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Unusual Chain Elongation at the Reversed Face in the Biosynthesis of (E,E)-Farnesol with the Enzyme System of Pisum sativum[1)]

Takayuki SUGA,* Rong-yu ZHENG, Shinji OHTA, Takamitsu YOSHIOKA, and Toshifumi HIRATA

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730

When (E,E)-farnesol was enzymatically synthesized from an equimolar mixture of 3,3-dimethylallyl pyrophosphate and (E)- or (Z)-[4-^{2}H]isopentenyl pyrophosphate (IPP) in the presence of iodoacetamide with a farnesyl pyrophosphate synthetase fraction prepared from Pisum sativum, the unusual addition of allylic residue to the re-re face of the carbon-carbon double bond of IPP was found to occur. This finding was the first example of the unusual chain elongation against Cornforth's stereochemical picture for the biosynthesis of isoprenoids.

During the elongation of (Z)-prenyl chain in the biosynthesis of polyprenols in higher plants,[2—4)] we have recently found the hydrogen elimination against Cornforth's basic picture[5—8)] for the stereochemistry of prenylation in the biosynthesis of isoprenoids. This finding prompted us to investigate stereochemical features of the prenyl chain elongation in the biosynthesis of isoprenoids in higher plants. In the course of the investigations, we found occurrence of the unusual addition of allylic residue to the re-re face of the C-C double bond of isopentenyl pyrophosphate (IPP) during the enzymatic formation of (E,E)-farnesol from an equimolar mixture of 3,3-dimethylallyl pyrophosphate (DMAPP; a primer in biosynthesis of isoprenoids) and IPP in the presence of iodoacetamide (IAA; an inhibitor of IPP isomerase) with the farnesyl pyrophosphate (FPP) synthetase fraction of Pisum sativum. In cases of uptake of an equimolar mixture of DMAPP and IPP and uptake of only IPP as substrates in the absence of IAA, on the other hand, the chain elongation occurred at the si-si face of the C-C double bond of IPP following Cornforth's basic picture. We here wish to communicate the new findings.

Incubation of DMAPP and regiospecifically deuteriated (E)-[4-^{2}H]IPP[9)] with the FPP synthetase fraction[12)] in the presence of 10 mM of IAA produced deuteriated farnesol. The presence of two deuteriums at C-4 and C-8 methylenes of the farnesol[13)] biosynthesized as above was proved by a decrease in the integral value of the ^{1}H NMR methylene signal at δ 2.05. Mass spectroscopy of the farnesol showed the deuterium enrichment of 91%. Then, the chirality at its C-4 and C-8 originating from the C-4 carbon atom of IPP was determined by measuring circular dichroism curves of [4,8-$^{2}H_{2}$]farnesal[14)] derived from the farnesol by active MnO_{2} oxidation and methyl

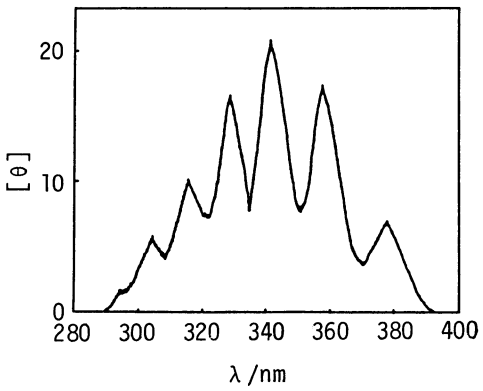


Fig. 1. CD curve of [4,8-$^2H_2$]-farnesal derived from [4,8-$^2H_2$]-farnesol biosynthesized from DMAPP and (E)-[4-^{2}H]IPP with the FPP synthetase fraction from P. sativum.

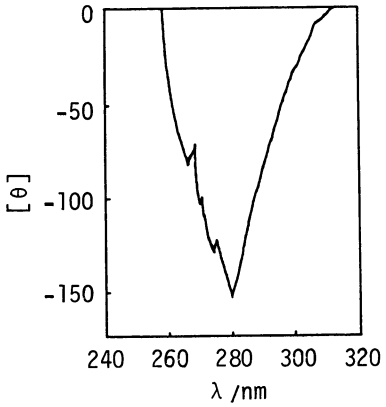


Fig. 2. CD curve of methyl [3-^{2}H]-levulinate resulted from [4,8-$^2H_2$]-farnesol biosynthesized from DMAPP and (E)-[4-^{2}H]IPP with the FPP synthetase fraction from P. sativum.

[3-^{2}H]levulinate[15] derived from the farnesal by ozonolysis followed by methylation. The [4,8-$^2H_2$]farnesal thus obtained exhibited a positive Cotton effect attributed to the $n \longrightarrow \pi^*$ transition of a carbonyl chromophore in the 290—390 nm region, as shown in Fig. 1 (see also Exp. 1 of Table 1). On the basis of the fact that this methyl [3-^{2}H]levulinate exhibited a negative Cotton effect in the 260—310 nm region as shown in Fig. 2, the chirality at C-3 of the methyl levulinate was found to be R.[16,17] Thus, this chirality at C-3 of the methyl levulinate definitely shows that the chirality at C-4 and C-8 of the deuteriated farnesol and farnesal are all R. Cornforth et al. have established the addition of allylic residue to the si-si face of the C-C double bond of IPP on the basis of the steric positions of ^{2}H at C-4 and C-8 of farnesol biosynthesized from (2R)-[2-^{2}H]mevalonic acid with the enzyme system of pig liver.[18] This Cornforth's stereochemical picture clearly indicates that, when farnesol was enzymatically synthesized from the equimolar mixture of

Table 1. CD maximum ($[\theta]_{343}$) of deuteriated farnesal enzymatically synthesized from the ^{2}H-labeled substrates

Exp. No.	Substrates	Incubation conditions[a]	Farnesal $[\theta]_{343}$
1 i)	DMAPP + (E)-[4-^{2}H]IPP	P	+21±0.4
1 ii)	DMAPP + (E)-[4-^{2}H]IPP	P	+20±0.1
2	DMAPP + (Z)-[4-^{2}H]IPP	P	-25±0.1
3	DMAPP + (E)-[4-^{2}H]IPP	A	-11±0.3
4	DMAPP + (Z)-[4-^{2}H]IPP	A	+24±2
5	(E)-[4-^{2}H]IPP	A	-20±3
6	(Z)-[4-^{2}H]IPP	A	+20±5

a) "P" denotes incubation in the presence of IAA. "A" denotes incubation in the absence of IAA.

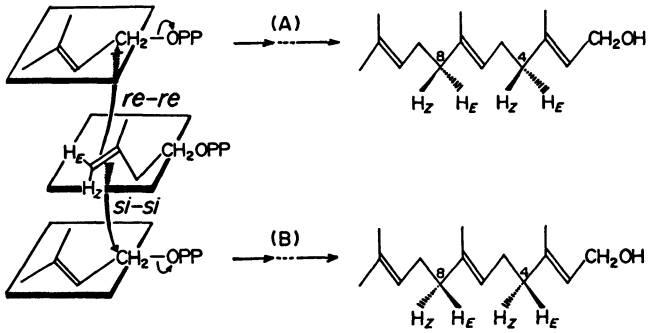
DMAPP and IPP in the presence of IAA, the addition of allylic residue to the C-C double bond of IPP at the chain elongation process in the biosynthesis of our (4R,8R)-[4,8-^{2}H]farnesol occured at the re-re face of the IPP, as shown in Scheme 1 (A).

Occurrence of the chain elongation at the reversed face (re-re face) of the C-C double bond of IPP was further supported by such a fact that deuteriated farnesal biosynthesized from DMAPP and (Z)-[4-^{2}H]IPP in the same manner as above exhibited a negative Cotton effect, as shown in Exp. 2 of Table 1, in contrast to the positive Cotton effect of the above-described deuteriated farnesal obtained from DMAPP and (E)-[4-^{2}H]IPP.

When farnesols were biosynthesized from an equimolar mixture of DMAPP and (E)-[4-^{2}H]IPP (Exp. 3 of Table 1) and from only (E)-[4-^{2}H]IPP (Exp. 5 of Table 1) in the absence of IAA, deuteriated farnesals derived from the farnesols exhibited a negative Cotton effect. This negative Cotton effect indicates that the chirality at C-4 and C-8 of the farnesol and farnesal are all S. In these cases, the chain elongation was found to occur at the si-si face of the C-C double bond of the IPP, as shown in Scheme 1 (B). This was supported a positive Cotton effect of the deuteriated farnesals obtained in incubations using (Z)-[4-^{2}H]IPP in contrast to (E)-[4-^{2}H]IPP used in the above cases, as shown in Exp. 4 and 6 of Table 1.

Occurrence of the addition of allylic residue to the si-si face in the chain elongation process in the biosynthesis of farnesol has been recognized for the biosynthesis of farnesol from DMAPP and (E)- and (Z)-[4-^{2}H]IPPs with the enzyme system from pig liver[10] and pumpkin.[19] We here reexamined the biosynthesis of farnesol from DMAPP and (E)- and (Z)-[4-^{2}H]IPPs with the enzyme system of pig liver and pumpkin in the presence of IAA under the conditions similar to those in the case of P. sativum, and we again confirmed occurrence of the addition of allylic residue to the C-C double bond of IPP following Cornforth's stereochemical picture, since the negative and the positive Cotton effect was observed for the deuteriated farnesal obtained, respectively.

The above-described new finding on the biosynthesis of farnesol with the FPP synthetase fraction of P. sativum in the presence of IAA is the first example of the unusual addition of allylic residue to the re-re face of the C-C



Scheme 1.

double bond of IPP, in opposition to Cornforth's stereochemical picture for the prenylation in the biosynthesis of isoprenoids.[18] This unusual chain elongation may be caused by attack of IAA to active sites of FPP synthetase of P. sativum. An approach to solve the mechanism is now in progress.

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9) (E)- and (Z)-[4-^{2}H]IPPs were synthesized following the method previously reported.[10,11] The isotopic purity of (E)- and (Z)-[4-^{2}H]IPPs obtained was 95% and 94%, respectively, as determined on the basis of the MS spectrum of each deuteriated isopentenol, which was obtained from the corresponding IPP by hydrolysis with phosphatase.
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12) Peas, P. sativum, (500 g) germinated at 25 °C for 2 d were macerated at 0 °C in a Waring Blender for 90 s with 100 mM Tris-HCl buffer solution (1 dm^3, pH 7.5) containing 450 mmol of sucrose and 10 mmol of 1,4-dithiothreitol and the resulting slurry on filtration through gauze yielded a homogenate, the homogenate was centrifuged at 14,000 × g for 30 min at 0 °C. The supernatant was used as a FPP synthetase fraction. The enzymatic reaction was carried out as follows: A mixture of 100 mmol of $MgCl_2$, 1 mmol of 1,4-dithiothreitol, 10 mmol of KF, 10 mmol of IAA, 500 μmol of DMAPP, 500 μmol of (E)- or (Z)-[4-^{2}H]IPP, and 16 g of the FPP synthetase fraction in 100 mM of Tris-HCl buffer solution (1 dm^3, pH 7.5) were incubated at 37 °C for 24 h and then treated with alkaline phosphatase as usual.
13) IR (neat) 2140 cm^{-1} (C-D); ^{1}H NMR ($CDCl_3$) δ=1.69 (6H, s); EI-MS m/z 224 (M^+).
14) IR (neat) 2150 cm^{-1} (C-D); ^{1}H NMR ($CDCl_3$) δ=1.69 (6H, s); EI-MS m/z 222 (M^+).
15) IR ($CHCl_3$) 2250 cm^{-1} (C-D); ^{1}H NMR ($CDCl_3$) δ=2.67 (3H, m); EI-MS m/z 131 (M^+).
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