The Enantiomeric Purity of (*S*)- and (*R*)-4-Methylfarnesols Synthesized from Artificial Substrates by Means of a Farnesyl Diphosphate Synthase Reaction

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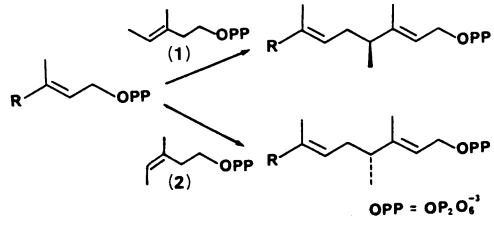
Synopsis. Both (*S*)- and (*R*)-4-methylfarnesols, synthesized respectively from (*E*)- and (*Z*)-3-methyl-3-pentenyl diphosphates by means of farnesyl diphosphate synthase reactions, were demonstrated to be of almost 100% enantiomeric purity.

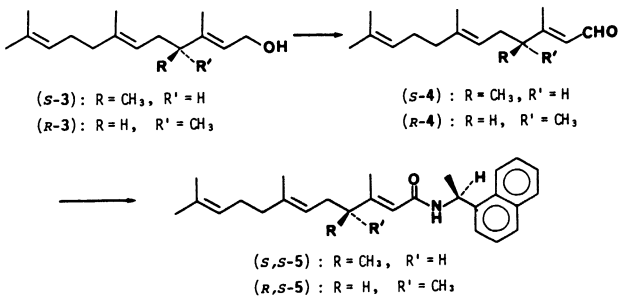
Although the utilization of enzymes to prepare chiral compounds of synthetic value is well documented,[1] only a limited number of studies have dealt with enzymes catalyzing carbon-to-carbon-bond formation. We have previously demonstrated the synthetic value of pig-liver farnesyl diphosphate synthase, showing that this enzyme can be used to prepare both (*S*)- and (*R*)-4-methylfarnesols and their homologues directly by the type of C-C bond-forming reactions[2] shown in Scheme 1. We have also synthesized insect metabolites and related compounds using some of these products as chiral synthons.[3-5] However, the enantiomeric purities of the 4-methylfarnesols synthesized by this enzymatic method remained to be determined accurately, though roughly opposite ORD spectra were previously observed for these alcohols.[2] We here wish to report evidence to show that the enantiomeric excesses of these alcohols are both almost 100%.

Results and Discussion

In order to determine the enantiomeric excess of the products derived from (*E*)- (**1**) and (*Z*)-3-methyl-3-pentenyl diphosphate (**2**), we first examined the conversion of racemic (2*E*,6*E*)-4-methylfarnesol (**3**) into diastereomeric derivatives that could be separated from each other. The racemic alcohol **3** was synthesized as follows: The Wittig reaction of 3-geranyl-2-butanone (**6**) with diethyl ethoxycarbonylmethylphosphonate gave a mixture of the *Z*,*E*- and *E*,*E*-isomers of ethyl 4-methylfarnesate, from which ethyl (*RS*)-(2*E*,6*E*)-4-methylfarnesate (**7**) was purified by silica-gel chromatography. Racemic **3** was then obtained by the $LiAlH_4$ reduction of **7**. The alcohol **3** was oxidized with MnO_2 to the aldehyde **4**, which was then oxidized with $NaClO_2$[6] to give (2*E*,6*E*)-4-methylfarnesic acid (**8**) in a quantitative yield. A chromatographic analysis of the methyl ester derived from **8** showed that no isomerization to the 2*Z* isomer had taken place during the oxidation. The oxidation of **4** was also carried out in D_2O so that epimerization could be monitored; it was confirmed by mass spectrometry that no deuterium was incorporated into the acid. The acid was then treated with (*S*)-1-(1-naphthyl)ethylamine in the presence of dicyclohexyl-



Scheme 1. Chiral synthesis by pig liver farnesyl diphosphate synthase.



Scheme 2. Conversion of (*S*)- and (*R*)-4-methylfarnesols to the corresponding acid amides. (1) MnO_2 (2) $NaClO_2$ (3) (*S*)-1-(1-Naphthyl)-ethylamine+DCC.

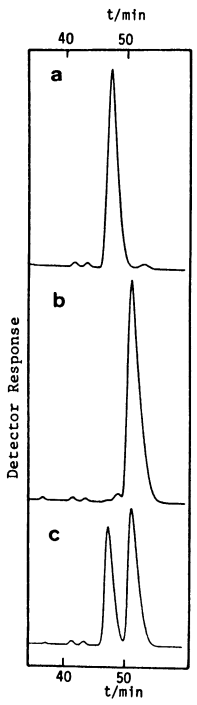


Fig. 1. HPLC of (a) (*R*,*S*)-**5**, (b) (*S*,*S*)-**5**, and (c) a mixture of (*R*,*S*)-**5** and (*S*,*S*)-**5**.

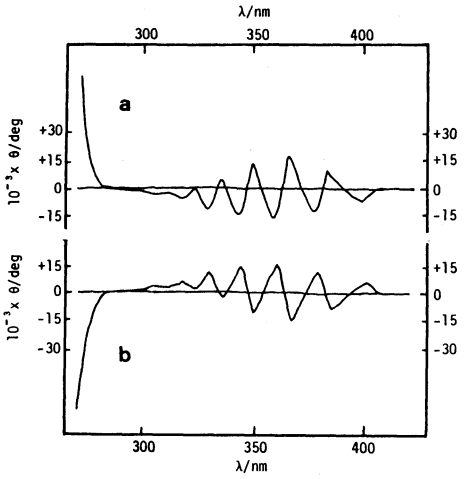


Fig. 2. CD spectra of (a) (*S*)-**4** (c=23.8 mol m^{-3}) and (b) (*R*)-**4** (c=19.8 mol m^{-3}).

carbodiimide to give a diastereomeric mixture of the corresponding amides, which could subsequently be well separated from each other by means of HPLC. Then, the (*S*)-4-methylfarnesol [(*S*)-**3**] and its *R* enantiomer [(*R*)-**3**], synthesized by the farnesyl diphosphate synthase method, were similarly converted into the corresponding amides, (*S,S*)-**5** and (*R,S*)-**5** respectively. As is shown in Fig. 1, the chromatography of the amides derived from the *S* and *R* isomers indicated that both (*S*)- and (*R*)-4-methylfarnesols had high enantiomeric purities. In other words, the introduction of a methyl group on either the *E* or the *Z* side of C-4 of isopentenyl diphosphate does not disturb the stereochemical course of the C-C bond formation.

The CD spectra of the (*S*)-4-methylfarnesal [(*S*)-**4**] and its *R* enantiomer [(*R*)-**4**], which are perfectly antipodal, also support the high optical purities of these compounds (Fig. 2).

Experimental

The (*S*)-1-(1-naphthyl)ethylamine was purchased from Aldrich. The alkaline phosphatase was obtained from Boehringer-Mannheim. The (*E*)-3-methyl-3-pentenyl diphosphate (**1**), its *Z* isomer (**2**), and geranyl diphosphate were prepared from the corresponding alcohols by diphosphorylation according to the method of Poulter et al.[7]

3-Geranyl-2-butanone (6). A solution of geranyl chloride (5.0 g, 28.9 mmol) in 30 ml of absolute ethanol was added, drop by drop, to a solution of the sodium compound of ethyl 2-methylacetoacetate (ethyl 2-methylacetoacetate, 4.59 g, 31.0 mmol; sodium ethoxide, 2.95 g, 43.4 mmol; and absolute ethanol, 170 ml) under a nitrogen atmosphere at 0 °C. After stirring has been continued at room temperature for 12 h, water was added and the mixture was extracted with hexane. The hexane extract was washed with saturated solutions of NH_4Cl and NaCl and dried over $MgSO_4$. The solvent was evaporated, and the residue was chromatographed on a column of silica gel. Elution with hexane/ethyl acetate (10/1) gave 6.75 g (83.4%) of ethyl 2-geranyl-2-methyl-3-oxobutanoate. A mixture of the ester (6.54 g, 23.2 mmol), 2 M KOH (150 ml; 1 M=1 mol dm^{-3}), and ethanol (25 ml) was stirred overnight at room temperature, after which the mixture was acidified and extracted with diethyl ether. The ether extract was worked up routinely to give **6** (4.82 g, 99%).

Ethyl (*RS*)-(2*E*,6*E*)-4-Methylfarnesate (7). To a suspension of NaH (1.06 g, 44 mmol) in dry benzene we added diethyl ethoxycarbonylmethylphosphonate (5.26 g, 23 mmol) and **6** (4.42 g, 21.3 mmol); the mixture was stirred at room temperature for 1 h and then at 65 °C overnight. Water and hexane were then added, and the mixture was shaken. The organic layer was treated as usual to give a mixture (22/3) of **7** and its 2*Z* isomer (1.50 g, 24%), from which the 2*E* isomer **7** was purified by HPLC on silica gel with hexane/ethyl acetate (200/1).

(*RS*)-4-Methylfarnesol (3). Ethyl 4-methylfarnesate (**7**, 194.2 mg, 0.7 mmol) was added under a nitrogen atmosphere to a suspension of $LiAlH_4$ (55.7 mg, 1.4 mmol) in diethyl ether (10 ml), after which the mixture was stirred at 0 °C. The reaction mixture was worked up routinely, and the product was purified by silica-gel chromatography with hexane/ethyl acetate (5/1). The yield of (*RS*)-**3** was 152.7 mg; 93%.

(*RS*)-4-Methylfarnesic Acid (8). A solution of $NaH_2PO_4 \cdot 2H_2O$ (107.0 mg, 0.68 mmol) and $NaClO_2$ (80.0 mg, 0.88 mmol) in water (0.81 ml) was added, drop by drop, to a stirred solution of (*RS*)-4-methylfarnesal (**4**, 21.3 mg), obtained by the MnO_2 oxidation of (*RS*)-**3** in *t*-butyl alcohol (1.9 ml) and 2-methyl-2-butene (0.5 ml). After being stirred at room temperature for 50 min, the mixture was diluted with water and extracted with hexane. The hexane extract was then purified by chromatography on silica gel with hexane/ethyl acetate (5/1) to give **8** in a quantitative yield.

For the reaction in D_2O, $NaD_2PO_4 \cdot 2D_2O$ and $(CH_3)_3COD$ were used instead of $NaH_2PO_4 \cdot 2H_2O$ and *t*-butyl alcohol.

***N*-[(*S*)-1-(1-Naphthyl)ethyl]-(*S*)-4-methylfarnesamide [(*S,S*)-5] and *N*-[(*S*)-1-(1-Naphthyl)ethyl]-(*R*)-4-methylfarnesamide [(*R,S*)-5].** (*S*)-1-(1-Naphthyl)ethylamine (7.5 mg, 0.044 mmol) was added to a solution of dicyclohexylcarbodiimide (9.9 mg, 0.048 mmol) and (*RS*)-4-methylfarnesic acid (**8**, 10.0 mg, 0.04 mmol) in dichloromethane, after which the mixture was stirred at room temperature overnight. The reaction mixture was then extracted with diethyl ether, and the extract was washed with saturated solutions of NH_4Cl, $NaHCO_3$, and NaCl. After the removal of the solvent, the residue was purified by silica-gel chromatography to give a diastereomeric mixture of the amides in a 23% yield.

Enzymatic Synthesis of (*S*)-4-Methylfarnesol [(*S*)-3] and Its *R* Enantiomer [(*R*)-3]. The enzymatic synthesis was carried out essentially by the same procedure as has been described previously,[2] except for the scale of incubation. The incubation volumes were 1.8 dm^3 and 7.2 dm^3 for the syntheses of (*S*)-**3** and (*R*)-**3** respectively. The yields of (*S*)-**3** and (*R*)-**3** were 24.8 mg and 47.0 mg respectively.

(*S*)-4-Methylfarnesal [(*S*)-4] and Its *R* Enantiomer [(*R*)-4]. Manganese dioxide (20 equiv) was added to a solution of (*S*)-**3** (24.8 mg) or (*R*)-**3** (47.0 mg) in dichloromethane, and the mixture was stirred at room temperature overnight. After the mixture had then been filtered, the filtrate was concentrated and the product was purified by silica-gel chromatography. The yields were both quantitative.

Conversion of (*S*)-4 and (*R*)-4 to Amides (*S,S*)-5 and (*R,S*)-5. (*S*)-4-Methylfarnesic acid [(*S*)-**8**, 15.4 mg] and its *R* enantiomer [(*R*)-**8**, 24.5 mg], which had been obtained respectively from (*S*)-**4** and (*R*)-**4** by $NaClO_2$ oxidation in D_2O, were treated with cyclohexylcarbodiimide as has been described for the reaction of (*RS*)-4-methylfarnesic acid (**8**).

HPLC Separation of (*S,S*)-5 and (*R,S*)-5. The separation of the diastereomeric amides, (*S,S*)-**5** and (*R,S*)-**5**, was performed by chromatography on a 4.6×250 mm column of Zorbax Sil with hexane/ethyl acetate saturated with water (30/1) at a flow rate of 2 ml min^{-1}. The enantiomeric

purities of (*S*,*S*)-**5** and (*R*,*S*)-**5** were both more than 98% ee.

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