The Formation of Trichodiene from All-*trans*-farnesyl Pyrophosphate by *Trichothecium roseum*

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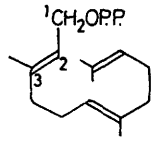
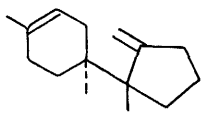
Summary The isomerization and cyclization of all-*trans*-farnesyl pyrophosphate (**1**) to trichodiene (**2**) by a cell-free system obtained from *Trichothecium roseum*, takes place with the loss of a pro-1(*S*) hydrogen atom of farnesyl pyrophosphate and its ultimate replacement with a pro-4(*S*)-hydrogen atom of NADPH.

TRICHODIENE (**2**) is the parent hydrocarbon of the sesquiterpenoid trichothecane group of fungal metabolites.[1,2] We have shown that it is formed from all-*trans*-farnesyl pyrophosphate (**1**).[2] During the formation of the six-membered ring of trichodiene by *Trichothecium roseum*, the 2,3-double bond of all-*trans*-farnesyl pyrophosphate must isomerize to the *cis*-configuration. Whilst the hydrogen atom attached to the double bond was retained in the isomerization, a hydrogen atom was lost from C(1) of all-*trans*-farnesyl pyrophosphate. We now report on the stereochemistry of this loss and on the origin of the added hydrogen atom.



(1)



(2)

[5(*R*)-5-^{3}H,2-^{14}C]-Mevalonic acid was prepared using mevaldate reductase[3] and converted with a pig-liver enzyme preparation to all-*trans*-[1,5,9-^{3}H,4,8,12-^{14}C]-farnesyl pyrophosphate (^{3}H : ^{14}C = 10·5 : 1). This was shown to be stereochemically homogeneous at C(1) by hydrolysis to farnesol (^{3}H : ^{14}C = 10·4 : 1) with alkaline phosphatase. Stereospecific oxidation of the alcohol with liver alcohol dehydrogenase led to the loss of one label (farnesal ^{3}H : ^{14}C = 7·13 : 1). Incubation of the farnesyl pyrophosphate with a cell-free preparation[2] from *Trichothecium roseum* gave trichodiene (**2**) (^{3}H : ^{14}C = 10·5 : 1). Thus a pro-5(*R*)-mevalonoid hydrogen atom is retained and thus it is a pro-5(*S*) hydrogen atom [≡ 1(*S*) hydrogen atom of farnesyl pyrophosphate] that is lost on the formation of the trichodiene.

A reduced pyridine nucleotide is an essential co-factor for trichodiene formation. [4(*S*)-4-^{3}H]-NADPH (56·44 μCi μmol^{-1}) was prepared from [1-^{3}H]-glucose by coupling hexokinase to glucose-6-phosphate dehydrogenase in the presence of NADP$^+$, ATP and magnesium chloride. Incubation of equimolar amounts of the labelled NADPH with all-*trans*-[4,8,12-^{14}C]-farnesyl pyrophosphate (38·7 μCi μmol^{-1}) gave trichodiene (^{3}H 434,028 d.p.m., ^{14}C 473,088 d.p.m.). The ^{3}H : ^{14}C ratio corresponds to the transfer of 63% of the label from the [^{3}H]-NADPH, some dilution of the labelled co-factor by endogenous pyridine nucleotide having presumably occurred. Thus the origin of the added hydrogen is from the 'B' face of NADPH. This is in contrast to the common redox pattern, for example with liver alcohol dehydrogenase, but it is the same as in squalene biosynthesis.[4]

Two recent reports are relevant to these results. Using a system derived from *Andrographis paniculata*, Overton[5] has shown that the pro-1(*S*) hydrogen atom is lost in the conversion of the free alcohol, all-*trans*-farnesol to 2-*cis*-farnesol. Farnesol is not a substrate for the *Trichothecium roseum* system. If the two isomerizations were to proceed by a similar mechanism, then there must be retention of configuration at C(1) during the displacement of the pyrophosphate. It is however possible that two different mechanisms are involved. On the other hand using intact cultures of *Helminthosporium sativum*, Imai and Marumo showed[6] that the pro-1(*R*) hydrogen atom of farnesol was abstracted in this isomerization. However, since these workers were using an intact culture, their results might not distinguish between a non-specific alcohol dehydrogenase (*cf.* the stereochemistry of liver alcohol dehydrogenase) and a system forming an integral part of an isomerization sequence.

(*Received*, 15*th January* 1975; *Com.* 046.)

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