Pharmaceutical Research, Vol. 7, No. 7, 1990

Review

Crystallographic Consequences of Molecular Dissymmetry

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The molecular chirality associated with an optically active molecule is manifested in the bulk crystallography of the compound. The historical development of optical activity was greatly aided by systematic studies of the habits of enantiomorphic crystals. The concepts of molecular dissymmetry, crystallography, and chirality are therefore linked. Racemic materials can be characterized by means of their melting-point phase diagrams, and this information used to design rational separations of racemic mixtures into their component enantiomers. Certain compounds are found to resolve spontaneously upon crystallization, and the enantiomers of these conglomerate species may be separated by direct crystallization. Compounds which crystallize as true racemates require resolution through the formation and separation of dissociable diastereomer species. The choice of resolution pathway is therefore determinable through an evaluation of the melting-point phase diagrams. When possible, resolution through direct crystallization represents the simplest, most cost-effective means of enantiomer resolution.

KEY WORDS: enantiomorphic crystals; molecular dissymmetry; crystallography; racemic mixtures; diastereomers; melting points; phase diagrams.

INTRODUCTION

The stereoselective actions associated with the enantiomeric constituents of a racemic drug can differ markedly in their pharmacodynamic or pharmacokinetic properties (1–4). These factors cause considerable amount of concern, since at the present time many chiral drugs are marketed as racemic mixtures. This situation is not invariably bad, but it is clear that a racemate should not be administered when a clear-cut advantage exists with the use of a resolved enantiomer (5). An excellent discussion regarding the possible selection of a resolved enantiomer over a racemate, from both a practical and a regulatory viewpoint, has been provided by De Camp (6).

When the decision is made to administer a drug as a resolved enantiomer, a significant aspect of the development work is to produce the material in large quantities with complete control over all chiral sites. This feat is usually accomplished through the formation and separation of dissociable diastereomer complexes, and a staggering number of such resolution procedures are available (7,8). Other procedures being actively recommended include chromatographic or biochemical separation. Almost neglected is the possibility that compound enantiomers can sometimes be separated through direct crystallization (9), at a considerable lower degree of effort.

In the latter process, the resolution of the chiral molecule takes place spontaneously during the course of crystallization. This type of enantiomeric resolution takes advantage of the fact that the molecular chirality of a given compound is expressed in its overall crystallography. In the present article the relation between molecular and crystallographic chirality is explored, and some of the consequences of these relationships are discussed.

QUARTZ AND THE CONCEPT OF HEMIHEDRAL CRYSTAL FACES

The most appropriate point to begin a discussion of molecular and crystallographic chirality is to discuss the case of quartz. To a first approximation, a typical quartz crystal consists of a hexagonal prism, capped at each end by a hexagonal pyramid. This geometrical figure is highly symmetrical, possessing 24 symmetry elements of various types.

It was noted by Hauy at the beginning of the nineteenth century that quartz crystals contained many small facets which considerably reduced the overall symmetry (10). These facets were found only on alternate corners of the crystal and were therefore described as being hemihedral. As shown in Fig. 1, the distribution of these hemihedral faces gives rise to two forms of quartz, identified at the time as being either left- or right-handed. The crystal still retains six elements of symmetry, however. It was noted that the two forms of quartz were mirror images of each other and that no amount of orientation permitted the superimposition of one form onto the other.

Herschel investigated the optical properties of quartz slabs, which were cut perpendicular to the long crystal axis (10). He found that crystals cut from left-handed quartz would invariably rotate the plane of linearly polarized light in a clockwise fashion, while crystals cut from right-handed quartz rotated the plane of linearly polarized light in a coun-

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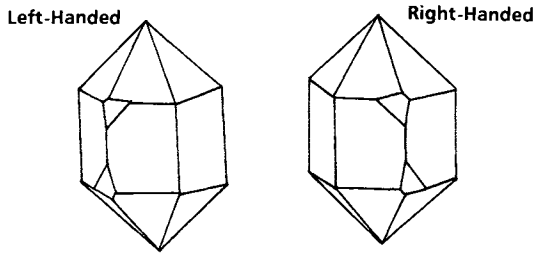

Fig. 1. The two handed crystal types of quartz, as distinguished by their hemihedral facets. The major crystal axis containing the 120° proper axis of rotation is vertical.

terclockwise direction. Elsewhere Biot was performing extensive studies on the optical rotatory properties of certain compounds dissolved in fluid solutions, and it was at this time that the connection between optical activity and crystallographic properties was made.

These observations were extremely important to Pasteur in that they permitted him to deduce requirements for chirality. He connected the concept of nonsuperimposable mirror images with the existence of chirality. Since quartz loses its optical rotatory power when dissolved or melted, he inferred that it was a helical arrangement of molecules in this particular solid which conveyed the given properties (10). He also understood that solids could possess certain elements of symmetry and still exhibit optical activity (i.e., chirality) and, therefore, coined the term "dissymmetry" to describe materials whose mirror images were not superimposable on each other.

PASTEUR AND THE TARTRATES

Although quartz loses its optical rotatory power when dissolved or melted, it was known that many organic compounds retained their ability to rotate the place of linearly polarized light even after being dissolved in a solvent. Tartaric acid in particular received extensive study, since it was found to exist in three chemically equivalent but physically nonequivalent, forms. Upon dissolution into water or other solvents, tartaric acid itself was found to exhibit the phenomenon of optical activity, while the racemic and paratartaric acids (as they were termed at the time) were found to be totally inactive.

Pasteur investigated the crystallographic properties of these acids and observed that while tartaric acid crystals exhibited hemihedral faces, crystals of racemic acid did not. The difference between these two forms is illustrated in Fig. 2. He extended his studies to include many simple and double salts of the same acids and verified that in every case optical activity was accompanied by the existence of hemihedral facets on the crystals.

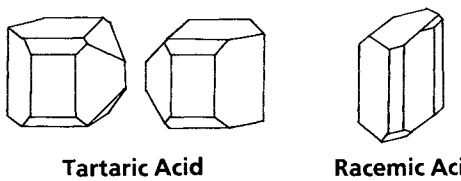

Fig. 2. Crystals of tartaric and racemic acids, illustrating the existence of hemihedral facets on the former and the lack thereof on the latter.

Pasteur then examined the crystals of optically inactive sodium ammonium paratartrate under the microscope and was very surprised to note that these crystals did contain hemihedral facets. Moreover, he observed that the overall assembly appeared to contain equivalent numbers of left-handed and right-handed crystals. He manually separated the two crystal types and examined these using a polarimeter. His observation was that the crystals hemihedral to the right rotated the place of linearly polarized light in a clockwise manner, while crystals hemihedral to the left rotated the linearly polarized light in the opposite fashion. It is significant to note that only the sodium ammonium salt could be separated in this manner, as all other salts of paratartaric acid yielded crystals devoid of hemihedral facets. Pasteur concluded that while racemic acid appeared to be a unique species, paratartaric acid actually consisted of a mixture of the two optically active tartaric acid forms.

MOLECULAR DISSYMMETRY

Pasteur's definition of dissymmetry as the existence of nonsuperimposable mirror images was therefore demonstrated to be applicable on both a crystallographic and a molecular basis. The modern requirement for the existence of chirality is based on symmetry considerations and group theory, and many excellent texts are available (11).

A symmetry element is defined as an operation that, when performed on an object, results in a new orientation of that object which is indistinguishable and superimposable on the original. There are five main classes of symmetry operations: (a) the identity operation (an operation which places the object back into its original orientation), (b) proper rotation (rotation of an object about an axis by some angle), (c) reflection plane (reflection of each part of an object through a plane bisecting the object), (d) center of inversion (reflection of every part of an object through a point at the center of the object), and (e) improper rotation (a proper rotation combined with either an inversion center or a reflection plane). Every object possesses some element or elements of symmetry, even if it only possesses the identity operation.

For instance, the ideal hexagonal bipyramid of quartz (ignoring the hemihedral facets) contains 24 symmetry elements: the identity, one 180°, two 120°, and two 60° proper axes parallel to the long crystal axis, six 180° proper axes perpendicular to the long crystal axis, one reflection plane perpendicular to the long crystal axis, six reflection planes parallel to the long crystal axis, and one 180°, two 120°, and two 60° improper axes parallel to the long crystal axis.

The rigorous, group theoretical requirement for the existence of chirality in a crystal or a molecule is that no improper rotation elements be present. This definition is often trivialized to require the absence of either a reflection plane or a center of inversion in an object, but these two operations are actually the two simplest improper rotation symmetry elements. It is important to note that a chiral object need not be totally devoid of symmetry (i.e., asymmetric) but that it merely be dissymmetric (containing no improper rotation symmetry elements). The tetrahedral carbon atom bound to four different substituents may be asymmetric, but the reason it represents a site of chirality is its dissymmetry.

Inclusion of the hemihedral facets on the hexagonal bipyramid lowers the total number of symmetry elements of quartz to only six: the identity, two 120° proper axes parallel to the long crystal axis, and three 180° proper axes perpendicular to the long crystal axis. The object contains no improper axes of rotation and is therefore chiral, but still is fairly symmetric.

The mirror images of a compound are denoted as enantiomers, and an equimolar mixture of the two enantiomers is termed a racemic mixture. The generally accepted configurational nomenclature for tetrahedral carbon enantiomers was devised by Cahn *et al.* and is based on sequencing rules (12). Enantiomers are identified as being either *R* or *S*, depending on the direction (clockwise or counterclockwise) of substituents after they have been arranged according to increasing atomic mass. Compounds containing more than one dissymmetric site are identified as diastereomers, and in compounds containing *n* dissymmetric centers the number of diastereomers will equal 2^n.

Many compounds are incompletely resolved into the component enantiomers, and therefore it is useful to define quantities which are used to specify the degree of resolution. The enantiomeric purity is a rigorous definition, being given by

$$\text{enantiomeric purity} = \frac{X_+ - X_-}{X_+ + X_-}$$

where X_+ and X_- are the mole fractions of the dextrorotatory and levorotatory enantiomers, respectively. Another commonly used term for enantiomeric purity is optical purity, which is only properly defined from specific rotation data:

$$\text{optical purity} = \frac{\text{specific rotation of the mixture}}{\text{specific rotation of pure enantiomer}}$$

When the enantiomeric composition of a compound is determined by means other than polarimetry (such as chiral chromatography or nuclear magnetic resonance), only the term enantiomeric purity should be used to identify the results.

Enantiomers will exhibit completely equivalent physical and chemical properties, with the exception that they will rotate the plane of linearly polarized light in opposite directions. Diastereomers can often exhibit quite different physical properties, and these differences are useful for their separation.

The nature of the tartaric acids studied by Pasteur and others can now be easily explained. Tartaric acid (containing two dissymmetric carbon atoms) is capable of existing as four diastereomers, identified as the (S,S), (R,R), (R,S), and (S,R) isomers. The paratartaric acid resolved by Pasteur represents an equimolar mixture of the (S,S) and (R,R) isomers of tartaric acid, while the racemic acid represents the (R,S), and (S,R) isomers. This latter isomer pair is rendered equivalent by a reflection plane and is, therefore, denoted the *meso*-isomer. The physical properties of the various tartaric acids are summarized in Table I (13). It is clear that the physical properties of the various tartrate diastereomers are significantly different, therefore demonstrating that the molecular chirality exerts a profound influence on the macroscopic level.

CRYSTALLOGRAPHY AND CHIRALITY

A crystalline solid is characterized by a high degree of internal order, consisting of a three-dimensional translational repetition of a basic structural pattern (denoted as the unit cell). For organic molecules, each unit cell may contain one or several molecules, the arrangement of which reflects the overall symmetry of the structure as a whole. Unit cells are characterized by their size and by the internal angles which are formed at the intersection of the unit cell axes. It has been shown that there are only 230 possible ways to combine all known symmetry elements in a crystal lattice, and therefore the crystal structures of all compounds must fall within one of these 230 space groups. These 230 space groups have been divided into 32 crystal classes and 7 overall crystal systems (14).

The arrangements of molecules in crystals are dictated by combinations of geometric and attractive forces. The packing of resolved enantiomers in a crystal must necessarily lead to the existence of an enantiomorphic solid, where the overall crystal structure is dissymmetric. Only 11 of the 32 crystal classes are enantiomorphic, and only 66 space groups are contained within these 11 classes.

Although enantiomers may crystallize in any one of these 66 space groups, it happens that only a few groups are actually encountered (8). Zorki (15) has compiled the space groups which have been observed for 430 resolved substances and has found that 95% of these compounds crystallize within three space groups. His particular breakdown was that 67% of the resolved enantiomers crystallized in the $P2_12_12_1$ space group (orthorhombic structure), 27% crystallized in the $P2_1$ space group (monoclinic structure), 1% in the C2 space group (triclinic structure), and 5% in the other 63 space groups.

A number of instances are known where an achiral compound or racemic mixture of enantiomers happens to crystallize in an enantiomorphic space group. In such cases, optical activity is observed in the crystal which is lost upon

Table I. Physical Properties of the Tartaric Acids[a]

Physical property	D-Tartaric acid	L-Tartaric acid	Paratartaric acid	Racemic acid
Isomer content	(S,S)	(R,R)	(S,S), (R,R)	(R,S), (S,R)
Melting point (°C)	170	170	206	160
Specific rotation	+12°	−12°	0	0
Solid density	1.760	1.760	1.697	1.737
Water solubility (g/L)	1390	1390	206	1250

[a] Data obtained from Ref. 13.

dissolution or fusion of the crystal. The optical activity of quartz, discussed earlier, is an example of this phenomenon. The chirality associated with such structures has been ascribed to three mechanisms (8): (a) stereochemically rigid molecules being arranged in a dissymmetric fashion within the crystal lattice [e.g., 1,2,5,6-dibenzanthracene (16)], (b) flexible molecules adopting a chiral conformation as a result of their crystal packing [e.g., benzil (17)], and (c) equimolar numbers of opposite enantiomers arranging themselves to form a chiral stack [e.g., *o*-tyrosine (18)].

The more general situation for a racemic compound is that it crystallizes in one of the 230 possible space groups which possess elements of inverse symmetry. Although 164 space groups exist which are not enantiomorphic, it has been observed that racemates tend to crystallize in only certain selected space groups. According to the compilation of Zorki (15), 56% of the racemates crystallized in the $P2_1/c$ space group (monoclinic structure), 15% crystallized in the C2/c space group (monoclinic structure), 13% in the $P\bar{1}$ space group (triclinic structure), and 16% in the other 161 achiral space groups.

The origin of the differing physical properties associated with the tartaric acids of Table I is now clear. The resolved enantiomers crystallize in the monoclinic $P2_1$ space group (19), while the racemic mixture (20) of these and the *meso* compound (21) crystallize in the triclinic $P\bar{1}$ space group (20). Although the racemate and *meso* compounds happen to crystallize in the same space group, the details of their unit cells are very different. Since the energetics associated with each crystal lattice must necessarily be different, the observation of different solid state properties for tartaric acid modifications is hardly surprising.

CRYSTALLINE RACEMATES AND THEIR PHASE DIAGRAMS

Not all racemic compounds yield crystals that exhibit hemihedral facets and, therefore, cannot be separated by the visual inspection method of Pasteur. However, racemic compounds must necessarily represent physical mixtures of the enantiomer components and, therefore, constitute a binary system. Such binary mixtures are governed by the phase rule and can be characterized as to their melting-point phrase diagrams. These are easily established through the use of differential scanning calorimetry (DSC), provided that the racemate and at least one resolved enantiomer are available.

An alternative method for the characterization of racemates is through the use of ternary phase diagrams, where one component is the solvent and the compound solubility is used as the observable parameter. However the full development of an appropriate solubility phase diagram is very time-consuming, since a number of solvents must be considered. The path of least resistance is to use DSC as a tool to identify the type of crystalline racemate, and to then identify a suitable enantiomer resolution procedure.

In 1899, Roozeboom (22) identified three basic racemate types on the basis of their melting-point phase diagrams, and two of these are illustrated schematically in Fig. 3.

The first racemate type is called a conglomerate, which is defined as an equimolar mixture of two crystalline enantiomers that are mechanically separable. This mixture would therefore melt as if it were a pure substance and will exhibit a eutectic in its melting-point phase diagram at the racemic composition. Conglomerate systems are formed only as a result of spontaneous resolution, which accompanies the crystallization step.

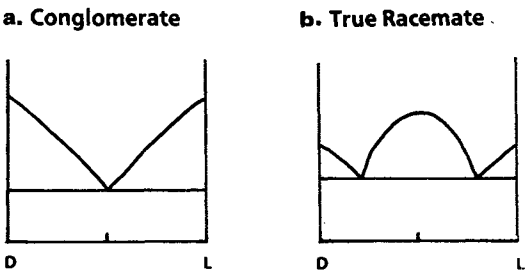

Fig. 3. Binary melting-point phase diagrams associated with the commonly encountered types of crystalline racemates: (a) conglomerate and (b) true racemate. Note that the eutectic point for the conglomerate occurs exactly at the equimolar point.

For a racemic compound to exhibit conglomerate behavior, the melting point of the resolved enantiomers must be at least 20°C higher than the melting point of the racemic mixture. Although a considerable number of conglomerate systems have been identified, and tabulations of these have been made available (8,23), it appears that the number of conglomerate systems is considerably underestimated. The best conclusion obtained to date is that about 5–10% of chiral crystalline compounds exist as conglomerates (8). Jacques *et al.* have concluded that the number of conglomerates is far more frequent among salts of chiral compounds than in the compounds themselves and that the number of compounds capable of undergoing spontaneous resolution is therefore much larger than currently estimated (24).

The second type of racemic mixture is termed a true racemate, where the two enantiomers are present in equimolar quantities in the crystal unit cell. This is certainly the most commonly encountered type of racemic mixture. Although the melting-point phase diagram shown in Fig. 3b is typical for a true racemate, the actual shape can vary considerably depending whether the melting point of the racemic mixture is greater or lower than that of the resolved enantiomers. The distinguishing mark of the true racemate is that it will exhibit two eutectic points in its melting-point phase diagram, although these may be difficult to detect if they are located at the extremes in the phase diagram.

The final racemic mixture type is termed a pseudoracemate and is formed when the two enantiomers form a solid solution or a mixed crystal. The occurrence of pseudoracemates is not common, with only about 40 ideal and 20 nonideal pseudoracemate systems being noted (8).

COMPLICATIONS INDUCED BY POLYMORPHISM

Many compounds are capable of existing in more than one crystal structure and, hence, exhibit the phenomenon of polymorphism. Should this material also be capable of existing as different solvates (each of which would most probably exhibit a different crystal structure), then this variation is termed pseudopolymorphism. When a compound is capa-

ble of exhibiting polymorphism, pseudopolymorphism, or both, the resulting phase diagram can be extremely complicated. Since knowledge of phase diagrams will be shown essential to the design of enantiomer separations, the complications induced by polymorphism can greatly confound a resolution.

Two types of interconversion among polymorphs are known. The first is enantiotropy, where the transition temperature for converting phase A into phase B is lower than the melting points of either of the two crystalline forms. Each form is stable within its specified temperature range, and the transition between these forms is reversible. The other situation is monotropy, where the transition temperature is higher than the melting points of either phase A or phase B and is, therefore, a virtual temperature. On phase is metastable with respect to the other, and any transformation is irreversible.

The possible phase interconversions which could be induced by polymorphism have been discussed in detail and summarized (8). Two conglomerate phases may interconvert, two enantiomer phases may interconvert, or two racemate phases may interconvert. A true racemate phase may convert into a conglomerate, or the reverse situation might take place. Finally, either a conglomerate or a racemate phase might convert to a solid solution phase, or vice versa. Not all of these polymorphic conversion situations have been observed, but a sufficiently large number of interconversions have been documented that the possibility must always be considered.

When polymorphism is possible for a given compound, the implications for enantiomer resolution are profound. In his original example, Pasteur mechanically separated the enantiomorphic crystals of sodium ammonium tartrate after their crystallization. When the same experiment was tried in the United States, crystals with hemihedral facets could not be obtained and the resolution could not be duplicated. It was ultimately learned that this particular crystal system existed as a conglomerate below 26°C and as a true racemate above 26°C. The observation of spontaneous resolution could be made only in European laboratories, whose ambient temperature was sufficiently low so as to permit formation of the conglomerate phase. In the warmer American laboratories, only the true racemate could be obtained. The validity of Pasteur's observations remained somewhat in doubt until the full phase diagram was obtained, and the discrepancy thus explained.

RESOLUTION OF ENANTIOMER MIXTURES

A resolution is a separation which begins with a racemic mixture and ends with the recovery of at least one of the optical isomers in its enantiomerically pure form. Such resolutions are essential to the production of a chiral therapeutic agent, since the generation of enantiomerically pure material requires the complete specification of chirality at some step during the chemical synthesis. This may be accomplished in a variety of different ways, such as incorporation of chiral synthons isolated from natural sources, asymmetric synthesis, kinetic resolution, enzymatic enantiomer activation, chromatographic resolution, resolution by direct crystallization, and resolution by means of diastereomer formation. The latter two processes entail a crystallization step of some type and, therefore, require some discussion regarding crystal and molecular chirality.

Resolution of Conglomerates

Compounds known to crystallize as conglomerates are much easier to resolve than true racemates, since the resolution step takes place spontaneously upon crystallization. There is no need to rely on the use of resolving agents, chromatographic solid supports, or the use of biological or chemical activation processes. When possible, the direct crystallization of one or both enantiomers represents the most economically attractive method for generation of enantiomerically pure substances.

Jacques and co-workers have provided extensive summaries of the methods whereby direct crystallization can be used to effect the resolution of a racemic mixture (8,9). Although these techniques are necessarily limited to racemates which crystallize as conglomerates, the efficiency with which the procedures may be implemented requires that the possibility of direct crystallization be examined.

The first method entails the mechanical separation of enantiomorphic crystals, formed simultaneously while the mother liquor remains racemic. This particular method is extremely time-consuming to perform, and impossible unless the crystals form with well-defined hemihedral faces. Nevertheless, it is often the method of choice to obtain the seed crystals required for other direct crystallization procedures. When a particular system has been shown by its phase diagram to be a conglomerate, and the crystals are not sufficiently distinct so as to be separated, polarimetry can often be used to establish the chirality of the enantiomer.

Even a few seed crystals, mechanically separated, can be used to produce larger quantities of resolved material. A second method of enantiomer separation by direct crystallization involves the localized crystallization of each enantiomer from a racemic, supersaturated solution. Seed crystals are placed in geographically separated locations in the crystallization vessel, and these serve as nuclei for the further crystallization of the like enantiomer. This procedure has been used to obtain both enantiomers of methadone, where approximately 50% total yield of enantiomerically pure material can be obtained (25). An apparatus has been described which permits the more automated use of localized crystallization and which has successfully been used to separate the enantiomers of hydrobenzoin (26).

Enantiomer separation may be practiced on the large industrial scale using the procedure known as resolution by entrainment (27). The method is based on the condition that the solubility of a given enantiomer be less than that of the corresponding racemate. To begin, a solution is prepared which contains a slight excess of one enantiomer. Crystallization is induced (usually with the aid of appropriate seed crystals), whereupon the desired enantiomer is obtained as a solid and the mother liquor is enriched in the other isomer. In a second crystallization step, the other enantiomer is obtained.

Resolution by entrainment is best illustrated through the use of an example, and the bench-scale resolution of hydrobenzoin by Brienne (9) is an excellent example. One

thousand one hundred milligrams of racemic material was dissolved along with 370 mg of (−)-hydrobenzoin in 85 g of 95% ethanol, and the solution cooled to 15°C. Ten milligrams of the (−)-isomer was added as seeds, and crystals were allowed to form. After 20 min, 870 mg of (−)-hydrobenzoin was recovered. Eight hundred seventy milligrams of racemic hydrobenzoin was then dissolved with heating, and the resulting solution cooled to 15°C and seeded with 10 mg of the (+)-isomer. Nine hundred milligrams of (+)-hydrobenzoin was recovered at this time. The process was cycled 15 times and ultimately yielded 6.5 g of (−)-hydrobenzoin and 5.7 g of (−)-hydrobenzoin. Each isomer was obtained as approximately 97% enantiomerically pure.

It is apparent that a resolution by entrainment can theoretically be carried out forever, yielding approximately equal amounts of resolved enantiomers. For example, approximately 13,000 tons of L-glutamic acid was produced from the synthetically manufactured racemate between 1963 and 1973 using an entrainment procedure (9). The method can be applied to any racemic mixture which crystallizes as a conglomerate, and the main complication which can arise is when the compound exhibits polymorphism. In that case, the entrainment procedure must be carefully designed to generate only the desired crystal form. It is evident that full knowledge of all phase diagrams is required, especially the solubility profile.

Resolution of True Racemates

When a compound forms a true racemate (as evidenced through its melting-point phase diagram), resolution by entrainment cannot be used to separate the enantiomers. True racemates can be separated only after the performance of a derivatization reaction. In most cases this procedure involves the formation of dissociable diastereomer species (28), which is often a simple salt. For instance, the first resolving agents introduced for acidic enantiomers were alkaloids, and hydroxyacids were used for the resolution of bases. Extensive tables of resolving agents and procedures are available (7,8,28).

For the specific case of the resolution of a racemic acid by a basic resolving agent (such as a chiral, resolved amine), the first step of the resolution procedure involves formation of diastereomeric salts:

Racemic mixture	Resolving agent	Diastereomer salts

$$\begin{bmatrix}(+)R\text{-COOH}\\(-)R\text{-COOH}\end{bmatrix} + 2\ NH_2\text{-}R'\ (+) \rightarrow \begin{bmatrix}(+)R\text{-COO-}NH_3\text{-}R'(+)\\(-)R\text{-COO-}NH_3\text{-}R'(+)\end{bmatrix}$$

According to convention (29), the (R,R) and (S,S) diastereomers are termed the *p* salts, and the (R,S) and (S,R) diastereomers are identified as the *n* salts. In the example used above, the (+)*R*-COO-NH_3-*R*′(+) diastereomer would correspond to the *p* salt, and the (−)*R*-COO-NH_3-*R*′(+) diastereomer would be the *n* salt. Since the *p* and *n* salts will generally exhibit different physical properties, they may be separated by ordinary physical means. Although these may be separated on a small scale by preparative chromatography, the most generally used method is that of fractional crystallization. The success of the resolution is critically related to this crystallization step.

The binary melting-point phase diagrams of diastereomeric salts are constructed in the same manner as had been described for enantiomers and racemates. Usually only a single eutectic point is observed, and an example of this type of phase diagram is shown in Fig. 4. The occurrence of the eutectic point at the equimolar point (i.e., mole fractions of 0.5 for both the *p* and the *n* salts) would be purely accidental. In fact, for the *p* salt to be separated from the *n* salt in a single crystallization step, the position of the eutectic should be substantially removed from the equimolar point. However, if the eutectic point is close or at the equimolar point, then the phase diagram would closely resemble that of a conglomerate. If so, it would be possible to devise an entrainment procedure for the separation of the *p* and *n* salts. It is concluded that no matter what form is obtained for the melting-point phase diagram, separation of the diastereomeric salts by crystallization will be possible.

Examples of experimentally determined melting-point phase diagrams of diastereomeric salts are available (30). It is also important to note that polymorphism can lead to complicated phase diagrams and to difficult diastereomer separations.

When suitable crystals of both diastereomer salts can be grown for a single crystal structure determination, it is usually found that the *p* and *n* salts crystallize in different space groups. In the sample of α-methylbenzylamine hydratropate, the *p* salt was found to crystallize in the $P2_1$ space group, while the *n* salt crystallized in the $P2_12_12_1$ space group (31). Although the molecular conformations in the two crystal forms are similar, the mode of packing to generate a crystal lattice is different.

Given that *p* and *n* salts generally crystallize in different space groups, it is hardly surprising that the diastereomeric salts would exhibit different physical properties. For example, the enthalpy of fusion of the *n* salt of α-methylbenzylamine mandelate was reported as 11.3 kcal/mol, while that of the *p* salt was only found to be 6.6 kcal/mol (30). Melting points of the diastereomeric salts are also known to differ as a result of the crystallography. The melting point of the *n* salt of α-methylbenzylamine mandelate was found to be 178°C, while the melting point of the *p* salt was reported as 109°C (32).

Since the solubility of a compound is strongly determined by its heat of fusion and melting point, it is to be anticipated that the *p* and *n* diastereomer salts would exhibit

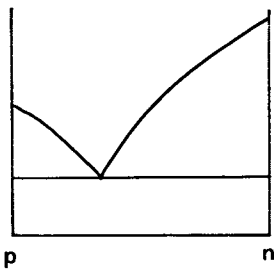

Fig. 4. Binary melting-point phase diagram as might be obtained for a typical diastereomeric salt. Note that the eutectic point is not located at the equimolar point.

differing solubilities. This fact is absolutely essential to the separation of diastereomers, since large solubility differences will yield more efficient resolutions. In the specific example of the resolution of a racemic acid using a basic resolving agent, the crucial second step can be written as follows.

Mixed diastereomer salts	Crystallization step	Separated diastereomer salts
$\begin{bmatrix}(+)R\text{-COO-NH}_3\text{-}R'(+)\\(-)R\text{-COO-NH}_3\text{-}R'(+)\end{bmatrix}$	$\rightarrow$	$(+)R\text{-COO-NH}_3\text{-}R'(+)$ + $(-)R\text{-COO-NH}_3\text{-}R'(+)$

For instance, the aqueous solubility of the *n* salt of α-methylbenzylamine mandelate has been reported as 49.1 g/liter, and the solubility of the *p* salt as 180 g/liter (33). The relative solubilities of *p* and *n* salt pairs can be greatly affected by the choice of temperature or solvent system. The path of diastereomer separation can therefore be manipulated by variations in experimental conditions, and it is often possible to crystallize either the *p* or the *n* salt depending on the parameters used. In many instances these quantities are determined empirically, but the development of a full solubility phase diagram can permit the more rational design of a crystallization procedure.

The efficiency of a resolution is evaluated by calculating the enantiomeric enrichment:

$$\text{enantiomeric enrichment} = \frac{X_D - 50}{50}$$

where X_D is the mole fraction of the desired enantiomer. When X_D is determined using polarimetry, use of the term optical enrichment is permissible.

Once either the *p* or the *n* salt (or both) is successfully crystallized, the diastereomer salt must be dissociated and the resolving agent separated. In the specific instance of acid resolution, the diastereomeric salt is cleaved by means of a hydrolysis reaction:

Separated diastereomer salts	Hydrolysis step	Separated enantiomers
$(+)R\text{-COO-NH}_3\text{-}R'(+)$	$\xrightarrow{HCl}$	$(+)R\text{-COOH} + (+)R'\text{-NH}_3Cl$
$(-)R\text{-COO-NH}_3\text{-}R'(+)$	$\xrightarrow{HCl}$	$(-)R\text{-COOH} + (+)R'\text{-NH}_3Cl$

The diastereomer cleavage must be simple and selective, take place in quantitative yield, must not racemize the resolved compound, and must leave the resolving agent in a form which is easily recovered. The recovery is usually effected by precipitating the resolving agent, or by extracting one product.

The economic factors associated with the resolution of compounds by means of dissociable diastereomers has been summarized (34). Important concerns are the price and availability of the resolving agent, ease and efficiency of recovery and recycling of the agent, stability of the *p* and *n* salts under the reaction conditions, overall yield of the reaction, and position of the resolution step in the reaction scheme. However, if the drug must be marketed as a resolved enantiomer and it does not exist as a conglomerate, then no other means of resolution is economically feasible.

SUMMARY

Observations on the chirality of crystals made it possible for Pasteur and others to identify dissymmetry as the true origin of optical activity. It was evident that the molecular chirality associated with a given compound was directly evident in the bulk crystallography of that compound.

Many chiral molecules have been observed to resolve spontaneously upon crystallization, forming enantiomorphic crystals which may be physically separated. These conglomerate systems are easily identified on the basis of their melting point phase diagrams. The racemic mixture of a conglomerate can be efficiently separated into its constituent enantiomers through direct crystallization procedures. When this method of resolution is available, it represents the most efficient and cost-effective method for enantiomer separation.

For compounds that crystallize as true racemates, no direct crystallization scheme is possible for the separation of enantiomers. In that case, the most efficient method of resolution involves the formation and separation of diastereomeric species. Resolution by means of dissociable diastereomers necessarily adds several steps, and their consequent expense, to a chemical synthesis.

With the ever-increasing administration of therapeutic agents as resolved enantiomers, methods for isolation of the desired species will be in constant development. It is clear that any worker seeking to resolve an enantiomeric pair would be well advised to first determine the melting point phase diagram, thus identifying the compound as being either a conglomerate or a true racemate. Should the compound in question happen to crystallize as a conglomerate, then enantiomer separation by direct crystallization would represent the preferred, cost-effective route. Separation of enantiomers by formation of dissociable diastereomers should be employed only if the direct method is not possible.

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