Pentalenene Biosynthesis and the Enzymatic Cyclization of Farnesyl Pyrophosphate. Anti Stereochemistry in a Biological S_E' Reaction

David E. Cane,* Christopher Abell, Rene Lattman, Charles T. Kane, Brian R. Hubbard, and Paul H. M. Harrison

Department of Chemistry, Brown University Providence, Rhode Island 02912

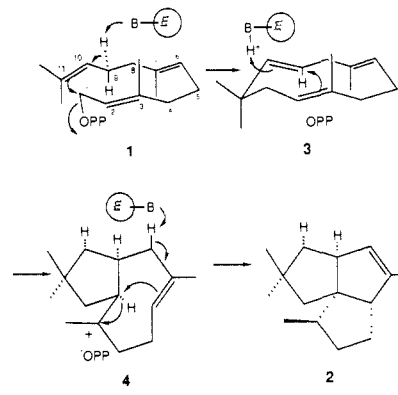
Received January 19, 1988

Pentalenene synthetase catalyzes the cyclization of *trans,trans*-farnesyl pyrophosphate (**1**) to pentalenene (**2**), the parent hydrocarbon of the pentalenolactone family of sesquiterpene antibiotics.[1,2] We have previously presented evidence supporting a mechanism in which ionization and cyclization of farnesyl pyrophosphate generates humulene (**3**) by electrophilic attack on the si-face of C-11 and deprotonation at C-9 (Scheme I). Reprotonation of humulene at C-10 and attack on the C-2,3 double bond is proposed to generate cation **4**, which can undergo hydride migration and further cyclization involving the C-6,7 double bond with loss of one of the H-8 protons of the farnesyl precursor to yield pentalenene.[1] Additional studies have reinforced this mechanistic picture and established that a single enzyme catalyzes conversion of farnesyl pyrophosphate to pentalenene, based on the demonstrated internal return of one of the original H-9 protons of farnesyl pyrophosphate to C-10 of the intermediate humulene.[3]

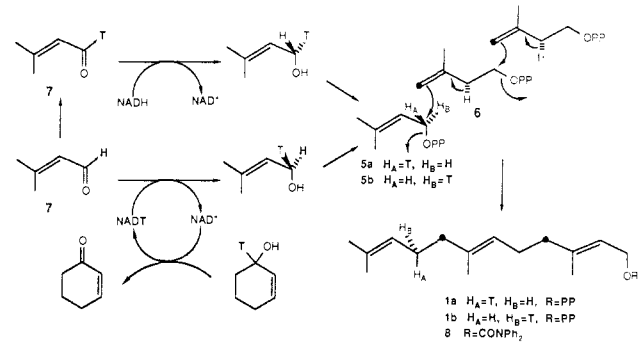
Farnesyl pyrophosphate itself is formed by the condensation of dimethylallyl pyrophosphate (DMAPP, **5**) with isopentenyl pyrophosphate (IPP, **6**).[4] Extensive studies of the prenyl transferase-catalyzed condensation reaction have shown that the key bond-forming steps take place by a stepwise sequence of ionization–electrophilic addition–elimination[5] with net stereochemistry and inversion of configuration at C-1 of the allylic pyrophosphate.[6] The first step in the cyclization of farnesyl pyrophosphate to pentalenene would appear to be the intramolecular analogue of the prenyl transferase reaction.[7] We now report that the ring-forming reaction, a formal intramolecular S_E' reaction, occurs with net anti stereochemistry.

The requisite substrates, (9*R*)- and (9*S*)-[9-^{3}H,4,8-^{14}C]farnesyl pyrophosphate (**1a** and **1b**), were prepared enzymatically from (1*R*)- and (1*S*)-[1-^{3}H]DMAPP (**5a** and **5b**), respectively, and [4-^{14}C]IPP (Scheme II). The (1*R*)-[1-^{3}H]DMAPP (**5a**) was prepared by reduction of 3-methyl-2-butenal (**7**) by using liver alcohol dehydrogenase (HLADH) coupled with catalytic NAD^+ and [1-^{3}H]cyclohexenol[8,9] followed by pyrophosphorylation of the resulting (1*R*)-[1-^{3}H]dimethylallyl alcohol.[10,11] Incubation of

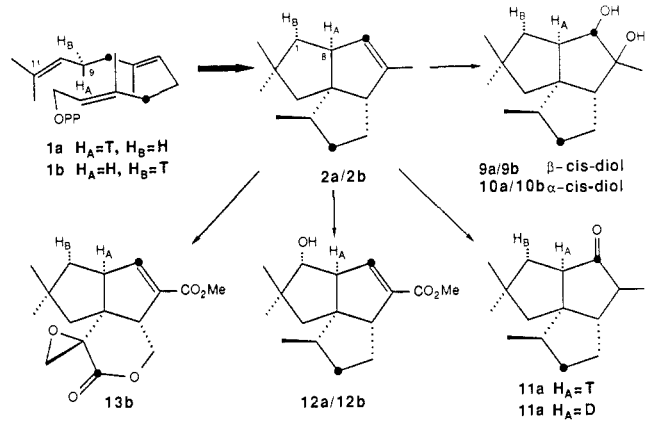
Scheme I



Scheme II



Scheme III



5a and [4-^{14}C]IPP with avian prenyl transferase[12a] gave **1a** which was treated with acid phosphatase. The resulting farnesol, after addition of inactive carrier, was converted to the diphenylurethane **8a**[11] which was recrystallized to constant activity and isotope ratio (Table I). For the synthesis of (1*S*)-[1-^{3}H]DMAPP (**5b**), [1-^{3}H]-3-methyl-2-butenal was first prepared by reduction of **7** with sodium [^{3}H]borohydride followed by reoxidation with PCC. Stereospecific reduction of [1-^{3}H]-**7** with HLADH and excess NADH gave (1*S*)-[1-^{3}H]dimethylallyl alcohol which was converted to DMAPP **5b**. Mixture with [4-^{14}C]IPP and incubation with prenyl transferase[12b] gave (9*S*)-[9-^{3}H,4,8-^{14}C]farnesyl pyrophosphate (**1b**) which was separated from DMAPP, IPP, and

(1) Cane, D. E.; Tillman, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 122.
(2) Seto, H.; Yonehara, H. *J. Antibiot.* **1980**, *33*, 92.
(3) Cane, D. E.; Abell, C.; Tillman, A. M. *Bioorg. Chem.* **1984**, *12*, 312.
(4) Poulter, C. D.; Rilling, H. C. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, pp 161–224.
(5) Poulter, C. D.; Rilling, H. C. *Acc. Chem. Res.* **1978**, *11*, 307. Poulter, C. D.; Argyle, J. C.; Mash, E. A. *J. Biol. Chem.* **1978**, *253*, 7227. Poulter, C. D.; Wiggins, P. L.; Le, A. T. *J. Am. Chem. Soc.* **1981**, *103*, 3926. Mash, E. A.; Gurria, G. M.; Poulter, C. D. *J. Am. Chem. Soc.* **1981**, *103*, 3927.
(6) Donninger, C.; Popjak, G. *Proc. R. Soc. London, Ser. B.* **1966**, *163*, 465. Cornforth, J. W.; Cornforth, R. H.; Donninger, C.; Popjak, G. *Proc. R. Soc. London, Ser. B.* **1966**, *163*, 492. Popjak, G.; Cornforth, J. W. *Biochem. J.* **1966**, *101*, 553. Cornforth, J. W.; Cornforth, R. H.; Popjak, G.; Yengoyan, L. *J. Biol. Chem.* **1966**, *241*, 3970.
(7) (a) Cane, D. E. *Tetrahedron* **1980**, *36*, 1109. (b) Davisson, V. J.; Neal, T. R.; Poulter, C. D. *J. Am. Chem. Soc.* **1985**, *107*, 5277.
(8) Cane, D. E.; Ha, H.-J.; Pargellis, C.; Waldmeier, F.; Swanson, S.; Murthy, P. P. N. *Bioorg. Chem.* **1985**, *13*, 246.
(9) The stereospecificity of the enzymatic reduction was confirmed by mixing (1*S*)-[1-^{3}H]dimethylallyl alcohol with [1-^{14}C]dimethylallyl alcohol and oxidation of the mixture with HLADH and excess NAD^+. Comparison of the $^3H/^{14}C$ ratios of the dinitrobenzoate ester of the alcohol and the aldehyde semicarbazone indicated a complete (99.8%) loss of tritium in the oxidation step, as expected. Hydrolysis of **5a** followed by similar analysis confirmed complete retention of configuration during the pyrophosphorylation reaction.
(10) Cramer, F.; Boehm, W. *Angew. Chem.* **1959**, *71*, 775.
(11) Cane, D. E.; Iyengar, R.; Shiao, M.-S. *J. Am. Chem. Soc.* **1981**, *103*, 914.
(12) (a) Homogenous prenyl transferase used in the preparation of **1a** was a generous gift from Professor C. D. Poulter. (b) Prenyl transferase for the preparation of **1b** was purified from chicken liver[13] through the hydroxylapatite step to a specific enzyme activity of 30 nmol/min/mg protein (0.03 U/mg). In a typical procedure, 1.26 nmol of (1*S*)-[1-^{3}H]DMAPP (**5b**) (64 μCi/μmol) and 1.05 nmol of [4-^{14}C]IPP (**6**) (50 μCi/μmol) were incubated with 0.13 U of prenyl transferase for 2 h at 37 °C in 6 mL of 20 mM bicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic acid buffer[14,15] (pH 7.0) in the presence of 1.0 mM $MgCl_2$, 10 mM β-mercaptoethanol, and 0.01% bovine serum albumin.

0002-7863/88/1510-4081$01.50/0

Table I. Conversion of (9*R*)-[9-^{3}H,4,8-^{14}C]Farnesyl Pyrophosphate (**1a**) and (9*S*)-[9-^{3}H,4,8-^{14}C]Farnesyl Pyrophosphate (**1b**) to Pentalenenes **2a** and **2b** by Pentalenene Synthetase and Distribution of the Label

compd	^{3}H/^{14}C	atom ratio	compd	^{3}H/^{14}C	atom ratio
1a	1.43	(1:2)[a]	**1b**	0.577 ± 0.007	(1:2)
8a	1.45 ± 0.02	1:2[b]	**8b**	0.497 ± 0.009	1:2
(**8a**)	1.28 ± 0.03	0.88:2[c]			
2a	1.48 ± 0.04	1:2	**2b**	0.502 ± 0.006	1:2
9a	1.49 ± 0.06	1:2	**9b**	0.486 ± 0.009	1:2
10a	1.47 ± 0.002	1:2	**10b**	0.478 ± 0.010	1:2
11a	1.46 ± 0.04	1:2			
11a	0.45 ± 0.02	0.31:2[d]			
12a	1.44 ± 0.04	1:2	**12b**	0.014 ± 0.006	0.03:2
			13b	0.525 ± 0.023	1.1:2

[a] Prepared by prenyl transferase reaction; based on the derived farnesyl diphenylurethane. [b] Prepared from farnesyl pyrophosphate (**1a**) reisolated from incubation with pentalenene synthetase. [c] Derived from farnesol reisolated from the pentalenene synthetase incubation and subjected to successive HLADH oxidation-borohydride reduction. [d] Exchanged with 0.2 N NaOD in D_2O-dioxane.[3] Predicted value 0.24:2 (cf. footnote 17).

geranyl pyrophosphate by reverse-phase ion-pairing HPLC.[16] The derived farnesyl diphenylurethane (**8b**) was recrystallized to constant activity (Table I).

For the conversion to pentalenene, (9*R*)-[9-^{3}H,4,8-^{14}C]farnesyl pyrophosphate (**1a**) was incubated with crude pentalenene synthetase,[3] and the resulting pentalenene (**2a**) was diluted with unlabeled pentalenene. Treatment of **2a** with OsO_4 gave diols **9a** and **10a**,[3] each of which was recrystallized to constant activity[17] (Table I). The incubation with (9*S*)-[9-^{3}H,4,8-^{14}C]farnesyl pyrophosphate (**1b**) was carried out by using 130-fold purified pentalenene synthetase which had been shown to be free of phosphatase, prenyl transferase, and isomerase activities.[18] Half of the resulting labeled pentalenene (**2b**) was diluted with inactive pentalenene, and the derived diols **9b** and **10b** were each recrystallized as before (Table I).

The precise location of the tritium in each sample of labeled pentalenene was established by a combination of chemical and microbiological methods[3] (Scheme III). Thus hydroboration-oxidation of **2a** gave the ketone **11a**, which lost greater than 92% of the predicted amount of label from C-8 upon base-catalyzed exchange (Table I). The absence of any tritium at H-1α of **2a** was established by feeding **2a** to intact cultures of *Streptomyces* UC5319 and isolation of the resulting labeled pentalenic acid methyl ester **12a** which had not lost any tritium.[3] By contrast, when the sample of **2b** was fed to cultures of *Streptomyces* UC5319, the derived **12b** had lost all tritium, whereas the co-metabolite *epi*-pentalenolactone F methyl ester (**13b**)[20] showed an unchanged ^{3}H/^{14}C ratio (Table I).

The above results establish that in the cyclization of farnesyl pyrophosphate to pentalenene, H-9re of **1** becomes H-8 of pentalenene, while H-9si undergoes net intramolecular transfer to H-1α of **2**, presumably by a deprotonation-reprotonation mechanism. Since the cyclization has already been shown to involve electrophilic attack on the si face of the 10,11-double bond of **1**,[21] the formal S_E' reaction takes place with net anti stereochemistry. This conclusion is completely consistent with the previously inferred RSR-CT conformation[3,21,22] of the cyclizing substrate, which prevents access by any enzymic base to the H-9re proton.

Acknowledgment. This work was supported by a grant from the National Institutes of Health, GM22172, and by postdoctoral fellowships from the Science and Engineering Research Council (C.A.) and the National Institutes of Health (C.T.K.).

(13) Reed, B. C.; Rilling, H. C. *Biochemistry* **1975**, *14*, 50.
(14) Mallette, M. F. *J. Bacteriol.* **1967**, *94*, 283.
(15) Bartlett, D. L.; King, C.-H. R.; Poulter, C. D. In *Methods in Enzymology*; Law, J. H., Rilling, H. C., Eds.; Academic Press: New York, 1985; Vol. 110, pp 171-184.
(16) Beyer, P.; Kreuz, K.; Kleinig, H. In *Methods in Enzymology*; Law, J. H., Rilling, H. C., Eds.; Academic Press: New York, 1985; Vol. 111, pp 248-252.
(17) As a control, a portion of the recovered farnesol resulting from the endogenous phosphatase activity in the pentalenene synthetase preparation was converted to the corresponding diphenylurethane (^{3}H/^{14}C 1.45), while the remainder was oxidized to farnesal by incubation with HLADH and NAD^+. Sodium borohydride reduction of farnesal and recrystallization of the derived diphenylurethane (^{3}H/^{14}C 1.28) established the presence of 12% of the tritium label at H-1re of the recovered farnesol, indicating that a portion of the DMAPP in the preparation of **1a** had been converted to (1*R*,5*R*,9*R*)-[1,5,9-^{3}H]farnesyl pyrophosphate by the combined action of endogenous DMAPP-IPP isomerase and prenyl transferase subsequently shown to be present in the crude pentalenene synthetase preparation. The proportion of tritium label at H-9re of the farnesyl pyrophosphate sample was therefore calculated to be 76%.
(18) Pentalenene synthetase, isolated as previously described,[19] was purified to a specific enzyme activity of 545 nmol/h/mg protein. For the preparative scale incubation, **1b** (0.40 nmol) was incubated for 1 h at 30 °C with 25 μg of pentalenene synthetase in 200 mM Tris, pH 8.4, containing 20 mM $MgCl_2$ and 5 mM β-mercaptoethanol.
(19) Cane, D. E.; Pargellis, C. *Arch. Biochem. Biophys.* **1987**, *254*, 421.
(20) Williard, P. G.; Sohng, J.-K.; Cane, D. E. *J. Antibiot.* **1988**, *41*, 130. Tillman, A. M.; Cane, D. E. *J. Antibiot.* **1983**, *36*, 170.
(21) Cane, D. E.; Rossi, T.; Tillman, A. M.; Pachlatko, J. P. *J. Am. Chem. Soc.* **1981**, *103*, 1838.
(22) Cane, D. E. *Acc. Chem. Res.* **1985**, *18*, 220.

Selective Binding of One Enantioface of Monosubstituted Alkenes to the Chiral Transition Metal Lewis Acid $[(\eta^5\text{-}C_5H_5)Re(NO)(PPh_3)]^+$

Gerardo S. Bodner, Jesús M. Fernández, Atta M. Arif, and J. A. Gladysz*

Department of Chemistry, University of Utah
Salt Lake City, Utah 84112
Received February 3, 1988

In recent years, dramatic advances have been made in methodology for the asymmetric hydrogenation and epoxidation of alkenes.[1,2] However, the best optical yields are obtained with functionalized alkenes that are capable of two-site binding to the reagent or catalyst. In the case of Rh(I)-catalyzed asymmetric hydrogenation, only alkenes that are substituted with polar groups, such as α-amino acrylic acid derivatives, are reduced in significant optical yields.[1] Similarly, Ti(IV)-catalyzed asymmetric epoxidation is most effective for allylic alcohols.[2] To our knowledge, no homogeneous binding agent exists that efficiently and predictably discriminates between the enantiofaces of simple monosubstituted alkenes $H_2C{=}CHR$.[3-5]

(1) This literature is extensive. For recent lead articles, see: (a) Landis, C. R.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1746. (b) Takaya, H.; Ohta, T.; Sayo, N.; Kumobayashi, H.; Akutagawa, S.; Inoue, S.-I.; Kasahara, I.; Noyori, R. *Ibid.* **1987**, *109*, 1596. (c) Alcock, N. W.; Brown, J. M.; Maddox, P. J. *J. Chem. Soc., Chem. Commun.* **1986**, 1532. (d) Zwick, B. D.; Arif, A. M.; Patton, A. T.; Gladysz, J. A. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 910. (e) Hayashi, T.; Kawamura, N.; Ito, Y. *J. Am. Chem. Soc.* **1987**, *109*, 7876.
(2) (a) Finn, M. G.; Sharpless, K. B. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1985; Vol. 5, Chapter 8. (b) Pedersen, S. F.; Dewan, J. C.; Eckman, R. R.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 1279.
(3) Consiglio has obtained quite high enantioface selectivities in the binding of monosubstituted alkenes to the ruthenium fragment $[(\eta^5\text{-}C_5H_5)Ru(L)(L')]^+$ (L, L' = chiral diphosphine), but propene and 3-methyl-1-butene appear to bind *opposite* faces, and structural characterization has not yet been possible: (a) Consiglio, G.; Pregosin, P.; Morandini, F. *J. Organomet. Chem.* **1986**, *308*, 345. (b) Consiglio, C.; Morandini, F. *Ibid.* **1986**, *310*, C66.

0002-7863/88/1510-4082$01.50/0