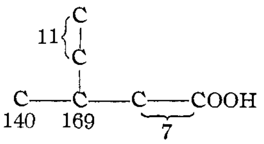
COMMUNICATIONS TO THE EDITOR

ISOLEUCINE BIOSYNTHESIS FROM THREONINE[1]

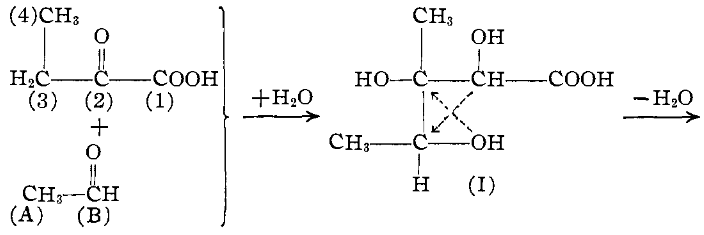
Sir:

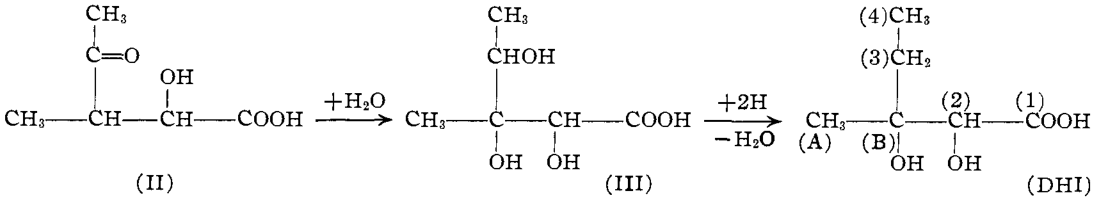
Recent studies with microörganisms[2] indicate that isoleucine is formed from the corresponding α,β-dihydroxy acid (DHI) by enzymatic dehydration and transamination. Part of the carbon skeleton of DHI is known to be derivable from certain C_4 compounds, including threonine.[3,4] In order to determine which of the isoleucine carbons are so derived, isotopic competition experiments[4] were carried out using *Neurospora crassa* and C^{14}-acetate as the source of labeled carbon. A washed mycelial pad of mutant No. 16117, which accumulates DHI, was incubated in minimal medium supplemented with C^{14}-1,2-acetate. A second pad was incubated in the identical medium except that 100 μM. of unlabeled L-threonine was added. At 48 hours, the pads were removed and the accumulated DHI isolated from each filtrate by ether extraction and paper chromatography. Both samples were then chemically degraded and the radioactivities of the various carbons compared. The results are shown below; the numbers indicate the radioactivity incorporated in the presence of unlabeled threonine, expressed as per cent. of the control (no threonine added)



The results show that threonine suppressed the incorporation of radioactivity into carbons 1, 2, 4 and 5 of isoleucine. In a separate experiment, C^{14}-1,2-threonine was found to be converted to C^{14}-1,2-DHI; thus isoleucine carbons 4 and 5 probably come from threonine carbons 3 and 4. The remaining two carbons of isoleucine may be derived from "active acetaldehyde," since Ehrensvard[5] has shown them to have the same derivation from acetate as the alpha and beta carbons of pyruvate.

Since threonine is probably deaminated to α-ketobutyrate prior to incorporation into isoleucine, it is attractive to postulate an aldol condensation between acetaldehyde and α-ketobutyrate followed by enolization and hydration. The resulting trihydroxy acid (I) would then be susceptible to a pinacol rearrangement, forming a keto-hydroxy acid (II). Hydration of II would yield a trihydroxy acid (III) which on reduction would become DHI





If, in the above scheme, pyruvate is substituted for α-ketobutyrate, the result would be synthesis of the dihydroxy acid precursor of valine.[2] Strassman, *et al.*,[6,7] have proposed similar mechanisms of valine and isoleucine biosynthesis involving ketol condensations. Final decision must await isolation of the actual intermediates and appropriate enzyme studies, since either type of condensation would be consistent with the available data.

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[1] Supported by a contract between the Regents of the University of California and the office of Naval Research.

[2] J. W. Myers and E. A. Adelberg, *Proc. Nat. Acad. Sci.*, **40**, 493 (1954).

[3] H. E. Umbarger and E. A. Adelberg, *J. Biol. Chem.*, **192**, 883 (1951).

[4] P. H. Abelson, *ibid.*, **206**, 335 (1954).

[5] G. Ehrensvard, private communication.

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

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

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RECEIVED JULY 26, 1954

INTRAMOLECULAR MIGRATION AND ISOLEUCINE BIOSYNTHESIS[1]

Sir:

In a previous study[2] the distribution of C^{14} in valines isolated from *Torulopsis utilis* grown on glucose in the presence of a variety of labeled substrates led to the suggestion that the carbon skeleton of this amino acid arises via a mechanism involving a ketol condensation of pyruvate and acetaldehyde to yield acetolactate, followed by an intramolecular migration of carbon 3 of the pyruvate moiety to carbon 1 of the acetaldehyde moiety. In the present communication evidence is presented

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[1] Aided by grants from the Atomic Energy Commission, contract No. AT(30-1)777, the American Cancer Society, and the National Cancer Institute of the Department of Health, Education, and Welfare.

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