Stereochemistry of the Decarboxylation Reaction catalysed by 3-Isopropylmalate Dehydrogenase from the Thermophilic Bacterium *Thermus thermophilus*

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The decarboxylation reaction catalysed by *threo*-D$_S$-3-isopropylmalate dehydrogenase from the thermophile *Thermus thermophilus* HB-8 has been firmly established to proceed with retention of configuration at the C-3 position of (2*R*,3*S*)-isopropylmalic acid.

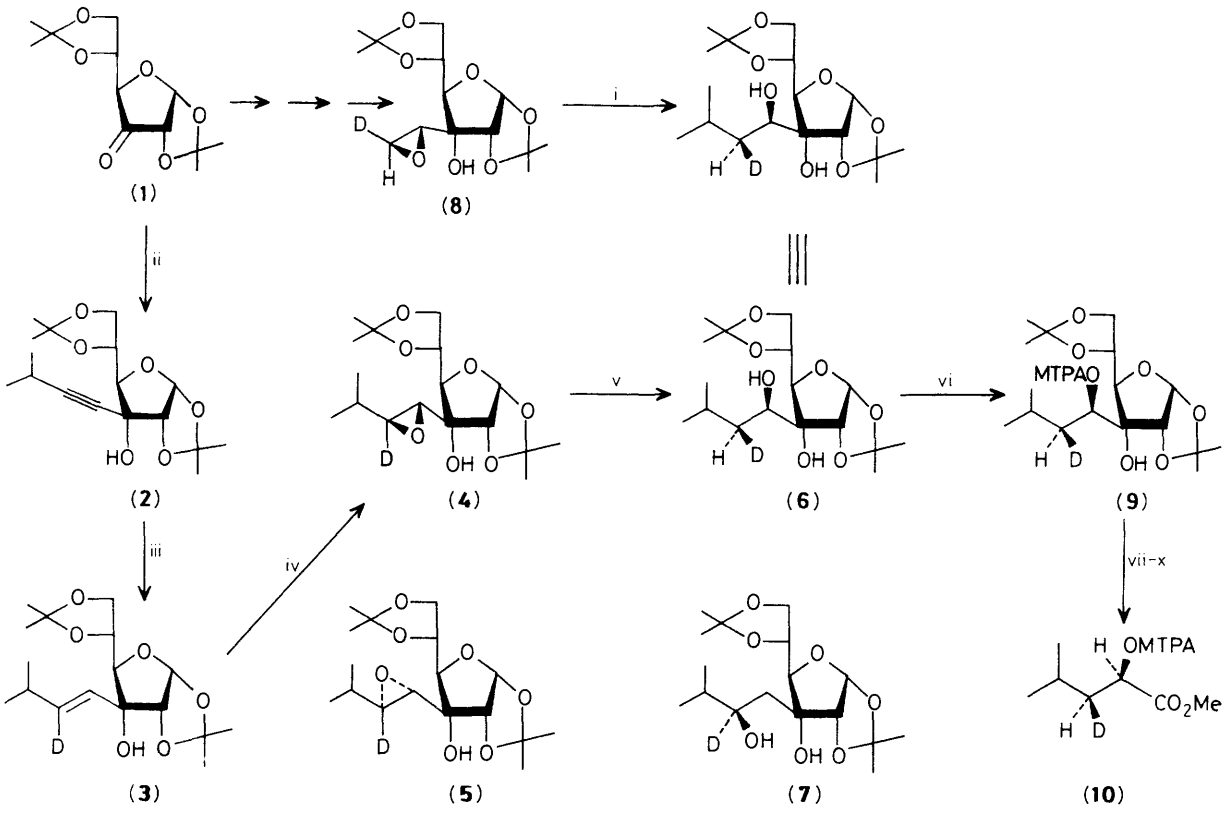
threo-D$_S$-3-Isopropylmalate dehydrogenase (IPMDH, E.C.1.1.1.85), which is involved in the biosynthesis of L-leucine,[1] has recently attracted much attention.[2] We have been studying IPMDH from the thermophilic bacterium *Thermus thermophilus* HB-8 with regard to its thermostable nature and evolution.[3] The hydride transfer reaction from the C-2 position of the substrate (2*R*,3*S*)-3-isopropylmalate (IPM) to the nicotinamide cofactor (NAD) has recently been elucidated to be A-specific.[4] Based on deuterium labelling experiments and ^{1}H n.m.r. analysis, we now report for the first time that the stereochemically cryptic decarboxylation reaction catalysed by IPMDH proceeds with retention of configuration at the C-3 position of IPM.

The deuterated substrate, (2*RS*, 3*SR*)-[3-^{2}H]-3-isopropylmalic acid, was prepared using a slight modification of the method previously described by (i) treating ethyl 2-(3,4-dimethoxyphenyl)-hydroxymethyl-3-methylbutanoate with lithium di-isopropylamide (LDA) (2 equiv.) in tetrahydrofuran (THF) to form a dianion, followed by quenching with D_2O, (ii) chromatographic separation of the resulting mixture of *threo*- and *erythro*-products, and (iii) further conversion [m.p. 116 °C; ^{1}H n.m.r. (200 MHz, D_2O): δ 0.85 (6H, d, *J* 6.8 Hz), 1.92 (1H, septet, *J* 6.8 Hz), and 4.70 (1H, s)].[5]

Incubation of the deuteriated substrate with the purified enzyme was carried out as described,[4] but the reaction medium was a 0.2 M phosphate buffer (pH 7.6), which is the optimal condition, rather than the specific 0.2 M carbonate buffer (pH 10.2),[4] thereby avoiding enolization and hydrogen exchange of the product, 2-oxoisocaproic acid. The enzyme reaction was terminated by direct addition of $NaBH_4$ to afford deuterated racemic 2-hydroxyisocaproic acid. This simultaneously introduced a new chiral centre, which facilitated the ^{1}H n.m.r. assignment of the methylene hydrogens at C-3. The ethereal extract was treated with CH_2N_2 and then converted into the corresponding (+) methoxy(trifluoromethyl)phenylacetyl (MTPA) esters, which were separated by preparative t.l.c. The more polar product was chromatographically identified with an authentic methyl (2*S*)-2-hydroxyisocaproate (+)-MTPA ester [δ_H 5.20 (d, *J* 3.7 Hz, H-2) and 1.68 (dd, *J* 3.8 and 9 Hz, H-3)], the less polar product being assigned to a derivative of (2*R*)-2-hydroxyisocaproic acid [δ_H 5.18 (d, *J* 10.4 Hz, H-2) and 1.82 (dd, *J* 4.5 and 10.4 Hz, H-3)]. Unconverted dimethyl (2*S*, 3*R*)-3-isopropylmalate (+)-MTPA ester was recovered in a slow moving fraction, and was distinguishable from dimethyl (2*R*, 3*S*)-3-isopropylmalate (+)-MTPA ester.

A stereochemical standard, (2*R*, 3*S*)-[3-^{2}H$_1$]-2-hydroxyisocaproate (+)-MTPA ester (**10**), has been synthesized chemically using our chirality transfer approach (Scheme 1).

1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (**1**) was reacted with 3-methylbutyn-1-ylmagnesium bromide



Scheme 1. *Reagents*: i, $Me_2CHMgBr$, CuBr, THF; ii, $Me_2CHC{\equiv}CMgBr$, THF; iii, $LiAlD_4$, THF; iv, *m*CPBA, CH_2Cl_2; v, $LiAlH_4$, THF; vi, (+)-MTPA-Cl, pyridine; vii, 1 M HCl–THF (1 : 2); viii, $NaBH_4$, MeOH; ix, $NaIO_4$; x, CH_2N_2.

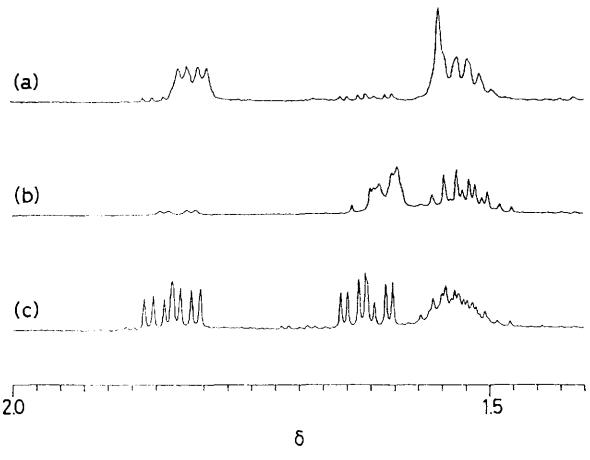
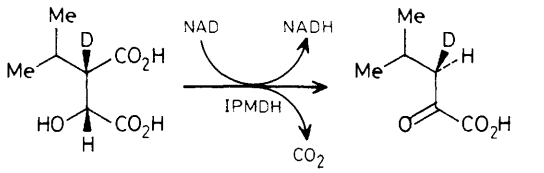


Figure 1. 500 MHz ^1H N.m.r. spectra ($CDCl_3$, Me_4Si) of methyl (2*R*)-2-hydroxyisocaproate (+)-MTPA esters. (a) The less polar product derived from the IPMDH reaction; (b) synthetic (2*R*,3*S*)-standard (**10**); (c) non-labelled standard. A signal at δ 1.55 in (a) is due to water contamination of the solvent.



Scheme 2

to afford 1,2 : 5,6-di-*O*-isopropylidene-3-*C*-(3-methylbutyn-1-yl)-α-D-allofuranose (**2**) with high stereoselectivity (m.p. 63—64 °C). $LiAlD_4$ reduction of (**2**) in THF afforded, with simultaneous regioselective introduction of deuterium, an (*E*)-alkene (**3**) [m.p. 90 °C; δ_H 5.34 (1H, br. s); δ_C 123.2 (s) and 139.3 (br.)]. The (*E*)-stereochemistry was confirmed by preparing the non-deuteriated analogue [δ_H 5.34 (d, *J* 16 Hz) and 5.91 (dd, *J* 16 and 6.3 Hz)]. Epoxidation of (**3**) with *m*-chloroperbenzoic acid (*m*CPBA) in CH_2Cl_2 gave (**4**) (54% yield; m.p. 116—117 °C) and (**5**) in a ratio of 13 : 2. The major epoxide (**4**) was reduced with $LiAlH_4$ in THF at room temperature to give, after chromatographic separation, a diol (**6**) having hydroxy groups on C-3 and C-1′ (46% yield; m.p. 133—134 °C) and a regioisomeric diol (**7**) (50% yield; m.p. 116—117 °C) having hydroxy groups at C-3 and C-2′. The absolute stereochemistry of (**6**) was determined by comparison of the ^1H n.m.r. spectrum with that of an authentic specimen synthesized by nucleophilic displacement of the stereochemically established epoxide (**8**) with isopropylmagnesium bromide in the presence of CuBr.[6,7]

Treatment of (**6**) with (+)-MTPA chloride in pyridine afforded a mono-MTPA ester (**9**) in 96% yield, which in turn was hydrolysed in 1 M HCl–THF (1 : 2) (70 °C, 60 h) to give a mixture of hemiacetals (70% yield). Further manipulation, *i.e.* $NaBH_4$ reduction, $NaIO_4$ oxidation, and the final esterification with CH_2N_2 were carried out without purification of the intermediates to give, after purification by preparative t.l.c., the desired methyl (2*R*,3*S*)-[3-$^2H_1$]-2-hydroxyisocaproate (+)-MTPA ester (**10**) [27% overall yield from (**9**)].

The ^1H n.m.r. spectra (500 MHz) of a non-labelled authentic (2*R*)-specimen, the stereochemical standard (**10**), and the less polar product obtained from the enzyme reaction

and derivatization *vide supra* were compared and a relevant region of the spectra is illustrated in Figure 1. The spectrum of the synthetic standard (**10**) clearly displays a broad multiplet at δ 1.54 due to the C-4 methine and a broad doublet at δ 1.63 due to the *pro-R* proton on C-3. No signal was observed at δ 1.83. In contrast, the spectrum of the less polar deuteriated (2*R*)-2-hydroxy-isocaproic acid derivative obtained from the enzyme reaction showed a doublet of doublets at δ 1.82 (*J* 4.5 and 10.4 Hz) due to the *pro-S* proton on C-3, but essentially no signal at δ 1.64. Since the stereochemistry of the C-3 position of 2-oxo-isocaproic acid was not altered at all during the operations, the configuration was determined unequivocally as *R*.

Decarboxylation catalysed by IPMDH from *T. thermophilus* HB-8 appears to take place with retention of configuration as depicted in Scheme 2.

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