ISOMERISM AND STEREOCHEMISTRY

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I. Basic Principles

Isomers are defined as molecules of identical atomic compositions (molecular formulas), but with different bonding arrangements of atoms or orientation of their atoms in space. Based on this definition, several types of isomerism are possible including constitutional, configurational, and conformational isomerism. Constitutional isomers (also called structural or positional isomers) are molecules with the same atomic composition but different bonding arrangements between atoms, as illustrated by the examples of catechol, resorcinol, and hydroquinone (Fig. 1). All of these compounds have the same atomic composition $(C_6H_60_2)$, but different bonding arrangements of atoms and are thus distinct chemical entities with different chemical and physical properties.

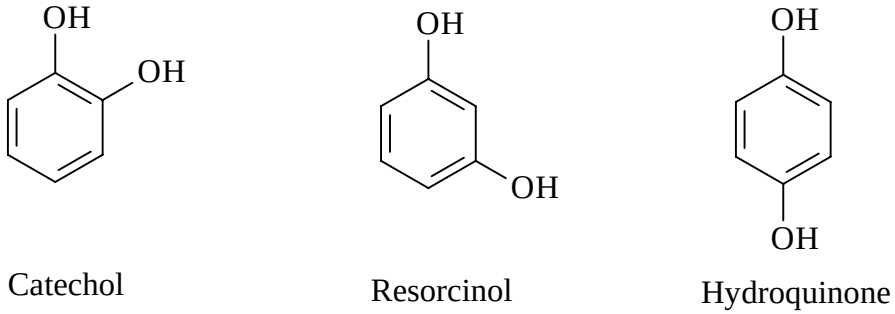


Figure 1. Constitutional isomers

Configurational isomers are defined as molecules of identical atomic composition and bonding arrangements of atoms, but different orientations of atoms in space, and these different orientations cannot interconvert freely by bond rotation. Since these types of isomers differ only in relative spatial orientations of atoms, they are commonly referred to as stereoisomers. Configurational stereoisomers are subcategorized as optical isomers (enantiomers) or geometric isomers (Fig. 2), depending upon the hybridization state and geometry of the atoms that impart the properties of stereoisomerism and the overall structure of the molecule. Stereoisomers of this type are distinct chemical entities that may have different chemical and physical 0roperties.

Conformational isomers (conformers) are stereoisomeric forms characterized by different relative spatial arrangements of atoms that result from rotation about sigma bonds. Thus, unlike configurational isomers, conformers are interconverting stereochemical forms of a single compound. The nature of conformational and configurational stereoisomerism, as well as the role of stereoisomerism in drug activity is the subject of this article.

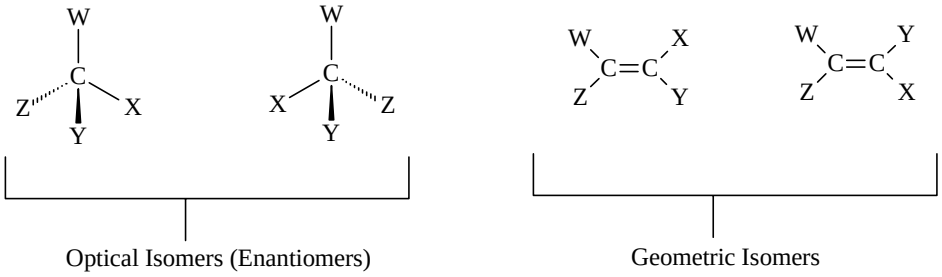


Figure 2. Stereoisomers

II. Optical Activity and Molecular Structure

Modern stereochemistry originated with the research of Malus in 1808 who discovered that plane-polarized light is generated when a beam of light is passed through calcium carbonate. In 1813, the mineralogist Biot reported that asymmetrically cut quartz crystals rotate the plane of a beam of polarized light. It also was noted that certain organic liquids, as well as solutions of certain organic compounds, can rotate the plane of polarized light. Biot attributed this effect on plane-polarized light to a property of the individual organic molecules through which the light is passed, a property now referred to as optical activity. The concept of optical activity was extended by Herschel in 1812 who observed that hemihedral quartz crystals, having odd faces inclined in one direction, rotated the plane of polarized light in one direction, whereas crystals whose odd faces were inclined in the opposite direction rotated plane-polarized light to the same extent but in the opposite direction.

Pasteur refined the observations of the mineralogists by proposing a link between optical activity and molecular structure. His landmark work of 1847 was based on earlier observations by Biot that chemically identical salts of tartaric acid rotated plane-polarized light differently. Pasteur discovered that two distinct crystalline forms of tartaric acid salt could be obtained from solutions of the optically inactive salt of "paratartaric acid" (also known as racemic acid), and that one crystal form has hemihedral faces that inclined to the right, whereas the other has faces that inclined to the left. He separated the distinct crystalline salts forms and observed that they, unlike paratartaric acid, are optically active; solutions of the left-handed crystals rotate the plane of polarized light to the right, and solutions of the right-handed crystals rotate the light to the same degree, but in the opposite direction. Pasteur further demonstrated that the left- and right-handed crystals were mirror images of each other and concluded that this property must reflect the handedness of the molecules that constitute the crystals.

The molecular basis for the left- and right-handedness of distinct crystals of the same chemical substance and the associated differences in optical rotation was developed from the hypothesis of Paterno (1869) and Kekule that the geometry about a carbon atom bound to four ligands is tetrahedral. Based on the concept of tetrahedral geometry, Van't Hoff and LeBel concluded that when four different groups or atoms are bound to a carbon atom, two distinct tetrahedral molecular forms are possible, and these bear a nonsuperimposable mirror-image relationship to one another (Fig. 3). This hypothesis provided the link between three-dimensional molecular structure and optical activity, and represents the foundation of stereoisomerism.

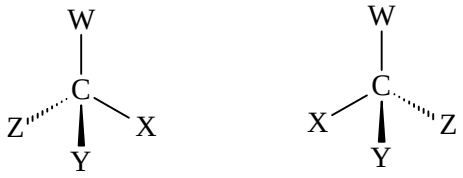
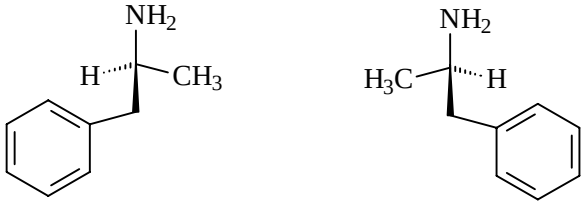


Figure 3. Tetrahedral geometry and optical isomerism

III. Chirality and Optical Isomers (Enantiomers)

The property of nonsuperimposability became known as chirality, and molecules containing asymmetrically substituted carbons are referred to as chiral molecules. The term chiral was derived from the Greek word meaning "hand" and was applied as a description of the left- and right-handedness of crystal structure resulting from molecular asymmetry. The individual mirror image forms of a chiral molecule are called optical isomers because they rotate the plane of polarized light (are optically active) and differ in structure only in the orientation of atoms or groups about the asymmetric carbon (are isomers). Today, optical isomers are more commonly referred to as enantiomers or an enantiomeric pair.

Generally, optical isomers or enantiomers have identical physical and chemical properties; for example, the enantiomeric forms of amphetamine (Fig. 4) have identical melting points, pKa, solubilities, etc. There are, however, two important differences in properties between the members of an enantiomeric pair. First, each member rotates the plane of polarized light to the same degree, but in opposite directions. The enantiomer rotating the plane to right (clockwise) is designated as the dextrorotatory (d) or (+)-enantiomer. The other enantiomer rotates the plane to the left (counterclockwise) and is designated as the levorotatory (1) or (-)-enantiomer. This is illustrated in Fig. 4 for the enantiomers of amphetamine, where



(+)-Amphetamine
$[\alpha] = +21.8^{o}$

(-)-Amphetamine
$[\alpha] = -21.8^{o}$

Figure 4. Amphetamine enantiomers

the enantiomer with the specific optical rotation of (+)-21.8^{E} is designated as dextrorotatory, whereas the mirror enantiomer with a specific rotation of (-)-21.8^{E} is called levorotatory. A second difference between enantiomers is their interactions with other chiral substances. For example, enantiomers may have different solubilities in chiral solvents, they may react at different rates in the presence of an optically active reagent or enzyme, and many have different affinities for chiral surfaces and receptors.

Most optically active drugs are chiral as a result of the presence of an asymmetrically substituted

tetrahedral carbon atom. However, chirality can result from other types of molecular asymmetry. For example, the presence of any asymmetrically substituted atom of tetrahedral geometry, such as silicon, quaternary nitrogen, and metals that form tetrahedral coordination complexes (manganese, copper, zinc, etc.) results in chirality. Similarly, compounds containing asymmetrically substituted tetrahedral phosphorus atoms are also chiral. The antineoplastic agent cyclophosphamide is one example of a compound with a chiral phosphorus moiety (Fig. 5).

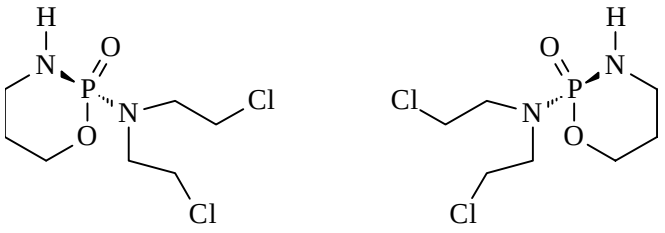


Figure 5. Optical isomers of cyclophosphamide

Chirality is also a property of compounds containing an asymmetrically substituted atom of pyramidal geometry. For example, secondary and tertiary amines bearing four different substituents (one "substituent" being the lone pair of electrons on nitrogen) are chiral. However, due to rapid pyramidal inversion (Fig. 6), the individual enantiomers are not usually separable (resolvable). On the other hand, amines that contain an asymmetrically substituted nitrogen atom in a ring system, particularly at a bridgehead position, may not undergo facile inversion, and thus it is possible to resolve distinct stereoisomers (Fig. 7). The same is true for asymmetrically substituted pyramidal sulfur derivatives such as sulfoxides, sulfonic esters, sulfonium salts, and sulfites. For example, the sulfur atom of the nonsteroidal antiinflammatory sulindac (Fig. 8) bears four difference substituents (one being a pair of electrons) and hence is chiral.

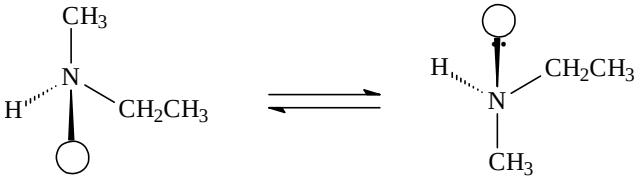


Figure 6. Pyramidal inversion of chiral nitrogen

Cyclic or multicyclic compounds with four different substituents projecting from the corners of the cycle have a center of asymmetry and are chiral if all four substituents are different. Such is the case for adamantine analogues, as shown in Fig. 9.

Certain biphenyl compounds may be chiral as a result of hindered rotation about the central bond and dissymmetric ring substitution. In this instance bond rotation may be inhibited by the presence of bulky groups at the ortho positions, forcing the two aromatic rings to lie in perpendicular, or near perpendicular, planes. If each aromatic ring of such a biphenyl system is dissymmetrically substituted, chirality results. This is illustrated by the three biphenyl derivatives (A, B, and C) in Fig. 10. In each of these compounds rotation is restricted as a result of the

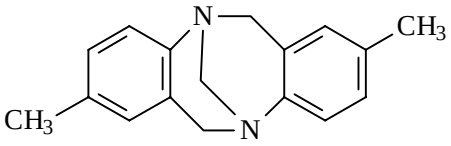


Figure 7. Troger's base

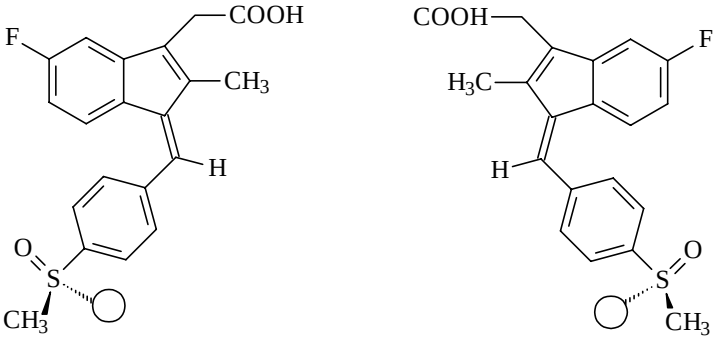


Figure 8. The chiral sulfoxide Sulindac

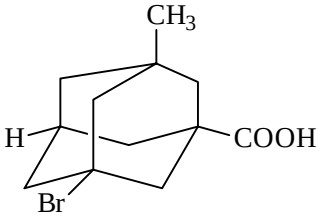


Figure 9. Chiral adamantine

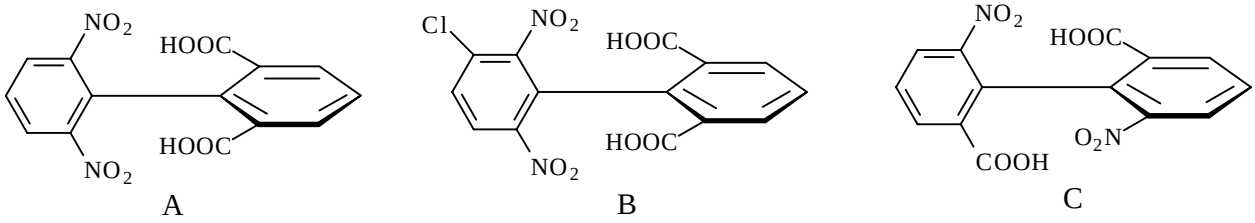


Figure 10. Biphenyl systems

presence of bulky ortho groups. However, only compound C is chiral because only this compound has two dissymmetrically substituted aromatic rings. The experimental antifertility and antiviral agent gossypol contains such a system and hence can exist in two enantiomeric forms (Fig. 11).

Finally, compounds that assume a helical shape, even a partial helix, may have left- and right-handed orientations and thus are chiral. The most notable examples of such chirality are the helices formed in polynucleotides (such as deoxyribonucleic acid) (DNA) and proteins.

However, drugs such as the benzodiazepine anxiolytics also exist as distinct helical forms (Fig. 12). Although these stereoisomers readily interconvert, only one helical form possesses affinity for the benzodiazepine receptor.

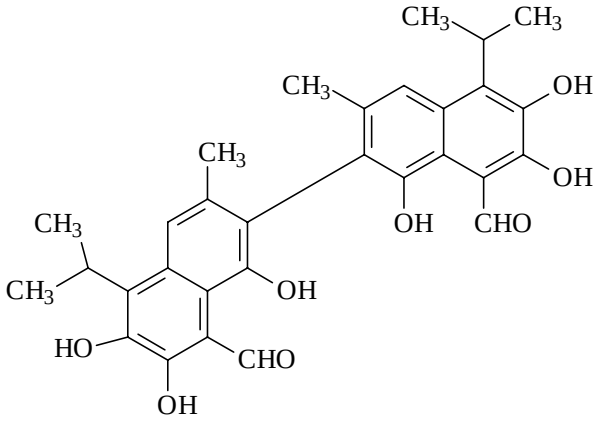


Figure 11. The chiral biaryl system gossypol.

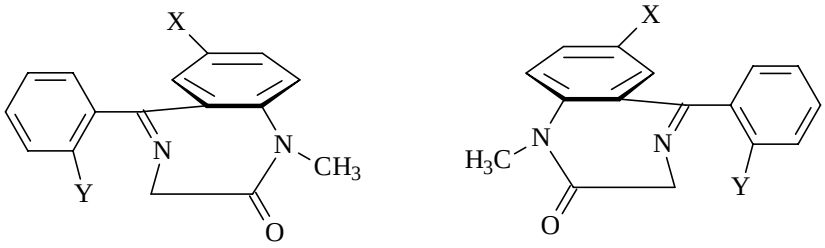


Figure 12. Helical benzodiazepine isomers

IV. Definitions and Nomenclature

As discussed in the preceding section, each member of an enantiomeric pair rotates the plane of polarized light to the same degree, but in opposite directions (dextrorotatory and levorotatory). However, the amount of optical rotation is not constant for an individual enantiomer but rather is dependent on the solvent, concentration, temperature, the wavelength of light used, and the path length of the sample cell employed to determine the rotation. Thus, meaningful optical rotation comparisons for chiral compounds are only possible when optical activities are determined under specified conditions. Such conditions are defined as specific rotations [or] and are expressed for solutions and neat liquids in Eqs. (1) and (2), respectively.

(1) $[\alpha]t,\lambda = 100\alpha/(l \times c)$

(2) $[\alpha]t,\lambda = \alpha/(l \times d)$

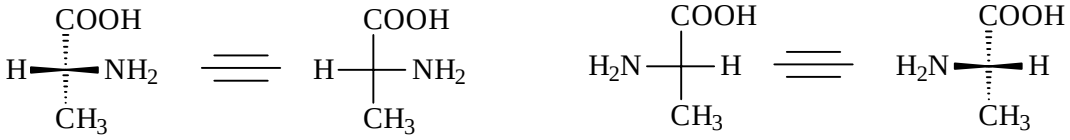
where $[\alpha]$ = measured rotation, t = temperature, λ = wavelength, c = concentration, d = density and l = length

Specific rotation data may assist in the identification of a specific enantiomer, or may be used to determine the optical purity (enantiomeric purity) of a mixture of enantiomers. Optical purity is defined as the percent excess of one enantiomer over another in a mixture and is expressed in Eq. (3).

(3) Optical purity = $([d] - [l])/([d] + [l]) \times (\alpha_{obs})/ \alpha_o$

Based on Eq. (3), a mixture consisting of equal amounts of each enantiomer would have no net optical rotation; the optical rotation of one enantiomer is cancelled by the rotation of the other enantiomer. Such a mixture is referred to as a racemic mixture or racemate.

Other terms commonly applied in discussions of optically active compounds include resolution and racemization. Resolution describes the processes whereby a racemic mixture is separated (resolved) into its component enantiomers. Racemization refers to the conversion of either enantiomer into equal parts (racemic mixture) of both enantiomers.



D-Alanine L-Alanine

Figure 13. Alanine and D/L configurational assignments

Over the years, several nomenclature systems have been developed to characterize the relationship between enantiomers. The system based on optical activity and the classification of enantiomers as dextrorotatory (d or (+)) or levorotatory (1 or (-)) already has been described. However, this system of nomenclature is of limited applicability because the sign of rotation, (+) or (-), does not predict the absolute configuration or the relative spatial arrangement of atoms in the enantiomers. In an attempt to designate the precise configurations about carbon centers of asymmetry, two nomenclature systems have been developed: the D/L system and the Cahn-Ingold-Prelog *R/S* system. The application of these systems requires that chiral molecules be oriented in a Fischer projection to reflect their tetrahedral geometry. In such a projection atoms or groups on the horizontal axis are projecting out of the plane of the paper, whereas those on the vertical axis are projecting behind the plane of the paper. When using the D/L system, the projection is oriented in such a way that the main carbon chain is positioned vertically, with the lowest numbered carbon positioned at the top. After the projection is aligned, the position of the principal substituent relative to the carbon chain is identified. If it is to the left of the vertical axis of the projection, the L-configuration is assigned. If the principal substituent is to the right of the vertical axis, the D-configuration is assigned. The application of this system is demonstrated in Fig.13 for the enantiomers of the amino acid alanine.

The D/L system was used widely in the past to assign the configurations of naturally occurring compounds such as amino acids and sugars. Its application to unnatural structures, such as most drugs, is limited because it can only be used when a compound contains a main carbon chain, and when an unambiguous choice of the principal substituents can be made. The use of this system is also complicated by the fact that the D- and L-absolute configurations are not related to the

directions that enantiomers rotate plane polarized light. For example, the D-enantiomer of glyceraldehyde rotates plane-polarized light to the right (dextrorotatory), while U-glyceraldehyde is levorotatory (Fig. 14). However, with the related enantiomers of glyceric acid, the reverse is true. Thus structurally related compounds with the same absolute configuration do not necessarily rotate the plane of polarized light in the same direction. This lack of correlation between the D/L and d-(+)/1-(-) nomenclature systems remains a source of confusion and error in the literature.

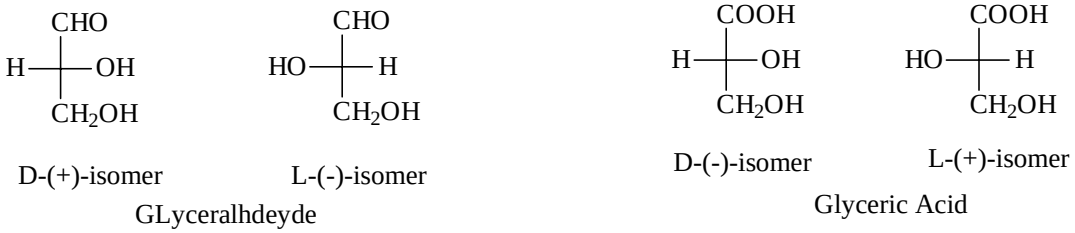


Figure 14. Configurations and optical rotations of enantiomers of glyceraldehyde and glyceric acid

The lack of a relationship between the sign of optical rotation (d or 1) and absolute configuration as designated by D/L, coupled with the uncertainties in assignments for primary substituents inherent to the D/L nomenclature system, led to the development of an unambiguous system for the designation of absolute configuration by Cahn, Ingold, and Prelog. In applying this method, the compound is oriented in a Fischer projection and the four groups or atoms bound to an asymmetric carbon are ranked by the following set of sequence rules:

1. Substituents are ranked (1, 2, 3, 4) by the atomic number of the atom directly joined to the chiral carbon.
2. When two or more of the atoms connected to the chiral carbon are the same, the atomic number of the next adjacent atom determines the priority. If two or more atoms connected to the second atom are the same, the third atom determines the priority, etc.
3. All atoms except hydrogen are formally given a valence of 4. When the actual valence is less than 4 (N, O), phantom atoms are assigned an atomic number of zero and therefore rank the lowest.
4. A tritium atom has a higher priority than deuterium, which has a higher priority than hydrogen. Similarly, any higher isotope has a higher priority than any lower one.
5. Atoms with double and triple bonds are counted as if they were connected by two or three single bonds. Hence a C==C is regarded as a carbon bound to two carbons; and a C==O is regarded as a carbon bound to two oxygens,

Once the four groups bound to the chiral carbon are ranked, the compound is oriented in such a way that the lowest priority group (4) is projected away from the observer. Then, if the other groups (1, 2, 3) are oriented by priority in a clockwise fashion, the molecule is designated as R (rectus), and if counterclockwise, as S (sinister). These sequence rules are applied in the assignment of the absolute configurations for the enantiomers of glyceraldehyde in Fig. 15. According to the first rule, the highest priority substituent (1) is the hydroxy group (OH) and the lowest priority group (4) is the hydrogen atom; since the atomic number of carbon is higher than

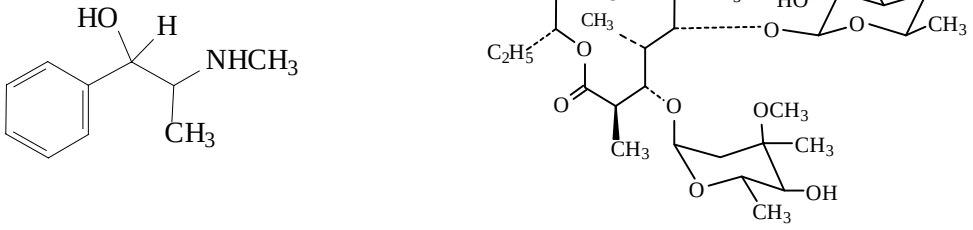
that of hydrogen but lower than that of oxygen, the two carbon substituents (CHO and CH_2OH) are assigned intermediate priorities. To determine the priority relative to the two carbon substituents, both the 2nd and 5th rules must be applied. The aldehyde carbon is part of a carbonyl (C=O) moiety which, by rule 5, is equivalent to a carbon bound to two oxygen atoms. The alcohol carbon (CH2OH) is bound to one oxygen and two hydrogens. The "two oxygens" of the aldehyde take priority over the single oxygen of the alcohol moiety; the aldehyde is assigned priority 2, and the alcohol priority 3. With all substituents ranked and the enantiomers oriented in such a way that the lowest priority group (4) is projected away from the observer, the configurations can be assigned. The enantiomer in which the substituents are oriented by priority in a clockwise fashion, is designated as R, and the enantiomer in which the substituents are oriented by priority in a counterclockwise direction is designated as S.

S-Glyceraldehyde R-Glyceraldehyde

Figure 15. Cahn-Ingold-Prelog sequence rule

V. Compounds with Multiple Centers of Asymmetry

Many stereoisomeric drugs contain more than one asymmetrically substituted atom. For example, ephedrine has two chiral centers, and the macrolide antibiotic eryth-romycin has 18 (Fig. 16). In such cases, a greater number of configurational isomers is possible;.the maximum number possible is $2^{n,}$ where n is the number of chiral atoms.



Ephedrine Erythromycin

Figure 16. Compounds with multiple chiral centers

For compounds with two chiral centers, the maximum number of configurational isomers possible is four, based on the 2^n rule. Since each chiral center may have an R- or S-configuration, the isomers possible include *RR, SS, RS,* and *SR,* as shown for ephedrine in Fig. 17. The *RS* and *SR* isomers are nonsuperimposable mirror images and hence are enantiomers. The same relationship exists for the *RR* and *SS* isomers. The relationship between each member of an enantiomeric pair and each member of the other enantiomeric pair is diastereomeric; they are

non-superimposable nonmirror images. Thus the *RS* isomer is a diastereomer of the *RR* and *SS* isomers, and the *SS* isomer is a diastereomer of the *RS* and *SR* isomers. In this case, the *SR* and *RS* enantiomers are referred to as ephedrines, whereas the *RR* and *SS* enantiomers are called pseudoephedrines. Diastereomers, unlike enantiomers, have' different physicochemical properties. Therefore the ephedrines have different solubilities, melting points, etc., than the pseudoephedrines.

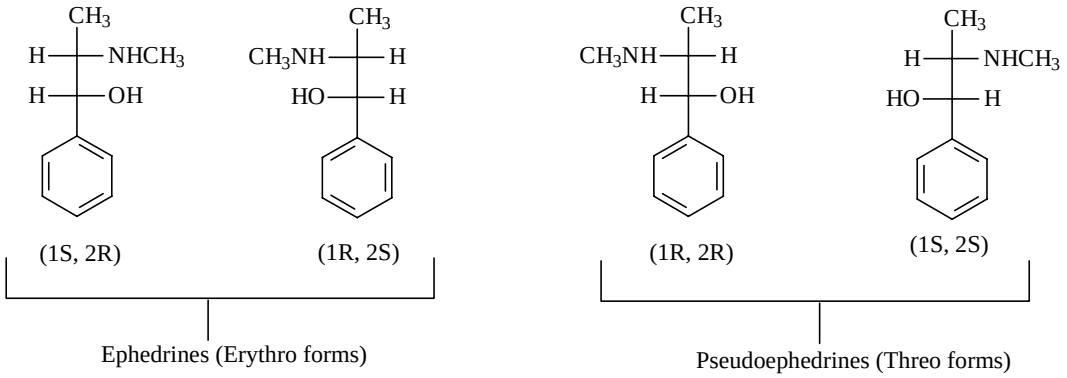


Figure 17. Ephedrine and pseudoephedrine stereoisomers.

In addition to the R and S designations, compounds with two chiral centers may also be identified by stereochemical nomenclature which describes the entire system. For example, the *erythro* and *threo* nomenclature derived from carbohydrate chemistry may be employed to describe the relative positions of similar groups on each chiral carbon. Thus, the ephedrines are designated as *erythro* forms since the similar groups (OH and NHCH3) are on the same side of the vertical axis of the Fischer projection, and the pseudoephedrines are designated as *threo* forms since like groups are on opposite sites of the vertical axis of the projection (Fig. 17).

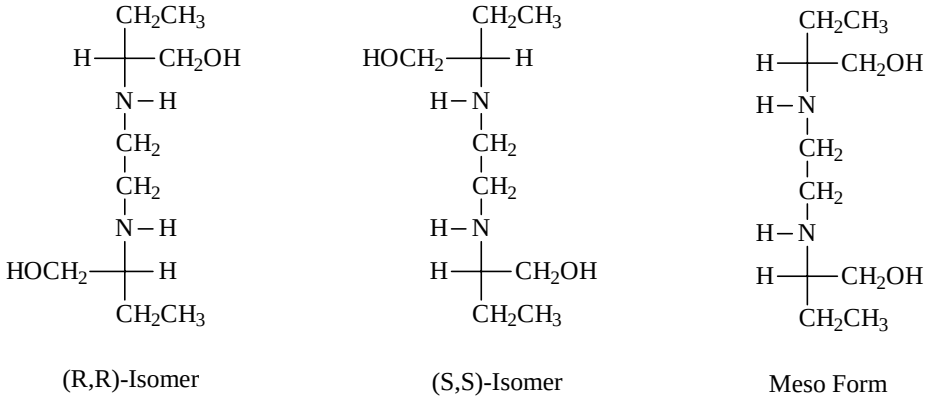


Figure 18. Ethambutol stereoisomers

It is important to realize that the 2^n rule predicts only the maximum number of stereoisomers possible in compounds with more than one center of chirality. For example, some compounds with two asymmetrically substituted carbon atoms may have only three stereoisomeric forms. This occurs when three of the substituents on one asymmetric carbon are the same as those on the other asymmetric carbon, as shown for the antitubercular ethambutol (Fig. 18). In this case, one

stereoisomer has a plane of symmetry even though two asymmetric atoms are present. Such an isomer is referred to as a *meso* compound and is optically inactive. Therefore, when a plane of symmetry is present in a compound with two centers of asymmetry, only three stereoisomeric forms are possible.

A number of drugs possess three or more asymmetrically substituted carbon atoms. For example, there are three chiral carbon atoms in the bicyclic ring system of the penicillin antibiotics (Fig. 19). Based on the 2' rule, eight stereoisomers are possible for the penicillins, but only the naturally biosynthesized *3S,5R,6R* en-antiomer displays significant antibacterial activity. Other antibiotics, such as the aminoglycosides and the tetracyclines, contain multiple chiral centers; the macrolide erythromycin has 18 chiral centers and thus there are 262,144 possible stereoisomers! (Fig. 16). These antibiotics are produced by microbes from chiral endogenous substances and thus a single stereoisomeric form is typically produced.

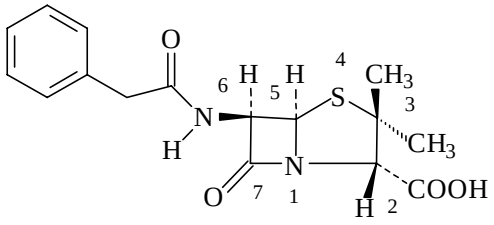


Figure 19. (3*S*, 5*R*, 6*R*)-Benzylpenicillin