The mechanism of photooxidation of **1-3** can be compared to the well-studied oxidation of sulfides to sulfoxides with singlet oxygen.[10] Both persulfoxide and thiadioxirane intermediates have been proposed for this oxidation.[10a] Similar intermediates can be proposed, as shown in Scheme I, for the reaction of the tellurapyrylium dyes with singlet oxygen (with the initial oxidized intermediate reacting with unoxidized dye). The final photoproducts **6** and **7** are hydrated forms of telluroxides. The hydration of telluroxides to give dialkyl and diaryl dihydroxy telluranes has been described and would be expected to be rapid in aqueous solvent.[11] Values of $k(^1O_2)$ for **1** are very sensitive to water concentration, increasing from 8×10^6 M^{-1} s^{-1} in 99% methanol to 1.5×10^6 M^{-1} s^{-1} in 50% aqueous methanol to 1.1×10^9 M^{-1} s^{-1} in water. These values suggest that water is involved in the rate-determining step of the photooxidation perhaps by adding to an initial pertelluroxide or telluradioxirane intermediate.

The photooxidation products **6** and **7** have been detected in vitro in cell cultures treated with tellurapyrylium dyes **1** and **2** and light. Extraction of cell cultures treated first with **1** or **2** followed by washing and irradiation gives detectable amounts of **6** or **7** by absorption spectroscopy. Yellow-green intracellular fluorescence (emission maximum $\approx$ 530 nm) has been observed with an epifluorescent microscope in cells cultured on multichamber tissue culture slides and treated with **2**.[3] We are actively investigating the relationship between the solution photochemistry of tellurapyrylium dyes and phototoxicity in vivo and in vitro.

(10) (a) Liang, J.-J.; Gu, C.-L.; Kacher, M. L.; Foote, C. S. *J. Am. Chem. Soc.* **1983**, *105*, 4717 and references cited therein. (b) Kacher, M. L.; Foote, C. S. *Photochem. Photobiol.* **1979**, *26*, 765. (c) Akasaka, T.; Kako, M.; Sonobe, H.; Ando, W. *J. Am. Chem. Soc.* **1988**, *110*, 494 and references cited therein.

(11) Detty, M. R. *J. Org. Chem.* **1980**, *45*, 274.

Pentalenene Biosynthesis and the Enzymatic Cyclization of Farnesyl Pyrophosphate. Inversion at C-1 during 11-Membered-Ring Formation

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Farnesyl pyrophosphate (FPP, **1**) is the universal biosynthetic precursor of the sesquiterpenes, which encompass a wide variety of carbon skeletal types.[1] Current biogenetic theory holds that cyclizations of FPP proceed through carbocationic intermediates resulting from loss of pyrophosphate from C-1, followed by electrophilic attack on the central or distal double bond. Further cyclizations and/or hydrogen or alkyl migrations and elimination or capture of the resulting carbocation by nucleophiles gives rise to the natural sesquiterpenes.[1]

For sesquiterpenes derived from attack on the central double bond, initial isomerization of FPP to nerolidyl pyrophosphate (NPP, **2**) is required to avoid formation of 6-membered rings containing a trans double bond.[1-3] Considerable evidence has accrued to support this concept, including several studies of the overall stereochemistry of reaction at C-1 of both FPP[1,4,5] and geranyl pyrophosphate in analogous monoterpene cyclizations.[1,6]

(1) (a) Cane, D. E. *Acc. Chem. Res.* **1985**, *18*, 220. (b) Cane, D. E. *Tetrahedron* **1980**, *36*, 1109.

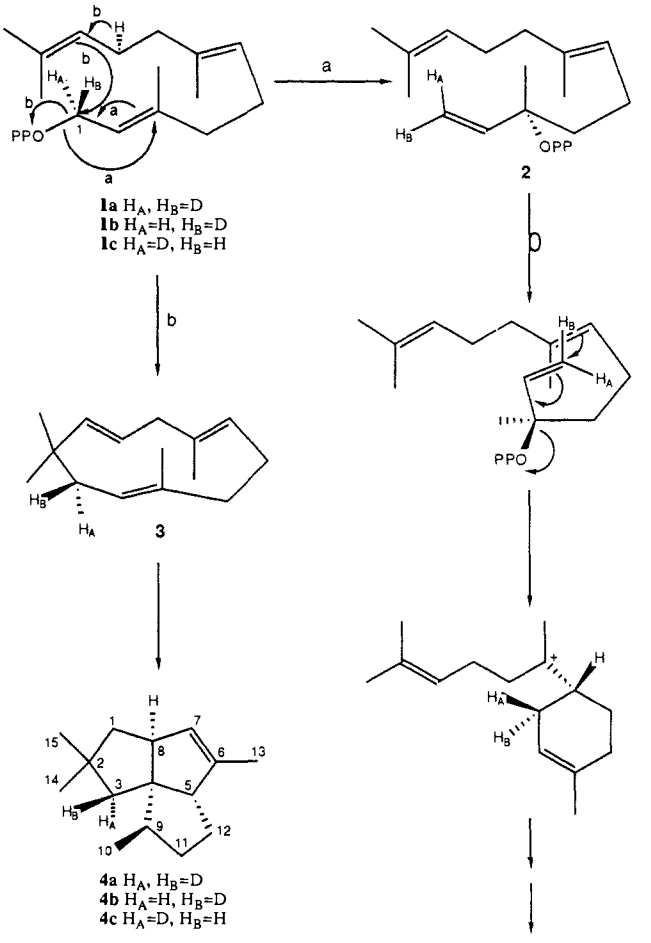
(2) Cane, D. E.; Iyengar, R.; Shiao, M.-S. *J. Am. Chem. Soc.* **1981**, *103*, 914.

(3) Cane, D. E.; Swanson, S.; Murthy, P. P. N. *J. Am. Chem. Soc.* **1981**, *103*, 2136.

(4) Cane, D. E.; Ha, H. J.; Pargellis, C.; Waldmeier, F.; Swanson, S.; Murthy, P. P. N. *Bioorg. Chem.* **1985**, *13*, 246.

(5) (a) Cane, D. E.; Ha, H. J. *J. Am. Chem. Soc.* **1986**, *108*, 3097. (b) Cane, D. E.; Abell, C.; Lattman, R.; Kane, C. T.; Hubbard, B. R.; Harrison, P. H. M. *J. Am. Chem. Soc.* **1988**, *110*, 4081.

Scheme I



In these cases, isomerization with a suprafacial 1,3-shift of the pyrophosphate group[2] must be followed by rotation about the C-2,3 bond in order to bring the two π systems (C-1,2 and C-6,7) into proximity for subsequent reaction (e.g., path a, Scheme I). This sequence results in displacement of pyrophosphate and formation of a new C-C bond with net retention of configuration at C-1 of FPP. There is no requirement for isomerization of the C-2,3 bond, however, when initial attack occurs at the C-10,11 double bond to generate, for example, humulene (**3**) (path b, Scheme I).[7,10,11] We now report direct proof of inversion at C-1 of FPP in the biosynthesis of pentalenene (**4**), the parent hydrocarbon of the pentalenolactone family of sesquiterpene antibiotics,[5b,12,13] based on deuterium NMR analysis of pentalenene derived from both (1*R*)- and (1*S*)-[1-^{2}H]FPP.

For the first experiment, [1,1-$^2H_2$;12,13-^{14}C]farnesyl pyrophosphate (**1a**)[14] was prepared by reduction of farnesal with

(6) Croteau, R.; Felton, N. M.; Wheeler, C. J. *J. Biol. Chem.* **1985**, *260*, 5956. Croteau, R. *Chem. Rev.* **1987**, *87*, 929.

(7) Isomerization is not required in the conversion of FPP to humulene[8] but there is strong indirect evidence for the intermediacy of NPP in formation of longifolene and sativene.[9] See: Croteau, R.; Cane, D. E. *Meth. Enzymol.* **1985**, *110*, 383.

(8) Croteau, R.; Gundy, A. *Biochem. Biophys.* **1984**, *233*, 838.

(9) Arigoni, D. *Pure Appl. Chem.* **1975**, *41*, 219.

(10) Hanson and co-workers have reported experiments that appear to support net inversion of configuration at C-1 of FPP in the biosynthesis of illudin M, based on an assumed retention of configuration during a subsequent oxidation: Hanson, J. R.; Marten, T.; Nyfeler, R. *J. Chem. Soc., Perkin Trans. 1* **1976**, 876. Cf.: Cane, D. E. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, pp 332-333.

(11) In the cyclization of FPP to longifolene via an initial 11-membered ring and sativene via an initial 10-membered ring, subsequent hydride shifts of the H-1 hydrogens precluded direct stereochemical analysis of the initial configurational changes at C-1.

(12) Cane, D. E.; Tillman, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 122.

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Table I. Carbon-13 and Proton Chemical Shifts for Pentalenene (4)

^{13}C δ (ppm)[a]	carbon	type[b]	^1H δ[c] (mult,J)[d]
140.57	6	C	
129.55	7	CH	5.15 (dqm, 1.9, 1.3)
64.73	4	C	
62.04	5[e]	CH	2.54 (br d, 8.9)
59.36	8[e]	CH	2.66 (m)
48.92	3[e]	CH_2 *re*	1.73 (dd, 12.9, <1)
		CH_2 *si*	1.35 (dd, 13.0, <1)
46.81	1[e]	CH_2 *re*	1.60 (ddd, 12.5, 9.1, 1.0)
		CH_2 *si*	1.17 (ddd, 12.6, 5.1, 0.7)
44.59	9	CH	1.84 (m)
40.51	2	C	
33.51	11[e]	CH_2	1.61 (m)
			1.27 (m)
29.94	14[f]	CH_3	0.98 (s)
29.11	15[f]	CH_3	0.99 (s)
27.59	12	CH_2	1.77 (m)
			1.33 (m)
17.01	10	CH_3	0.89 (d, 7.1)
15.50	13	CH_3	1.61 (m,small)

[a] 100.6-MHz ^{13}C NMR spectrum in $CDCl_3$ with solvent reference at 77.00 ppm. [b] Based on INEPT and $\{^1H\}$-^{13}C NOE. [c] 400-MHz ^1H NMR spectrum in $CDCl_3$ with internal TMS at 0.00 ppm. [d] Multiplicities (mult) and coupling constants (*J*, in Hz). [e] Assignments for these ^{13}C NMR signals differ from those of ref 13; see also ref 24. [f] These assignments may be reversed.

sodium borodeuteride, oxidation to [1-^{2}H]farnesal with MnO_2, and reduction with $NaBD_4$. Addition of [12,13-^{14}C]farnesol as an internal standard and pyrophosphorylation with the procedure of Poulter[15] gave **1a**. Incubation with a cell free preparation of pentalenene synthetase from *Streptomyces UC5319*[16] for 1 h at 25 °C gave 90 nmol of pentalenene (**4a**). The deuterium NMR spectrum of **4a**, isolated after addition of unlabeled carrier pentalenene and purification by silica gel chromatography, is shown in Figure 1A and demonstrates incorporation of deuterium at both H-3*re* and H-3*si*.

Assignments for the ^{13}C and ^1H signals of **4** were made from ^1H–^1H COSY,[17] ^1H–^{13}C heteronuclear shift correlation,[18] and NOE experiments (Table I). Most importantly, H-3*re* showed an NOE (4%) upon irradiation of CH_3-10, while H-3*si* exhibited an NOE (2%) upon irradiation of H-5. The observed NOE's are in accord with conformational analysis of **4** based on MM-2 calculations with the MacroModel program.[19]

Both (1*S*)- and (1*R*)-[1-^{2}H]FPP (**1b** and **1c**) were next prepared. For the (1*S*)-isomer, [1-^{2}H]farnesal was reduced with horse liver alcohol dehydrogenase (HLADH) and NADH;[20] [12,13-^{14}C]farnesol was added and the mixture pyrophosphorylated by using the method of Cramer and Böhm in order to preserve stereochemical integrity at C-1.[2,21] After addition of (1*RS*)-[1-^{3}H]FPP to aid in monitoring the purification, the resulting mixture of oligophosphates was separated by ion-exchange chromatography to afford **1b**. For the (1*R*)-isomer, farnesal was reduced with HLADH coupled with catalytic NAD^+ and [1-^{2}H]cyclohexenol.[4,22] Pyrophosphorylation with the same protocol as for **1b** gave **1c**. Both **1b** and **1c** were converted to pentalenene (**4b**, 585 nmol and **4c**, 170 nmol) by incubation with a cell free preparation of pentalenene synthetase[16] for 3 days at 4 °C.[23]

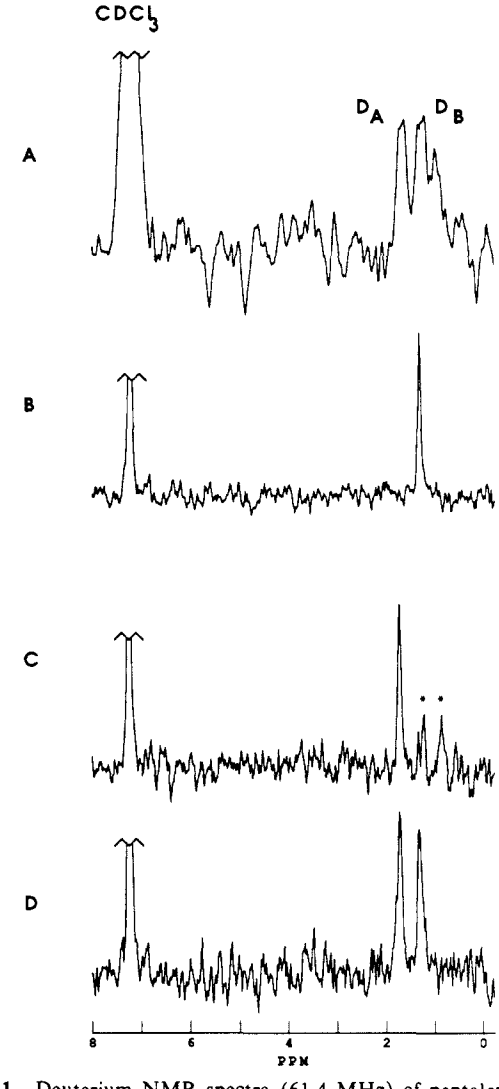

Figure 1. Deuterium NMR spectra (61.4 MHz) of pentalenene (**4**) derived from: A, [1,1-$^2H_2$]FPP (**1a**); B, (1*S*)-[1-^{2}H]FPP (**1b**); C, (1*R*)-[1-^{2}H]FPP (**1c**); and D, an equimolar mixture of **4b** and **4c**. Shifts are relative to natural abundance $CHCl_3$ at δ 7.24. Peaks marked with an asterisk are from natural abundance hexane and are removed after repeated concentration from $CHCl_3$ (see D).

The deuterium NMR spectra of the resulting samples of **4b** and **4c** are shown in spectra B and C in Figure 1. The results clearly show that the (1*S*)-isomer **1b** incorporates deuterium only into the 3*si* position of pentalenene (**4b**), while the (1*R*)-isomer **1c** gives **4c** with a single resonance corresponding to the 3*re* position. Therefore, inversion has occurred at C-1 during cyclization of farnesyl pyrophosphate to pentalenene. This result can be compared to prenyl transferase which can be viewed as the intermolecular analogue of terpenoid cyclases[1,24] and has been shown to catalyze bond formation with inversion at C-1 of the allylic pyrophosphate substrate.[25] Our results are completely consistent with the previously inferred *RSR*-CT conformation of the cyclizing substrate[1a,16a,26] and the intermediacy of the 11-membered-ring humulene in the enzymic cyclization.

Acknowledgment. This work was supported by a grant from the National Institutes of Health, GM22172.

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(15) Poulter, C. D.; Dixit, V. M.; Laskovics, F. M.; Noall, W. *J. Org. Chem.* **1981**, *46*, 1967.
(16) (a) Cane, D. E.; Abell, C.; Tillman, A. M. *Bioorg. Chem.* **1984**, *12*, 312. (b) Cane, D. E.; Pargellis, C. *Arch. Biochem. Biophys.* **1987**, *254*, 421.
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(18) Bax, A.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 501.
(19) The MacroModel structure of **4** was solved for the minimum energy conformation with a MM2 force field to indicate observable and unambiguous NOE's. Normal molecular models also clearly show that the observed NOE's are consistent only with the given assignment.
(20) 90 atom % D in the farnesol by ^1H NMR.
(21) Cramer, F.; Böhm, W. *Angew. Chem.* **1959**, *71*, 775.
(22) 99 atom % D in the farnesol by ^1H NMR.
(23) Control experiments showed that V_{max} at 4 °C was 0.25 that at 30 °C. However, enzyme half-life was extended from <30 min to >3 days by lowering the temperature, presumably due to retardation of proteases present in the preparation, thus allowing an increased yield of 4.
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