Stereochemistry of 2-Oxopantoate Formation by Oxopantoate Hydroxymethyltransferase

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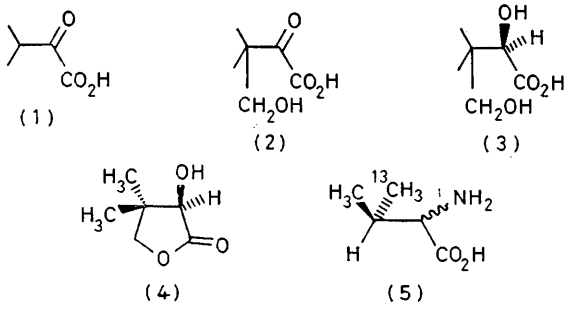
Summary The biosynthesis of 2-oxopantoate from 2-oxoisovalerate proceeds with retention of configuration at C-3 of 2-oxoisovalerate, in contrast to earlier conclusions.

THE initial stages of the biosynthesis of coenzyme A involve the reaction of 2-oxoisovalerate (**1**) with N^5N^{10}-methylenetetrahydrofolate to give 2-oxopantoate (3-hydroxymethyl-3-methyl-2-oxobutanoic acid) (**2**), catalysed by the enzyme oxopantoate hydroxymethyltransferase;[1] the product of this reaction is subsequently reduced to (*R*)-pantoate (**3**).

Evidence has recently been presented[2] that the biosynthesis of 2-oxopantoate (**2**) proceeds with inversion of configuration at C-3 of 2-oxoisovalerate. This conclusion was based on the assignment of the downfield methyl signal in the ^{13}C n.m.r. spectrum of pantolactone (**4**) to the methyl group *cis* to the hydroxy group; when [4-^{13}C]-(2*RS*,3*S*)-valine (**5**) was administered to cells of an appropriate auxotroph of *E. coli*, the downfield methyl signal of pantolactone (**4**) subsequently isolated, showed a considerable enhancement in intensity. We have also been interested in the stereochemistry of this conversion, and had provisionally assigned the upfield methyl signal as due to the *cis*-methyl group. This assignment is strongly supported by previous evidence that steric crowding, particularly by vicinal oxygen substituents, leads to an upfield shift in ^{13}C n.m.r. spectra.[3] This assignment has now been confirmed by a lanthanide shift reagent study.



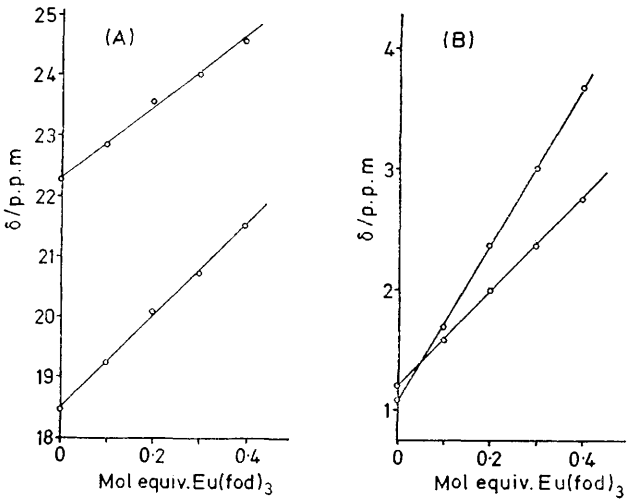


FIGURE. Effect of addition of $Eu(fod)_3$ on (A) ^{13}C n.m.r. spectrum (20 MHz) and (B) ^1H n.m.r. spectrum (100 MHz) of pantolactone (**4**). All spectra were recorded on 0·5 M solutions of pantolactone in $CDCl_3$.

The effect of increasing additions of $Eu(fod)_3$ on the positions of the two methyl signals in the proton noise decoupled ^{13}C n.m.r. spectrum of pantolactone (**4**) is shown in the Figure (A). If complexation occurs predominantly at the hydroxy group,† the greater shift of the upfield signal [δ 18·43 p.p.m., in absence of $Eu(fod)_3$] supports its assignment to the *cis*-methyl group. This was confirmed by correlation with ^1H n.m.r. data. The effects of $Eu(fod)_3$ on the methyl signals in the ^1H n.m.r. spectrum are shown in the Figure (B). The resonance at δ 1·06 can thus be assigned to the *cis*-methyl group, and that at δ 1·21 to the *trans*-substituent. When the ^{13}C n.m.r. spectrum in the presence of 0·4 mol equiv. of $Eu(fod)_3$ was recorded with specific proton decoupling at δ 2·7 (*i.e.*, the *trans*-methyl group), the downfield methyl group (δ 24·54) appeared as a singlet, whilst the upfield signal (δ 21·52) retained residual coupling appearing as a quartet. Equally, specific proton irradiation at δ 3·7 (the *cis*-methyl group) caused the upfield signal to appear as a singlet, with the downfield one as a quartet.

The evidence above, together with the biological studies reported earlier, using stereospecifically labelled valine,[2] leads to the conclusion that oxopantoate hydroxymethyltransferase forms 2-oxopantoate from 2-oxoisovalerate with retention of configuration at C-3. Oxopantoate hydroxymethyltransferase is thought to be a Class 2 aldolase[1] and its stereochemistry of action is therefore the same as all other aldolases which have been studied; the possible mechanistic significance of this retention mode has been discussed.[4] The stereochemistry is also the same as the serine hydroxymethyltransferase reaction;[5] this enzyme catalyses the reaction between glycine and N^5N^{10}-methylenetetrahydrofolate to form serine, but involves pyridoxal phosphate as cofactor, so the stereochemical analogy with oxopantoate formation may be less significant. It becomes of interest, however, to compare these two processes with respect to the stereochemistry of formation of the primary alcohol function.[6]

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† The resonance of the secondary alcohol position shows the greatest downfield shift with $Eu(fod)_3$ in both ^{13}C and ^1H n.m.r. spectra.

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