Biosynthesis of L-Valine in *Salmonella typhimurium*: Origin of the Diastereotopic Methyl Groups

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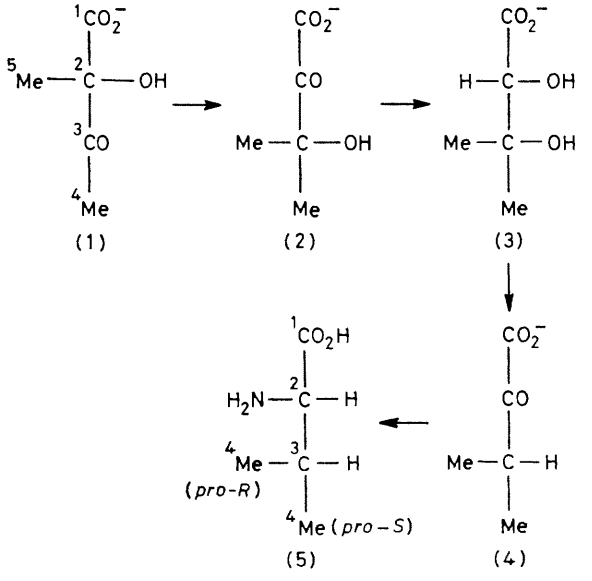
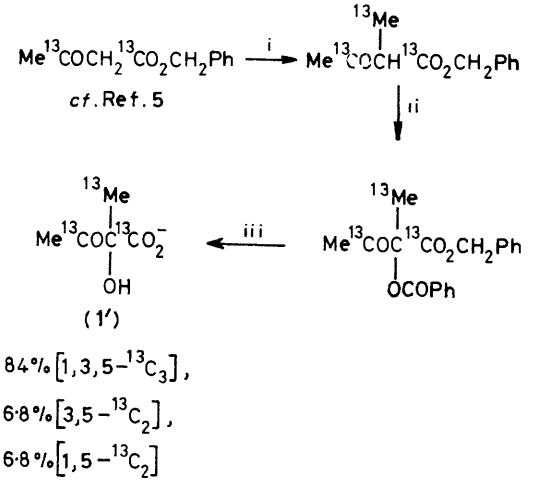
Summary When [1,3,5-$^{13}C_3$]-α-acetolactate {[1,3,5-$^{13}C_3$]-2-hydroxy-2-methyl-3-oxobutanoate} (**1′**) was incubated with a cell-free system from *Salmonella typhimurium*, the valine (**5′**) produced was labelled in the C-4 *pro-S* position, proving that during the tertiary ketol rearrangement catalysed by the enzyme reductoisomerase, the methyl group transfer is to the *re* face of the trigonal centre at C-3 of α-acetolactate (**1′**).

DURING valine biosynthesis, the enzyme reductoisomerase (ketol-acid reductoisomerase, EC 1.1.1.86) catalyses the conversion of (*S*)-α-acetolactate [(*S*)-2-hydroxy-2-methyl-3-oxobutanoate] (**1**)[1] into (*R*)-2,3-dihydroxy-3-methylbutanoate (**3**)[2] *via* 3-hydroxy-3-methyl-2-oxobutanoate (**2**). The dihydroxy acid (**3**) is converted into 2-oxo-3-methylbutanoate (**4**) by the enzyme αβ-dihydroxyacid dehydratase and the synthesis is completed by conversion of the keto acid (**4**) into L-valine (**5**) by the enzyme transaminase B (Scheme 1).[3]



SCHEME 1

In order to define further the stereochemistry of the reductoisomerase reaction we have investigated the origins of the C-4 methyl groups of the keto acid (**2**). Thus [1,3,5-$^{13}C_3$]-α-acetolactate (**1′**) was prepared as shown in Scheme 2 and incubated with a cell-free system from

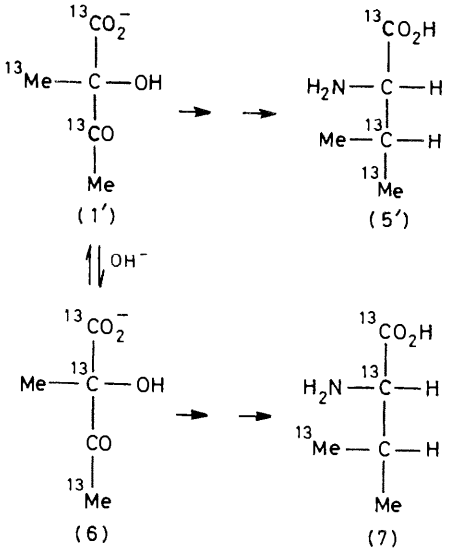


SCHEME 2. Reagents i, Na, ^{13}MeI; ii, Na, $(PhCO_2)_2$; iii, NaOH–H_2O–MeOH

S. typhimurium strain ilvA112 (deficient in threonine dehydratase). A ^{13}C label was introduced at the migration terminus (C-3) of the α-acetolactate (**1′**) (Scheme 3) so that the ^{13}C n.m.r. signal due to the ^{13}C-labelled migrating group in the product (**5′**) would be clearly revealed by ^{13}C–^{13}C coupling.

TABLE. ^{13}C N.m.r. spectra of DL-valine, and L-valine derived from [1,3,5-$^{13}C_3$]-α-acetolactate (**1′**) and from {(**1′**) + [1,2,4-$^{13}C_3$]-α-acetolactate (**6**)}.

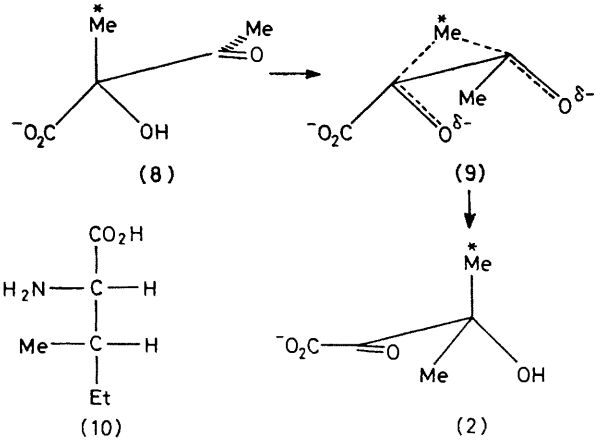
	DL-valine			[$^{13}C_3$]-L-valine (**5′**) from (**1′**)			[$^{13}C_3$]-L-valine [(**5′**)+(**7**)] from [(**1′**) + (**6**)]		
	Chem shift (p.p.m.)	Mult	Rel. peak height	Chem. shift (p.p.m.)	Mult. (*J*/Hz)	Rel. peak height	Chem shift (p.p.m.)	Mult. (*J*/Hz)	Rel. peak height
C-1	175·1	S	34	175·1	S	46	175·1	D (54·3)	22
							175·1	S	45
C-2	61·2	S	97	61·25	S	13	61·2	D (53·1)	100
							61·25	S	36
C-3	29·9	S	100	30·0	D (34·8)	100	30·0	D (34·2)	93
				29·9	S	15	29·9	S	31
C-4(*pro*-R)	18·8	S	66	18·8	S	11	18·8	S	91
C-4(*pro*-S)	17·5	S	72	17·4	D (34·5)	79	17·4	D (35·4)	77
				17·5	S	16	17·5	S	30



SCHEME 3

In the ^{13}C n.m.r. spectrum of the L-valine (**5'**) (Scheme 3) produced (Table), the natural abundance upfield signal due to the *pro-S* methyl carbon at C-4 (δ 17·5 p.p.m.)[4] was flanked by an intense doublet due to coupling of the labelled methyl carbon with the ^{13}C- labelled C-3, whereas the downfield signal due to the C-4 *pro-R* methyl carbon appeared as a natural abundance singlet. The corresponding coupling was seen in the signal (δ 30·0 p.p.m.) due to ^{13}C-3. Other signals (Table) were as expected for (2*S*,4*S*)-[1,3,4-$^{13}C_3$]valine (**5'**).

A complementary result was obtained by incubating [1,2,4-$^{13}C_3$]-α-acetolactate (**6**) with the cell-free system. The required substrate was obtained as an equilibrium mixture with [1,3,5-$^{13}C_3$]-α-acetolactate (**1'**) by base-catalysed tertiary ketol rearrangement of the latter, which proceeds with migration of the carboxylate ion[5] (Scheme 3). When this precursor [(**1'**) + (**6**)] was incubated in the cell-free system, the ^{13}C n.m.r. spectrum of the L-valine produced showed, in addition to signals attributable to (2*S*,4*S*)-[1,3,4-$^{13}C_3$]valine (**5'**), signals attributable to (2*S*,4*R*)-[1,2,4-$^{13}C_3$]valine (**7**). The signal due to the C-4 *pro-R* methyl carbon (δ 18·8 p.p.m.) appeared as an intense singlet, and the signals due to ^{13}C-1 and ^{13}C-2 appeared as doublets (Table). Since the conversion of the dihydroxy-acid (**3**) into valine (**5**) (Scheme 1) has been shown to take place with overall retention of configuration at C-3,[6] the present results prove that during the rearrangement step of the reductoisomerase reaction, the migrating methyl group is delivered to the *re* face of the trigonal centre at C-3. The conformation of α-acetolactate (**8**) at the active site of the enzyme must therefore be as shown in Scheme 4, and the rearrangement must proceed *via* a transition state (**9**) in which the oxygen substituents at C-2 and C-3 have a *syn* relationship.



SCHEME 4

The later stages in the biosynthesis of L-isoleucine (**10**) are catalysed by the same enzymes as those operating in the valine pathway.[7] Thus the methyl group migrating during valine biosynthesis occupies the same stereochemical position in the product (**5**) as the migrating ethyl group in isoleucine (**10**) biosynthesis.

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[1] F. B. Armstrong, C. J. R. Hedgecock, J. B. Reary, D. Whitehouse, and D. H. G. Crout, *J. Chem. Soc. Chem Commun*, 1974, 351.
[2] F. B. Armstrong, U. S. Muller, J. B. Reary, D. Whitehouse, and D. H. G Crout, *Biochim Biophys. Acta*, 1977, **498**, 282.
[3] V. W. Rodwell, 'Metabolic Pathways,' vol. III, ed. D. M. Greenberg, Academic Press, New York and London, 1969, p. 354.
[4] H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Am. Chem. Soc.*, 1973, **95**, 6149.
[5] D. H. G. Crout and C. J. R. Hedgecock, *J. Chem. Soc., Perkin Trans.* 1, 1979, 1982.
[6] R. K. Hill, S. Yan, and S. M. Arfin, *J. Am. Chem. Soc.*, 1973, **95**, 7857.
[7] Ref. 3, p. 351.