Stereochemistry of the Reductoisomerase and αβ-Dihydroxyacid Dehydratase-catalysed Steps in Valine and Isoleucine Biosynthesis. Observation of a Novel Tertiary Ketol Rearrangement

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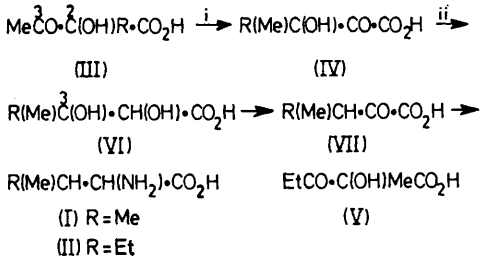
Summary The reductoisomerase of *Salmonella typhimurium* has a requirement for the 2*S*-isomer of acetolactate (III, R = Me) while the αβ-dihydroxy-acid dehydratase has a requirement for the 2*R* configuration but is not stereoselective with respect to the configuration at C-3.

FINAL stages in the biosynthesis of valine (I) and isoleucine (II) include the transformations shown in Scheme 1. The tertiary ketol rearrangement and reduction [steps (i) and (ii)] are mediated by the enzyme reductoisomerase and dehydration by the enzyme αβ-dihydroxyacid dehydratase.[1]

In assays with cell-free preparations from strains of

Salmonella typhimurium, (±)-2-hydroxy-2-methyl-3-oxobutanoic acid (acetolactic acid) (III, R = Me) was found to be active, but the 2*R*-isomer[2] was inactive. It was concluded that the reductoisomerase has a requirement for the 2*S*-isomer of acetolactate.



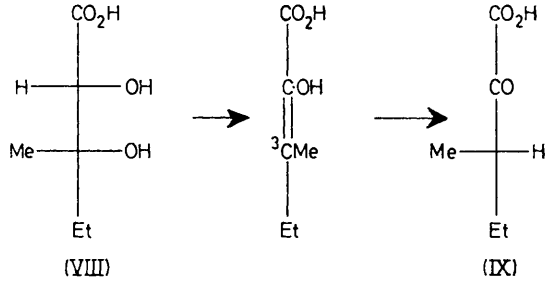
SCHEME 1

It was observed that acetolactate (III, R = Me) underwent racemisation in dilute alkali (c.d.). A probable mechanism of this racemisation *via* reversible tertiary-ketol rearrangement with migration of the methyl group as in the biosynthetic pathway [step (i), Scheme 1] was eliminated by the observation that 3-hydroxy-3-methyl-2-oxobutanoic acid (IV, R = Me) was not converted into acetolactate by dilute alkali.[3] Evidence that the racemisation nevertheless involved tertiary-ketol rearrangement was deduced from the observations that in NaOH–D_2O the acetyl methyl protons of acetolactate (III, R = Me) were rapidly exchanged and that the protons of the quaternary methyl groups were exchanged more slowly and at a rate similar to that of racemisation under comparable conditions. Further, the homologue (III, R = Et), the precursor of isoleucine, (II), underwent rearrangement in dilute alkali into the corresponding isomer (V). It was concluded that rearrangement took place with migration of the carboxylate anion. This was proved by treating [3-^{14}C]acetolactate (III) with dilute alkali. The reaction was quenched (before equilibrium had been attained) by reduction with sodium borohydride. Periodate cleavage of the resulting dihydroxy-acid gave acetaldehyde (70% of the activity of the dihydroxy-acid) and pyruvic acid (30% activity). These results prove that during the rearrangement the carboxylate group migrated from C-2 to become attached to the labelled atom, C-3. An analogous migration of a carboxylate ion in a benzilic acid rearrangement has very recently been demonstrated.[4]

The following isomers of the dihydroxy-acid (VI, R = Et) were prepared: (±)-*erythro* (2*R*,3*R* + 2*S*,3*S*), (±)-*threo*, 2*R*,3*R*[5] and 2*R*,3*S*. In growth studies with isoleucine–valine mutants of *S. typhimurium* only the (±)-*erythro* and 2*R*,3*R*-isomers were found to be significantly active. A corresponding result was obtained with the valine precursor (VI, R = Me): the 2*R*-isomer was fully active whereas the 2*S*-isomer was inactive.

In vitro enzymatic assays with cell-free preparations from *S. typhimurium* revealed that the (±)-*erythro*, (±)-*threo*, 2*R*,3*R*, and 2*R*,3*S*-isomers of the acid (VI, R = Et) were equally active under saturation conditions. The common element in these substrates was the presence of a component with the 2*R* configuration. The 2*R*-isomer[6] of the valine precursor (VI, R = Me) was active but the 2*S*-isomer was inactive. The dehydratase therefore has a rigid requirement for the 2*R* configuration of the substrate but can accommodate either a 3*R* or a 3*S* configuration in the isoleucine precursor (VI, R = Et).

The mechanism of the dehydration probably involves β-elimination of water followed by ketonisation of the intermediate enol.[7] The observation that both the 2*R*,3*R* and 2*R*,3*S*-isomers of the acid (VI, R = Et) were active in the enzyme assay, whereas only the 2*R*,3*R*- isomer was able to support the growth of the isoleucine–valine mutant indicates that both the elimination and ketonisation steps are stereospecific, and that the 2*R*,3*R*-isomer (VIII, Scheme 2)



SCHEME 2

gives the 3*S*-ketoacid (IX) corresponding to L-isoleucine (Scheme 2) whereas the 2*R*,3*S*-isomer gives the 3*R*-ketoacid corresponding to the biologically inactive L-alloisoleucine. This was confirmed by the observation that the 3*S*-isomer (VII) supported growth of the isoleucine-valine mutant whereas the corresponding 3*R*-isomer was inactive.

These results prove that the elimination and protonation steps (Scheme 2) are stereospecific and that the enol–keto conversion takes place with proton addition at C-3 to the same face of the enol as that of the departing hydroxy-group.

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[1] V. W. Rodwell, in 'Metabolic Pathways,' ed., D. M. Greenberg, vol. III, 3rd Edition, Academic Press, New York and London, 1969, p. 351.
[2] D. J. Robins and D. H. G. Crout, *J. Chem. Soc.* (*C*), 1970, 1334.
[3] We thank Professor R. K. Hill for an exchange of information on the racemisation of acetolactate.
[4] H. Rode-Gowal and H. Dahn, *Helv. Chim Acta*, 1973, **56**, 2070.
[5] R. K. Hill and S. Yan, *Bioorg. Chem.*, 1971, **1**, 446; D. H. G. Crout and D. Whitehouse, *J.C.S. Chem. Comm.*, 1972, 398.
[6] B. E. Nielsen, P. K. Larsen, and J. Lemmich, *Acta Chem. Scand.*, 1969, **23**, 967.
[7] S. M. Arfin, *J. Biol. Chem.*, 1969, **244**, 2250.