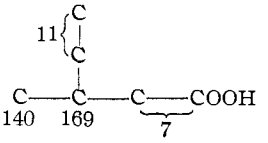
COMMUNICATIONS TO THE EDITOR

ISOLEUCINE BIOSYNTHESIS FROM THREONINE[1]

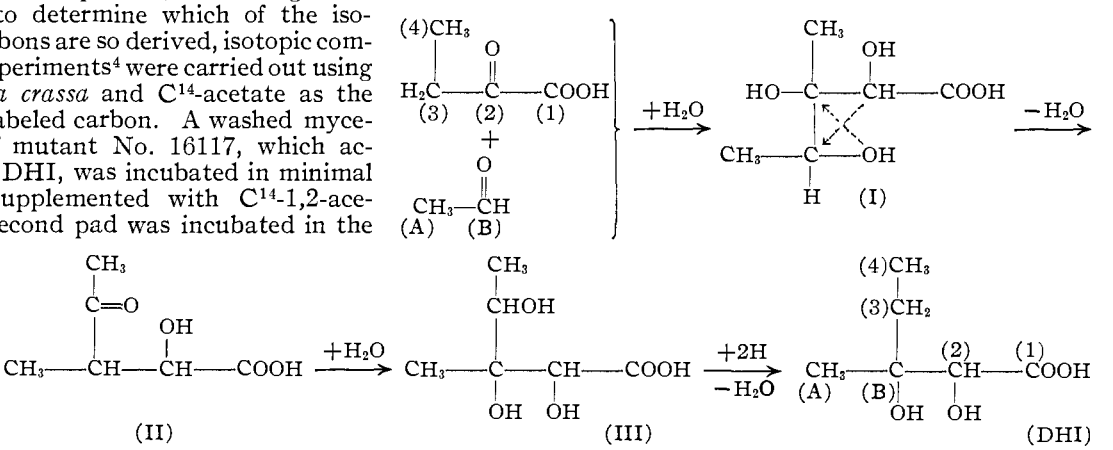
Sir:

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.

(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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INTRAMOLECULAR MIGRATION AND ISOLEUCINE BIOSYNTHESIS[1]

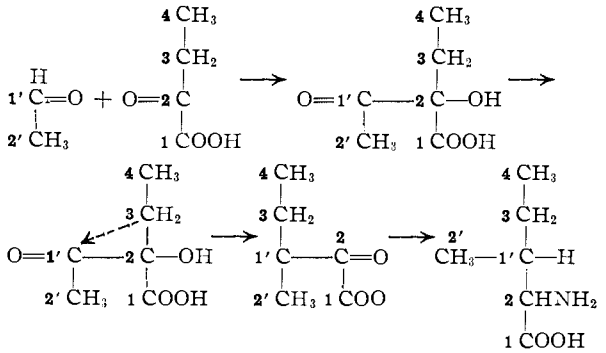
Sir:

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

(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).

that a similar intramolecular migration occurs in the biosynthesis of isoleucine isolated from the same experiments. The postulated sequence of events, shown in the figure, is a condensation of acetaldehyde with α-ketobutyric acid to yield α-aceto-α-hydroxybutyrate, followed by migration of the ethyl group from carbon 2 of the butyrate moiety to carbon 1′ of the acetaldehyde moiety.



Postulated mechanism of isoleucine biosynthesis from acetaldehyde and α-ketobutyrate

On the assumption that aspartic acid, derived from 4-carbon acids of the citric acid cycle, provides the carbon chain of α-ketobutyrate (presumably via homoserine and threonine[3]), it is possible to calculate from previously outlined considerations[4] that isoleucine synthesized in the presence of labeled acetates by the reaction sequence shown in the figure would have the distribution of acetate methyl and carboxyl carbon shown in the table.

DISTRIBUTION OF ACETATE CARBONS IN ISOLEUCINE

Values are in per cent. of total radioactivity present

	Isoleucine carbon number					
	5 CH_3—	4 CH_2—	3 CH—	6 (CH_3)—	2 $CHNH_2$—	1 COOH
Acetate Methyl Calculated	17	33	0	0	33	17
Acetate Methyl Observed	18	19	4	3	39	17
Acetate Carboxyl Calculated	50	0	0	0	0	50
Acetate Carboxyl Observed	46	0	3	3	1	47

Pure isoleucine, isolated from the yeast hydrolysates by chromatography on Dowex-50, was degraded chemically and the activities of each individual carbon were determined. The remarkable similarity between the observed and calculated distribution patterns shown in the table is regarded as strong evidence of the essential correctness of the mechanism shown in the figure. Distributions of lactate carboxyl, α and β carbons in isoleucine were also in accord with this postulation, based on known mechanisms of conversion of lactate to acetate, acetaldehyde and oxalacetate. These findings are in complete accord with those of Adelberg published in an accompanying report.[5]

Though intramolecular migrations of the type required by this formulation have not hitherto been observed in biological systems, this reaction is plausible from an organic chemical standpoint, being similar to the pinacol rearrangement. It is conceivable that alkyl group migration may play an important role generally in the biosynthesis of branched carbon chains.

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

(4) K. F. Lewis and S. Weinhouse, THIS JOURNAL, **73**, 2500 (1951).

(5) E. A. Adelberg, *ibid.*, **76**, 4241 (1954).

(6) Postdoctoral Fellow of the National Institutes of Health, Department of Health, Education, and Welfare.

EFFECTS OF RING SIZE ON ELECTRON DISTRIBUTION IN SATURATED HETEROCYCLIC COMPOUNDS[1]

Sir:

Measurements of the basicities of cyclic imines toward trimethylboron,[2] of cyclic ethers toward chloroform and methanol-*d*,[3,4] and of cyclic sulfides toward boron trifluoride,[5] have shown that the basicity changes with ring size in the order: 4 > 5 > 6 > 3-membered rings. Two differing interpretations of these observations have been suggested.

An interpretation based on steric factors was proposed by Brown and Gerstein[2] to account for the dissociation of the addition compounds of the cyclic imines with trimethylboron. According to this view the observed order of basicity results from a combination of F-strain which is more pronounced for the larger rings, and I-strain which is most important in the 3-membered ring. These strains are considered to be steric interactions which occur because of the association between donor and acceptor molecules.

On the other hand, steric factors alone do not account adequately for the results of the hydrogen bonding studies with cyclic ethers[3,4] nor are they a likely explanation for the interaction of cyclic sulfides with boron trifluoride.[5] It was suggested that the basicity differences were due rather to differences in electron availability caused *inherently* by the different sizes of the rings—that is, the electron distribution is altered by ring size.

Direct physical evidence that the electron distribution does depend significantly on ring size has now been obtained by observing the chemical shifts in the proton magnetic resonance[6] of the cyclic imines, ethers and sulfides. These shifts are a sensitive measure of differences in the electronic environment of nuclei. The δ-values[6] observed for the cyclic compounds are given in Table I, and it is seen that there are relatively large variations with ring size for the hydrogens in both the α and β CH_2 groups.

In each series of cyclic compounds, the δ-values of the 3-membered rings are consistently among

(1) This work was supported by the Office of Naval Research and by a Grant-in-Aid from E. I. du Pont de Nemours and Company.

(2) H. C. Brown and M. Gerstein, THIS JOURNAL, **72**, 2926 (1950).

(3) S. Searles and M. Tamres, *ibid.*, **73**, 3704 (1951).

(4) S. Searles, M. Tamres and E. R. Lippincott, *ibid.*, **75**, 2775 (1953).

(5) M. Tamres, S. Searles and R. F. Vance, Paper 39, presented before the Division of Organic Chemistry, 123rd meeting of the American Chemical Society, Los Angeles, California, March, 1953.

(6) L. H. Meyer, A. Saika and H. S. Gutowsky, THIS JOURNAL, **75**, 4567 (1953); prior publications discussing the method in detail are cited there.