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Artificially elicited capabilities of enzymes and their application in natural product chemistry

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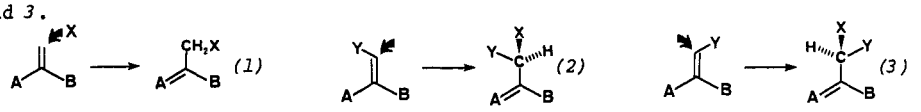
Abstract - By exploiting the stereospecificity of farnesyl diphosphate synthase reaction, both the S and R isomers of E,E-4-methylfarnesol and their derivatives, including faranal, 4-MeJH I, and their congeners were synthesized from E- (1) and Z-3-methyl-3-pentenyl diphosphate (2), respectively. Compound 1 acted as substrate in the reaction with various allylic diphosphate substrates catalyzed by undecaprenyl diphosphate synthase of Bacillus subtilis giving enantiomerically pure products. The reaction, however, stopped at the stage of a single condensation, thereby forming S-enantiomers of 2-Z-methylprenyl derivatives. Both 1 and 2 were accepted by nonaprenyl diphosphate synthase obtained from Micrococcus luteus condensing with allylic substrates to give S- and R-enantiomers of various polyprenyl derivatives, respectively.

INTRODUCTION

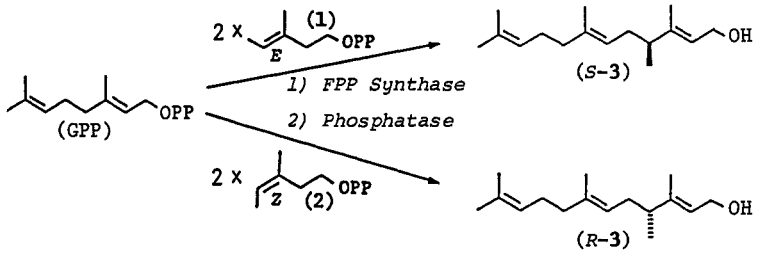
The abilities of enzymes to discriminate between enantiomers of chiral molecules or enantiotopic loci of prochiral molecules are well documented, and such abilities are utilized to prepare chiral compounds. In particular, oxidoreductases and hydrolases have been studied extensively from such points of view.
If the metabolic function of an enzyme is to catalyze the following type of reaction *1*, it may be possible to produce either enantiomer of a chiral compound at will by applying the same enzyme to artificial substrates modified within the range of substrate tolerance as shown in reactions *2* and *3*.



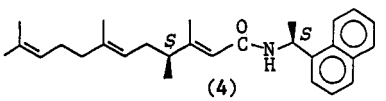
Prenyltransferases, which catalyze the fundamental chain elongation in isoprenoid biosynthesis, meet this condition. These enzymes are also interesting from mechanistic and synthetic viewpoints, because the enzyme-catalyzed reactions are repeated and stop at certain chain lengths depending on the specificities of individual enzymes. This paper is concerned with capabilities of several prenyltransferases which can be elicited by using artificially modified substrates. Application of such capabilities in natural product chemistry is also described.

CHIRAL SYNTHESIS BY FARNESYL DIPHOSPHATE SYNTHASE

Using pig liver farnesyl diphosphate synthase (FPP synthase), we have shown that both S- and R enantiomers (S-3 and R-3) of 4-methylfarnesol can be synthesized from E- (1) and Z-3-methyl--3-pentenyl diphosphate (2), respectively (ref. 1). This fact indicates that these artificial substrates react with geranyl diphosphate (GPP) in the same stereochemical manner as demonstrated for the natural substrate, isopentenyl diphosphate (IPP) (ref. 2).



The enantiomeric purities of the products were determined by chromatographic separation of the corresponding diastereomeric amides **4** and **5** derived from *S*-**3** and *R*-**3**, respectively. The amides were each eluted as a single peak on HPLC without cross contamination, indicating that both of them are enantiomerically pure. These results show that the introduction of a methyl group on either E- or Z side of C-4 of IPP does not disturb the stereochemical direction of the C-C bond formation.



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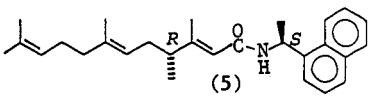
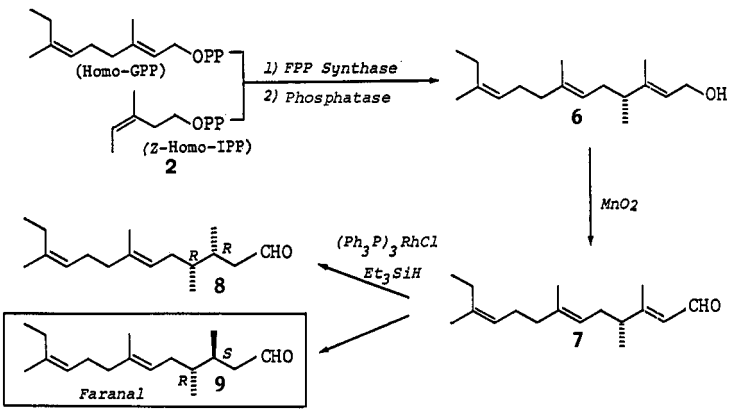


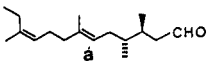
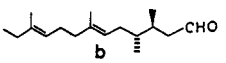
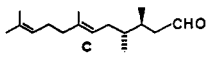
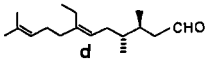
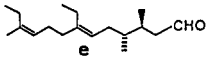
(5)

SYNTHESIS OF FARANAL AND ITS CONGENERS

Taking advantage of broad substrate specificity of FPP synthase with regard to allylic substrates (ref. 3), we applied the FPP synthase method to the chiral synthesis of faranal, the trail pheromone of the Pharaoh's ant, to determine its absolute configuration. The synthesis and bioassay of the four possible optical isomers led to the conclusion that the pheromone has the 3S,4R configuration (ref. 4). The synthetic scheme is given below. The enantiomers corresponding to **8** and **9** were also synthesized by using **1** in place of **2**, but neither of them showed pheromone activity at all even when assayed at high concentrations. This fact also shows that the stereospecificity of this synthetic method is extremely high.



Knowing that the 3-S,4R structure is essential for the pheromone activity, we synthesized several structural congeners of faranal and examined their activities (ref. 5).
It seems not very crucial whether the substituent at C-11 is an ethyl or a methyl, in contrast to the 7-methyl, which cannot be replaced by an ethyl group without losing the activity.

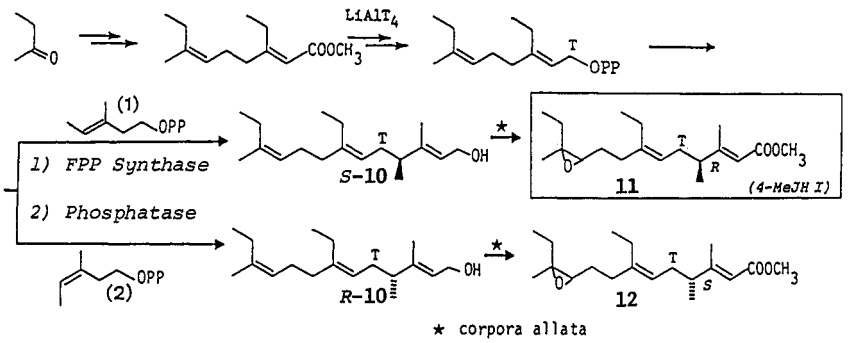
Compound	Concentration* (ng/cm)	Activity
 a	0.05	+
	0.5	+++
 b	0.05	−
	0.5	+++
 c	0.05	−
	0.5	++
 d	0.05	−
	0.5	−
 e	0.05	−
	0.5	−

(-) No ant follows the trail. (+) A few ants follow the trail.
(++) Many ants follow the trail. (+++) All ants follow the trail.

* Each compound contains the the corresponding 3R epimer in an amount as indicated below.
The contents (3S,4R/3R,4R) of **a**, **b**, **c**, **d**, and **e** are 45/55, 40/60, 40/60, 40/60, and 45/55, respectively.

SYNTHESIS AND ABSOLUTE CONFIGURATION OF 4-MeJH I

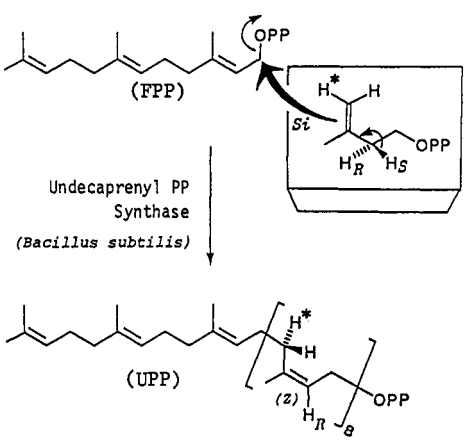
4-MeJH I is a juvenile hormone isolated from embryos of tobacco hornworm (ref. 6). The absolute configuration at C-4 is of particular interest in relation to that of faranal. In order to determine the configuration of 4-MeJH I, we took a biogenetic approach by combining the FPP synthase method and biotransformation by cultured organs. The strategy was as follows. If S- (*S*-**10**) and R-4-methyldihomofarnesol (*R*-**10**) were fed to corpora allata, one of them should be metabolized to a substance identical with natural 4-MeJH I. To facilitate the investigation, the chiral 4-methyldihomofarnesols were labeled with tritium. The labeled alcohols were fed to corpora allata of tobacco hornworm, and the metabolites were analyzed by gaschromatography. The material biotransformed from the S enantiomer (*S*-**10**) comigrated with the natural product, whereas the metabolite derived from the R enantiomer (*R*-**10**) had a little longer retention time. Thus, 4-MeJH I was demonstrated to have the 4-S configuration (ref. 7).



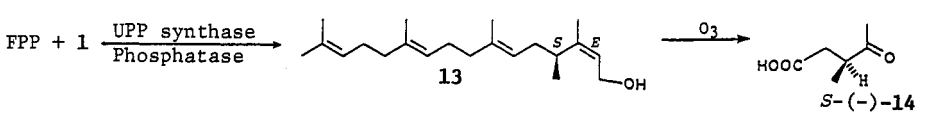
Since *S*-**10** and *R*-10 were metabolized efficiently without detectable amounts of a diastereomeric epoxide, we presume that the stereochemical effect of the 4-methyl group has no influence on the approach of epoxidation enzyme to the 10,11-double bond. If the unnatural 4R isomer had been metabolized poorly, or given the two diastereomers, it coud be argued that the stereochemistry at C-4 could affect that at C-10,11. It therefore seems likely that the epoxy group has the same congiguration as that of JH III. It is worth noting that the configuration of the 4-methyl group of 4-MeJH I is opposite to that of faranal.

CHIRAL SYNTHESIS BY UNDECAPRENYL DIPHOSPHATE SYNTHASE

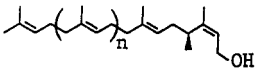
Undecaprenyl diphosphate synthase (UPP synthase) of Bacillus subtilis (ref. 8) catalyzes the consecutive condensation of eight molecules of IPP with FPP as the primer to give E,E-farnesyl-all-E-octaprenyl diphosphate (undecaprenyl diphosphate) in such a stereochemical manner as shown below (ref. 9). The stereochemical parallels and contrast between FPP synthase and UPP synthase led us to be interested in a topological comparison of the active sites of these enzymes as well as in a synthetic application of of UPP synthase.



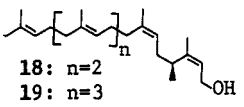
Compound 1 was accepted as substrate by UPP synthase reaction with FPP. Interestingly, however, the reaction stopped at the stage of a single condensation to give a C_{21} compound. The alcohol product was identified with chemically synthesized 4-methyl-Z,E,E-geranylgeraniol, and the absolute configuration was determined to be S by converting it to (-)-4-methyllevulinic acid (**13**), which was also synthesized by the FPP synthase method. The exclusive formation of 13 indicates the the reaction of **1** occurs in the same stereochemical course as that of IPP. However, the Z isomer **2** was not accepted at all as substrate or even inhibitor. This is also in a sharp contrast with the case of FPP synthase, which accepts both **1** and **2** to produce even compounds with longer chains than that of the normal product.



Compound 1 acted with not only FPP but also various allylic substrates, regardless of the configuration of the double bond at C-2, forming the corresponding 4-methylprenyl derivatives resulting from a single condensation of **1**. As a result, (4R) compounds **15** - **19** were synthesized.



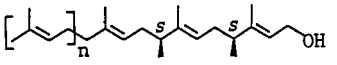
15: n=1
16: n=2
17: n=3



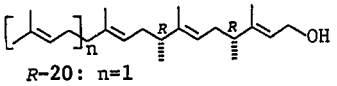
18: n=2
19: n=3

CHIRAL SYNTHESIS BY NONAPRENYL DIPHOSPHATE SYNTHASE

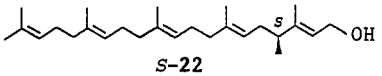
The natural function of nonaprenyl diphosphate synthase (NoPP synthase) of Micrococcus luteus (ref. 10) is the synthesis of C_{40} and C_{45} polyprenyl diphosphates with all E-double bonds. This enzyme catalyzed the reaction of **1** and **2** with GPP in a similar way to that of FPP synthase in terms of stereochemistry but in a different way in substrate-product specificity. Both **1** and **2** were good substrates reacting with various lenghs of all-E-prenyl diphosphates, but the reaction stopped after one or two condensations were completed. In contrast to UPP synthase, which was inactive for the combination of 4-methyl derivatives of allylic substrates and IPP, NoPP synthase accepted the 4-methyl derivatives when the partner is IPP. This implies that chiral methyls can be introduced at will not only at C-4 but also at C-4n positions of polyprenyl compounds. These results are also interesting from a standpoint of the reaction mechanism of enzyme which regulates the beutiful C-C bond forming reactions. The following chiral compounds were obtained by NoPP synthase reactions.



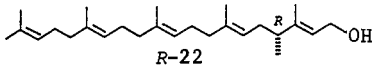
S-20: n=1
S-21: n=2



R-20: n=1
R-21: n=2



S-22



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