this was also the case with Pasteur's inactive malic acid). Thus, that the enantiomer of sarcolactic acid might be present in the inactive milk-lactic acid was never revealed. One can only conclude that Pasteur's observations in 1860, and earlier, had not had the profound effect on the 'geometrical' speculations of organic chemists that we might suppose from our present perspective.