IX: bp 54 °C (0.07 mm); IR (neat) 1690, 1750 cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 1.25 (t, J = 6.8 Hz, 6 H, $COOCH_2CH_3$, $COSCH_2CH_3$), 1.24 (d, J = 6.8 Hz, 3 H, CH_3), 2.36 (dd, J = 15.8 and 7.9 Hz, Ha) 2.80 (dd, J = 15.8 and 7.9 Hz, Hb), 2.88 (q, J = 6.8 Hz, 2 H, $COSCH_2CH_3$), 3.13 (heptet, J = 6.8 Hz, Hx), 4.15 (q, J = 6.8 Hz, 2 H, $COOCH_2CH_3$); mass spectrum m/e 204 (M^+, not observed), 159 (P^+, $M^+ - 45$, OEt), 143 ($M^+ - 61$, SEt), 115 (143 − 28, CO), 87 (115 − 28, CO), 92 (M^*, 92 × 143 = 115[2]). Anal. Calcd for $C_9H_{16}O_3S$: C, 52.91; H, 7.89; S, 15.69. Found: C, 52.95; H, 7.83; S, 15.55.

(18) P. Dowd, B. K. Trivedi, M. Shapiro, and L. K. Marwaha, *J. Am. Chem. Soc.*, **98**, 7875 (1976), have found that the acrylate group migrates in a nonenzymic model reaction of methylitaconate ⇄ α-methyleneglutarate isomerization. We thank Professor Dowd for a copy of this preprint.

(19) The role of the Co atom of B_{12} in the rearrangement after initial cleavage of the cobalt-carbon bond is still obscure.[20]

(20) B. T. Golding and L. Radom, *J. Am. Chem. Soc.*, **98**, 6331 (1976); R. B. Silverman and D. Dolphin, *ibid.*, **98**, 4626 (1976); K. L. Brown and L. L. Ingraham, *ibid.*, **96**, 7681 (1974).

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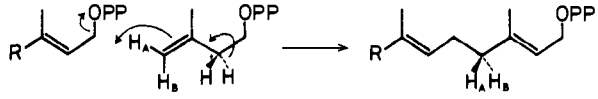
Construction of a Chiral Center by Use of the Stereospecificity of Prenyltransferase

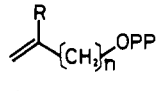
Sir:

The substrate specificity of prenyltransferase (farnesyl PP[1] synthetase EC 2.5.1.1) is not very stringent with respect to the structure of the allylic PP, and about 30 homologues of farnesyl PP have been synthesized by the action of farnesyl PP synthetase.[2] However, the specificity for the non-allylic PP is relatively high and only 3-ethylbut-3-enyl PP (**1b**) and 4-methylpent-4-enyl PP (**1c**) have been shown to be reactive as substrates in place of the natural substrate, isopentenyl PP (**1a**).[3] The stereochemistry of prenyltransferase is well established as shown in Scheme I by elegant works of Cornforth,

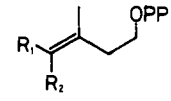
Scheme I



Popjak, and their collaborators.[4] Therefore, the examination of *E*- (**2a**) and *Z*-3-methylpent-3-enyl PP (**2b**) seems attractive



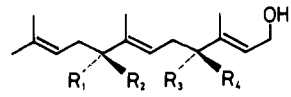
1a: n=2, R=CH_3
b: n=2, R=C_2H_5
c: n=3, R=CH_3



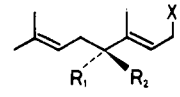
2a: R_1=CH_3, R_2=H
b: R_1=H, R_2=CH_3

and worth doing, because they are expected, if accepted as substrates in place of **1a**, to give chiral molecules which are enantiomeric with each other, and because such an anticipation, however, is dangerous since some modifications of a substrate may cause an abnormal reaction as exemplified by the case of **1b** and **1c** in the reaction catalyzed by isopentenyl PP isomerase[5] and prenyltransferase,[6] respectively. In this paper we now report that both **2a** and **2b** react stereospecifically to give new farnesyl PP homologues having chiral centers at which the new C-C bond is constructed during the enzymatic condensation.

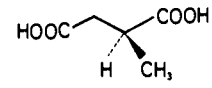
Compounds **2a** and **2b** were prepared from the corresponding alcohols[5] by the phosphorylation as usual. The incubation mixture for the enzymatic reaction contained, in a final volume of 5 mL, 100 μmol of Tris-HCl buffer, pH 7.7, 25 μmol of $MgCl_2$, 500 nmol of dimethylallyl PP or geranyl PP (**4d**), 500 nmol of **2a** or **2b**, and 0.5 mg of farnesyl PP synthetase (specific activity: 68.0 nmol of **1a** incorporated min^{-1} mg^{-1}) purified from pig liver.[2a] The mixture was incubated at 37 °C for 60 min and was then treated with alkaline phosphatase for more than 10 h. The hydrolysates were extracted with light petroleum and subjected to GLC-mass spectrometric analysis.[7] The products derived from dimethylallyl PP with **2a** showed two peaks. The major one emerged at 18.4 min (retention volume relative to that for *E,E*-farnesol (**3a**), 1.04; yield based on **2a**, 12.6%) and the mass spectrum for this material exhibited a parent ion at m/e 250 ($C_{17}H_{30}O$) with an intensity of 0.9% relative to the base peak at 69 (C_5H_9). Peaks were also observed at 232 (M − 18), 219 (M − 31), 189 (M − 18 − 43), 181 (M − 69), 163 (M − 18 − 69), which were reasonable for 4,8-dimethylfarnesol (**3d** or **3e**). The other minor component had a retention time of 7.3 min (1.05 relative to that for geraniol (**4a**), 3.9% yield) and the mass spectrum showed peaks at m/e 168 (M, $C_{11}H_{20}O$), 150 (M − 18), 137 (M − 31), 125 (M − 43), 81 (M − 18 − 69), and 69 (C_5H_9), indicating that the product was 4-methylgeraniol (**4b** or **4c**). The material derived from **4d** and **2a** showed a single peak at a retention volume of 1.02 relative to that for **3a** (19.2% yield) and the mass spectrum exhibited peaks at m/e 236 (M, $C_{16}H_{28}O$), 218 (M − 18), 205 (M − 31), 175 (M − 18 − 43), and 69 (C_5H_9) which was the base peak. These results indicate that the product was 4-methylfarnesol (**3b** or **3c**). The geometry of the newly formed double bond was proved to have *E* configuration by the NMR spectrum as shown later.[8] The *Z*-isomer **2b** was also enzymatically reactive and the products of condensation with dimethylallyl PP or **4d** were not distinguishable in GLC-mass spectrometric analysis from those obtained by the condensation of **2a** with dimethylallyl PP or **4d**.[9] The rates of condensation with **4d** of **2a** and **2b** relative to that of **1a** were 0.39 and 0.15, respectively.



3a: R_1=R_2=R_3=R_4=H
b: R_1=R_2=R_3=H, R_4=CH_3
c: R_1=R_2=R_4=H, R_3=CH_3
d: R_1=R_3=H, R_2=R_4=CH_3
e: R_1=R_3=CH_3, R_2=R_4=H



4a: R_1=R_2=H, X=OH
b: R_1=H, R_2=CH_3, X=OH
c: R_1=CH_3, R_2=H, X=OH
d: R_1=R_2=H, X=OPP
e: R_1=H, R_2=CH_3, X=OPP
f: R_1=CH_3, R_2=H, X=OPP



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Then, incubations of preparative scale (ca. 80-fold of the usual) were made to determine the configuration of the products and the free alcohols liberated by the treatment with alkaline phosphatase were purified by TLC.[10] The 4-methylfarnesol[11] formed by the condensation of **4d** and **2a** gave a negative ORD curve ($[\alpha]_D$ −10.7 ± 2.1°),[12] and conversely, the alcohol derived from **4d** and **2b** was found to be dextrotatory as expected ($[\alpha]_D$ +10.0 ± 8.3°). When these alcohols were converted to the corresponding aldehydes with active MnO_2, the signs of the ORD curves were both reversed. These results indicate that they were enantiomeric with each other. The 4,8-dimethylfarnesol[13] derived from dimethylallyl PP and **2a** also showed a negative ORD curve ($[\alpha]_D$ −11.4 ± 5.1°). The 4-methylfarnesal and the 4,8-dimethylfarnesol both of which were derived from **2a** were degraded by ozonolysis followed by hypoiodite oxidation to methylsuccinic acid.[4] Both samples of methylsuccinic acid were found to be levorotatory, indicating that the methylsuccinic acid was the *S* isomer (**5**).[14] Consequently the 4-methylfarnesyl PP from **2a** and **4d** and 4,8-dimethylfarnesyl PP from **2a** and dimethylallyl PP were both assigned to have *S* configuration, **3b-PP** and **3d-PP**, respectively. It is apparent that the products obtained by the

reaction of **2b** with dimethylallyl PP and with **4d** are the *R* isomers, **3c-PP** and **3e-PP**, respectively, since they show opposite behavior in ORD.

Consequently, it was concluded that the new artificial homologues (**2a** and **2b**) of isopentenyl PP (**1a**) could be substrates for farnesyl PP synthetase in place of **1a** to condense with the allylic substrates in the same stereochemical manner with that demonstrated for the natural substrates by Cornforth et al.[4] In other words, these homologues served as probes to distinguish the side of the double bond of **1a** from which the C-C bond is newly formed; namely, this is an example of the visualization of the stereospecificity latent in an enzyme-catalyzed prochiral process.

References and Notes

(1) PP stands for pyrophosphate.
(2) (a) G. Popják, P. W. Holloway, and J. M. Baron, *Biochem. J.*, **111**, 325 (1969); (b) G. Popják, J. L. Rabinowitz, and J. M. Baron, *ibid.*, **113**, 861 (1969); (c) K. Ogura, T. Nishino, T. Koyama, and S. Seto, *J. Am. Chem. Soc.*, **92**, 6036 (1970); (d) T. Nishino, K. Ogura, and S. Seto, *ibid.*, **93**, 794 (1971); (e) *Biochim. Biophys. Acta*, **235**, 322 (1971); (f) *J. Am. Chem. Soc.*, **94**, 6849 (1972); (g) *Biochim. Biophys. Acta*, **302**, 33 (1973); (h) C. D. Poulter, D. M. Satterwhite, and H. C. Rilling, *J. Am. Chem. Soc.*, **98**, 3376 (1976).
(3) (a) K. Ogura, T. Koyama, and S. Seto, *J. Chem. Soc., Chem. Commun.*, 881 (1972); (b) T. Koyama, K. Ogura, and S. Seto, *Chem. Lett.*, 401 (1973); (c) K. Ogura, A. Saito, and S. Seto, *J. Am. Chem. Soc.*, **96**, 4037 (1974); (d) A. Saito, K. Ogura, and S. Seto, *Chem. Lett.*, 1013 (1975).
(4) J. W. Cornforth, R. H. Cornforth, G. Popják, and L. Yengoyan, *J. Biol. Chem.*, **241**, 3970 (1966).
(5) **1b** is converted into unexpected **2a**; T. Koyama, K. Ogura, and S. Seto, *J. Biol. Chem.*, **248**, 8043 (1973).
(6) The reaction of **1c** with **4d** results in an exclusive formation of the *Z*-homologue of farnesyl PP.[3c]
(7) A Shimadzu-LKB GLC–mass spectrograph type 9000 was used with a 1-m column of 5% Carbowax 20M at linear programmed temperature at a rate of 4 °C/min from 130 to 220 °C.
(8) R. B. Bates and D. M. Gale, *J. Am. Chem. Soc.*, **82**, 5749 (1960). See footnotes 11 and 13.
(9) The yield of 4,8-dimethylfarnesol, 4-methylgeraniol, and 4-methylfarnesol was 3.4, 0.8, and 7.6% on the basis of **2b**, respectively.
(10) Silica gel TLC in a system of benzene–ethyl acetate (4:1) in which the R_f for **3a** is 0.51.
(11) Yield, 3.5 mg; R_f, 0.48 on TLC; NMR (CCl_4) δ 1.00 (d, 3 H), 1.62 (s, 9 H), 1.68 (s, 3 H), 1.99 (br s, 7 H), 4.03 (d, 2 H), 5.02 (m, 2 H), and 5.34 ppm (t, 1 H).
(12) Optical activities were measured in hexane except for methylsuccinic acid (in water) with JASCO ORD Recorder Model ORD/UV-5 in a cuvette of 1-mm light path.
(13) Yield, 3.7 mg; R_f, 0.47 on TLC; NMR (CCl_4) δ 0.97 (d, 3 H), 1.03 (d, 3 H), 1.62 (s, 3 H), 1.66 (s, 6 H), 1.71 (s, 3 H), 2.07 (br s, 6 H), 4.08 (d, 2 H), 5.06 (m, 2 H), and 5.39 ppm (t, 1 H).
(14) A. Fredga, J. P. Jennings, W. Klyne, P. M. Scopes, B. Sjöberg, and S. Sjöberg, *J. Chem. Soc.*, 3928 (1965).

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Resonance Raman Spectra of Cytochrome $P450_{cam}$

Sir:

We present the first well-resolved resonance Raman spectra of the heme protein, cytochrome $P450_{cam}$. This protein, isolated from the bacterium, *Pseudomonas putida*, is instrumental in the methylene hydroxylation of D-(+)-camphor and belongs to a general class of hydroxylating heme proteins involved in detoxification, drug metabolism, carcinogenesis, and steroid biosynthesis. Cytochromes of the P450 type are identified by a unique Soret band (ca. 450 nm) of the reduced and carbon monoxide complexed heme. The P450 cytochromes share some properties of the heme iron with other proteins. The principal difference lies in their ability to serve both the oxygen binding role of heme proteins and the ferrous-ferric redox role of cytochromes, and also to bind selectively their carbon substrates. We limit this report to a discussion of the native (ferric) enzyme in the presence of a saturating level of substrate (camphor).

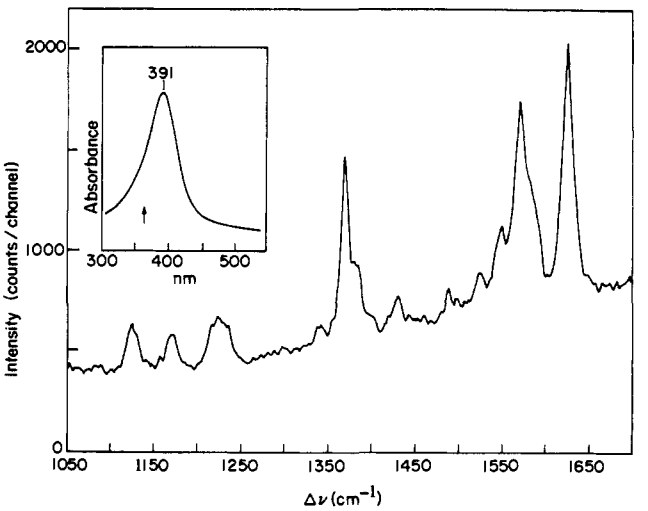

Figure 1. Resonance Raman spectrum of cytochrome $P450_{cam}$. The laser frequency is 3638 Å with 20 mW power at the sample. Slit widths are 4 cm^{-1} and counting time is 10 s/channel, 1 cm^{-1} step size. The insert shows the optical absorption spectrum of the reaction state under study (oxidized $P450_{cam}$ plus camphor). The arrow denotes the position of the laser excitation frequency.

Table I. Resonance Raman Peak Positions of $P450_{cam}$

Δν (I, pol)[a]	Δν (I, pol)	Δν (I, pol)	Δν (I, pol)
318 (w, u)	785 (w, u)	1224 (m, u)	1525 (m, p)
351 (m, p)	797 (w, u)	1339 (w, u)	1549 (m, u)
377 (w, u)	822 (w, u)	1368 (s, p)	1570 (s, p)
675 (w, u)	978 (w, u)	1379 (m, u)	1584 (m, p)
691 (w, u)	1003 (w, u)	1398 (w, u)	1623 (s, dp)
720 (w, u)	1125 (m, u)	1429 (m, u)	
754 (m, dp)	1170 (m, u)	1488 (m, p)	

[a] Δν is Stokes shift in cm^{-1} (± 2 cm^{-1}) relative to the excitation wavelength (3637.8 Å); I corresponds to relative intensity of peaks: s = strong, m = medium, w = weak; pol refers to the polarization of peaks: p = polarized ($I_\perp/I_\parallel \lesssim 1/8$), dp = depolarized ($I_\perp/I_\parallel \simeq 3/4$), u = undetermined.

Three main experimental findings arise from this preliminary study: (1) the optical absorption on the high energy side of the Soret band (Soret maximum at 391 nm) is strongly coupled to low frequency modes (<500 cm^{-1}) normally associated with iron-nitrogen vibrations; (2) a large number of Raman peaks appear in the region 650-850 cm^{-1}, possibly reflecting the excitation of a ligand vibrational mode; (3) peak positions in the region 1050-1700 cm^{-1} are rather unusual and indicate a weakening of prophyrin ring bond strengths.

The Raman spectra are obtained using an excitation wavelength of 3637.8 Å from a Coherent Radiation Model CR12 argon ion laser. The power at the sample in all runs is close to 20 mW. The quartz sample container allows accumulation of both (right angle) Raman scattering and optical absorption data. The Raman data are collected by means of a Spex 1401 double monochromator equipped with photon counting apparatus and digital data storage. The spectra are averaged to reduce statistical fluctuations and plotted using a small computer. The optical absorption measurements are performed routinely before and after each run using a Cary 14 spectrophotometer. During the Raman runs the sample is kept at approximately 4 °C by means of a thermoelectric cooling unit. The polarization of the Raman scattered light is determined with a polarization filter and a polarization scrambler is used to ensure equal response of the monochromator to both polarizations.