THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 235, No. 10, October 1960
Printed in U.S.A.

The Nature of Substrate Asymmetry in Stereoselective Reactions*

H. HIRSCHMANN

From the Departments of Medicine and Biochemistry, School of Medicine, Western Reserve University, Cleveland, Ohio

(Received for publication, December 22, 1959)

During the preparation and review of the paper on glycerol dissimilation by Stjernholm and Wood (1), it became evident that the term "symmetrical 3 carbon compound" is ambiguous. This ambiguity arises because the term symmetry has acquired a dual meaning in biochemical discussions. There is a type of symmetry which prevents the existence of optically active forms and there is a type which prevents the differentiation of identical groups in chemical reactions. Glycerol traditionally has been considered a symmetrical molecule because it is optically inactive, but it is not symmetrical when judged by the reactivity of its primary carbinol groups which are differentiated in biological systems, as was demonstrated by Schambye *et al.* (2), and by Swick and Nakao (3). It is the main purpose of this communication to define the asymmetric characteristic which in glycerol and many other compounds permits the differentiation of seemingly identical groups.

The possibility of such discrimination was foreseen by Ogston (4), who showed that it could be effected by a three point attachment of a substrate like glycerol to the surface of an enzyme. Although this model of the substrate-enzyme complex clearly demonstrates a steric difference between the two carbinol groups, it apparently gave rise to the notion that this difference was not inherent but was acquired only upon attachment to the enzyme. The concept of a "biological asymmetry" which ascribes to the enzyme a unique role in the process of discrimination became untenable when Schwartz and Carter (5) demonstrated that the discrimination of structurally identical groups could still occur if a simple asymmetric reagent like 1-α-phenethylamine was substituted for an enzyme.[1] As in the case of glycerol, the substrate in this experiment was of the structural type Caabc which signifies a central carbon atom substituted with four *symmetrical* groups of which two (a) are identical, but different from the two other dissimilar groups (b and c). The steric nonequivalence of the (a) groups which was shown by their discrimination by the asymmetric reagent can also be detected by inspection of molecular models (5). Schwartz and Carter suggested several ways by which this fact could be recognized, including the following observation. Although the molecule Caabc possesses a plane of symmetry which bisects the central carbon atom as well as the (b) and (c) groups, the resulting halves are not identical since they cannot be superimposed upon each other. Since this situation resembles the one prevailing in the *meso* form of a compound possessing a plane of symmetry which bisects the molecule into two nonsuperimposable halves, the central carbon of Caabc has been designated as a *meso*-carbon atom (5). It represents the simplest structural type which permits the differentiation of the two (a) groups. Any further simplification of the structure Caabc, such as the substitution of (c) either by an (a) or by a (b) group, results in a compound which no longer allows the discrimination of the (a) groups (5). The concept of the *meso*-carbon atom is one of great utility, because whenever a structure contains one or more *meso*-carbon atoms, the differentiation of their like substituents is possible. However, it is not a universal criterion, because there exist structures which permit the differentiation of structurally identical groups that are not substituents of *meso*-carbon atoms. Illustrations of this fact will be presented in the "Survey" given below. Since Schwartz and Carter could account for the asymmetric behavior of like substituents of *meso*-carbon atoms by the asymmetric location of each (a) group relative to the remainder of the molecule (Cabc-), they thought it would be desirable to distinguish these sterically distinct positions by appropriate designations, but refrained from making specific suggestions. A nomenclature which would serve this purpose will be proposed in this communication.

Before this analysis (5), Wilcox (7) had presented the problem of differentiation from another point of view. If one of the (a) groups in Caabc is isotopically labeled as in Caa*bc, the structure no longer has a plane of symmetry if the two isotopes are considered as distinct atoms. Such compounds were termed "isotopic pseudoasymmetric" by Wilcox and "isotopically asymmetric" by Karnovsky *et al.* (8). Wilcox's attempt to explain differentiation by this variant of the usual asymmetry was not completely successful because his criterion (7), although not limited to the structural type Caa*bc, does not describe all structures which permit differentiation.[2] Moreover, the concept of isotopic asymmetry tends to obscure the cause of asymmetric behavior by focusing attention on a tool which can, but frequently need not, be used (5, 11, 12) to demonstrate the phenomenon of differentiation. Even when applied, the success of isotopic labeling in detecting differentiation depends on the fact

---

* Supported by Grant C-1679 of the National Institutes of Health, United States Public Health Service.

[1] This experiment has been criticized on the grounds that the process was reversible (6). Although this would have a bearing on the mechanism of discrimination, it does not alter the fact that discrimination occurred. Moreover, this writer has learned from Dr. Carter that unpublished control experiments established the irreversibility of the process.

[2] One structural type Caa(+b)(−b) has been noted previously (9). Additional cases in which the rule of Wilcox does not predict differentiation are exemplified by the pairs of hydrogen atoms attached to C-4 in citric acid and to C-2 or to C-4 in cyclohexanol (*XI*). Another criterion was proposed by Racusen and Aronoff (10), but this rule was shown to have exceptions (5, 9).

that the enzyme or reagent is unable to detect the label (7, 8, 13) and, therefore, ignores the asymmetry which has been introduced into the molecule with the isotope.

ROTATIONAL AND REFLECTIVE SYMMETRY

Two objects can be regarded as identical only if they can be superimposed upon each other. With the use of this principle one can arrive at a general criterion for the steric equivalence or difference of structurally identical groups (or atoms) (9). To facilitate the discussion, these identical groups will be designated by the distinct symbols (a') and (a''). If a molecular model of a compound containing such groups can be superimposed upon itself in such a way that the (a') group of one representation coincides with the (a'') group of the other, and every other atom of the first representation coincides with a like atom of the other, then obviously these two arrangements are indistinguishable and no reagent can react preferentially with either the (a') or (a'') group. Conversely, if such superposition is impossible it will be equally impossible to superpose the *product*[3] resulting from the interaction of a reagent R with one of the (a) groups upon the *product* resulting from its interaction with the other. The two products, therefore, are not identical but may be mirror images if R is a symmetrical reagent. If they are mirror images,[4] no differentiation can occur because both products possess equal probability of formation and equal stability. However, if R is optically active (*e.g.* $(+)R$), the two products cannot be optical antipodes because the mirror image of the product $X(+)R$ is not $X'(+)R$ but $X'(-)R$, if X' indicates the mirror image of X. Since different compounds which are not optical antipodes are likely to differ at least to some extent in the ease of their formation and in their stability, differentiation of the two (a) groups is possible. If the reagent is an enzyme, differentiation whenever possible is likely to be complete, although this is not invariably the case (14). We conclude, therefore, that a compound permits the differentiation of two structurally identical groups (a') and (a'') if and only if it is impossible to superpose a model of the compound upon itself in such a way that the (a') and (a'') groups coincide and the two arrangements are otherwise indistinguishable. Application of this test to glycerol (*I*) and to dihydroxyacetone (*II*) (Fig. 1) shows that the primary carbinol groups of glycerol can be differentiated, but those of dihydroxyacetone cannot.

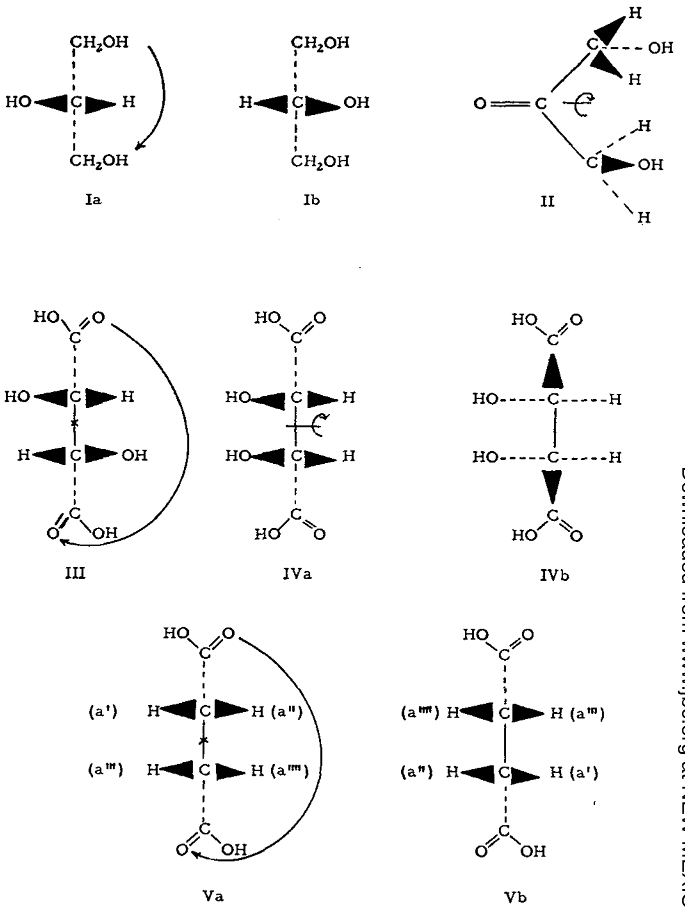


FIG. 1. Examples of rotational symmetry and asymmetry. Rotation of dihydroxyacetone (*II*) around an axis coincident with the carbonyl bond permits superposition of the carbinol groups, whereas glycerol (*Ia*) if rotated, *e.g.* 180° around an axis perpendicular to the plane of the paper, gives *Ib* which is different from *Ia*. D-Tartaric acid (*III*) and succinic acid (*V*) can be superposed upon themselves by such a rotation, but *meso*-tartaric acid (*IVa*) cannot. If the latter is rotated around an axis in the plane of the paper as indicated, *IVb* results which is again different from *IVa*.

In Figs. 1 to 3 solid triangles signify bonds which project from the bases towards the reader, whereas broken lines extend from the central carbon atoms away from the reader.

In general, a finite rigid object is termed symmetrical if it can be superposed: (*1*) on its mirror image, or (*2*) upon itself in a position which differs from the original one. Although the meaning of symmetry as usually used in chemistry is restricted to that defined by the first criterion, it is evident that both types of symmetry are of importance in this field. If a molecular model can be superposed upon its mirror image, resolution into optical antipodes is impossible. If the model can be superimposed upon itself in a novel arrangement, the identical groups (a') and (a'') which thereby come into coincidence cannot be differentiated in any reaction. It follows, therefore, that the differentiation of the primary carbinol groups of glycerol is indeed caused by a lack of symmetry in its structure, but of a symmetry which differs in kind from the one which prevents the resolution of glycerol into optical antipodes.

To distinguish the two types of symmetry which characterize structures that can be superimposed either on their mirror images or in a new way upon themselves, the terms reflective and rotational symmetry have been used (15).[5] These terms refer to diagnostic tests for such symmetries. Mathematical analysis

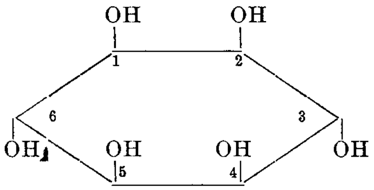
---

[3] The term "product" is used here in a broad sense. It may refer to a compound, to the association of two or more substances as in a substrate-enzyme complex, or to a transition state.

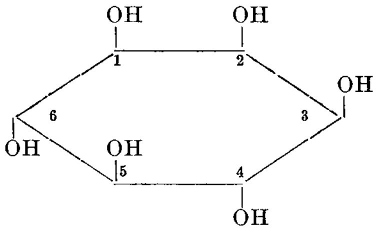
[4] Even if R has no optical activity, the products are not invariably mirror images of each other, and if they are not, differentiation is possible. For example, the two hydrogens at C-2 in cyclohexanol (*XI*) can be differentiated by symmetrical reagents as in the stereospecific eliminations to form cyclohexene. The structural requirements for differentiation by optically inactive reagents are stated in footnote 14.

[5] These two terms are shorter and probably better suited for discussions such as the one given by Stjernholm and Wood (1) than the customary designations in terms of axes of symmetry. To avoid any ambiguity, the term "rotational symmetry" as used in this paper corresponds to the presence of a simple axis of symmetry greater than one, and the term "reflective symmetry" to the presence of an alternating axis of symmetry. (In current terminology "axis of rotatory inversion" is preferred to the more familiar "alternating axis of symmetry" (16).)

has shown that two identical rigid objects (or two positions of the same object) with the same center of gravity can always be superimposed by a rotation. Furthermore, any rigid object which can be superimposed upon its mirror image can always be made to yield an arrangement indistinguishable from the original by means of a reflection in a plane (which produces the mirror image) to be followed, if necessary, by a rotation (which brings this mirror image into coincidence with the original). Therefore, a structure has *reflective symmetry* if an indistinguishable arrangement can be obtained either by a reflection alone or by a reflection in combination with a rotation.[6] Such a structure can be superimposed upon its mirror image and, therefore, cannot exist in an optically active form. A structure has *rotational symmetry* if another arrangement indistinguishable from the first can be obtained by a rotation alone. Structurally identical groups which can be brought into coincidence by such a rotation cannot be differentiated in any reaction.[7] Both types of symmetry can coexist in the same compound and either of them can occur without the other. For example, dihydroxyacetone (*II*) has both; D-tartaric acid (*III*) lacks reflective symmetry but has rotational symmetry and its identical groups (carboxyl, carbinol, hydrogen), which all can be superimposed (Fig. 1), therefore cannot be differentiated; *meso*-tartaric acid (*IV*), on the other hand, which has reflective symmetry but no rotational symmetry permits the differentiation of its structurally identical groups. It should be emphasized that rotational symmetry prevents the differentiation only of those groups which can be brought into coincidence by operating the test for such symmetry. For example, the rotation which demonstrates the rotational symmetry of succinic acid (*V*) permits the superposition of the methylene and the carboxyl carbons and of two pairs of hydrogen atoms (labeled (a') and (a''''); (a'') and (a''')). No rotation, however, allows the superposition of (a') or (a'''') on either (a'') or (a''') and these pairs, therefore, can react at different rates.[8] Similarly, the two carbinol groups of dihydroxyacetone are indistinguishable, but the two carbon-bound hydrogens at each carbinol are sterically distinct. The utility of the rotation test is particularly apparent in the case of the inositols. It shows almost at a glance that *muco*-inositol (*VI*) contains three pairs of carbon atoms (numbered 1 and 4, 2 and 5, and 3 and 6) which cannot be differentiated. These pairs are sterically distinct from each other as are all six carbon atoms of *myo*-inositol (*VII*).



VI



VII

NOMENCLATURE

Two systems of nomenclature have been proposed for labeled glycerol. Schambye *et al.* (2) have suggested that the isomer of labeled glycerol which when fed to rats gives rise to glucose-3,4-C^{14} be called glycerol-1-C^{14} in conformity with lactate-1-C^{14}, which also is converted to glucose-3,4-C^{14}. Karnovsky *et al.* (8) determined the configuration of this type of labeled glycerol and called it L-glycerol-1-C^{14} and thereby gave to glycerol-3-C^{14} the designation D-glycerol-1-C^{14}. Neither system provides a satisfactory nomenclature for all purposes. The D-L nomenclature is limited to isotopically labeled componds. It therefore gives no opportunity to express the fundamental fact that the two primary carbinol groups of unlabeled glycerol are sterically distinct. Obviously, if the differentiation of "identical" groups is demonstrated without isotopes (11, 17), this D-L system cannot be used. The numbering system of Schambye *et al.* (2) does not disclose the steric structure. It is based on a metabolic reaction as effected by a specified organism. In this case, experimental details may influence the numbering since it cannot be taken for granted that different enzymes act in the same manner.[9] Every extension of this system to other compounds requires similar specifications of the enzyme system and of the reaction product which is used for numbering the precursor.

The system now proposed is based on steric structure and is applicable to unlabeled compounds if they contain identical groups which can be differentiated. If atoms of such groups are customarily designated by number (*e.g.* carbon atoms) conventional numbering permits two ways to number a given atom. The proposed system determines which of these numbers is to be used. Where numbering is not customary, as in the case of hydrogen atoms, it is suggested to differentiate the two distinct orientations by the symbols (*A*) and (*P*) (derived from the Latin words *ante*, in front of, and *post*, behind). To make the necessary conventions as few in number and as widely applicable as possible, it seemed desirable to use some of the rules of the (*R*)-(*S*) system which has been devised by Cahn *et al.* (18) for designating asymmetric configurations. Although their system at times ignores close structural similarities, it possesses the great advantage of universal applicability.

---

[6] The two main criteria for this type of symmetry, the plane and the center of symmetry, are special cases of this general rule and can readily be derived from it. If no rotation is required the structure has a plane of symmetry. If an indistinguishable arrangement results from a reflection in a plane followed by a rotation through 180° around an axis perpendicular to this plane, the structure has a center of symmetry.

[7] The converse statement that a compound with rotational asymmetry cannot have indistinguishable groups is strictly true only if the compound can be adequately represented by a rigid molecular model (9). Minor complications can arise from rotations around single bonds and are common to all stereochemistry. This problem is met most readily if we consider groups as indistinguishable, also if they can be superposed by a rotation around a single bond which yields an otherwise indistinguishable arrangement. For example, although creatinine lacks rotational symmetry, the three hydrogen atoms of its methyl group are sterically equivalent as shown by a rotation around the methyl-nitrogen bond.

[8] As yet, this differentiation has not been observed (14).

[9] Recently, Moore described a rat liver preparation which apparently converts glycerol directly to D-glyceraldehyde (17). A conversion of labeled glycerol to glucose which would proceed by this pathway should result in a labeling different from the one observed under the conditions of Schambye *et al.* (2), (*e.g.* labeling at 1 and 6 instead of 3 and 4).

In the (*R*)-(*S*) system of Cahn *et al.* one determines the priority sequence of the substituents of an asymmetric atom. This sequence is in the order of decreasing atomic numbers of the *atoms* bound to the asymmetric center. If the relative priority of two or more atoms cannot be established in this manner, a similar comparison of the atomic numbers of their substituent atoms is made. If there is still no difference one continues on outward until a decision can be reached. For proper interpretation of this and of a few supplementary rules, the original paper (18) should be consulted. For the examples given here and for many others of biochemical interest, it will be sufficient to recognize the following priority order of substituents:

OH, NH_2, COOH, ketone, aldehyde, tertiary, secondary, primary carbinol, tri-, di-, monoalkylated CH_3, CH_3, and H.

If the sole difference between two groups lies in their geometry, *cis* has priority over *trans*. If they differ only in their configuration, the one assigned the (*R*) takes precedence over the one assigned the (*S*) configuration. These configurations are determined by viewing a three-dimensional model from the side opposite to the substituent with the lowest priority. They are called (*R*) and (*S*), respectively, if the sequence of the remaining three substituents in the order of their priorities describes a clockwise (*rectus*, right) or counterclockwise (*sinister*, left) turn (Fig. 2).

If this procedure is applied to a structure exemplified by Caabc, no configuration can be determined because the two (a) groups have the same priority. The system, however, can still be utilized for our purposes if we arbitrarily assign a *fixed* configuration to such compounds. We then can reverse the process and determine a priority order for the structurally identical substituents (a). In order to avoid a numbering system different from the one chosen for glycerol on biogenetic grounds (2), the (*S*) configuration has been adopted as the frame of reference. The system can be formalized by two rules.

Rule 1—The symbols for the four substituents (a), (a), (b), (c) are chosen so that (b) has a higher priority than (c) in the Cahn-Ingold-Prelog system (18). The molecular model is viewed from the side opposite to (c). Starting from (b) in a counterclockwise direction, the first (a) group reached is assigned, as appropriate, either the lower number or the (*A*) orientation (Fig. 3). An alternative formulation of this rule, which is suitable chiefly for aliphatic compounds, may be more convenient in this field. A Fischer projection of Caabc which shows the higher priority substituent (b) to the *left* and the (c) substituent to the right has the (a) group with the lower number or (*A*) orientation *above* the central carbon. Conversely, if the (b) group is *above* and the (c) below the central atom, the (a) group with the lower number is on the *left*.[10]

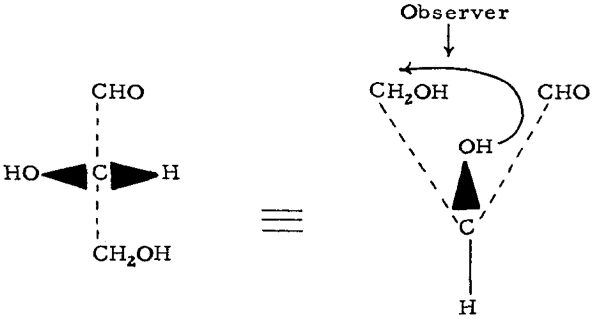


FIG. 2. L-Glyceraldehyde. Priority sequence: OH, CHO, CH_2OH, H. The compound has the (*S*) configuration.

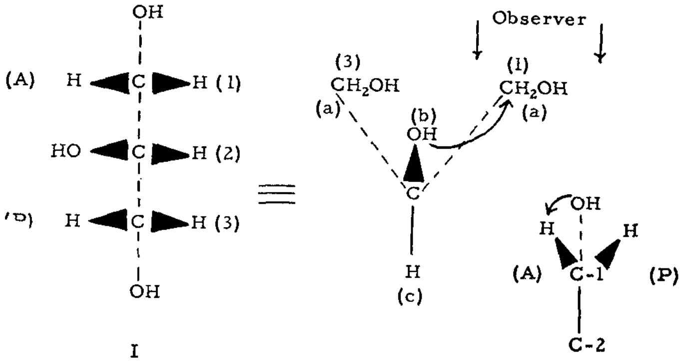


FIG. 3. Designation of the sterically distinct positions in glycerol.

Rule 2—In internally compensated structures the asymmetric center with the (*R*) configuration is given the lower number. If there are two or more pairs of asymmetric centers, that numbering system is chosen which results in the (*R*) configuration for the asymmetric carbon atom with the lowest number.[11]

The use of these rules will be illustrated by examples given in the next section.

SURVEY OF STRUCTURAL TYPES WITH REFLECTIVE SYMMETRY WHICH PERMIT DIFFERENTIATION[12]

1. Differentiation of Like Substituents of Meso-Carbon Atoms—As shown by the rotation test, the two structurally identical groups attached to a *meso*-carbon atom (Fig. 1, *I*) can be differentiated. Examples of *meso*-carbon atoms are C-2 of glycerol (Fig. 3), C-3 of citric acid (*VIII*),[13] C-1 of cyclohexanol (*XI*), and the two methylene carbon atoms of succinic acid (*V*). The stereonomenclature for these compounds is determined by Rule

---

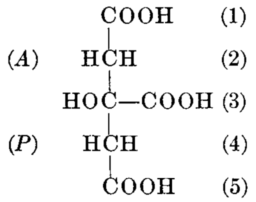
[10] If a Fischer projection is not presented in such a manner that this rule can be applied, it should be rotated in the plane of the paper by 180° but not by 90°. All projection formulas given (*VIII*–*X*) are Fischer projections. The term implies that the substituents above or below a central carbon atom are farther from the observer than those shown to the right or left.

[11] Although it would have been more in keeping with the rules of Cahn *et al.* to select the asymmetric center nearest the plane or center of symmetry as the one determining the direction of numbering, the above rule was chosen since it can be applied not only to aliphatic structures but also to such cyclic compounds as the inositols. In the numbering system of the inositols by Fletcher *et al.* (19), two "stereochemically equivalent carbon atoms" are chosen as number 1. These alternate choices for C-1, however, are sterically not equivalent since they possess antipodal configurations. Application of Rule 2 unambiguously selects one of these alternatives and results in a numbering system identical with the one used by Magasanik (11) in summarizing the action of *Acetobacter suboxydans* (Table I in reference (11)) and by Cahn *et al.* (18).

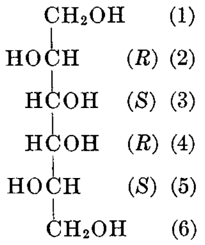
[12] This survey is not exhaustive and is limited to structural types that have been or might be encountered in biochemical studies.

[13] Martius and Schorre (13) have proposed a configuration for the dideuterocitric acid obtained from oxalocitramalic acid lactone. This configuration is based on the application of the lactone rule to the optical rotation of this lactone and indicates that C-4 in *VIII* is converted to the keto group of α-ketoglutaric acid. The numbering which results from the application of Rule 1 does not agree with the one chosen by Mosbach *et al.* (20). Also, it should be noted that Martius and Schorre (13) present L-dideuterocitric acid in a projection formula which does not conform to the Fischer convention (5).

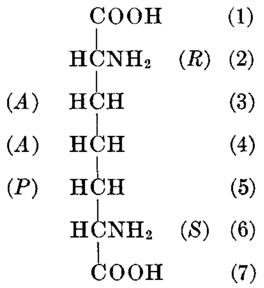
1. The carbon-bound hydrogen atoms of succinic acid which in *V* are labeled (*a*′) and (*a*′′′′) have the (*A*) orientation; the two others, the (*P*) orientation.



VIII



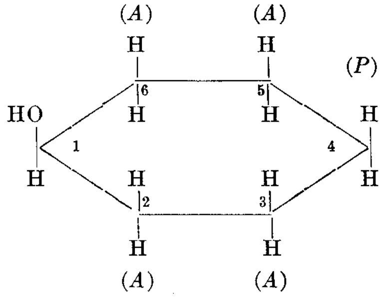
IX



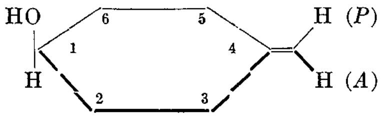
X

2. Differentiation of Other Like Groups in Compounds Containing Meso-Carbon Atoms—Schwartz and Carter (5) in defining a *meso*-carbon atom specified that every substituent group of such an atom possesses a plane of symmetry. It is readily seen that C-1 and C-3 in glycerol (Fig. 3) are not *meso*-carbon atoms, because one of their substituents (—CH(OH)·CH$_2$OH) has no plane of symmetry. Similarly, C-2 and C-4 of citric acid (*VIII*) and C-2 to C-6 of cyclohexanol (*XI*) are not *meso*-carbon atoms. Nevertheless, the rotation test shows that their like substituents (hydrogen atoms) also can be differentiated from each other. It is desirable to distinguish these centers from *meso*-carbon atoms because the latter but not the former require optically active reagents for the differentiation of their structurally identical substituents.[14]

The orientations of the hydrogen atoms of the methylene groups of *I* (Fig. 3), *VIII*, and *XI* can be assigned by means of Rule 1. This is possible even for the hydrogens at C-4 in cyclohexanol (*XI*), if one takes into account that C-3 has a higher priority than C-5. However, in the case of cyclic structures the (*A*)-(*P*) nomenclature is not needed since it is more convenient to designate the orientations of the various hydrogen atoms of cyclohexanol in the conventional manner by stating their *cis* or *trans* relationship to the hydroxyl group.



XI



XII

3. Differentiation in Compounds with Asymmetric Carbon Atoms—These structures are exemplified by *meso*-tartaric acid (*IV*), *muco*-inositol (*VI*), *myo*-inositol (*VII*), dulcitol (*IX*), and *meso*-2,6-diaminopimelic acid (*X*) (12). In these *meso* compounds the direction of numbering of the carbon atoms is determined by Rule 2. The assignment of the (*A*) and (*P*) orientations for the carbon-bound hydrogens in *X* requires the use of Rule 1.

4. Differentiation in Compounds with Rotational Asymmetry of the Axial[15] *Type*—Although the exocyclic carbon atom in *XII* is not a *meso*-carbon atom, the two hydrogens attached to it can be differentiated, since the structure lacks rotational symmetry. As explained by Cahn *et al.* (18) the four substituents to be considered in this case are the hydroxyl (b) and the alkyl-bound hydrogen (c) at C-1 and the two hydrogens (a) attached to the olefinic carbon. Use of Rule 1 leads to the assignment shown in *XII*.

NOMENCLATURE OF ISOTOPICALLY LABELED COMPOUNDS WHICH PERMIT DIFFERENTIATION

Ordinarily, the same numbering system is used for isotopically labeled compounds as for their normal analogues. It is justified, therefore, to refer to D- and L-glycerol-1-C^{14} also as glycerol-3-C^{14} and glycerol-1-C^{14}, respectively. In the D-L system the position of the labeled atom is held constant and the orientations of the hydroxyl and hydrogen at C-2 are used as the index of isomerism. This is a logical nomenclature for chemical studies such as the preparation of the isomers from an enantiomorphic pair like D and L-glyceric acid-1-C^{14} (8). In the system now proposed, the orientations of the hydrogen and of the hydroxyl are held constant and the position of the labeled carbinol group is used as index of isomerism. The two isomers of labeled glycerol when adsorbed on a stereospecific enzyme must show the same orientations of the four substituents (reacting, nonreacting primary carbinol, hydroxyl, and hydrogen) relative to the enzyme surface. If the orientations were different for the two isomers, the enzyme would be able to detect the position of the labeled atom which, as was pointed out above, is not the case. Therefore, the system

---

[14] If a molecule has structurally identical groups (*a*′) and (*a*″) *which can be differentiated according to the rotation test*, their differentiation requires an optically active reagent, only if the molecule has reflective symmetry and if (*a*′) and (*a*″) can be brought into coincidence by operating the test for such symmetry. For example, cyclohexanol (*XI*) has no rotational symmetry and all of its hydrogen atoms, therefore, can be differentiated. The molecule has a plane of symmetry which bisects C-1 and C-4. The hydrogens at C-2 and at C-6 which are *cis* to the hydroxyl occupy corresponding positions across the plane of symmetry. They are brought into coincidence by reflection in this plane and, therefore, can be differentiated only by optically active reagents. However, the two hydrogens at C-2, both of which lie on the same side of the plane of symmetry, or the ones at C-4, which lie within this plane, are not mutually superposed by reflection in the plane of symmetry and therefore allow differentiation by optically inactive reagents. Similarly, in a *meso* compound Caa(+b)(−b) the differentiation of the (a) groups is possible with any reagent, whereas a reagent differentiating between the (b) groups must be optically active.

[15] The term "axial" is used here in the same sense as employed by Cahn *et al.* (18).

which fixes the *orientations of the substituents* rather than the *position of the isotope* is the logical one to describe metabolic studies. Consequently, if we designate the two isomers as glycerol-1-C^{14} and glycerol-3-C^{14} we can present experimental data with greater clarity and succinctness. For example, this system allows one to summarize the observations that L-glycerol-1-C^{14} phosphorylates at C-3 and D-glycerol-1-C^{14} at C-1 (8) with the statement that regardless of the site of labeling, glycerol is phosphorylated at C-3.

SUMMARY

The symmetry properties which determine whether a compound can have structurally identical substituents that cannot be differentiated in chemical reactions, are distinct from the symmetry properties which determine whether it can have optical activity. The two types of symmetry are termed rotational and reflective symmetry. A structure has reflective symmetry if it can be superposed on its mirror image. Rotational symmetry is present if a rotation of the molecule produces another indistinguishable arrangement. All groups (or atoms) which can be brought into coincidence by such a rotation react at identical rates, whereas those which cannot be superposed in an indistinguishable arrangement can be differentiated by their reactivity. In many, but not all cases, this differentiation requires an optically active reagent such as an enzyme. A rule is given which states when differentiation by optically inactive reagents can occur.

A nomenclature has been proposed which allows one to designate by different numbers or symbols, structurally identical groups which can be differentiated. It is applicable to labeled and unlabeled compounds.

Acknowledgment—The author is greatly indebted to Drs. H. E. Carter, H. G. Wood, and D. Y. Curtin for valuable discussions during the preparation of this manuscript.

REFERENCES

1. Stjernholm, R., and Wood, H. G., *J. Biol. Chem.*, **235,** 2757 (1960).
2. Schambye, P., Wood, H. G., and Popják, G., *J. Biol. Chem.*, **206,** 875 (1954).
3. Swick, R. W., and Nakao, A., *J. Biol. Chem.*, **206,** 883 (1954).
4. Ogston, A. G., *Nature (London)*, **162,** 963 (1948).
5. Schwartz, P., and Carter, H. E., *Proc. Natl. Acad. Sci., U. S.*, **40,** 499 (1954).
6. Wolfrom, M. L., *Proc. Natl. Acad. Sci., U. S.*, **40,** 794 (1954).
7. Wilcox, P. E., *Nature (London)*, **164,** 757 (1949).
8. Karnovsky, M. L., Hauser, G., and Elwyn, D., *J. Biol. Chem.*, **226,** 881 (1957).
9. Hirschmann, H., In S. Graff (Editor), *Essays in biochemistry*, John Wiley and Sons, Inc., New York, 1956, p. 156.
10. Racusen, D. W., and Aronoff, S., *Arch. Biochem. Biophys.*, **34,** 218 (1951).
11. Magasanik, B., In S. Graff (Editor), *Essays in biochemistry*, John Wiley and Sons, Inc., New York, 1956, p. 181.
12. Antia, M., Hoare, D. S., and Work, E., *Biochem. J.*, **65,** 448 (1957).
13. Martius, C., and Schorre, G., *Ann. Chem. Liebigs*, **570,** 143 (1950).
14. Englard, S., and Colowick, S. P., *J. Biol. Chem.*, **221,** 1019 (1956).
15. Weyl, H., *Symmetry*, Princeton University Press, Princeton, 1952, p. 66, 43, 44.
16. Woodward, P., *Chem. & Ind. (London)*, 1599 (1955).
17. Moore, B. W., *J. Am. Chem. Soc.*, **81,** 5837 (1959).
18. Cahn, R. S., Ingold, C. K., and Prelog, V., *Experientia*, **12,** 81 (1956).
19. Fletcher, H. G., Jr., Anderson, L., and Lardy, H. A., *J. Org. Chem.*, **16,** 1238 (1951).
20. Mosbach, E. H., Phares, E. F., and Carson, S. F., *Arch. Biochem. Biophys.*, **33,** 179 (1951).