genated over a period of four hours at 45 p.s.i. The solution was then filtered to remove both the catalyst and the insoluble product. This mixture was extracted in a Soxhlet extractor with the original ether. On separation of the ether, 0.6 g. (60%) of a yellow compound was obtained. Benzene extraction removed the color (oxidized product) and left a white residue; m.p. 209–211°.

Anal. Calcd. for $C_8H_{10}ClN_4$: C, 48.8; H, 4.58. Found: C, 48.6; H, 4.72.

2-Methyl-6,7-dimethylpteridinol-4.—2-Methyl-4,5-diamino-6-hydroxypyrimidine[22] (3 g.) was suspended in 150 ml. of an aqueous mixture containing 0.5 g. of sodium sulfite and 2.5 g. of biacetyl. The suspension was refluxed (15 minutes) to effect solution which in turn was then evaporated to dryness. The residue was extracted with two 30-ml. portions of boiling absolute alcohol and the combined extracts were concentrated to one-half the volume and set in the refrigerator overnight. With further concentration the total yield was 2.5 g. (62%) of a white powder; m.p. 256–259°. Recrystallization from absolute alcohol gave m.p. 261–262°.

Anal. Calcd. for $C_9H_{10}N_4O$: C, 56.83; H, 5.30. Found: C, 56.6; H, 5.31.

(22) A. Maggiolo, A. P. Phillips and G. H. Hitchings, THIS JOURNAL, **73**, 106 (1951).

CORVALLIS, OREGON

[CONTRIBUTION FROM THE LANKENAU HOSPITAL RESEARCH INSTITUTE AND THE INSTITUTE FOR CANCER RESEARCH]

The Biosynthesis of Isoleucine[1]

BY MURRAY STRASSMAN,[2] ALICE J. THOMAS, LILLIAN A. LOCKE AND SIDNEY WEINHOUSE

RECEIVED JULY 1, 1955

In previous publications evidence from isotope tracer studies in *Torulopsis utilis* was reported indicating that pyruvic acid may be a direct precursor of the valine carbon chain. A mechanism for the synthesis of valine in this organism was proposed, involving a condensation of acetaldehyde and pyruvic acid to yield acetolactic acid, followed by an intramolecular migration of a methyl group to yield the branched carbon chain of valine. In the present paper data are presented which indicate that the synthesis of the structurally similar isoleucine molecule probably proceeds by an analogous series of reactions, involving a condensation of acetaldehyde with α-ketobutyric acid instead of pyruvic acid, to yield a homolog of acetolactic acid, α-aceto-α-hydroxybutyric acid. Migration of the ethyl group in this intermediate is presumed to produce the carbon skeleton of isoleucine.

In previous isotopic tracer studies of valine synthesis in the yeast, *Torulopsis utilis*,[3,4] evidence was obtained which indicated that this branched chain amino acid is produced *via* a ketol condensation of acetaldehyde and pyruvic acid to yield acetolactic acid, followed by an intramolecular migration of the α-methyl group. Once a reaction of this type was conceived, it seemed probable that the biosynthesis of the homologous, branched-chain amino acid, isoleucine, might proceed by a similar pathway. As shown in Fig. 1, the condensation of acetaldehyde with the homolog of pyruvic acid, α-ketobutyric acid, would yield α-keto-α-hydroxybutyric acid. A shift of the ethyl group from carbon 2 to carbon 3, the carbonyl carbon of the acetaldehyde moiety, would produce the carbon skeleton of isoleucine. In a preliminary note,[5] evidence was presented in support of this mechanism. In the present communication, detailed experimental procedures are described, and more extensive data are reported which further confirm the mechanism of isoleucine biosynthesis shown in Fig. 1.

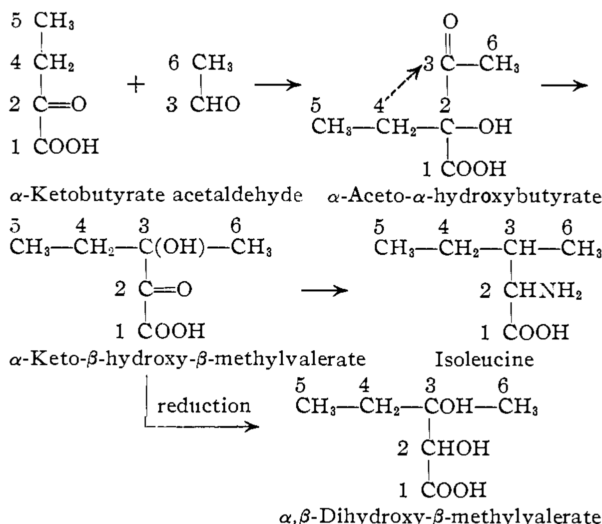

Fig. 1.—Postulated mechanism of isoleucine biosynthesis from acetaldehyde and α-ketobutyric acid. Numbering of carbon atoms is arranged to give isoleucine with the numbering shown in Table II.

Experimental

Complete details concerning cultivation of the organism, radioactivity assays, the isolation of amino acids and other experimental procedures are given in previous publications concerned with lysine[6] and valine[4] biosynthesis.

Isolation of Isoleucine.—Using the procedure previously described[4] a mixture of isoleucine and leucine, containing traces of methionine, was obtained by chromatography on Dowex 50. The two acids were separated from one another in pure form by rechromatographing the mixture on a starch column, 45 × 3.8 cm., following the procedure of Aqvist.[7] This was accomplished by dissolving the mixture in 25–35 ml. of butanol saturated with water, placing the solution on the starch column, and eluting under 14 mm. pressure with butanol saturated with water at such a rate that one 14-ml. fraction was collected every hour. Usually, the isoleucine was completely contained in frac-

(1) This work was done under contract with the United States Atomic Energy Commission, Contract No. AT(30-1)777, and was aided by grants from the American Cancer Society, recommended by the Committee on Growth of the National Research Council, and the National Institutes of Health, Department of Health, Education and Welfare.

(2) Post-doctoral Fellow of the National Institutes of Health, Department of Health, Education and Welfare.

(3) M. Strassman, A. J. Thomas and S. Weinhouse, THIS JOURNAL, **75**, 5135 (1953).

(4) M. Strassman, A. J. Thomas and S. Weinhouse, *ibid.*, **77**, 1261 (1955).

(5) M. Strassman, A. J. Thomas, L. A. Locke and S. Weinhouse, *ibid.*, **76**, 4241 (1954).

(6) M. Strassman and S. Weinhouse, *ibid.*, **75**, 1680 (1953).

(7) S. E. G. Aqvist, *Acta Chem. Scand.*, **5**, 1031 (1951).

tions 60–80. These were evaporated to dryness, and tested microbiologically to make sure of the absence of leucine, using *Streptococcus faecalis* according to the procedure of Toennies and Shockman.[8]

The chromatographically pure isoleucine thus obtained was diluted with an appropriate amount of inactive isoleucine for chemical degradation.

Degradation of C^{14}-Labeled Isoleucine.—The activity of each of the individual carbon atoms of isoleucine was determined by means of the reactions listed in Fig. 2.

Decarboxylation of Isoleucine with Ninhydrin (Reaction 1).—Approximately 2 mmoles of isoleucine was dissolved in 35 ml. of water containing 0.15 ml. of 80% phosphoric acid, and was decarboxylated with ninhydrin as described previously in the degradation of valine.[4]

Oxidation of α-Methylbutyraldehyde to α-Methylbutyric Acid (Reaction 2).—The residual ninhydrin reaction mixture was decanted from the precipitate and, after diluting to 50 ml., the α-methylbutyraldehyde was distilled into a flask cooled in ice. Five grams of $MgSO_4 \cdot 7H_2O$ was added to the distillate and, while stirring rapidly, 5 ml. of 1.5 N $KMnO_4$ was added slowly. The mixture was stirred for about one hour, then 100 ml. of water, 30 g. of $MgSO_4 \cdot 7H_2O$ and 10 ml. of 50% H_2SO_4 were added, and the acid was distilled with steam. It was collected in six or seven 50-ml. fractions, each being titrated with 0.1 N NaOH. The acid, characterized by means of a Duclaux curve, was obtained in a yield of about 70%.

Decarboxylation of α-Methylbutyric Acid (Reaction 3).—The sodium α-methylbutyrate was evaporated to dryness, dissolved in 0.7–0.8 ml. of 100% H_2SO_4, and decarboxylated with sodium azide as described previously for the degradation of isobutyric acid.[4] The secondary butylamine formed in the reaction was liberated by adding 8 ml. of 5 N NaOH to the reaction mixture and distilling into 7 ml. of 0.35 N H_2SO_4.

Oxidation of *sec*-Butylamine to Methyl Ethyl Ketone (Reaction 4).—The solution of amine sulfate was diluted to 20 ml., cooled in ice and treated with an amount of 1.0 N NaOH equivalent to the H_2SO_4 used to trap the amine. Nine ml. of 1.5 N $KMnO_4$ was added, and the solution allowed to stand for about 5 hours at room temperature. The ketone was then distilled with steam, collected in two 50-ml. portions and converted to the hydrazone by adding a solution of 300 mg. of 2,4-dinitrophenylhydrazine in 4.5 ml. of alcohol containing 0.6 ml. of concd. H_2SO_4. The 2,4-dinitrophenylhydrazone of methyl ethyl ketone thus obtained after recrystallization had the correct melting point of 114–115° and did not depress that of an authentic sample.

Degradation of Methyl Ethyl Ketone with Sodium Hypoiodite (Reaction 5).—The 2,4-dinitrophenylhydrazone was suspended in 30 ml. of 9 N H_2SO_4, and the mixture was distilled into an iced solution of 3.2 g. of sodium hydroxide in 25 ml. of water. When about 20–25 ml. of distillate had been collected, 9.0 ml. of 1 N iodine–KI solution (25 g. of KI and 12.5 g. of I_2 in 100 ml. of water) was added dropwise with stirring. The solution was allowed to stand at room temperature for 20 minutes, then cooled in ice and centrifuged. The supernatant solution containing sodium propionate was separated from the iodoform, and the latter oxidized to CO_2 with chromic acid solution as previously described.[4]

The solution of sodium propionate was acidified, and the acid steam distilled. The distillate was titrated with 0.1 N NaOH. The propionic acid thus obtained gave a Duclaux curve characteristic of the pure acid, indicating an exclusive split between the methyl and carbonyl carbons of the ketone. If, as in some instances, the yield of propionic acid was lower than 0.4 mmole, the material was diluted with an exact amount of inactive sodium propionate to obtain at least 0.6 millimole with which to carry the degradation to completion.

Degradation of Propionic Acid (Reactions 6–9).—The sodium propionate was evaporated to dryness, dissolved in 0.4–0.5 ml. of 100% H_2SO_4, and the acid was decarboxylated by the Schmidt reaction.[4] The resulting ethylamine was isolated by steam distillation, as in reaction 3, and collected in 6 ml. of 0.26 N H_2SO_4. The solution of amine sulfate was diluted to 16 ml., neutralized to pH 11 with 1.0 N NaOH, and 5 ml. of 1.5 N $KMnO_4$ was added. After the solution had remained at room temperature for 3 hours, it was heated to 80–90° for 15 minutes. Then, 30 g. of $MgSO_4 \cdot 7H_2O$ and 10 g. of 50% H_2SO_4 were added and the acetic acid was steam distilled. The acetic acid was shown to be pure by means of a Duclaux curve, and was subsequently degraded as described previously.[4]

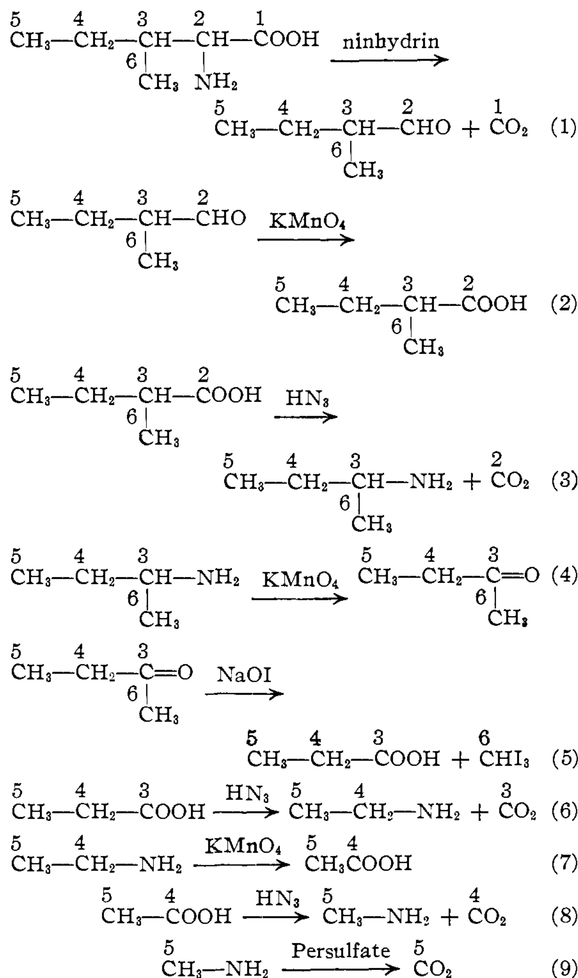

Fig. 2.—Reactions employed in degradation of labeled isoleucines.

Results and Discussion

The activities of the isoleucines obtained from yeast grown in the presence of variously labeled acetates and lactates are reported in Table I. Both acetate carbons, and the α and methyl carbons of lactate were readily incorporated into isoleucine. On

TABLE I

SPECIFIC ACTIVITIES OF ISOLEUCINE SAMPLES SYNTHESIZED BY *T. Utilis* IN THE PRESENCE OF VARIOUS LABELED SUBSTRATES

Activities of isoleucines are given in counts/min./7.5 cm.[2] dish at "infinite thickness," corrected for original activities of 10 μcuries in the substrates.

Labeled substrate	Activity of isoleucine, c.p.m.	Labeled substrate	Activity of isoleucine, c.p.m.
Acetate-1-C^{14}	1266	Lactate-1-C^{14}	156
Acetate-2-C^{14}	1235	Lactate-2-C^{14}	1998
		Lactate-3-C^{14}	1142

(8) G. Toennies and G. D. Shockman, *Arch. Biochem. Biophys.*, **45**, 447 (1953). We are indebted to Dr. Shockman and Mr. Kolb of this Institute for their helpful suggestions in adapting this procedure to fit our specific needs.

the other hand, the carboxyl carbon of lactate was only slightly incorporated. These results are in marked contrast with those for valine,[4] in which all three lactate carbons, but neither of the acetate carbons was readily incorporated. These samples of valine and isoleucine were isolated from batches of yeast obtained in experiments conducted over a period of about four years. Thus, we cannot attach too much significance to the absolute activities observed. These are regarded only as rough estimates of the order of magnitude of incorporation.

Calculation of Acetate Carbon Distribution of Isoleucine.—It is now apparent that α-ketobutyrate is most likely derived from the carbon chain of oxalacetic acid *via* the reaction sequence: aspartate → homoserine → threonine → α-aminobutyric acid.[9,10] On the basis of a direct conversion of aspartate to α-ketobutyrate without fission of the carbon chain, as shown in Fig. 3, coupled with the reasonable assumption that the aspartate is derived directly from oxalacetate, it is possible to calculate a distribution of acetate carbons among those of isoleucine derived *via* the reaction mechanism shown in Fig. 1. This may be done from the following considerations.

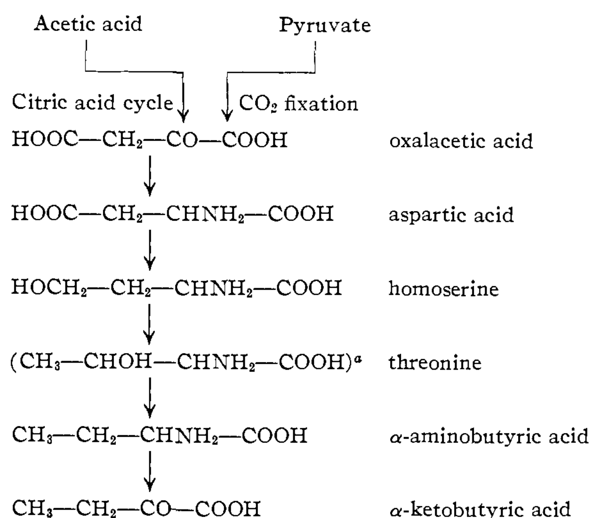

Fig. 3.—Biosynthesis of α-ketobutyric acid. [a] It is possible threonine is not on the direct pathway of α-aminobutyric acid formation.)

It can be seen easily, by tracing acetate carbons through the citric cycle,[6] that the 4-carbon acids so derived will have 3 of their carbons, one carboxyl, and the two central carbons, derived from an acetate methyl carbon, and one carboxyl derived from an acetate carboxyl. Having passed through a symmetrical stage (succinate and fumarate), oxalacetate produced in the citric acid cycle will thus have twice as much acetate methyl carbon in its two central carbons as in its carboxyl carbons. Also, it will have equal amounts of acetate carboxyl carbon in its carboxyls, but none in its two central carbons.

On the basis of the series of reactions shown in Fig. 1, carbons 1 and 2 of isoleucine would be derived, respectively, from the α-carboxyl and the adjacent carbonyl carbon of oxalacetate; carbons 4 and 5 of isoleucine (the ethyl group) would come from oxalacetate carbons 3 and 4 (the acetyl moiety); and carbon 3 and carbon 6 (the methyl carbon) would come, respectively, from the carbonyl and methyl carbons of acetaldehyde. Thus, acetate carboxyl carbon would be present equally and exclusively in isoleucine carbons 1 and 5; and acetate methyl carbons would be present in carbons 1, 2, 4 and 5, with twice as much in 2 and 4 as in 1 and 5. On the whole, as shown in Table II, the observed values agree quite well with those calculated. Thus, acetate carboxyl carbon was present equally and almost entirely in carbons 1 and 5; carbons 2 and 4 together had almost twice the acetate methyl activity present in carbons 1 and 5 together. However, carbon 2 had a somewhat higher than calculated activity and carbon 4 somewhat lower, making the distribution in these presumed aspartate-derived carbons approximately 1:2:1:1 rather than 1:2:2:1. This is not regarded as a serious reflection on the mechanism of Fig. 1. It is probable that in growing cells, the citric acid cycle is not the only important source of aspartic acid carbon, and hence the distribution of acetate carbons therein might differ considerably from that calculated on the basis of exclusive formation *via* the cycle. That the distribution of pyruvate carbon 2 in aspartate differs from that predicted on the basis of the citric acid cycle as an exclusive source has been shown by Wang, *et al.*,[11] and will be dealt with in the next section. The possibility that other mechanisms exist whereby 4-carbon acids are formed from acetate is not excluded. It is therefore not surprising that the acetate distribution differed from the theoretically calculated values. Despite these deviations the over-all similarity in the observed and calculated distribution patterns constitutes grounds for belief in the essential correctness of the mechanism in Fig. 1.

Incorporation of Lactate Carbon in Isoleucine.—As predicted by the mechanism of Fig. 1, the activity of isoleucine produced from carboxyl-labeled lactic acid was of low order (Table I); hence degradation of this sample was not carried out. Three main pathways may be considered by which lactate carbons 2 and 3 may be incorporated into isoleucine. These are: *via* pyruvate and acetaldehyde, *via* acetate and *via* CO_2 fixation with pyruvate. Without knowing the extent of each of these reactions, we cannot calculate a precise theoretical distribution for lactate carbons as was done for acetate. However, assuming that all three of these processes occur, a rough estimation may be made on the basis of these known pathways. Lactate-2-C^{14}, on CO_2-fixation, would directly produce oxalacetate-2-C^{14}, and in view of its ready conversion to acetate-1-C^{14}, lactate-2-C^{14} would also be expected to yield oxalacetate-1,4-C^{14} *via* the citric acid cycle. On direct decarboxylation, lactate-2-C^{14} would yield acetaldehyde-1-C^{14}. From the mechanisms of Figs. 1 and 3, one would thus anticipate that lactate carbon 2 would be incorporated highly into isoleucine carbons 2 and 3, to a lesser ex-

(9) P. H. Abelson, E. Bolton, a R. Britten, D. B. Cowie and R. B. Roberts, *Proc. Natl. Acad. Sci.*, **39**, 1020 (1953).

(10) S. Black and N. G. Wright, *Federation Proc.*, **13**, 184 (1954).

(11) C. H. Wang, R. C. Thomas, V. H. Cheldelin and B. E. Christensen, *J. Biol. Chem.*, **197**, 663 (1952).

TABLE II

PERCENTAGE C^{14}-DISTRIBUTION IN ISOLEUCINE FROM ACETATE AND LACTATE

Values are calculated on basis of total activities of 100%.

Labeled compound	Isoleucine carbon no. 5 CH_3	4 CH_2	3 CH	6 (CH_3)	2 $CHNH_2$	1 COOH
Acetate						
Methyl (calcd.)	17	33	0	0	33	17
Methyl (obsd.)	18	19	4	3	39	17
Acetate						
Carboxyl (calcd.)	50	0	0	0	0	50
Carboxyl (obsd.)	46	0	3	3	1	47
Lactate						
Methyl (predd.)	Low	High	Low	High	Intermediate	Low
Methyl (obsd.)	7	27	5	41	15	5
Lactate						
α-Carbon (predd.)	Intermediate	Low	High	Low	High	Intermediate
α-Carbon (obsd.)	7	4	36	1	43	9

tent in carbons 1 and 5, and least in carbons 4 and 6. On the same grounds, lactate carbon 3 would be incorporated most highly in carbons 4 and 6, less highly in carbon 2, and least in carbons 1, 3 and 5. The agreement of these predicted with the observed distributions, shown in Table II, is regarded as strong confirmatory evidence of the correctness of the mechanism in Fig. 1.

Further evidence of the participation of aspartic acid in isoleucine biosynthesis is provided by a comparison of the distribution of lactate carbon 2 in the isoleucine carbons considered to be derived from aspartate, *viz.*, carbons 1, 2, 4 and 5, with the distribution of pyruvate carbon 2 in aspartic acid isolated from yeast by Wang, *et al.*[11] From the observed distribution of lactate carbon 2 among isoleucine carbons 1, 2, 4 and 5, we calculated its distribution in aspartic acid, and in Table III have compared our values with those given by Wang, *et al.* The close correspondence between the two independent sets of data provides, we believe, further corroboration of the participation of aspartate in isoleucine biosynthesis and thus of the essential correctness of the mechanism in Fig. 1.

TABLE III

COMPARISON OF DISTRIBUTION OF PYRUVATE α-CARBON IN ASPARTATE WITH DISTRIBUTION OF LACTATE α-CARBON IN THE PRESUMED ASPARTATE-DERIVED PORTION OF ISOLEUCINE

	% distribution of C^{14} in aspartic acid carbons HOOC— 4	CH_2— 3	$CHNH_2$— 2	COOH 1
Distribution found by Wang, *et al.*[11] from α-C^{14}-pyruvate	23	7	55	16
2-C^{14}-lactate distribution calcd. from aspartate moiety, carbons 4, 5, 2, 1 of isoleucine	11	6	68	14

A biochemical relationship between the two structurally similar amino acids, valine and isoleucine, was first indicated in studies by Bonner, Tatum and Beadle,[12] who found a single mutant strain of *Neurospora* which required both acids for growth. Further mutant studies[13-15] led to the discovery of two α,β-dihydroxy acids having the carbon chains of valine and isoleucine, which were identified as precursors of the respective amino acids. Another point that may be cited in support of the plausibility of the mechanism of Fig. 1 is that it provides a simple explanation of the formation of these dihydroxy acids; *viz.*, by direct reduction of the α-keto-β-hydroxy acid postulated as the direct rearrangement product, as shown in Fig. 1.

Still other isoleucine-requiring mutants have been discovered which can grow on the 4-carbon acids, α-ketobutyric acid, α-aminobutyric acid and threonine.[15-17] Also, Abelson,[18] in isotopic competition experiments with *E. coli* showed that the addition of these 4-carbon acids to growth media containing glucose labeled with C^{14} suppressed the radioactivity incorporated into isoleucine. Using C^{14}-labeled acetic acids, Tatum and Adelberg[19] found that the *Neurospora* mutant incorporated C^{14} into the dihydroxy analogs of isoleucine and valine. In agreement with our data, these workers showed that the distal carbon of the ethyl group of isoleucine originates from the carboxyl carbon of acetate. They also found that the α- and β-carbons of isoleucine were produced from acetic acid carbons, and proposed a mechanism involving multiple condensations of acetic acid molecules to account for the distribution of activity. Also in accord with our results are the data of Ehrensvard and co-workers,[20] who found that the carboxyl of acetate gives rise to the carboxyl carbon of isoleucine, and that the acetate carboxyl activity in this position is about five times that in the rest of the molecule.

Further isotopic data which are in accord with the mechanism here proposed have been provided

(12) D. Bonner, E. L. Tatum and G. W. Beadle, *Arch. Biochem.*, **3**, 71 (1943).

(13) E. A. Adelberg and E. L. Tatum, *ibid.*, **29**, 235 (1950).

(14) E. A. Adelberg, D. M. Bonner and E. L. Tatum, *J. Biol. Chem.*, **190**, 837 (1951).

(15) H. E. Umbarger and E. A. Adelberg, *ibid.*, **192**, 883 (1951).

(16) J. W. Myers and E. A. Adelberg, *Proc. Natl. Acad. Sci.*, in press.

(17) H. J. Teds, Oak Ridge National Laboratory, Rep. No. 164, 1948.

(18) P. H. Abelson, *J. Biol. Chem.*, **206**, 335 (1954).

(19) E. L. Tatum and E. A. Adelberg, *ibid.*, **190**, 843 (1951).

(20) G. Ehrensvard, L. Reio, E. Saluste and R. Stjernholm, *ibid.*, **189**, 93 (1951).

recently by Adelberg.[21] In isotopic competition experiments with an isoleucine-requiring mutant of *Neurospora*, he found that the presence of unlabeled threonine considerably reduced the incorporation of acetate-1,2-C^{14} into the dihydroxyisoleucine analog. Whereas isoleucine carbons 3 and 6 were undiluted in their activity, carbons 1, 2, 4 and 5 were diluted to a considerable degree. The conclusion that carbons 1, 2, 4 and 5 are derived from threonine carbons was supported by a subsequent experiment, in which he found that administration of threonine-1,2-C^{14} resulted in 1,2-C^{14} labeling in the dihydroxyisoleucine analog. Adelberg suggested a mechanism for isoleucine synthesis which also involves an intramolecular migration, differing in some detail from the one we have proposed. The mechanism proposed by us was chosen because of its analogy with known chemical and biological processes, as discussed previously with regard to its participation in valine biosynthesis.[4] At present, no data are available to choose between these, and final conclusions will have to await identification of intermediates in these biosynthetic processes.

(21) E. A. Adelberg, THIS JOURNAL, **76**, 4241 (1954).

PHILADELPHIA, PA.

[CONTRIBUTION FROM THE BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

Synthesis of Unsaturated α-Lecithins.[1] I. L-α-(Dioleyl)-lecithin

BY ERICH BAER, DMYTRO BUCHNEA AND ALAN G. NEWCOMBE

RECEIVED JULY 5, 1955

A procedure permitting the synthesis of the optically pure enantiomeric forms of unsaturated α-lecithins has been developed. The synthesis of L-α-(dioleyl)-lecithin is described and its infrared spectrum, solubilities and other physical data are reported. The catalytic reduction of the L-α-(dioleyl)-lecithin offers a new route for the preparation of L-α-(distearoyl)-lecithin.

The isolation from natural sources of lecithins containing either two identical saturated[2-4] or unsaturated fatty acids[5] established the existence of these two types of phosphatides in nature and thus invalidated an earlier hypothesis that all natural lecithins contain one molecule each of a saturated and unsaturated fatty acid. In order to establish the structure and configuration of natural phosphatides, and to make pure representatives of these compounds readily accessible, methods for their synthesis were developed in this Laboratory. Our procedure for the synthesis of the enantiomeric forms of fully saturated α-lecithins has already been reported.[6-8] This procedure, employing phenylphosphoryl dichloride as the phosphorylating agent, unfortunately cannot be applied to the synthesis of unsaturated lecithins, since the phenyl group of the phenyl ester of the unsaturated lecithin, which would be an intermediate in this procedure, could not be removed by catalytic hydrogenolysis without the simultaneous reduction of the fatty acid double bond. Since in our experience phenylphosphoryl dichloride as a phosphorylating agent is superior in several respects to phosphorus oxychloride, it was deemed desirable to devise a procedure that would permit its use in the synthesis of unsaturated lecithins. Obviously, in this case, the removal of the protective phenyl group by catalytic hydrogenolysis would have to precede the introduction of the unsaturated fatty acid substituents into the glycerol moiety of the phosphatide. With this in mind a sequence of reactions was devised by means of which the synthesis of a fully unsaturated α-lecithin, namely, L-α-(dioleyl)-lecithin finally has been achieved. This compound was selected for synthesis not only because of the presence of oleic acid in many of the naturally occurring glycerolphosphatides,[9] but also in the hope that the unsaturated lecithin would prove to be a good substitute for beef heart lecithin in the serodiagnosis of syphilis.[10]

The synthesis was carried out as follows (see Reaction Scheme): D-acetone glycerol (I) was phosphorylated by means of phenylphosphoryl dichloride and quinoline, and the resulting acetone L-α-glycerylphenylphosphoryl chloride (II) was esterified with ethylene chlorohydrin in the presence of pyridine. The acetone L-α-glycerylphenylphosphoryl ethylene chlorohydrin (III) thus formed was freed of its phenyl group by catalytic hydrogenolysis and of its acetone group by mild acid hydrolysis, and the L-α-glycerylphosphoryl ethylene chlorohydrin (IV) was isolated in the form of its barium salt. Treating the barium salt with oleyl chloride and pyridine in anhydrous dimethylformamide gave the barium salt of L-α-dioleylglycerylphosphoryl ethylene chlorohydrin

(1) The feasibility of the procedure reported in this communication was tested by Dr. A. G. Newcombe by preparing a known saturated lecithin. The procedure was improved and applied to the synthesis of the unsaturated lecithin by Dr. D. Buchnea.

(2) A. Lesuk and R. J. Anderson, *J. Biol. Chem.*, **139**, 457 (1941).

(3) S. J. Thannhauser, J. Benotti, J. and N. F. Boncoddo, *ibid.*, **166**, 669 (1946).

(4) S. J. Thannhauser and N. F. Boncoddo, *ibid.*, **172**, 135 (1948).

(5) D. J. Hanahan and M. E. Jayko, THIS JOURNAL, **74**, 5070 (1952).

(6) E. Baer and M. Kates, *ibid.*, **72**, 942 (1950).

(7) E. Baer and J. Maurukas, *ibid.*, **74**, 158 (1952).

(8) E. Baer, *ibid.*, **75**, 621 (1953).

(9) The isolation of the dipalmitoleyllecithin from brewer's yeast had not then been reported by Hanahan and Jayko.

(10) The serological investigation of the unsaturated lecithin is being carried out by Drs. D. B. Tonks and R. H. Allen and Miss Evelyn Fowler, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa. Some of their findings were reported at the "Symposium on Recent Advances in the Study of Venereal Diseases," Washington, D. C., U. S. Public Health Service, 1955 (B. David Tonks, H. Rovelle Allen and Evelyn Fowler, The Use of a Synthetic Unsaturated (Dioleyl)-L-α-lecithin in Cardiolipin Antigens for the Serodiagnosis of Syphilis. A Comparison with Other Lecithins, Natural and Synthetic).