liquor from the first crop. This material showed the same behavior as the first crop of crystals when examined by paper chromatography. In cold runs, the identity of the methylation product was substantiated further by preparation of the acetone solvated crystal form and comparison of its X-ray pattern with that of an authentic sample of erythromycin. An infrared spectrum obtained from the methylation product was indistinguishable from that obtained from erythromycin of known identity.

INDIANAPOLIS, INDIANA

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[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

# Studies on Diastereoisomeric α-Amino Acids and Corresponding α-Hydroxy Acids. III. Configuration of the β-Asymmetric Center of Isoleucine

BY MILTON WINITZ, SANFORD M. BIRNBAUM AND JESSE P. GREENSTEIN

RECEIVED NOVEMBER 26, 1954

By virtue of the greater susceptibility of L-acyl-L-amino acids over D-acyl-L-amino acids to the action of renal acylase I, a method of determining the optical configuration of the β-asymmetric center of isoleucine has been developed. For this purpose, *dl*-α-methylbutyryl-L-norleucine was subjected to the action of the enzyme, yielding as a hydrolytic product a mixture of free (+) and (−)α-methylbutyric acids in which the former predominated. Since (+)α-methylbutyric acid is derivable from L-isoleucine (and D-alloisoleucine), the β-asymmetric center of these stereomers has been assigned the L-configuration. On the basis of the known configuration of the β-asymmetric centers of the isomeric isoleucines, threonines and phenylserines, a correlation between the configuration of this center and the extent of susceptibility of the α-asymmetric center to various L- and D-directed enzyme systems has been noted.

Previous papers of this series[1,2] were concerned, in part, with the utilization of the partial molar rotation data of the α- and ω-asymmetric centers of several diasymmetric amino acids for the assignment of configuration to the α-asymmetric center. Such amino acids with two dissimilar centers of optical asymmetry which have thus far been demonstrated in proteins include threonine, hydroxyproline, isoleucine and hydroxylysine. Although these amino acids may exist as four optical stereoisomers, the employment of physical, chemical and biological criteria has, in every instance, led to the assignment of the L-configuration to the α-asymmetric center of the naturally occurring form.[3,4] Demonstration of a D-configuration for the ω-asymmetric center of L-threonine[5] and L-hydroxyproline[6] has been made, whereas the configuration of the δ-asymmetric center of L-hydroxylysine remains uncertain at this time. The present communication is concerned with the configuration of the β-asymmetric carbon atom of isoleucine.

In 1907, Ehrlich[7] demonstrated the presence of (−)α-methylbutanol-1 as a yeast fermentation product of L-isoleucine. Oxidation of this material to the corresponding aldehyde, (+)α-methylbutyraldehyde,[7] and acid, (+)α-methylbutyric acid,[8] was readily effected. The synthesis of a mixture of L-isoleucine and D-alloisoleucine from (+)α-methylbutyraldehyde by Ehrlich,[7] and from (−)α-methylbutanol-1 by White and Smith,[9] *via* methods that did not lead to Walden inversion of the asymmetric center provide unequivocal evidence that the degradation of L-isoleucine by yeast proceeded with retention of configuration of the β-center of asymmetry.[10] The configurational relationship between the β-asymmetric center of L-isoleucine (also D-alloisoleucine) and (+)α-methylbutyric acid, as well as its reduction products, was thereby established.[11]

On the basis of the foregoing discussion, the assignment of a configuration to (+)α-methylbutyric acid relative to some reference compound, as L-serine in the amino acid series or D-glyceraldehyde in the carbohydrate series, will in turn assign the L- or D-configuration to the β-asymmetric carbon atom of each of the four stereoisomeric isoleucines.[12] However, since the asymmetric carbon atom of α-methylbutyric acid is linked to two alkyl substituents, the assignment of a D- or an L-designation to this compound could become quite arbitrary when based solely on absolute configuration. Examination of Fig. 1 will readily reveal how such ambiguity could arise.

In Fig. 1, the configuration of a typical L-amino acid, written in the conventional Fischer diagram, is compared with the levo- and dextrotatory forms of α-methylbutyric acid. These forms were so represented to conform to the findings of recent reports, derived from X-ray[13] and chemical evi-

(1) J. P. Greenstein, N. Izumiya, M. Winitz and S. M. Birnbaum, THIS JOURNAL, **77**, 707 (1955).

(2) M. Winitz, S. M. Birnbaum and J. P. Greenstein, *ibid.*, **77**, 716 (1955).

(3) A. Neuberger, *Advances in Protein Chem.*, **4**, 297 (1948).

(4) J. P. Greenstein, *ibid.*, **9**, 121 (1954).

(5) C. E. Meyer and W. C. Rose, *J. Biol. Chem.*, **115**, 721 (1936); D. P. Shoemaker, J. Donohue, V Schomaker and R. B. Corey, THIS JOURNAL, **72**, 2328 (1950).

(6) A. Neuberger, *J. Chem. Soc.*, 429 (1945); J Zussman, *Acta Cryst., Camb.*, **4**, 72 (1951).

(7) F. Ehrlich, *Ber.*, **40**, 2538 (1907).

(8) W. Marckwald, *ibid.*, **37**, 1038 (1904).

(9) H. C. White and H. T. Smith, Abstracts, 126th American Chemical Society Meeting, 3C (1954).

(10) Further evidence in support of this view was provided by the report of W. S. Fones (THIS JOURNAL, **76**, 1377 (1954)) that the degradation of L-isoleucine and D-alloisoleucine with ninhydrin led to the formation of (+)α-methylbutyraldehyde, whereas comparable treatment of their respective optical antipodes resulted in the production of the levorotatory aldehyde.

(11) It has long been the practice to label commercially available synthetic isoleucine as DL-isoleucine, regardless of the proportion of alloisoleucine present. To our knowledge no preparation of pure DL-isoleucine is now available on the American market, but only mixtures consisting (a) of nearly equal amounts of L-isoleucine and D-alloisoleucine, and (b) of DL-isoleucine and DL-alloisoleucine in unknown relative proportions. Identification of pure DL-isoleucine and of the mixtures cited may be made by determination of the melting points of their respective acetyl derivative; *cf.* J. P. Greenstein, L. Levintow, C. G. Baker and J. White, *J. Biol. Chem.*, **188**, 647 (1951); W. A. H. Huffman and A. W. Ingersoll, THIS JOURNAL, **73**, 3366 (1951).

(12) J. P. Greenstein, L. Levintow, C. G. Baker and J. White, *J. Biol. Chem.*, **188**, 647 (1951).

(13) J. Trommel, *Proc. Koninkl. Ned. Akad. Wetenschap.*, Series B, **56**, 272 (1953); *ibid.*, Series B, **57**, 364 (1954).

dence,[14] on the absolute configuration of the β-asymmetric center of isoleucine relative to L-glyceraldehyde.

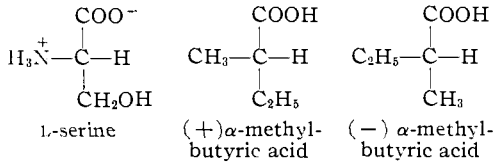



Fig. 1.—Configuration of α-methylbutyric acid.

Figure 1 demonstrates that either the levo- or the dextrotatory form of α-methylbutyric acid could be assigned an L-configuration depending upon whether the ethyl or the methyl group is considered to occupy a position equivalent to that of the amino group of the reference amino acid. When viewed solely in the light of their absolute configuration, the selection of a D- or an L-designation for those acids wherein two alkyl substituents are linked to the asymmetric carbon atom therefore becomes meaningless. Such designation for compounds of this type can nonetheless become quite meaningful when they are considered in regard to their biological behavior.[15] Thus, the assignment of a D- or an L-configuration to α-methylbutyric acid can assume significance if the behavior of this compound, or derivatives thereof, could be related to the behavior of compounds of known configuration in biological systems of known stereochemical specificity.

**The Enzymatic Susceptibility of DL-α-Methylbutyryl-L-norleucine.**—In order to relate the configuration of each of the isomeric α-methylbutyric acids to its biological behavior, it was deemed most expedient to subject a susceptible amino acid derivative of these compounds to the action of a peptidase of established stereospecificity. The intracellular carboxypeptidase, renal acylase I, which has been extensively used in a general procedure for the resolution of racemic amino acids,[16,17] appeared uniquely suited to this purpose. Recent studies[18] in this Laboratory demonstrated that the antipodal specificity exhibited by this enzyme toward acylated amino acids was not confined solely to residues on the carboxyl end of the substrate, but was also evident, though to a less marked degree, with asymmetric acyl substituents. Thus, various acylated amino acids with acyl substituents of the L-configuration were hydrolyzed at greater rates than the corresponding substrates with D-acyl substituents.

Since previous data[18] suggested that the difference between the rates for the L- and D-acyl substituents increased, in some instances, with increasing length of the side chain of the terminal free-carboxy L-amino acid, the employment of an α-methylbutyryl derivative of an amino acid with a fairly long side chain appeared desirable. For this purpose, L-norleucine was the amino acid of choice. Conversion of DL-α-methylbutyric acid to the acid chloride by treatment with thionyl chloride, followed by the coupling of this intermediate with L-norleucine *via* the usual Schotten–Baumann procedure, yielded the diastereomeric mixture, DL-α-methylbutyryl-L-norleucine, with a specific rotation of −9.8° (*c* 2% in absolute ethanol). In order to ascertain the relative proportions of each diastereomer in the mixture, it was subjected to acid hydrolysis and the liberated α-methylbutyric acid subsequently isolated. Lack of optical rotation of the liberated acid was indicative of the absence of fractionation of diastereomers during synthesis. The diastereomeric mixture, DL-α-methylbutyryl-L-norleucine, was therefore composed of a 1:1 mixture of each of the component diasteromers.

Since L-acyl-L amino acids are cleaved at a more rapid rate than the corresponding D-acyl-L derivatives, inspection of the hydrolytic rate curve for DL-α-methylbutyryl-L-norleucine would readily reveal whether the difference in susceptibility of its component diastereomeric forms to the action of acylase I is of sufficiently large magnitude to be distinguishable. Such a rate curve is presented in Fig. 2.

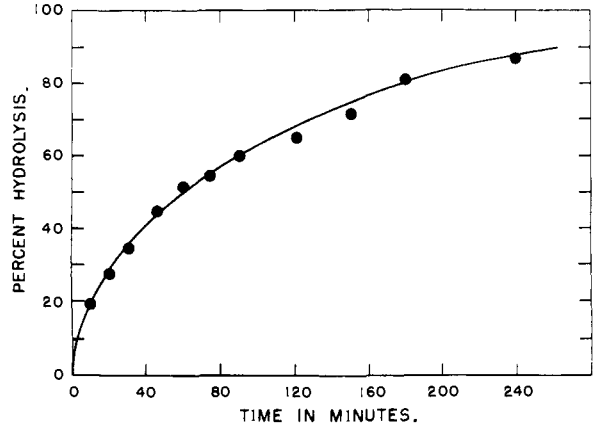



Fig. 2.—Hydrolysis of DL-α-methylbutyryl-L-norleucine by acylase I. Digests consisted of equal volumes of 0.05 molar neutralized substrate, 0.1 molar phosphate buffer at *p*H 7 and enzyme solution at 1 mg. N per ml.

Figure 2 demonstrates a rapid decrease, with time, in the rate of hydrolysis of DL-α-methylbutyryl-L-norleucine. Since previous experience with this enzyme indicated zero-order kinetics for all substrates tested,[16] this phenomenon strongly suggested the presence of two substrates which were hydrolyzed at different rates. With curves of this type, neither the actual nor the relative magnitudes of the hydrolytic rates of the component substrates can be readily determined. However, the rapid rate of change exhibited by the slope at about 50% hydrolysis was indicative of an appreciable depletion of the more susceptible isomer at this point. Such data suggested that the isolation of α-methylbutyric acid with demonstrable optical activity could be achieved if the enzymic digestion was terminated prior to 50% hydrolysis.

For the isolation of optically active α-methylbu-

(14) S. Stälberg-Stenhagen and E. Stenhagen, *Arkiv. Kemi Mineral. Geol.*, **24B**, 1 (1947).

(15) Configurational assignment here is analogous to the situation previously encountered with the optical antipodes of isovaline.[2]

(16) S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952).

(17) J. P. Greenstein, S. M. Birnbaum and M. C. Otey, *ibid.*, **204**, 307 (1953).

(18) S-C. J. Fu, S. M. Birnbaum and J. P. Greenstein, THIS JOURNAL, **76**, 6054 (1954).

tyric acid, an enzymic digest of a 0.025 molar solution of DL-$\alpha$-methylbutyryl-L-norleucine, at *p*H 7.2, and terminated at 40% of complete hydrolysis,[19] was employed. Successive treatment of the enzymic hydrolysate, which included removal of protein followed by evaporation *in vacuo* at *p*H 8.5, acidification to *p*H 1.8 and extraction with ether, yielded a solution composed of a mixture of liberated $\alpha$-methylbutyric acid as well as unhydrolyzed substrate. Concentration of the ethereal solution followed by the addition of petroleum ether effected separation of the former, which remained in solution, from the latter, which precipitated. Fractional distillation of the isolated $\alpha$-methylbutyric acid yielded an analytically pure product with a boiling point of 69° (at 10 mm. pressure) and an $[\alpha]^{25}D$ +6.0°.

An early communication by Marckwald[11] reported an $[\alpha]D$ +17.5° for (+)$\alpha$-methylbutyric acid obtained by oxidation of the precursor alcohol. These data suggested that the enzymic cleavage product, obtained above, was composed of a mixture of approximately 34% optically active (+) form and 66% racemate. On the assumption that no fractionation occurred during the isolation procedure, a twofold greater rate of cleavage occurred with (+)$\alpha$-methylbutyryl-L-norleucine than with its diastereomer. In any event, a greater susceptibility to acylase I is indicated for the former.

As additional confirmation of the foregoing relative rates of hydrolysis exhibited by the two diastereomers, acid hydrolysis of the residual unhydrolyzed substrate from the enzymic digest, followed by isolation of the liberated $\alpha$-methylbutyric acid with a levorotation, was effected.

On the basis of the known stereochemical specificity requirements of acylase I,[18] the foregoing results permit the assignment of an L- and a D-configuration, respectively, to the dextro- and levorotatory antipodes of $\alpha$-methylbutyric acid. The assignment of such designations, based on the stereochemical specificity requirements of hydrolytic enzymes, assumes validity when viewed in the light of the mass of cumulative data on this subject.[20-22] Since the configuration of the $\beta$-asymmetric center of the four stereoisomeric isoleucines is known relative to the optical forms of $\alpha$-methylbutyric acid,[10] a configurational designation can be given to the $\beta$ asymmetric carbon of each of the corresponding antipodes of isoleucine. Thus, the assignment of an L-configuration to the $\beta$-asymmetric center of L-isoleucine and D-alloisoleucine, as well as the designation of a D-configuration to the $\beta$-carbon atom of their corresponding diastereomers, becomes permissible.[23]

**The Biological Significance of Optical Configuration.**—Studies on the stereochemical specificity exhibited toward amino acids, or derivatives thereof, by such enzymes as the oxidases, decarboxylases, transaminases and proteases, have formed the subject of widespread investigation. However, such studies, almost without exception, have been concerned with the $\alpha$-asymmetric center of the amino acid involved. The existence, in nature, of several amino acids which contain a secondary center of asymmetry invites attention to the influence, if any, of these $\omega$-asymmetric centers on susceptibility of the $\alpha$-asymmetric centers to enzymic action. Table I presents data, previously observed in this Laboratory,[16,17,24] which are pertinent to this question.

TABLE I

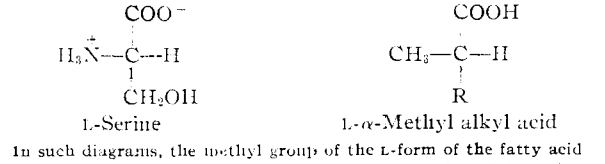
INFLUENCE OF THE $\beta$-ASYMMETRIC CENTER ON THE SUSCEPTIBILITY OF THE $\alpha$-ASYMMETRIC CENTER OF DIASTEREOMERIC AMINO ACIDS TO VARIOUS ENZYMES

| | Oxidation by amino acid oxidases[a] | | | | Hydrolysis by renal acylase I[d] | |
|---|---|---|---|---|---|---|
| | $\alpha$-D-Amino acid | | $\alpha$-L-Amino acid | | | |
| | $\beta$-Carbon | Rate[b] | $\beta$-Carbon | Rate[c] | Acyl-L amino acid | Glycyl-L amino acid |
| Threonine | L | 0.3 | D | 0 | 720[e] | .. |
| Allothreonine | D | 2.6 | L | 0.9 | 2580[e] | .. |
| Isoleucine | D | 14.8 | L | 71 | 340[f] | 132 |
| Alloisoleucine | L | 5.9 | D | 0.8 | 148[f] | 88 |
| Phenylserine | L | 0 | D | 0.7 | ... | .. |
| Allophenylserine | D | 0 | L | 140 | ... | .. |

[a] Rates in terms of micromoles of oxygen consumed per hour per mg. of N. [b] With acetone powder of hog kidney. [c] *C. adamanteus* venom. [d] Rates in terms of micromoles of substrate hydrolyzed per hour per mg. of N. [e] N-Chloroacetyl derivative. [f] N-Acetyl derivative.

Examination of Table I reveals that the rate of action of L-amino acid oxidase[17] on L-threonine,[5] L-phenylserine[25] and L-alloisoleucine, in which the configuration of the $\beta$-asymmetric carbon atom is D, is in every instance slower than that of the corresponding $\beta$-L-diastereomer. In like manner, the rate of action of D-amino acid oxidase[17] on D-allothreonine and D-isoleucine, wherein the $\beta$-asymmetric center is of the D-configuration, proceeds at a faster rate than that of their respective $\beta$-L-stereoisomers. A comparable situation is observed with respect to the action of the $\alpha$-L-directed renal acylase I toward N-acylated L-allothreonine and L-isoleucine which proved more susceptible than either the L-threonine or L-alloisoleucine derivatives. It should be noted that these data consistently indicate that for

tive to L-serine in the amino acid series is shown in the typical Fischer diagram below.




L-Serine L-$\alpha$-Methyl alkyl acid

In such diagrams, the methyl group of the L-form of the fatty acid may be considered to occupy a position equivalent to that of the amino group of L-serine. This relation can be made on the basis of previously reported data on the absolute configuration of D-isoleucine[13,14] and as a result of the biological data reported in the present communication; *cf.* W. Klyne, *Biochem. J.*, **53**, 378 (1953).

(19) It should be noted that for substrate solutions of 0.1 molar concentration or greater, a marked inhibition of enzymatic action was observed. This inhibition, which increased with an increase in substrate concentration, was not observed at concentrations below 0.1 molar.

(20) M. Bergmann and J. S. Fruton, *Advances in Enzymol.*, **1**, 63 (1941).

(21) D. M. Greenberg and T. Winnick, *Ann. Rev. Biochem.*, **14**, 11 (1945).

(22) B. Helferich, *The Enzymes*, **1**, 79 (1950).

(23) The configuration of the $\alpha$-asymmetric center of compounds of the type R—CH(CH₃)COOH (where R is an alkyl substituent), relative to L-serine in the amino acid series is shown in the typical Fischer diagram below.

(24) K. R. Rao, S. M. Birnbaum, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **198**, 507 (1952).

(25) W. S. Fones, *Arch. Biochem. Biophys.*, **36**, 486 (1952).

primarily α-L-directed enzymes, an L-configuration in the β-asymmetric center is more conducive to enzymic action than the β-D-configuration, whereas the opposite situation obtains for primarily α-D-directed enzymes. A recent study of the enzymatic decarboxylation of the stereoisomeric phenylserines is consistent with this viewpoint.[26]

## Experimental

**DL-α-Methylbutyryl-L-norleucine.**—A mixture of 200 g. of DL-α-methylbutyric acid with 155 ml. of thionyl chloride and 2 ml. of phosphorus trichloride was allowed to stand at 25° for 2 hours until the reaction diminished, and was then refluxed for 3–4 hours on the steam-bath. The clear liquid was first distilled at 43° under reduced pressure, and subsequently at 115–115.5° at ordinary pressure. Rupe reported a boiling point of 115–116° for this acid chloride.[27] A solution of 20 g. of L-norleucine[16] in 150 ml. of chilled 1 *N* KOH was shaken at 0° with alternate portions totalling 12.1 g. of DL-α-methylbutyryl chloride and 100 ml. 1 *N* KOH. At the end of the reaction the clear solution was treated with concd. HCl to *p*H 1.7. The mixture was twice extracted with ether, the extracts combined and dried over anhydrous sodium sulfate, and the solvent evaporated leaving a colorless oily residue. This was rubbed with petroleum ether, the supernatant liquid discarded, and the residual oil taken up several times in ether and precipitated each time with an excess of petroleum ether. The oil was finally converted to a mass of crystals which weighed 12 g. when dried. The material was not recrystallized in order to avoid the possibility of fractionation of the diastereomers. It melted over the range of 80–90°; $[\alpha]^{25}D$ −9.8° (*c* 2 in abs. ethanol).

*Anal.* Calcd. for $C_{11}H_{21}O_3N$: C, 61.4; H, 9.8; N, 6.5. Found: C, 60.9; H, 10.0; N, 6.6.

**Action of Acylase I on DL-α-Methylbutyryl-L-norleucine.**—A solution of 54 g. (0.25 mole) of DL-α-methylbutyryl-L-norleucine was treated with lithium hydroxide to *p*H 7.2 and then diluted to a volume of 10 l. After the addition of 1.5 g. of acylase I powder,[18] the mixture was incubated at 37°. Aliquots, withdrawn periodically over a period of about 14 hours, were analyzed for free amino acid by the manometric ninhydrin–$CO_2$ method. Termination of the reaction at 40% of complete hydrolysis was effected by the addition of 2 *N* hydrochloric acid to *p*H 5. After the addition of Norit A, the digest was filtered to remove the major portion of the protein. Readjustment to *p*H 8.5 was followed by concentration *in vacuo* to a volume of approximately 500 ml.

**Optical Characteristics of Methylbutyric Acid after Enzymic (Acylase I) Hydrolysis and after Acid Hydrolysis of the Substrate.**—The enzymic digest concentrate of DL-α-methylbutyryl-L-norleucine, as described above, was acidified to *p*H 1.7 with concd. HCl, twice extracted with ether and the combined extracts dried over anhydrous sodium sulfate. Removal of the solvent left a thick oily residue which was a mixture of the free α-methylbutyric acid and residual α-methylbutyryl-L-norleucine. These were separated by dissolving the residue in a small amount of dry ether and adding a large excess of petroleum ether. The free acid remained in solution, whereas the acylated amino acid separated as a white, oily mass. The residue was again dissolved in ether and precipitated again with excess petroleum ether. Both supernatant solutions were combined and the solvent removed by a stream of air. The residual oil was twice fractionated by distillation at 10 mm., the final product which amounted to 4.3 g. boiling at 69°.[28] The $\alpha_D$ at 25° was +11.25° for a 2-dcm. tube, and using a $d_{25}$ value of 0.94, the calculated $[\alpha]^{25}D$ was +6.0°.

*Anal.* Calcd. for $C_5H_{10}O_2$: C, 58.8; H, 9.8. Found: C, 58.2; H, 9.8.

The residual α-methylbutyryl-L-norleucine which had been precipitated from ether solution by excess petroleum ether was refluxed with 200 ml. of 2 *N* HCl for 2.5 hours. After cooling to 25°, the solution was extracted with ether as above and the residual oil subjected to fractional distillation at 10 mm. pressure. The clear liquid boiled at 69–69.5°, and after the second distillation amounted to 6.2 g. The $\alpha_D$ at 25° was −5.5° for a 2-dcm. tube and using a $d_{25}$ value of 0.94, the calculated $[\alpha]^{25}D$ was −3.0°.

*Anal.* Calcd. for $C_5H_{10}O_2$: C, 58.8; H, 9.8. Found: C, 58.5; H, 9.9.

Twenty grams of DL-α-methylbutyryl-L-norleucine was refluxed for 3 hours with 400 ml. of 2 *N* HCl. The solution was cooled, extracted twice with ether, and the combined extracts dried over anhydrous sodium sulfate. Removal of the solvent left a yellowish residual oil amounting to about 5.5 g. The oil was twice fractionated *in vacuo* at 10 mm., boiling at 68.5–70° the first time, and rather sharply at 70° the second time. The yield of colorless liquid was 4 g. Measured in a 2-dcm. tube it showed no measurable optical rotation.

*Anal.* Calcd. for $C_5H_{10}O_2$: C, 58.8; H, 9.8. Found: C, 58.6; H, 10.0.

(26) W. J. Hartman, R. S. Pogrund, W. Drell and W. G. Clark, THIS JOURNAL, **77**, 816 (1955).

(27) H. Rupe, *Ann.*, **369**, 338 (1909).

(28) The fractionations were kindly performed by Dr. S-C. J. Fu.

BETHESDA, MARYLAND

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE]

# Steric Relationship between Threonine and Isoleucine as Indicated by an Antimetabolite Study[1]

BY M. RABINOVITZ, MARGARET E. OLSON AND DAVID M. GREENBERG

RECEIVED DECEMBER 16, 1954

O-Methylthreonine but not O-methylallothreonine is a competitive inhibitor of the incorporation of radioactive isoleucine into proteins of the Ehrlich ascites carcinoma. The incorporation of radioactive leucine is also inhibited, but non-competitively, and this inhibition is relieved by isoleucine. The steric requirement for isoleucine antagonism is one which may be expected from the configuration of isoleucine as determined by chemical and X-ray evidence.

Isoleucine, an amino acid which is a constituent of proteins, may exist in two possible diastereoisomeric forms. One of these structures represents isoleucine, the other alloisoleucine, an amino acid which is not a constituent of proteins. It has, moreover, been established recently by both chemical[2a] and X-ray techniques[2b] that the α-amino and β-methyl carbons of isoleucine have the erythro configuration. The configurations of threonine and allothreonine have been established[3] earlier and the methyl ethers of these compounds assume

(1) Presented at the Autumn, 1954, meeting of the Division of Biological Chemistry, American Chemical Society, New York. Aided by grants from the Damon Runyon Memorial Fund for Cancer Research and the Cancer Research Funds of the University of California.

(2a) S. Ställberg-Stenhagen and E. Stenhagen, *Arkiv. Kemi, Mineral. Geol.*, **24B**, No. 9, 1 (1947).

(2b) J. Trommel, *Proc. Konikl. Nederl. Akad. van Wetenschappen*, **B56**, 272 (1953); *ibid.*, **57**, 364 (1954).

(3) C. E. Meyer and W. C. Rose, *J. Biol. Chem.*, **115**, 721 (1936).