Bergamotene Biosynthesis and the Enzymatic Cyclization of Farnesyl Pyrophosphate

David E. Cane,* Douglas B. McIlwaine, and Paul H. M. Harrison

Department of Chemistry, Brown University Providence, Rhode Island 02912

Received September 26, 1988

Sesquiterpene synthetases catalyze the cyclization of farnesyl pyrophosphate (FPP, **1**), the universal biosynthetic precursor of the sesquiterpenes.[1] Ionization of the allylic pyrophosphate and intramolecular attack on the central or distal double bond, followed by further cyclization or rearrangement and elimination or capture of the carbocation by nucleophiles, gives rise to over 200 sesquiterpenes.[1]

The sesquiterpene *β-trans*-bergamotene (**2**) is the parent hydrocarbon of the ovalicin[2] and the fumagillin[3] family of antibiotics. Rearrangement of FPP to the tertiary allylic isomer nerolidyl pyrophosphate (**3**) and cyclization will give the bisabolyl cation **4**, which is proposed to undergo further cyclization and elimination to form the pinene type skeleton of **2**. We now report the identification of a new sesquiterpene cyclase, bergamotene synthetase, from a cell free preparation of *Pseudeurotium ovalis*, which catalyzes the cyclization of FPP to β-bergamotene. We have also shown that the cyclization proceeds with net retention of configuration at C-1 of FPP based on deuterium NMR analysis of β-bergamotene derived from both (1*R*)- and (1*S*)-[1-^{2}H]FPP.

Incubation of 2.02 μmol of [12,13-^{14}C]FPP (5 μM, 5.1 × 10^5 dpm/μmol)[4] with 190 mL of a cell-free extract from *P. ovalis*[5] and 210 mL of 50 mM, pH 7.2, phosphate buffer containing 1 mM EDTA, 10% glycerol, and 5 mM dithioerythritol (DTE) for 4 h at 30 °C followed by quenching with 100 mL of ethanol and extraction with pentane gave labeled bergamotene which was purified by flash chromatography after dilution with 5 mg of synthetic (±)-β-bergamotene.[7] The activity of the recovered bergamotene (1.18 × 10^5 dpm) corresponded to a turnover of 232 nmol of FPP.

To determine the location of the ^{14}C activity, the labeled bergamotene was diluted with additional carrier to a total of 245 mg and converted in 60% overall yield to the diastereomeric mixture of 10,11-diols **5** (Scheme I) by selective epoxidation with *m*-chloroperbenzoic acid, followed by treatment with 3% $HClO_4$. A portion of the diol mixture was derivatized as the corresponding bis-dinitrobenzoate ester **6** which was recrystallized from ethanol to constant specific activity (8.84 × 10^4 dpm/mmol). Cleavage of remaining **5** with $NaIO_4$ gave inactive aldehyde **7**[8] and acetone trapped as the semicarbazone derivative **8**. Recrystallization of **8** to constant specific activity (8.40 × 10^4 dpm/mmol) from 85% ethanol revealed that all the radioactivity resided in the terminal methyl groups as expected.

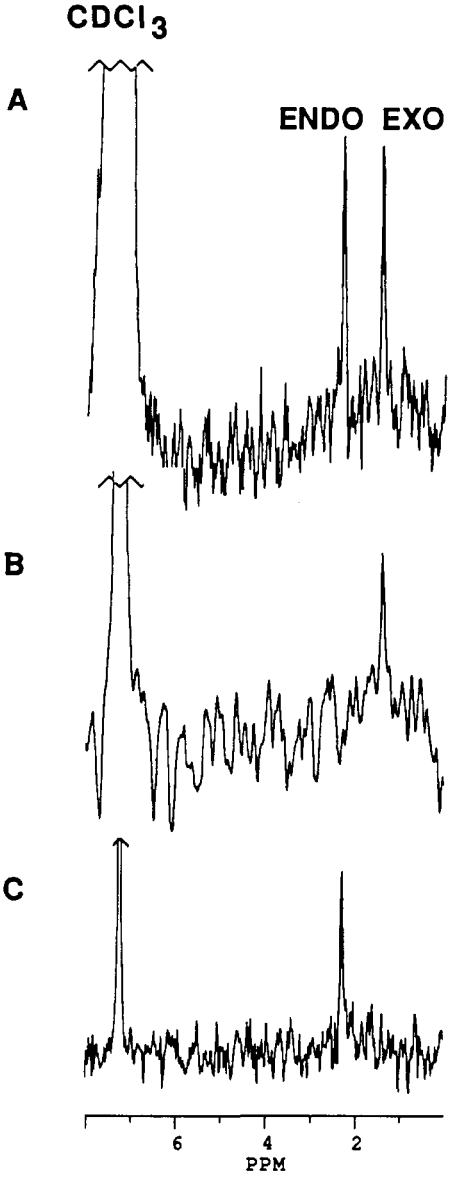
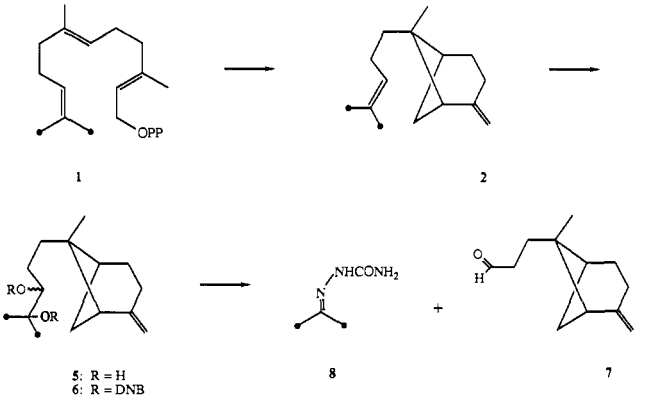


Figure 1. Deuterium NMR spectra (61.4 MHz) of β-bergamotene derived from (A) [1,1-$^2H_2$]FPP (**1a**), (B) (1*S*)-[1-^{2}H]FPP (**1b**), and (C) (1*R*)-[1-^{2}H]FPP (**1c**). Shifts are relative to natural abundance $CHCl_3$ at δ 7.26.

Scheme I



Having established the viability of the β-bergamotene synthetase preparation, we next examined the stereochemistry of the cyclization of FPP. The critical assignment of the ^{1}H NMR signals corresponding to the H-6 protons of **2** was achieved by a combination of ^{1}H-^{1}H COSY, ^{13}C-^{1}H heteronuclear shift correlation and NOE experiments. The signals at δ 2.31 (ddd, *J* = 5.5, 5.5,

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(2) (a) Cane, D. E.; King, G. S. S. *Tetrahedron Lett.* **1976**, 4737. (b) Cane, D. E.; McIlwaine, D. B. *Tetrahedron Lett.* **1987**, 6545.

(3) Nozoe, S.; Kabayashi, H.; Morisaki, N. *Tetrahedron Lett.* **1976**, 4625.

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(5) The cell free system was prepared from the mycelia obtained from 2.5 L of *P. ovalis* culture, grown as previously described[6] for 5 days. The cells were washed successively with 1.0 M KCl, 0.8 M NaCl, and potassium phosphate buffer (50 mM, pH 7.2) containing 1 mM EDTA, 10% glycerol, and 5 mM DTE and then resuspended in buffer and ruptured by rapid stirring for 6 min in 15-s on/off intervals with 0.5 mm glass beads in a 250 mL jacketed cell at 0 °C. The resultant slurry was centrifuged at 13000 g to remove glass beads and the supernatant (0.181 mg protein/mL) recentrifuged at 100000 g for 1 h at 4 °C to remove microsomal particulates.

(6) (a) Cane, D. E.; Levin, R. H. *J. Am. Chem. Soc.* **1975**, *97*, 1282. (b) Cane, D. E.; Levin, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 1183.

(7) (a) Corey, E. J.; Desai, M. C. *Tetrahedron Lett.* **1985**, 3535. (b) Snider, B.; Kulkarni, Y. S. *J. Org. Chem.* **1985**, *50*, 2809.

(8) Counted as its semicarbazone derivative.

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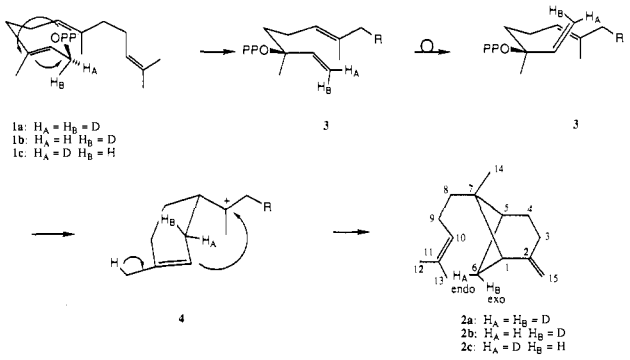
Scheme II



10 Hz) and δ 1.43 (d, J = 10 Hz) were assigned to H-6_{endo} and H-6_{exo}, respectively, based on the reported magnetic anisotropy of the puckered cyclobutane ring of the pinene skeleton.[9] In support of these assignments was the absence of vicinal coupling between H-6_{exo} and either H-1 or H-5, consistent with the 100° dihedral angle between the neighboring protons.[10] The assignments were confirmed unambiguously by the observed NOE enhancement of the δ 2.31 signal (H-6_{endo}) upon irradiation of the H-8 protons of the proximal side chain.

Incubation of [1,1-$^2H_2$]FPP (**1a**)[11] with crude bergamotene synthetase at 4 °C for 48 h yielded 110 nmol of β-bergamotene (**2a**, Scheme II) which, after dilution with 5 mg of (±)-β-bergamotene as carrier and purification by column chromatography, was analyzed by 61.4 MHz ^2H NMR revealing the expected signals at δ 1.41 and 2.30 (Figure 1A). Incubation of (1*S*)-[1-$^2H_1$]FPP (**1b**)[11] at 30 °C for 4 h with bergamotene synthetase yielded 92 nmol of β-bergamotene (**2b**). ^2H NMR analysis showed deuterium enrichment only in the H-6_{exo} position at δ 1.41 (Figure 1B). Finally, two incubations of (1*R*)-[1-$^2H_1$]FPP (**1c**)[11] yielded 146 nmol of β-bergamotene (**2c**) which showed deuterium enrichment only in the complimentary H-6_{endo} position at δ 2.30 (Figure 1C).

The above results clearly demonstrate that FPP can be converted into β-bergamotene by a cell-free preparation from *Pseudeurotium ovalis*, mediated by a new sesquiterpene cyclase, bergamotene synthetase. Further, the results show that this cyclization occurs with net retention of configuration of C-1 of FPP.[12] Similar results have also been obtained for the cyclization of FPP to the sequiterpene hydrocarbon trichodiene.[16] Since cyclization of FPP to form 6-membered rings requires initial isomerization to nerolidyl pyrophosphate (NPP, **3**) to avoid formation of a ring with a trans double bond,[1] present work is directed toward investigating the intermediacy of NPP in the cyclization of FPP to β-*trans*-bergamotene.

Acknowledgment. This work was supported by grants from the National Institutes of Health (GM22172 and GM30301). We would like to thank Dr. John S. Oliver for the preparation of (1*S*)-[1-^2H]farnesyl pyrophosphate.

A Dissociative Pathway for Equilibration of a Hydrido $CoL(H)^{2+}$ Complex with CO_2 and CO: Ligand-Binding Constants in the Macrocyclic [14]Dienecobalt(I) System

Carol Creutz,* Harold A. Schwarz,* James F. Wishart, Etsuko Fujita, and Norman Sutin

Chemistry Department, Brookhaven National Laboratory, Upton, New York 11973

Received September 27, 1988

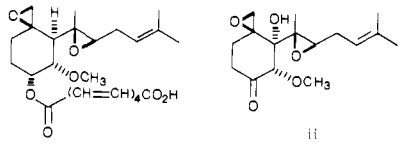
Metal hydride complexes are central to many catalytic processes, and their reactivity is of interest in its own right.[1] Metal carbon dioxide complexes and metallocarboxylates are often intermediates in CO_2 reduction and water-gas shift systems.[2] Despite the importance of these complexes, few thermodynamic and kinetic data for their formation exist. Here we report the results of pulse-radiolysis experiments yielding equilibrium and kinetic data for the binding of H^+,[3] CO_2,[4,5] and CO to the low-spin d^8 macrocyclic[6] cobalt(I) complex[7] CoL^+ in aqueous solutions. In the CoL^+ system (which has found application in both the photoreduction of water[8] to H_2 and electroreduction[9] of CO_2 to

(9) Saito, S.; Nakagawa, N.; Suzuki, A.; Itoh, M. *Tetrahedron Lett.* **1967**, 1003.

(10) The MacroModel structure of **2** was solved for the lowest energy conformation with a MM2 force field to give the dihedral angles H1,C1,C6,H-6_{exo} = 99.2°, and H5,C5,C6,H-6_{exo} = 100.7°.

(11) Harrison, P. H. M.; Oliver, J. S.; Cane, D. E. *J. Am. Chem. Soc.* **1988**, *110*, 5922.

(12) This conclusion is based upon the reasonable assumption that β-*trans*-bergamotene has the absolute configuration as illustrated, consistent with the known absolute configurations of fumigillin[13] (i) and ovalicin[14] (ii), the



demonstrated conversion of β-*trans*-bergamotene to ovalicin,[2b] and the expectation that the introduction of oxygen at C-1 of ovalicin proceeds with retention of configuration.[15] The absolute configuration of β-*trans*-bergamotene is currently under investigation.

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(2) (a) Eisenberg, R.; Hendrickson, D. E. *Adv. Catal.* **1979**, *28*, 79. (b) Sneeden, R. P. A. In *Comprehensive Organometallic Chemistry*; Wilkinson, G., Ed.; Pergamon Press: Oxford, 1982; Vol. 8, Chapter 50.4. (c) Ito, T.; Yamamoto, A. In *Organic and Bio-organic Chemistry of Carbon Dioxide*; Inoue, S., Yamazaki, N., Eds.; Wiley: New York, 1982; Chapter 3. (d) Darensbourg, D. J.; Kudaroski, R. A. *Adv. Organomet. Chem.* **1983**, *22*, 129. (e) Walther, D. *Coord. Chem. Rev.* **1987**, *79*, 135. (f) Inoue, S.; Koinuma, H. *Rev. Inorg. Chem.* **1984**, *6*, 291. (g) Palmer, D. A.; Van Eldik, R. *Chem. Rev.* **1983**, *83*, 651. (h) *Catalytic Activation of Carbon Dioxide*; Ayers, W. M., Ed.; ACS Symposium Series American Chemical Society: Washington, DC, 363; 1988. (i) Ford, P. C.; Rokicki, A. *Adv. Organomet. Chem.* **1988**, *28*, 139.

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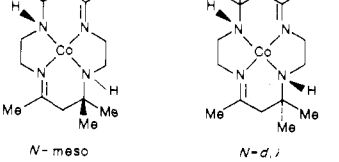
(6) (a) L = 5,7,7,12,14,14-$(CH_3)_6$-1,4,8,11-tetraazacyclotetradeca-4,11-diene. *N-d,l*-$CoL(H_2O)(ClO_4)_2$[6b-e] was prepared via published methods.



(b) Goedken, V. L.; Kildahl, N. K.; Busch, D. H. *J. Coord. Chem.* **1977**, *7*, 89. (c) A number of isomers are possible for ML complexes;[6d,e] in this work those arising from "primary" and "secondary" substitution of the "*d,l*" configuration are important. Primary denotes the face on which the 1,8-N-H protons are located; secondary, that on which the 7,14-axial CH_3 groups are found. (d) Warner, L. G.; Rose, N. J.; Busch, D. H. *J. Am. Chem. Soc.* **1968**, *90*, 6938; **1967**, *89*, 703. (e) Schwarz, C. L. Ph.D. Thesis, Wayne State University, 1988.

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