Stereochemistry of the Conversions *in vivo* of L- and D-Threonine into 2-Oxobutanoic Acid by the L- and D-Threonine Dehydratases of *Serratia marcescens*

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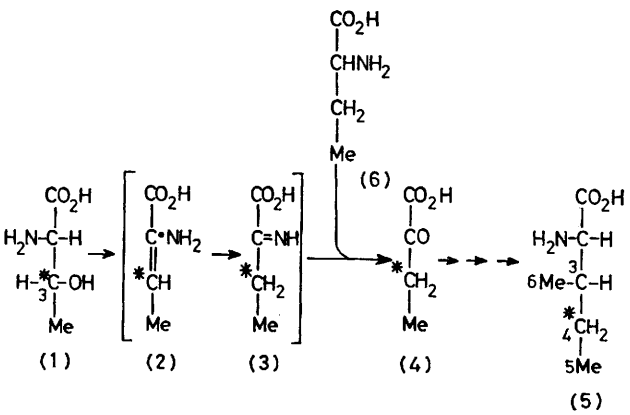
Summary The conversions of L-[3-^{2}H]-(**8**) and D-[3-^{2}H]-threonine (**9**) and of DL-(3*S*)-[3-^{2}H]-2-aminobutanoic acid (**10**) into L-isoleucine (**5**) by mutant strains of *Serratia marcescens* were used to demonstrate that the conversions of L- (**1**) and D-threonine (**7**) into 2-oxobutanoic acid by the respective dehydratases take place with overall retention of configuration.

THE first specific step in the pathway of L-isoleucine (**5**) biosynthesis in micro-organisms and higher plants is the conversion of L-threonine (**1**) into 2-oxobutanoic acid (**4**), a transformation catalysed by the enzyme L-threonine dehydratase (E.C. 4.2.1.16) (Scheme).[1] This transformation has been postulated to proceed *via* 2-aminobut-2-enoic acid (**2**) and 2-iminobutanoic acid (**3**) (Scheme).[2] Since the final transformation must take place in a minimum of two discrete steps (*e.g.* hydration followed by elimination), the conversion of L-threonine into 2-oxobutanoic acid by this



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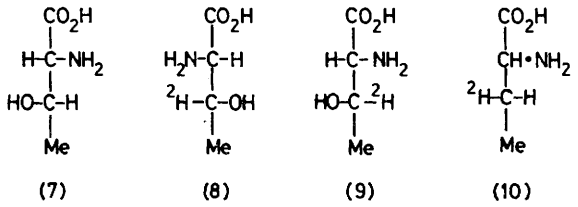
mechanism may involve four or more distinct enzyme-catalysed reactions. In order to obtain information on the degree of control exerted by the enzyme in this overall transformation, we have examined the stereochemistry of the changes taking place at C-3 in L-threonine (**1**) during conversion into L-isoleucine (**5**) by *Serratia marcescens*.

Biosynthetic investigations in whole organisms with precursors labelled with stable isotopes are often made difficult by low concentrations of the investigated metabolite and by dilution with endogenously produced material. By using bacterial mutants with feedback-resistant and de-repressed enzymes, these problems can be circumvented and efficient conversions of precursors into products can be achieved with relatively low dilution by endogenous material.

S. marcescens strain IHr313 possesses a feedback-resistant L-threonine dehydratase and accumulates L-isoleucine (**5**) when incubated with excess of L-threonine (**1**).[3] When this strain was grown in the presence of L-[3-^{2}H]threonine (**8**) {prepared from [1-^{2}H]acetaldehyde and *N*-pyruvylideneglycinatoaquocopper(II) dihydrate,[4] followed by resolution of the *N*-phthaloyl derivative as the brucine salt[5]}, L-[4-^{2}H]isoleucine (as **5**) was produced. In the 220 MHz n.m.r. spectrum of L-isoleucine (**5**), the signals due to the diastereotopic protons at C-4, a prochiral centre derived from C-3 of L-threonine (**1**), appear as two completely separated multiplets [H-4u and H-4d, Figure (a)].



In the spectrum of L-isoleucine (**5**) biosynthesized from L-[3-^{2}H]threonine (**8**) [Figure (b)], the signal due to H-4d was considerably diminished. By comparing multiple integrations of the signals due to H-4u and H-4d with those due to the protons at C-3, C-5, and C-6 [cf. (**5**)], the deuterium label was shown to be located entirely in H-4d (Table).† *S. marcescens* strain 149 lacks L-threonine dehydratase[6] and also converts both stereoisomers of 2-aminobutanoic acid (**6**) into L-isoleucine (**5**) *via* 2-oxobutanoate (Scheme).[7] When DL-(3*S*)-[3-^{2}H]-2-aminobutanoic acid (**10**) {prepared from (*R*)-[1-^{2}H]ethanol[8] *via* the tosylate and sodiodiethylacetamidomalonate, followed by hydrolysis} was incubated with this strain, the deuterium in the resulting L-isoleucine (**5**) was located entirely in H-4d [Figure (c), Table].‡ Since both L-[3-^{2}H]threonine (**8**) and DL-(3*S*)-[3-^{2}H]-2-aminobutanoic acid (**10**) gave L-isoleucine (**5**) labelled in the same diastereotopic hydrogen at C-4, it was concluded that the

TABLE. Per cent deuterium content of H-4u and H-4d in L-isoleucine (**5**) derived from deuteriated precursors, as determined by 220 MHz n.m.r. spectroscopy.

Precursor	H-4d	H-4u
L-[3-^{2}H]Threonine	57 ± 2	3 ± 4
DL-(3*S*)-[3-^{2}H]-2-Aminobutanoic acid	71 ± 3	5 ± 5
D-[3-^{2}H]Threonine	3 ± 8	76 ± 4

conversion of L-threonine (**1**) into 2-oxobutanoic acid (**4**) (Scheme) took place with overall retention of configuration.

S. marcescens has an inducible D-threonine dehydratase.[9] Strain 149 accumulates L-isoleucine when grown in the presence of D- or DL-threonine.[10] When this strain was grown in the presence of DL-[3-^{2}H]threonine [(**8**) and (**9**)], the deuterium label in the resulting L-isoleucine (**5**) was found to be located exclusively in H-4u [Figure (d), Table], demonstrating that, as with the L-threonine dehydratase, the D-threonine dehydratase of *S. marcescens* converts D-threonine (**7**) into 2-oxobutanoic acid (**4**) with overall retention of configuration at C-3.

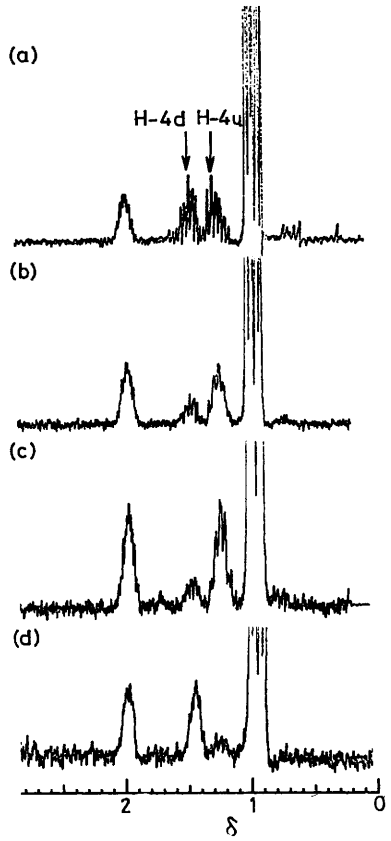


FIGURE. Partial 220 MHz n.m.r. spectra of L-isoleucine (**5**) and biosynthetically-produced samples of L-isolecuine (**5**) partially deuteriated at C-4.

† In this and subsequent experiments, the labelled L-isoleucine (**5**) was accompanied by endogenous, unlabelled material. The deuterium contents of all samples of L-isoleucine (**5**) as determined by n.m.r. spectroscopy were in agreement with values estimated by mass spectrometry.

‡ Preliminary experiments with [3-^{2}H]-2-aminobutanoic acid were carried out to check that non-stereospecific exchange of the C-3 hydrogen did not occur under the incubation conditions. Thus DL-(3*S*)-[3-^{3}H]-2-aminobutanoic acid (**6**) (^{3}H/^{14}C 2·72) was converted into L-isoleucine (**5**) (^{3}H/^{14}C 2·64). 2-Aminobutanoic acid reisolated from the growth medium had ^{3}H/^{14}C = 2·63. Unless a large tritium isotope effect was operating these results demonstrated the absence of non-stereospecific exchange at C-3 in 2-aminobutanoic acid (**6**) during conversion into L-isoleucine (**5**).

Although the threonine dehydratases of *S. marcescens* have not been rigorously studied, it is probable that, like other bacterial threonine dehydratases, they are pyridoxal-dependent.[11] The results reported here are therefore consistent with the general pattern of retention of configuration in pyridoxal catalysed elimination–addition reactions.[12]

The results described above for the transformation of D-threonine (**7**) into 2-oxobutanoic acid (**4**) reveal the same stereochemical course as that recently reported for the conversions of D-serine[13] and D-threonine[14] into pyruvic acid and 2-oxobutanoic acid (**4**) respectively by the D-serine dehydratase of *Escherichia coli*. They imply, if the proposed mechanism (Scheme) is correct and applicable to both L- and D-threonine dehydratases, that protonation at C-3 of the intermediate (**2**) derived from L-threonine (**1**) and D-threonine (**7**) is stereospecific and takes place on the *re* and *si* faces respectively.

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