Tetrahedron Letters, Vol.31, No.29, pp 4189-4190, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

PRENYLTRANSFERASE REACTION INVOLVING ENANTIOMERIC DISCRIMINATION. ENZYMATIC SYNTHESIS OF (S)-10,11-EPOXYFARNESOL FROM RACEMIC 6,7-EPOXYGERANYL DIPHOSPHATE AND ISOPENTENYL DIPHOSPHATE

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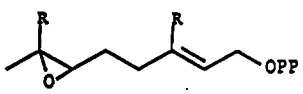
SUMMARY: (S)-10,11-Epoxyfarnesol was formed in 66% ee from racemic 6,7-epoxy geranyl diphosphate and isopentenyl diphosphate by farnesyl diphosphate synthase reaction followed by phosphatase hydrolysis.

It has previously been shown that pig liver farnesyl diphosphate synthase (FPP synthase) can be used to synthesize both (S)- and (R)-4-methylfarnesols and their homologues[1]. This method has been successfully applied in the determination of the absolute configurations of faranal[2] and 4-methyl juvenile hormone[3]. In these cases isopentenyl diphosphate (IPP) homologues such as (E)- and (Z)-3-methylpent-3-enyl diphosphates were used. The principle of this method of chiral synthesis is based on the stereospecificity of the reaction of the homoallylic diphosphates in carbon-to-carbon bond formation. However, it has not yet been examined whether enantioselectivity due to the substrate specificity for allylic substrates can be exploited to prepare chiral molecules. This paper reports the first example of prenyltransferase reaction with artificial substrates designed from such a viewpoint.

6,7-Epoxygeranyl diphosphate (1) and 6,7-expoxybishomogeranyl diphosphate (2), which were synthesized by diphosphorylation[4] of the corresponding epoxy alcohols[5], were examined to see whether they would be accepted as substrates to give chiral epoxides with juvenoid skeletons. The standard incubation mixture for enzymatic reaction contained, in a final volume of 1 mL, 20 μmol of Tris-HCl buffer, pH 7.4, 5 μmol of $MgCl_2$, 25 nmol of [1-^{14}C]IPP, an epoxy substrate (1 or 2), and 3.5 mg of pig liver FPP synthase[6]. After incubation at 37°C for 30 min, the product was extracted with 1-butanol and its amount was determined by counting the radioactivity in the extract.

Both 1 and 2 were reactive and showed reactivity profiles as shown in Fig. 1, from which their apparent Km values were estimated to be 55 μM and 27 μM, respectively.



1 : R = CH_3
2 : R = C_2H_5

OPP = O-P(O$^-$)-O-P(O$^-$)-O$^-$ (O$^-$)

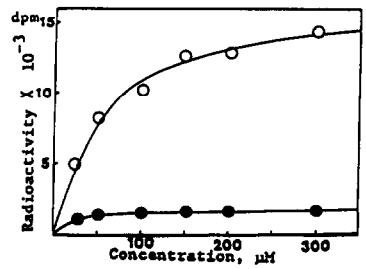
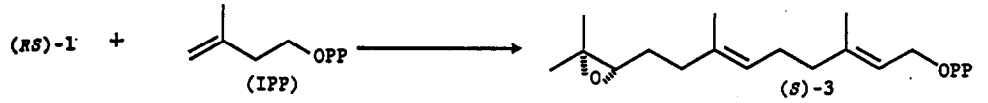


Fig. 1. Reactivities of 1 (○) and 2 (●) in the reaction catalyzed by FPP synthase

The butanol extract of the reaction mixture obtained in the experiment carried out with 200 μM of 1 was treated with alkaline phosphatase to cleave the diphosphate esters completely, and the hydrolysate was subjected to reversed phase TLC (KC-18 plate, Whatman, acetone:H_2O = 9:1). The TLC showed a single radioactivity peak with a slightly larger Rf value than that of E,E-farnesol. Radio-HPLC[7] also showed a single peak at a slightly

shorter retention time than that of E,E-farnesol. The GLC-mass spectrum[8] of the product was identical with that of chemically synthesized (RS)-10,11-epoxyfarnesol. The conversion was 16% based on IPP.



In order to estimate the extent of chiral discrimination, the radioactive epoxyfarnesol was mixed with (RS)-10,11-epoxyfarnesol, and the mixture was subjected to chiral phase HPLC by which the enantiomers could be separated from each other[9]. As a result, 83% of the radioactivity was associated with the faster eluting enantiomer, which was shown to have the S configuration by its negative optical rotation[10]. The rest of the radioactivity was associated with the R-(+) enantiomer. These results indicate that the enzyme catalyzes the condensation between (RS)-1 and IPP with a preference of the S enantiomer, thereby affording (10S)-10,11-epoxyfarnesyl diphosphate (S-3) with 66% ee. Since the diphosphate product was hydrolyzed quantitatively, the enantiomeric purity attained is attributable entirely to the substrate specificity of FPP synthase. Although the enatiomeric purity is not satisfactory from a synthetic point of view, it is noteworthy and interesting in terms of enzyme mechanism that the enzyme recognizes the chiral center located as far as five bonds away from the reaction center, at which the carbon-to-carbon bond formation takes place. This is in contrast to the case of well studied hydrolases which resolve enantiomers by recognizing chiral centers adjacent to reaction centers. The enantiomeric purity varied only slightly depending on the concentration of 1 in the enzyme reaction.

Although 2 was also accepted as substrate, its reactivity was too low for the product to be analyzed further.

ACKNOWLEDGMENT: This work was supported by the Asahi Glass Foundation for the contribution to industrial technology, special coordination funds of the Science and Technology Agency, and Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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(5) For the synthesis of (RS)-(2E, 6cis)-6,7-epoxy-3-ethyl-7-methyl-2-nonenol (6,7-epoxybishomogeraniol), 7-methyl-6-nonen-3-one [Dahm, K. H.; Trost, B. M.; Roller, H. J. Am. Chem. Soc. 1967, 89, 5292-5294] was treated with m-chloroperbenzoic acid to give the epoxide, which was then subjected to the Wittig reaction with diethyl ethoxycarbonyl-methylphosphonate followed by chromatographic separation to yield ethyl (RS)(2E, 6cis)-3-ethyl-7-methyl-6,7-epoxy-2-nonenoate. [NMR: δ 0.99(t, 3H), 1.08(t, 3H), 1.22(t, 3H), 1.27(S, 3H), 1.60(m, 4H), 2.28(q, 2H), 2.62(q, 2H), 2.72(t, 1H), 4.14 (q, 2H), 5.62(S, 1H)]
(6) Holloway, P. W.; Popják, G. Biochem. J. 1967, 104, 57-70. specific activity; One mg protein produces 7.36 x 10^{-2} nmol of FPP/min.
(7) The radio-HPLC was carried out with a 0.8 x 10 cm column of μ-Bondapak C_{18} (Waters, U.S.A.) using methanol as solvent with a flow rate of 1.1 mL/min.
(8) GLC was carried out at linear programmed temperature at a rate of 16°C/min from 130 to 180°C on a 1 m silicon OV-1 column with He gas at 50 mL/min. MS;m/z 220 (M-18), 202(M-18-18), 135(M-18-85), 85, 59 and 43.
(9) The enantiomers were resolved by HPLC carried out on a 0.46 x 25 cm column of CHIRALCEL OB L (Daicel Chemical Industries, Japan) with hexane:isopropanol = 99.7:0.3 (v/v) at a flow rate of 0.5 mL/min.
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(Received in Japan 5 June 1990)