Stereochemistry of Isopentenyl Pyrophosphate Isomerase

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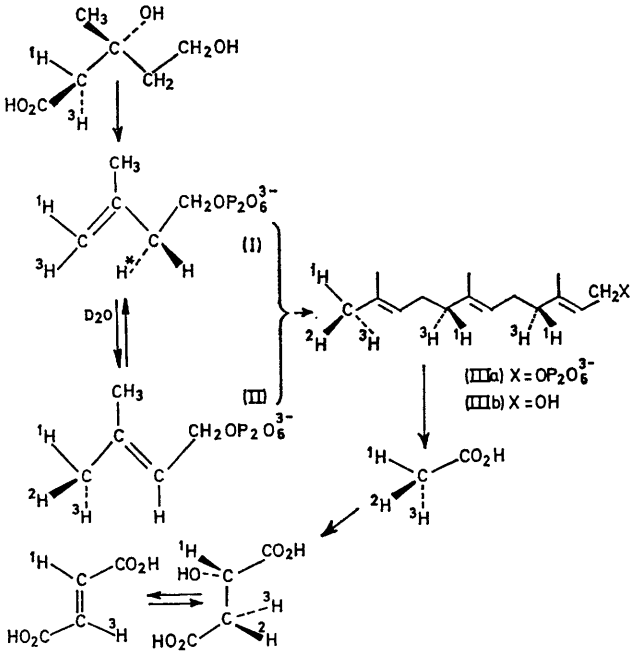
Summary On the enzyme isopentenyl pyrophosphate isomerase, a proton is added to the *re* side of the double bond of 3-methyl-3-butenyl pyrophosphate.

THE enzyme isopentenyl pyrophosphate isomerase (E.C.5.3.3.2) mediates a reversible transformation of 3-methyl-3-butenyl pyrophosphate (I) into 3-methyl-2-butenyl pyrophosphate (II) and thus produces the initiating species (II) for polyisoprenoid biosynthesis. Partially purified enzyme has been obtained from yeast[1] and from pig liver.[2,3] It has been shown[4] that the isomerization (I → II) removes the 2-*pro*-*R*-hydrogen[5] (H*) of (I) but the stereochemistry of the accompanying addition of a proton from the medium to C-4 of (I) has not been determined until now.

A preparation[6] of soluble enzymes from pig liver, dissolved in deuterium oxide, was incubated with 2*R*,3*R*-[2-$^3H_1$]-mevalonate and with 2*S*,3*R*-[2-$^3H_1$]mevalonate[7] (introduced as racemates). Farnesyl pyrophosphate (IIIa) was formed and it was cleaved by alkaline phosphatase to farnesol (IIIb). The terminal C_5 unit of farnesol prepared from unlabelled mevalonate in the same conditions was found by mass spectrometry to be mainly the expected monodeuteriated species but was accompanied by significant amounts of di- and tri-deuteriated species evidently generated by multiple isomerizations. Similarly some deuterium was found elsewhere in the farnesol molecule and could be attributed to 3-methyl-3-butenyl pyrophosphate deuteriated at C-4 by the same process.

Labelled farnesol, after dilution with carrier, was ozonized in ethyl chloride at −70° and aqueous potassium iodide–iodine, followed by sodium carbonate, were added before warming to room temperature. In this way acetone from the terminal isopropylidene group was converted into iodoform and acetate ion without an opportunity for exchange of methyl hydrogens with the medium (this was checked by control experiments). Acetic acid was isolated by steam distillation, purified by partition chromatography on silica, and examined for chirality by a method already described in detail:[8] it was converted enzymatically, *via* acetylcoenzyme A and condensation with glyoxylate on malate synthase, into 2*S* malate; this was incubated with fumarate hydratase and the percentage of tritium remaining bound to carbon was measured by scintillation counting. When treated in this way, [$^2H_1$,$^3H_1$]acetate of *R* chirality affords a malate retaining most of its tritium on equilibration with fumarate hydratase, whereas a malate from *S* acetate loses most of its tritium.



SCHEME

The sequence 2*R*-[2-$^3H_1$]mevalonate → farnesol → acetate → malate yielded a malate that retained 63·4% of its

tritium with fumarate hydratase. Symmetrically, 2*S*-[2-$^3H_1$]mevalonate gave malate that lost 63·5% of its tritium. It follows that from 2*R*-[2-$^3H_1$]mevalonate *R* acetate had been generated and from 2*S*-[2-$^3H_1$]mevalonate *S* acetate. But 2*R*-[2-$^3H_1$]mevalonate is known[7] to generate, with the soluble enzyme system from liver, *Z*-[4-$^3H_1$]-3-methyl-3-butenyl pyrophosphate (I), whereas 2*S*-[2-$^3H_1$]-mevalonate generates the *E* isomer. Thus for an *R* methyl group to be generated in deuterium oxide, addition of the deuteron must have been to the *re* face[5] of the double bond. The result with 2*S*-[2-$^3H_1$]mevalonate confirms this conclusion; the preferred stereochemical sequence from 2*R* mevalonate is shown in the Scheme. Thus the prototropic change mediated by the isomerase, superficially similar to the process of association of C_5 units when polyisoprenoids are formed, is stereochemically different. The stereochemical relation between the incoming and outgoing hydrogen atoms suggests a concerted addition–abstraction rather than the consecutive 1,2-addition and 1,2-elimination advanced[7] as consistent with the stereochemistry of polyisoprenoid chain extension. Experiments regarded[2] as inconsistent with a concerted mechanism will be examined in detail elsewhere.

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