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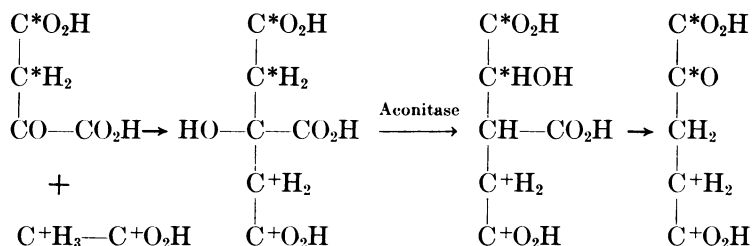
A NONENZYMATIC ILLUSTRATION OF "CITRIC ACID TYPE"  
ASYMMETRY: THE MESO-CARBON ATOM\*

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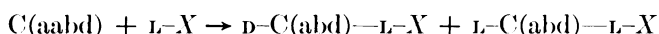
*Communicated March 3, 1954*

It has been clearly demonstrated that in the enzymatic synthesis and degradation of citric acid the two  $-\text{CH}_2\text{CO}_2\text{H}$  groups behave differently.<sup>1-11</sup> One arises from acetate and yields the carboxymethyl group of the derived  $\alpha$ -ketoglutarate<sup>3, 4, 5, 7, 9</sup>. The other is produced from the carboxymethyl group of oxaloacetate and yields the oxalyl group of  $\alpha$ -ketoglutarate:<sup>1, 2, 6, 8</sup>:



Thus one of the groups in citric acid is described as the "oxaloacetate-derived, aconitase-active group" and the other as the "acetate-derived, aconitase-inactive group." Ogston<sup>12</sup> has attempted to explain this specific behavior of the two "identical" groups in a symmetrical molecule on the basis of a "three-point" attachment of the citrate molecule to the enzyme surface. Wilcox<sup>13</sup> has also advanced a theoretical explanation of this behavior.

In view of the unexpected results obtained in these enzymatic reactions, it seemed highly interesting to determine whether an analogous behavior could be demonstrated in a nonenzymatic, homogeneous reaction system, or, in other words, to discover whether an unequal amount of the two diastereoisomeric products would result from the reaction of an asymmetric substance  $L-X$  with a molecule of the type  $C(aabd)$  in which all four groups are symmetrical (i.e., possess at least one plane of symmetry), and two ( $aa$ ) are identical but differ from the two other, dissimilar groups ( $bd$ ):

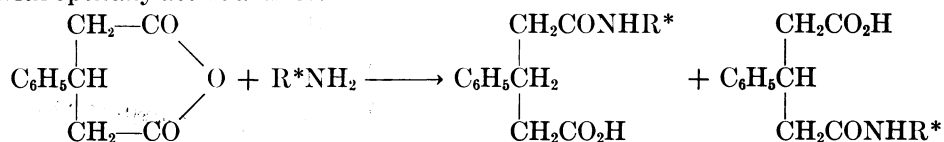


Since enzymatic reactions characteristically show a high stereochemical specificity as compared with simpler systems, it seemed unlikely that any nonenzymatic reac-

tion would yield a highly disproportionate amount of the two products. It was essential, therefore, to select a clean-cut reaction giving a high yield of the two diastereoisomeric products. Otherwise, analysis of the reaction mixture and interpretation of the results would be highly complicated. In particular, it seemed desirable to employ as the C(aabd) molecule one in which the reaction of one (a) group would hinder or prevent reaction of the other.

After considering several possible substances,  $\beta$ -phenylglutaric anhydride was selected as the most promising. This compound has some similarity to citric acid in that it is a  $\beta$ -substituted glutaric acid derivative. It reacts smoothly with amines, and reaction of one of the (a) groups destroys the other. The monoamides produced are usually nicely crystalline solids.

A study was therefore undertaken of the reaction of  $\beta$ -phenylglutaric anhydride with optically active amines:



The first amine studied [*d*-desoxyephedrine,  $\text{C}_6\text{H}_5\text{CH}_2\text{CH}(\text{NHCH}_3)\text{CH}_3$ ] gave an almost 50-50 ratio of the two products. However, with *l*- $\alpha$ -phenethylamine [ $\text{C}_6\text{H}_5\text{CH}(\text{NH}_2)\text{CH}_2$ ], highly significant results were obtained. The conditions finally selected for optimum yield and convenience are illustrated by the following reaction, which represents one of a number of similar runs.

#### EXPERIMENTAL

To 7.6 gm. (0.04 mole)  $\beta$ -phenylglutaric anhydride, dissolved by warming on the steam cone in 44 ml. of hot benzene (about  $50^\circ$ ), was added dropwise, with a pipette, an equimolecular amount (4.84 gm., 0.04 mole) of *l*- $\alpha$ -phenethylamine. The amine adhering to the walls of the weighing vessel was washed in with 6 ml. of benzene. The solution quickly became viscous, and started to deposit crystals within several seconds; within a few minutes the mass became semisolid. The mixture was allowed to stand at room temperature for several hours. It was then diluted with 50 ml. of benzene:hexane, 3:1 v/v. The mixture was placed in the cold room for 48 hours to complete the crystallization. The fine, powdery precipitate was then filtered with suction; on the funnel it packed down to a hard cake. This was washed with a little cold benzene and dried *in vacuo* over concentrated sulfuric acid. The yield was 11.7 gm. (95 per cent).

*Analysis:* Calculated for  $\text{C}_{19}\text{H}_{21}\text{O}_3\text{N}$ : C, 73.28; H, 6.81; N, 4.52; neutralization equivalent, 311.4. Found: C, 73.20; H, 6.95; N, 4.50; neutralization equivalent, 311.

In other similar runs the yields ranged from 11.4 to 11.7 gm. (93-95 per cent) (neutralization equivalents, 311-314;  $[\alpha]_D^{27} = -69^\circ.5$  to  $-70^\circ$  [2.5 per cent solution in methanol]).

These products were used in a series of fractionation studies which finally led to the separation of each of the isomers in the pure state. Repeated extraction of the mixtures with warm ( $40^\circ$ ) ethyl acetate left a residue from which the less soluble isomer (A) was obtained by three recrystallizations from hot ethyl acetate (30

volumes) in the form of platelets melting at 184°–185° (microblock, uncorrected). The ethyl acetate-soluble material was recrystallized from acetone and then from ether, giving the more soluble isomer (B). It was subsequently found that, once crystals of pure A and B were available, the entire process could be accomplished from ethyl acetate by stepwise concentration and alternate seeding with A and B crystals. The B-enriched fractions (m.p., 130°–160°) obtained in this way were recrystallized from 70 volumes of hot ethyl acetate, giving pure B (rectangular plates) melting at 169°–170° (microblock, uncorrected). The average specific rotations of the various materials are summarized below:

Isomer A:	$[\alpha]_D^{27} = -83^{\circ}.3$ (range, $-83^{\circ}.1$ to $-83^{\circ}.5$ );
Isomer B:	$[\alpha]_D^{27} = -49^{\circ}.4$ (range, $-49^{\circ}.2$ to $-49^{\circ}.5$ );
Total products:	$[\alpha]_D^{27} = -69^{\circ}.8$ (range, $-69^{\circ}.5$ to $-70^{\circ}$ ).

From these data it can be calculated that the total product contained 60 per cent of isomer A and 40 per cent of isomer B. If all the remaining 5 per cent of unaccounted-for reaction product were isomer B, the ratio would be 57-43. A solubility analysis of the reaction product showed exactly 60 per cent of the less soluble isomer to be present in the reaction product. (The authors wish to express their appreciation for this analysis to R. J. Herberg, Physiochemical Research Division, Lilly Research Laboratories.)

#### DISCUSSION

These data clearly show that asymmetric reactions of the citric acid type can occur in homogeneous solution, without requiring attachment to, or complexing with, an enzyme surface. Furthermore, the production in these reactions of two diastereoisomeric substances possessing different thermodynamic properties demands the conclusion that the two (a) groups from which they were derived are themselves not stereochemically equivalent. This concept, that the two (a) groups per se are stereochemically different without regard to isotope labeling or attachment to an enzyme surface, has not been clearly stated and, in certain cases at least, has not even been recognized. As a matter of fact, the citrate-enzyme model of Ogston is actually a demonstration of the nonequivalence of the two  $-\text{CH}_2\text{CO}_2\text{H}$  groups, rather than an explanation of the difference in behavior of two "identical" groups when combined with an enzyme surface.

The stereochemical nonequivalence of the two (a) groups in the molecule C(aabd) can be clearly shown in two or three ways. Such molecules<sup>14</sup> possess a plane of symmetry passing through the carbon atom C and bisecting groups (b) and (d) (see Fig. 1). The two halves are mirror images as required by the definition of a plane

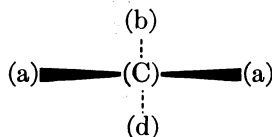


FIG. 1

of symmetry, but they are *not superimposable*, and the (a) groups bear an antipodal relationship to each other. Thus the molecule C(aabd), although it does not contain an asymmetric carbon atom and does possess a plane of symmetry, neverthe-

less contains two groups (aa) which are asymmetrically located with respect to the remainder of the molecule and which bear a mirror image relationship to each other.

In discussing the various "degrees" of asymmetry of the saturated carbon atom two unique types must be considered, namely, C(abde) and C(aabd). The former is the "asymmetric carbon atom," a term whose stereochemical implications are thoroughly understood. If two groups of an asymmetric carbon atom are made identical, the resulting molecule [C(aabd)] has a plane of symmetry, and the carbon atom is no longer asymmetric. However, the mirror-image halves are *not superimposable*, and the two (a) groups, which are asymmetrically related to the remainder of the molecule, will react at different rates with an asymmetric reagent. Since these are the only two types of carbon atoms showing specific behavior toward an asymmetric reagent (any further simplification gives molecules whose mirror-image halves are superimposable), it seems desirable to describe the C(aabd)-type carbon atom by a term reflecting its stereochemical characteristics. In this connection, the close similarity between a carbon atom of this type and the *meso* form of a substance containing two like asymmetric carbon atoms is of interest. Each possesses a single plane of symmetry separating mirror-image halves which are not superimposable. In each, the symmetry results from the internal compensation of two enantiomorphically related groups. The main difference is that in the *meso* form the two groups are themselves asymmetric, whereas in the C(aabd) molecule they are symmetrical groups asymmetrically located in the molecule. In view of this close similarity we suggest that a carbon atom of the type C(aabd) be designated as a "*meso*-carbon atom." Such a term provides a convenient classification for this type of asymmetry and clearly emphasizes the most important stereochemical property of such a carbon atom, namely, that each of the two (a) groups bears an asymmetric relationship to the remaining three groups and thus can be expected to show some degree of stereochemical specificity in reacting with an asymmetric substance.

It should be noted that most substances containing a *meso*-carbon atom will have but a single plane of symmetry, separating nonsuperimposable halves. However, a molecule containing two like *meso*-carbon atoms, C(aab)-C(aab), possesses two planes of symmetry each separating superimposable halves. Yet such a molecule, just as the simpler C(aabd) type, would be expected to yield unequal amounts of diastereoisomers on reaction with an appropriate asymmetric reagent.<sup>15</sup>

This example emphasizes the necessity of considering the nature of the individual carbon atoms involved, rather than the over-all symmetry of the molecule, in evaluating the reaction of a symmetrical molecule with an asymmetric reagent. In any molecule containing one (or more) *meso*-carbon atoms, reaction of the two (a) groups with an asymmetric reagent will proceed at different rates, yielding unequal amounts of diastereoisomeric products. In the case of a highly asymmetric reagent, such as an enzyme, the difference in rates is usually such as to give exclusively one diastereoisomer, whereas with less complex systems the discrimination may be very much less.

A second method of illustrating the stereochemical nonequivalence of the two (a) groups arises from a consideration of the relationship of each of the (a) groups to the remaining three groups of the molecule (Fig. 2). If one looks from the "right" (a) toward the central carbon atom, the remaining three groups are seen in the

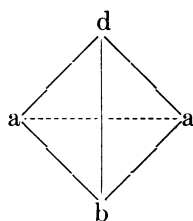


FIG. 2

clockwise order (a)  $\rightarrow$  (b)  $\rightarrow$  (d), whereas from the "left" (a) the clockwise order is reversed (a)  $\rightarrow$  (d)  $\rightarrow$  (b). Furthermore, this same difference is observed in a rearward approach to either of the faces opposite the (a) groups. Therefore, an asymmetric reagent will encounter an asymmetric environment whether it reacts directly with (a) or displaces (a) by an inversion reaction from the rear. Or, to state the situation in another way, a group (a) attached to a carbon atom containing three dissimilar groups is asymmetrically located regardless of whether one of the three groups is also an (a) group.

This method of viewing the situation provides a basis for describing each (a) group uniquely. A simple method of approaching the problem is to orient the molecule as in the Fischer projection formulas, with one unlike group—assume it to be (d)—below, and the other—(b)—above (Fig. 3). The two (a) groups could

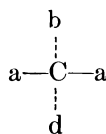
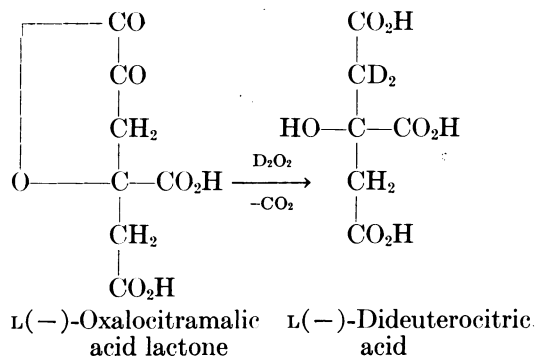


FIG. 3

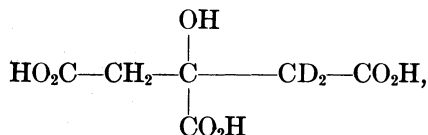
then be designated by an appropriate symbol or word indicating their configuration. There is one major difficulty in this approach, and that is the formulation of a satisfactory general basis for orienting the two unlike groups "up" and "down" (or "right" and "left") respectively. It seems premature to attempt a solution of this problem in the present paper. However, as a basis for discussion, two specific examples (citric acid and glycerol) will be discussed.

Martius and Schorre<sup>16</sup> have synthesized L(-) $\alpha,\alpha$ -dideuterocitric acid by the following method:

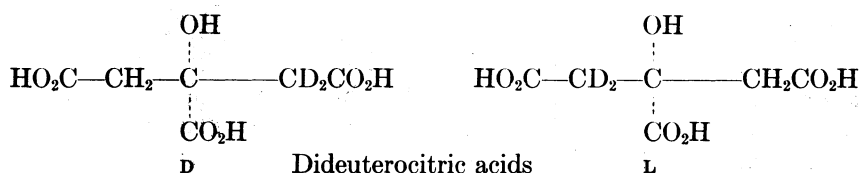


The D isomer was prepared in the same manner from D(+) oxalocitramalic acid lactone. On enzymatic degradation,<sup>11</sup> the  $\alpha$ -ketoglutaric acid obtained from the L isomer retained the deuterium label, whereas that from D(+)-dideuterocitric acid contained no deuterium. These results with the two isomers eliminate the possibility that an "isotope effect" was responsible for the observed differences.

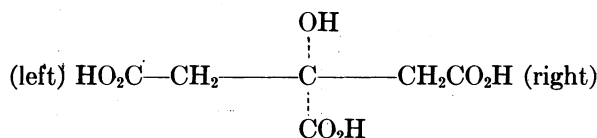
The two dideuterocitric acid isomers, written according to the Fischer conventions, are shown below. (The original authors<sup>11</sup> wrote the L isomer as



but if this molecule is written according to the Fischer convention [with top and bottom groups projecting behind the plane of the paper], it must be formulated as shown.)

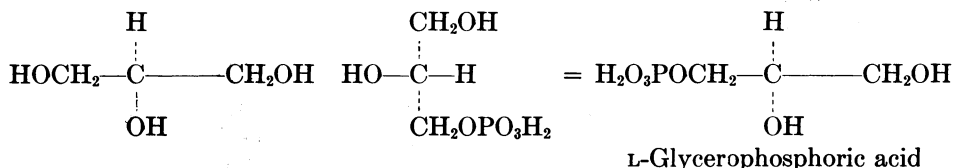


In the L isomer the deuterium was present in the acetate-derived, aconitase-inactive group, whereas in the D isomer the deuterium was present in the oxaloacetate-derived, aconitase-active group. Therefore, if citric acid is written with the OH "up" and the  $\text{CO}_2\text{H}$  "down" the oxaloacetate-derived group is the "right" group, while the acetate-derived group is the "left" group:



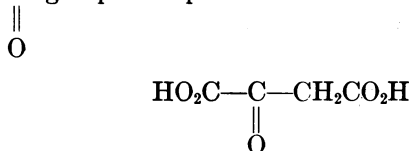
It is possible, therefore, to indicate either of the two  $-\text{CH}_2\text{CO}_2\text{H}$  groups in citric acid specifically by an appropriate word or letter or by a projection formula.

Glycerol contains a *meso*-carbon atom, and, since the configuration of the glycerophosphoric component of phospholipides is known to be L, the  $-\text{CH}_2\text{OH}$  group carrying the phosphate group becomes the "left" group when the molecule is oriented as shown below:

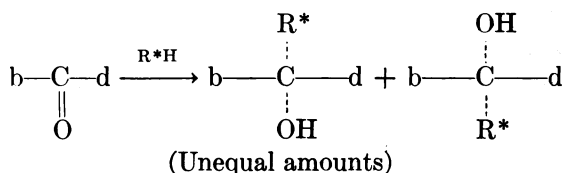


An interesting extension of the concept of the *meso*-carbon atom arises from a consideration of the reaction of oxaloacetate and acetate to yield citrate. In this reaction, acetate gives only the "left"  $-\text{CH}_2\text{CO}_2\text{H}$  group of citric acid. Obviously

this could not be the case unless oxaloacetate itself possessed asymmetric elements. If one examines the oxaloacetate molecule, it can be seen that the molecule possesses a plane of symmetry separating nonsuperimposable halves and that the carbonyl carbon atom conforms to the definition of a *meso*-carbon atom in which the two bonds of the  $-C-$  group correspond to the two like (a) groups:



Thus the two bonds of the carbonyl group are asymmetrically related to the remainder of the molecule, and their reaction with an asymmetric substance should proceed stereospecifically to yield unequal amounts of the stereoisomeric products:



b and d = Symmetrical groups (i.e., contain at least one plane of symmetry).

R\* = Asymmetric group (D or L form).

The behavior of ketones can be generalized to include also compounds of the type  $b-c-d$ , where  $X = O, S, NR, CR_2$ . In such compounds, reactions involving

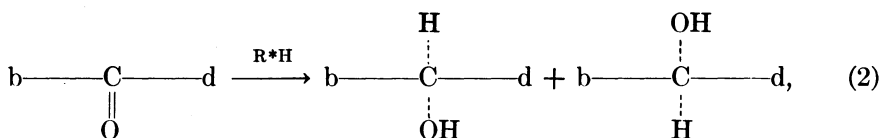
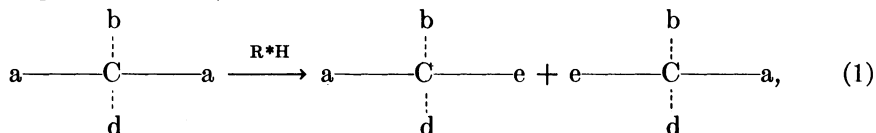


the double bond and an asymmetric substance would be expected to give unequal amounts of stereoisomeric products, since the two bonds of the double bond are not stereochemically equivalent. Most of the known asymmetric transformations of *meso*-carbon atoms are of this type. The enzymatic reduction of pyruvic acid and of other ketones to optically active alcohols is well known. In the nonenzymatic field, examples include the partially asymmetric reduction of ketones by asymmetric Grignard reagents<sup>17</sup> and by aluminum-2-butoxide in (+)-2-butanol<sup>18</sup> (Meerwein-Ponndorf-Verley reduction), the reaction of benzaldehyde with hydrogen cyanide in the presence of quinine,<sup>19</sup> and the Reformatsky reaction of a ketone with *l*-menthyl bromoacetate.<sup>20</sup> It is interesting to note that many of the so-called "asymmetric syntheses" involve the reaction of a *meso*-carbon atom with an asymmetric reagent, yet, as far as the authors are aware, it has not been pointed out that the stereochemical nonequivalence of the two (a) groups is the fundamental factor responsible for the asymmetric course of the reaction. Much of the extensive literature on asymmetric synthesis could have been more simply presented from this viewpoint.

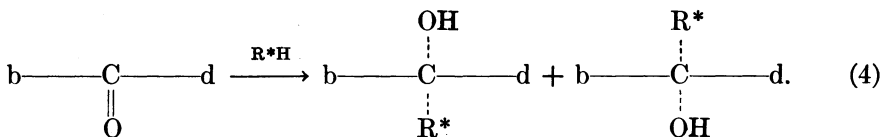
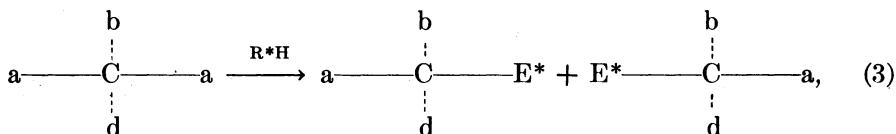
In conclusion, it should be pointed out that the reactions of *meso*-carbon atoms with asymmetric reagents can be classified into two groups. In one, the *meso*-carbon atom is converted into an asymmetric carbon atom; in the other, to a different *meso*-carbon atom. A brief discussion of these two types is presented below.

A. *Conversion of a Meso-Carbon Atom to an Asymmetric Carbon Atom.*—If an

(a) group of a *meso*-carbon atom [C(aabd)] is converted into a group other than (b) or (d), the carbon atom becomes asymmetric. If the asymmetric reagent is incorporated into the final product, diastereoisomers will result; if not, enantiomorphs will be produced. In each instance, however, the two possible products will form in unequal amounts, and in the case of enzymatic reactions the inequality may be so marked that only one isomer can be detected. These reactions are illustrated in equations (1)–(4), below.

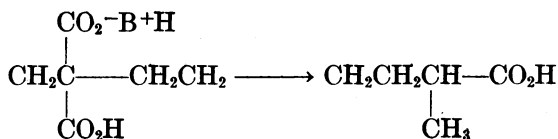


Enantiomorphs (unequal amounts)



Diastereoisomers (unequal amounts)

Several reactions of the type shown in equations (2) and (4) have been presented previously. Examples of reactions of the C(aabd) type (eqs. [1] and [3]) are very rare. The best known is the decarboxylation of the brucine salt of methyl ethyl malonic acid to yield partially optically active methyl ethyl acetic acid:<sup>21</sup>



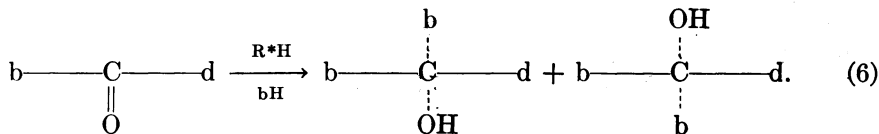
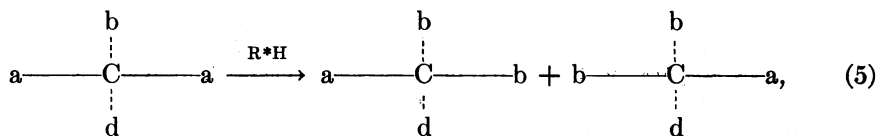
B = Brucine

Formally, this reaction corresponds to the type shown in equation (1). However, the uncertainty as to the mechanism by which the optically active acid is produced<sup>22, 23</sup> makes it difficult to evaluate this reaction. The conversion of 4-methyl cyclohexanone to an optically active 2-oximino derivative by *d*-2-octyl nitrite<sup>24</sup> is likewise subject to question on the basis of the yield obtained and uncertainty as to reaction mechanism.

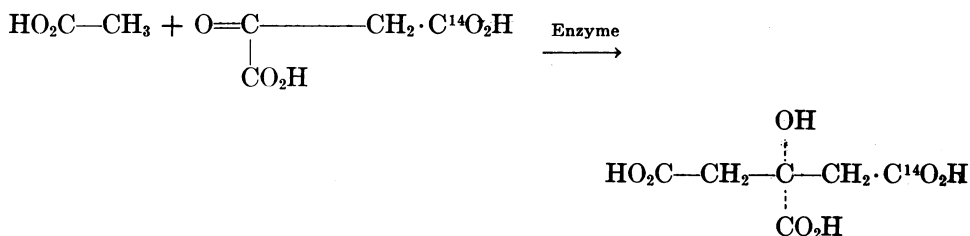
The only clean-cut example of reaction (4) of which the authors are aware is that reported in this paper.

B. *Conversion of a Meso-Carbon Atom to a Different Meso-Carbon Atom.*—





Demonstration of the stereochemical specificity of the (a) groups in a reaction of this type is difficult, since the two reaction products are, of course, identical. Therefore, it is possible to show the different behavior of the (a) groups only by introducing an isotopic label into either the original or the newly formed (b) group. In this case the label will be unequally distributed between the two (b) groups, and this inequality may be detected by an appropriate asymmetric reagent. The synthesis of citric acid from oxaloacetic acid is an outstanding example of this type of reaction:



Further examples of this type, however, are not likely to be numerous in view of the obvious experimental difficulties involved.

\* Part of the material in this paper was taken from the thesis submitted by Pearl Schwartz to the Graduate College of the University of Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry.

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<sup>1</sup> E. A. Evans, Jr., and L. Slotkin, *J. Biol. Chem.*, **136**, 301 (1940).

<sup>2</sup> H. G. Wood, C. H. Werkman, A. Hemingway, and A. O. Nier, *J. Biol. Chem.*, **142**, 31 (1942).

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<sup>5</sup> S. Weinhouse, G. Medes, and N. F. Floyd, *J. Biol. Chem.*, **168**, 691 (1946).

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<sup>10</sup> P. E. Wilcox, C. Heidelberger, and V. R. Potter, *J. Am. Chem. Soc.*, **72**, 5019 (1950).

<sup>11</sup> C. Martius and G. Schorre, *Ann.*, **570**, 143 (1950).

<sup>12</sup> A. G. Ogston, *Nature*, **162**, 963 (1948).

<sup>13</sup> P. E. Wilcox, *Nature*, **164**, 757 (1949).

<sup>14</sup> In the discussion which follows, the molecules will usually be written as in the Fischer projection formulas—i.e., the groups at top and bottom, indicated by dotted lines, are considered to be projecting behind the plane of the paper, while the groups on either side are considered to be projecting forward in front of the plane of the paper.

<sup>15</sup> This type of molecule, containing two like *meso*-carbon atoms, possesses a twofold axis of symmetry and offers an exception to the rule of Racusen and Aronoff (D. W. Racusen and S. Aronoff, *Arch. Biochem. Biophys.*, **34**, 218 [1951]) that "discrimination of identical groups or atoms by an asymmetric agent (enzyme, optical antipode, etc.) is possible only in molecules which do not possess a twofold (or greater) axis of symmetry."

<sup>16</sup> Martius and Schorre, *op. cit.*, p. 140.

<sup>17</sup> H. S. Mosher and E. La Combe, *J. Am. Chem. Soc.*, **72**, 3991 (1950).

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<sup>21</sup> W. Marckwald, *Berichte*, **37**, 349, 1368 (1904).

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## A NOTE ON RECENT DEVELOPMENTS IN AUDITORY THEORY

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Communicated April 8, 1954

First in 1928, and again in further detail in a series of papers beginning in 1941, Békésy<sup>1</sup> reported the results of direct observations on the form of response in the cochlea. In fresh human temporal bones in which suitable exposures had been made, he examined the motions of the basilar membrane and related structures under the microscope with stroboscopic illumination. These motions were found to take the form indicated in Figure 1.

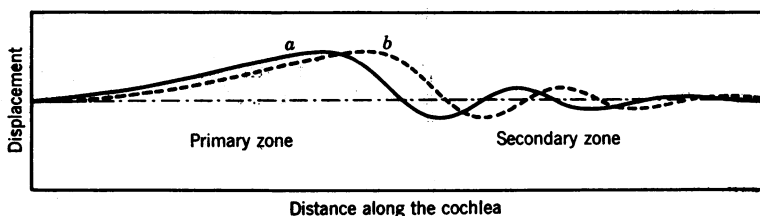


FIG. 1.—The pattern of displacement of the basilar membrane for a tone of middle frequency. Curve *a* represents the pattern as seen at one moment, and curve *b* the pattern a moment later. From Békésy (see note 1).

The pattern illustrated is that produced by a tone of intermediate frequency. A large portion of the membrane, extending from the basal end to well beyond the middle of the cochlea, is set in vigorous vibration, and then, in the more apical regions, the amplitude of the displacement changes rapidly and finally falls to zero. The phase of the motion of different parts of the membrane varies only moderately over the main portion of the excited region, which may be called the "primary zone," and then varies greatly beyond the maximum, where the amplitude grows small. The patterns for higher tones are similar, except that they are displaced toward the basal end of the cochlea and hence involve smaller amounts of the basilar membrane in vigorous motion.