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# SOLANESYL DIPHOSPHATE SYNTHASE REACTION WITH ARTIFICIAL SUBSTRATES. FORMATION OF *R* AND *S*-ENANTIOMERS OF 4- AND 8-METHYL DERIVATIVES OF GERANYLGERANYL DIPHOSPHATE

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**Abstract:** *(E)*- and *(Z)*-3-Methyl-3-pentenyl diphosphates acted as artificial substrates in the reaction with geranyl diphosphate catalyzed by solanesyl diphosphate synthase of *Micrococcus luteus*. The reactions of the *E*- and *Z*-isomers proceeded in the same stereochemical manner as that with the natural substrate but stopped at the stage of two steps of condensation, forming $C_{16}$- and $C_{22}$-prenyl diphosphates having extra one and two methyl groups at 4- and 4,8-positions, respectively.

The reaction catalyzed by prenyltransferase is unique and attractive from a synthetic point of view in that the polymerization of isoprene units proceeds stereospecifically and terminates precisely when the chains reach certain lengths. Solanesyl diphosphate synthase [EC 2.5.1.11] catalyzes the consecutive condensation of seven molecules of isopentenyl diphosphate (IPP) with geranyl diphosphate (GPP) to give (all-*E*)-nonaprenyl (solanesyl) diphosphate (SPP)[2].

We have previously shown that undecaprenyl diphosphate (UPP) synthase, which catalyzes the consecutive condensation of eight molecules of IPP with (*E,E*)-farnesyl diphosphate (FPP) as the primer to yield (*E,E*)-farnesyl-(all-*Z*)-octaprenyl diphosphate (UPP), accepts (*E*)-3-methyl-3-pentenyl diphosphate (**1**) in place of IPP. However, the reaction of the $C_6$ homolog with FPP makes a full stop at the stage where a single condensation is completed, yielding (*S*)-(2*Z*,6*E*,10*E*)-4-methylgeranylgeranyl diphosphate. Moreover, it does not accept the *Z*-isomer (**2**) as a substrate[3]. On the other hand, both **1** and **2** can be substrates for FPP synthase to condense with an allylic substrate in the same manner as that of the natural substrate, IPP[4,5]. This reaction is exploited to construct chiral molecules with either the *S*- or *R*-configuration[5] and has been applied successfully to chiral synthesis of insect metabolites including faranal[6] and 4-methyljuvenile hormone[7].

In order to expand the scope of utility of prenyltransferases in organic synthesis, and also to learn the substrate specificities of prenyltransferases, we examined the reactivities of **1** and **2** with respect to SPP synthase. At first, the reaction of the IPP homologs with GPP as the allylic substrate was examined as follows. The incubation mixture (final volume, 1 mL) contained 25 $\mu$M of the homolog (**1** or **2**) to be examined, 25 $\mu$M of GPP, 20 mM of Tris-HCl buffer, pH 7.7, 5 mM of $MgCl_2$, 750 $\mu$g protein of HMF (high molecular mass fraction

of *M. luteus*[8]) and 40 $\mu$g of SPP synthase. Both protein fractions were partially purified from *M. luteus*[8] and were free from any other prenyltransferases. After incubation at 37°C for 1 h, the products were extracted with 1-butanol and the extract was treated with acid phosphatase[9]. The hydrolysate was extracted with pentane and subjected to GC-MS analysis[10].

The products derived from the reaction with **1** showed two peaks on GC. The mass spectrum of the major product[11] was indistinguishable from that of 4-methylfarnesol, which was obtained by use of FPP synthase[5]. The other product[12] showed a mass spectrum corresponding to 4,8-dimethylgeranylgeraniol. The yields of 4-methylfarnesol and 4,8-dimethylgeranylgeraniol based on the allylic substrate, GPP, were 12 and 6%, respectively. In order to determine the stereochemistry of the products, they were oxidized to the corresponding aldehydes with active $MnO_2$ in dichloromethane, and the aldehydes were purified by HPLC. The 4-methylfarnesal had the same retention time (6.23 min) on GC[13] as that of chemically synthesized (*E,E*)-4-methylfarnesal, showing a negative Cotton curve with $\Delta\varepsilon_{\lambda = 247} = -0.64 \pm 0.24$. As an authentic specimen of (4*S*)-(*E,E*)-4-methylfarnesal, which was synthesized from **1** and GPP by the FPP synthase reaction[5] followed by $MnO_2$ oxidation, showed a negative Cotton curve with $\Delta\varepsilon_{\lambda = 247} = -0.75 \pm 0.13$, the absolute configuration of the enzymatic products of the SPP synthase reaction was thus demonstrated to be 4*S*. The enantiomeric purity appeared to be almost 100%[14]. Similarly it is reasonable to assign the other enzymatic product to (4*S*,8*S*)-(*E,E,E*)-4,8-dimethylgeranylgeranyl diphosphate (**4S**)[15].

The products derived from the reaction of **2** with GPP also showed two peaks corresponding to those observed in the reaction of **1** with GPP on GC-MS analysis. However, the yields of 4-methylfarnesol and 4,8-dimethylgeranylgeraniol based on GPP were 7.3 and 1.2%, respectively, which were lower than those in the case of **1**. The aldehydes formed by $MnO_2$ oxidation of these alcohols were shown to have the *E*-structures by comparing the retention times with those of authentic samples. In addition, both of the aldehydes showed positive Cotton curves, indicating that the absolute configurations are reverse to those derived from the reaction of **1** with GPP. These results indicate that the SPP synthase reaction of **1** or **2** with GPP proceeds in the same stereochemical manner as that of the natural substrate, IPP. However, the chain elongation reaction stops at the stage at which GPP condensed with two molecules of **1** or **2** to form a chiral $C_{22}$ compound, **4S** or **4R**.

The reaction of **1** or **2** was also examined using other allylic substrates, FPP or (*E,E,E*)-geranylgeranyl diphosphate (GGPP). Radio-labeled substrates, [1-$^{14}$C]FPP and [1-$^{3}$H]GGPP were employed for this purpose. The reaction mixture was made of the same constituents as described above except that 1.3 nmol of [1-$^{14}$C]FPP (spec. act., 21.6 mCi/mol) or 4.6 nmol of [1-$^{3}$H]GGPP (spec. act., 10 mCi/mol) was employed instead of GPP. After incubation at 37°C overnight, the enzymatic products were treated with acid phosphatase as usual[9], and the hydrolysate was subjected to TLC analysis[16]. In the case of the reaction of FPP with **1**, three radioactivity peaks were observed as shown in Figure 1A.

The fastest-migrating one corresponds to [1-$^{14}$C]farnesol. The next peak, which showed an Rf value of 0.53, a little smaller than that of (*E,E,E*)-geranylgeraniol, can be reasonably assigned to (*S*)-(*E,E,E*)-4-methylgeranylgeraniol. The third one, Rf 0.41, is similarly assigned to (4*S*, 8*S*)-(*E,E,E,E*)-4,8-dimethylpentaprenol.

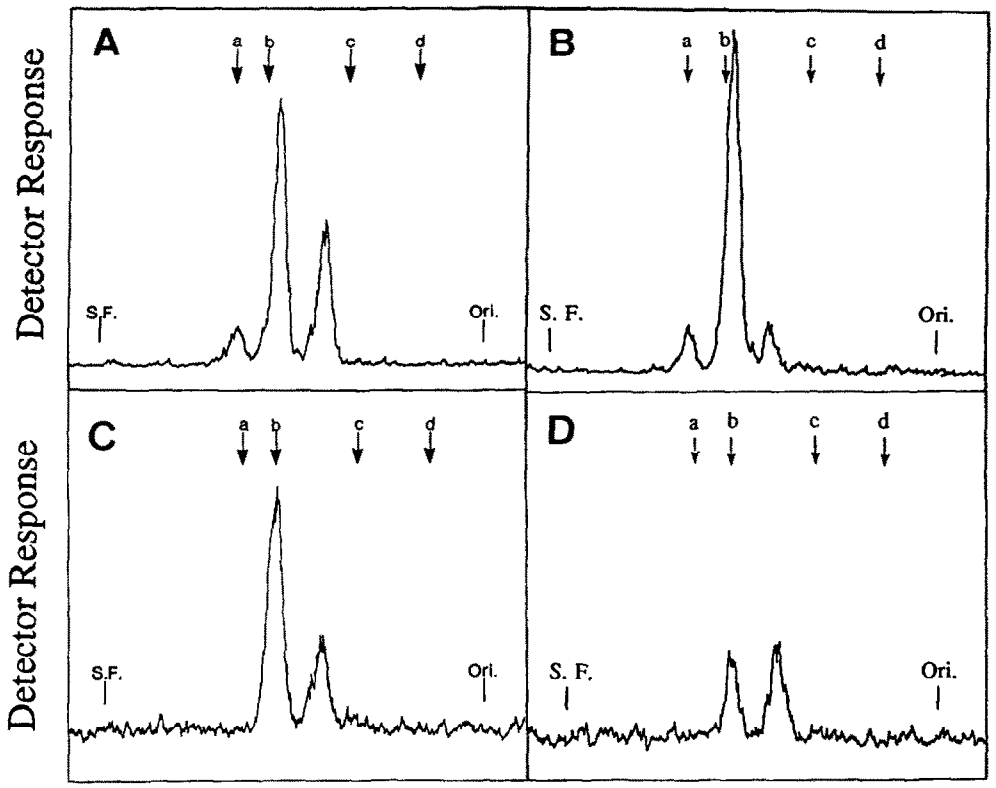


Figure 1. TLC-radiochromatograms of the alcohols derived from SPP synthase reactions with artificial substrates. A, Products derived from the reaction of [1-$^{14}$C]FPP with **1**; B, Products derived from the reaction of [1-$^{14}$C]FPP with **2**; C, Products derived from the reaction of [1-$^{3}$H]GGPP with **1**; D, Products derived from the reaction of [1-$^{3}$H]GGPP with **2**. Arrows indicate the migration positions of reference polyprenols: a, (*E,E*)-farnesol; b, (*E,E,E*)-geranylgeraniol; C, (2*Z*,6*Z*,10*E*,14*E*,18*E*)-hexaprenol; d, solanesol.

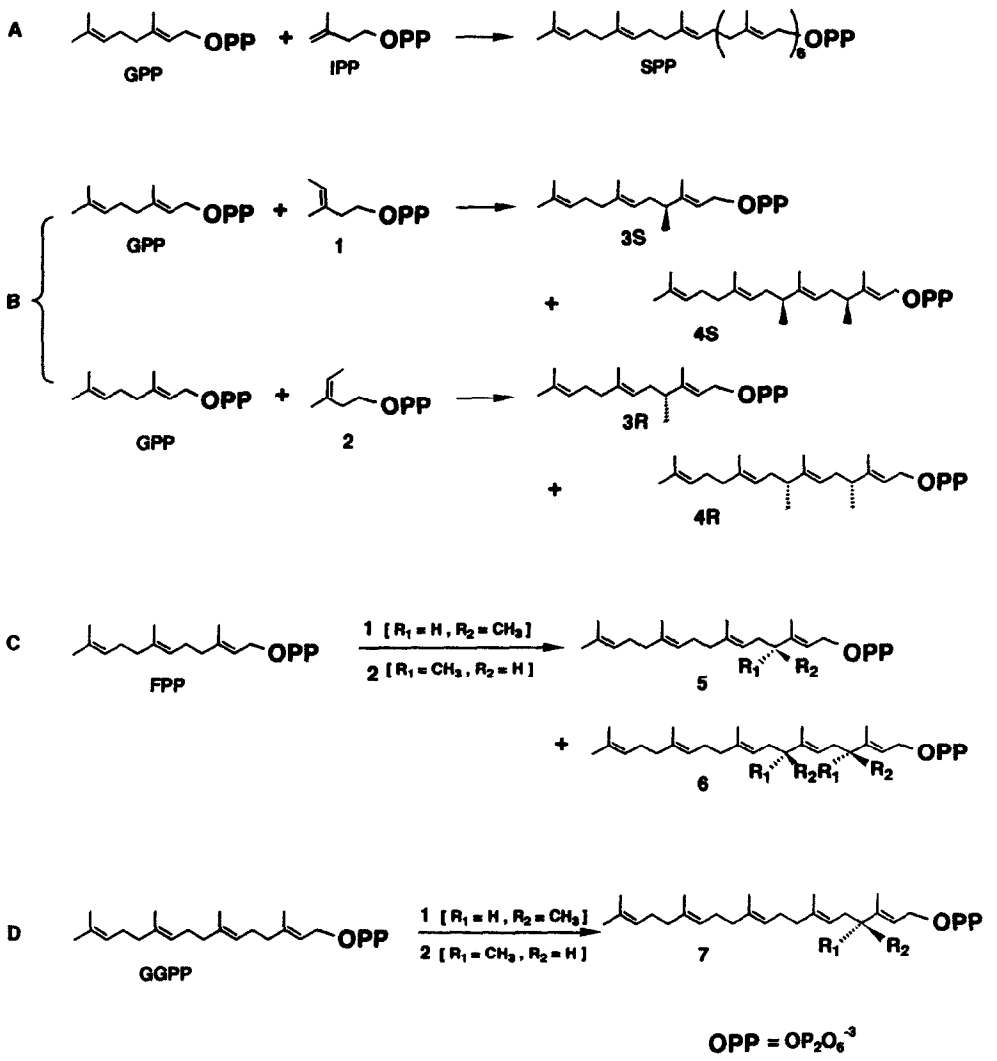


Figure 2. SPP synthase reactions with **1** or **2** as the artificial substrate. A, Reaction of natural substrates, GPP and IPP; B, Reaction of **1** or **2** with GPP as the allylic primer; C, Reaction of **1** or **2** with FPP as the allylic primer; D, Reaction of **1** or **2** with GGPP as the allylic primer.

As shown in Figure 1B, three alcohols were obtained from the reaction of **2** with FPP. They are also assignable to farnesol (Rf 0.64), *(R)*-*(E,E,E)*-4-methylgeranylgeraniol (Rf 0.53), and (4*R*,8*R*)-*(E,E,E,E)*-4,8-dimethylpentaprenol (Rf 0.43).

On the other hand, when [1-$^3$H]GGPP was employed as the allylic substrate, *(E,E,E,E)*-4-methylpentaprenol (Rf 0.43) was the only product in the reaction of either **1** or **2** (Figures 1C and 1D). The peak at Rf 0.55 corresponded to geranylgeraniol.

It is noteworthy that SPP synthase catalyzes a sequential condensation of two molecules of either **1** or **2** with FPP to yield *(S)*- or *(R)*-4,8-dimethyl-*(E,E,E,E)*-pentaprenyl diphosphate ($C_{27}$, **6**) while UPP synthase catalyzes a single condensation of only one molecule of **1** with FPP to give *(S)*-*(2Z,6E,10E)*-4-methylgeranylgeranyl diphosphate ($C_{21}$). The latter enzyme cannot accept **2** as a substrate[3]. These results, taken together with the properties of FPP synthase[5], indicate that the enzyme responsible for *E*-chain elongation has a more tolerant substrate binding site than does the enzyme for *Z*-chain elongation.

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**References and Notes**

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10. A JEOL GC-MS spectrometry system type JMS-DX 300 was used with a 1-m 20% Carbowax 20M column at 220°C. Helium gas was used as a carrier at a flow rate of 30 mL/min. The potential of the ionizing electron beam was 70 eV.

11. MS; m/z 236 (0.9), 218 (0.5), 205 (2.1), 175 (1.4), 167 (0.7), 149 (1.4), 136 (5.0), 121 (4.3), 107 (7.1), 93 (10), 81 (28) and 69 (100).
12. MS; m/z 318 (2.0), 300 (1.3), 287 (2.1), 249 (1.1), 231 (4.3), 218 (10), 181 (7.8), 163 (51), 149 (17), 137 (25), 121 (55), 107 (70), 93 (25), 81 (100), 69 (100).
13. A Shimadzu GC–chromatograph type GC–4CPF was used at 200°C on a 1–m 20% Carbowax 20M column at He gas flow rate of 30 mL/min. The retention time of (2*Z*,6*E*)–4–methylfarnesal was 5.36 min.
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15. The 4,8–dimethylgeranylgeranial derived from the product showed a negative Cotton curve with $\Delta\varepsilon_{\lambda = 247} = -0.65 \pm 0.5$.
16. The radioactive product alcohols were analyzed with a pre–coated reversed–phase LKC–18 plate (Whatman) with a solvent system of acetone/$H_2O$ (8:1). Authentic polyprenol standards, (*E,E*)–farnesol, (*E,E,E*)–geranylgeraniol, (2*Z*,6*Z*,10*Z*,14*E*, 18*E*)–hexaprenol, and solanesol (all–*E*–nonaprenol) were visualized with iodine vapor. The distribution of radioactivity was determined by scanning the plate with an Aloka radiochromatoscanner.