graphic comparison to the natural product establishes their identity. This synthesis provides further confirmation of the structural assignment which was based primarily upon spectral analysis.[2b]

The dramatic effect of an oxygen substituent on the regioselectivity of this Pd(2+)-catalyzed cyclization via isomerization indicates the complexity of the mechanism of this unusual reaction. What the role of oxygen may be can only be speculated upon until more definitive mechanistic work is available. Nevertheless, it appears that by use of proper substituents, either 1,3- or 1,4-dienes may be selectively available—a major broadening of the synthetic potential. This first short (eight steps from **8**) synthesis of an isolactarane, sterepolide, illustrates the potential that such a broadening can have. The applicability of this reaction toward developing effective approaches to a broad array of polyhydrindanes such as the illudanes, sterpuranes, and marasmanes[1] is a goal of future work.

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Supplementary Material Available: Characterization of **2**, **3**, **4a**, **5**, **6**, and **7** (3 pages). Ordering information is given on any current masthead page.

Stereochemistry of the Carbon to Carbon Bond Formation in the Biosynthesis of Polyprenyl Chains with *Z* Double Bonds. Studies with Undecaprenyl-Pyrophosphate Synthetase

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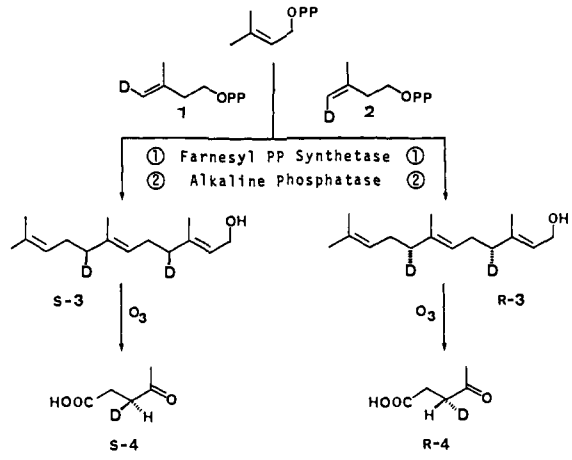
Since Cornforth, Popják, and their co-workers[1,2] established the stereochemistry of fundamental chain elongation processes involved in isoprenoid biosynthesis, the stereochemistry of the proton elimination from isopentenyl pyrophosphate (IPP) during the condensation with allylic pyrophosphates has been well documented with enzymes from various organisms.[3] However, nothing other than pig liver prenyltransferase catalyzing the synthesis of (*E*,*E*)-farnesyl pyrophosphate[2] has been studied in terms of stereochemical direction of the C–C bond formation with respect to the face of the double bond of IPP. Therefore, the stereochemistry of enzymatic C–C bond formation leading to a (*Z*)-prenyl chain has been a problem of particular interest to be solved. We here report evidence to show that allylic pyrophosphates add to the *si* face of IPP during the *Z* chain elongation catalyzed by undecaprenyl-pyrophosphate synthetase.

Cornforth et al.[2] have determined the steric course of farnesyl-pyrophosphate synthetase reaction by identifying the absolute configuration of [2-^{2}H]succinic acid derived from [4,8,12-$^2H_3$]-farnesol biosynthesized from mevalonic acid labeled chirally with deuterium at C-2. On the basis of their work, we examined a direct and feasible method of analysis for the stereochemistry of prenyltransferase reactions. The strategy was as follows: A prenyltransferase reaction with an allylic pyrophosphate and (*E*)-(**1**) or (*Z*)-[4-^{2}H]IPP (**2**) would give a product labeled chirally with deuterium in the prenyl moiety derived from **1** or **2**. Ozonolysis of this product would give (*S*)- or (*R*)-[3-^{2}H]levulinic acid, which could be correlated with a specimen derived from the chiral [4,8-$^2H_2$]farnesol [(*S*)-**3** or (*R*)-**3**] synthesized from dimethylallyl pyrophosphate (DMAPP) and **1** or **2** by the pig liver farnesyl-pyrophosphate synthetase reaction.[2]

Scheme I



Substrates **1** and **2** were synthesized by pyrophosphorylation of the corresponding alcohols obtained as follows: 3,4-Dibromo-3-methyl-1-butanol obtained by bromination of 3-methyl-3-buten-1-ol was dehydrobrominated with KOH to give (*E*)- and (*Z*)-4-bromo-3-methyl-3-buten-1-ol, which were separated from each other by silica gel chromatography. The bromo derivatives were converted into the corresponding lithium compounds, which were then quenched with ^2H_2O to give (*E*)- and (*Z*)-[4-^{2}H]-3-methyl-3-buten-1-ol. The NMR spectra of the *E* and *Z* isomer showed absorptions for the olefinic protons at δ 4.70 (s) and 4.74 (s), respectively. The deuterium contents of these alcohols were both 93% as determined by mass spectrometry.

In order to clarify the relation between the configuration of [3-^{2}H]levulinic acid and its optical rotation, (*R*)- and (*S*)-[3-^{2}H]levulinic acids were synthesized as shown in Scheme I by large-scale incubations as follows: Two flasks, each containing, in a final volume of 1 L, 20 mmol of Tris-HCl buffer, pH 7.7, 10 mmol of $MgCl_2$, 7 mmol of 1,4-dithiothreitol, 300 μmol of DMAPP, 300 μmol of **1**, and 100-mg protein of pig liver farnesyl-pyrophosphate synthetase[4] were incubated at 37° C for 24 h and then treated with alkaline phosphatase as usual. The reaction mixtures were extracted with pentane, and the extracts were purified by silica gel chromatography to give 17.8 mg of (4*S*,8*S*)-[4,8-$^2H_2$]farnesol [(*S*)-**3**, $[\alpha]_{320}$ + 3.0 ± 1.3°, ^{2}H content 92%], yield 26.0% based on **1**. (4*R*,8*R*)-[4,8-$^2H_2$]Farnesol [(*R*)-**3**, $[\alpha]_{320}$ −3.8 ± 0.5°, ^{2}H content 92%] was also obtained by similar incubations using **2** in place of **1**, yield 15.6 mg, 48% based on **2**. The ozonolysis of (*S*)-**3** and (*R*)-**3** under conditions similar to those reported[2] gave (*S*)-[3-^{2}H]levulinic acid [(*S*)-**4**, ^{2}H content 92%, $[\alpha]_{320}$ +63 ± 17°], and the *R* isomer [(*R*)-**4**, ^{2}H content 92%, $[\alpha]_{320}$ −82 ± 6°], respectively. Thus, the former and the latter were found distinguishable from each other, showing positive and negative Cotton effects, respectively.

Then this method was applied to the stereochemical analysis of undecaprenyl-pyrophosphate synthetase reaction. Five flasks, each containing in 1 L, 100 mmol of Tris-HCl buffer (pH 8.5), 500 μmol of $MgCl_2$, 50 mmol of NH_4Cl, 5 g of Triton X-100, 25 μmol of (*E*,*E*)-farnesyl pyrophosphate, 100 μmol of **1**, and partially purified undecaprenyl-pyrophosphate synthetase obtained from 25 g of *Bacillus subtilis*,[5] were incubated at 37 °C for 24 h. The products were treated with acid phosphatase by the method of Fujii et al.,[6] and the hexane extracts were purified on TLC and

(1) Cornforth, J. W.; Cornforth, R. H.; Donninger, C.; Popják, G. *Proc. R. Soc. London, Ser. B* **1965**, *163*, 492–514.
(2) Cornforth, J. W.; Cornforth, R. H.; Popják, G.; Yengoyan, L. *J. Biol. Chem.* **1965**, *241*, 3970–3987.
(3) Poulter, C. D.; Rilling, H. C. In "Biosynthesis of Isoprenoid Compounds"; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; p 165.
(4) Holloway, P. W.; Popják, G. *Biochem. J.* **1967**, *104*, 57–70.
(5) Takahashi, I.; Ogura, K. *J. Biochem. (Tokyo)* **1982**, *92*, 1527–1537.
(6) Fujii, H.; Koyama, T.; Ogura, K. *Biochim. Biophys. Acta* **1982**, *712*, 716–718.

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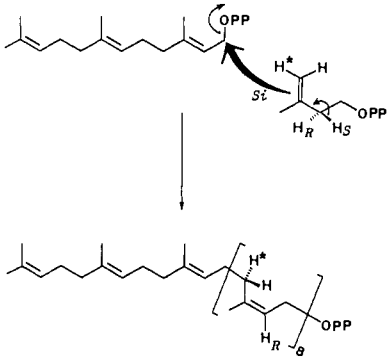
Scheme II



HPLC to give 5.2 mg of [4,8,12,16,20,24,28-$^2H_7$]-(*E,E*)-farnesyl-(*all-Z*)-heptaprenol (decaprenol), $[\alpha]_{320}$ + 5.9 ± 1.2°, ^2H content 93%, and 14.2 mg of [4,8,12,16,20,24,28,32-$^2H_8$]-(*E,E*)-farnesyl-(all-*Z*)-octaprenol (undecaprenol), $[\alpha]_{320}$ +6.3 ± 0.5°, ^2H content 93%. These polyprenols were combined and subjected to ozonolysis. The levulinic acid thus obtained showed a positive Cotton curve ($[\alpha]_{320}$ +42 ± 14°, $[\theta]_{280}$ +81 ± 9°), indicating that the configuration was *S*. Conversely, (*R*)-[3-^2H]levulinic acid ($[\alpha]_{320}$ −51 ± 14°, $[\theta]_{280}$ −74 ± 14°) was obtained by similar experiments using **2** in place of **1**. Therefore, it was evidenced that the C–C bond formation took place at the *si* face of the double bond of IPP.

The present observations, taken in conjunction with the previous finding that the 2-*pro-S* hydrogen of IPP is eliminated during the undecaprenyl-pyrophosphate synthetase reaction,[5] indicate that the stereochemical course of the condensation forming *Z* double bonds is similar to that of (*E,E*)-farnesyl-pyrophosphate synthetase reaction in that the C–C bond is formed on the same side as the C–H bond to be cleaved (Scheme II).

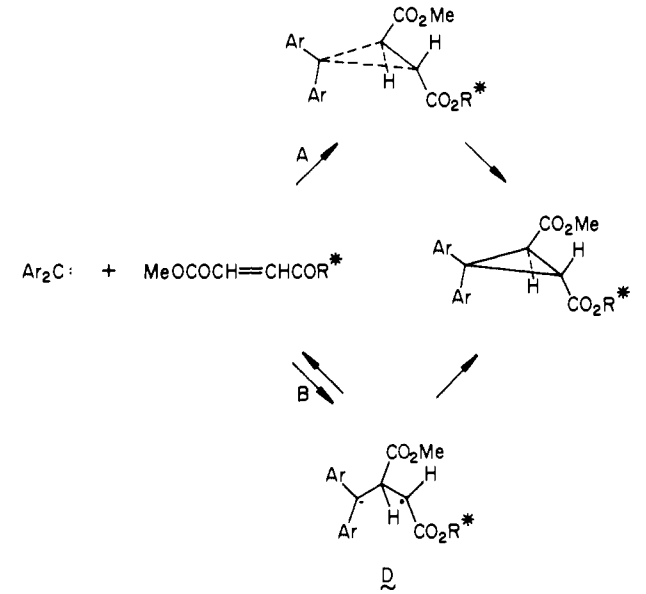


Figure 1. Synchronous vs. asynchronous pathway.

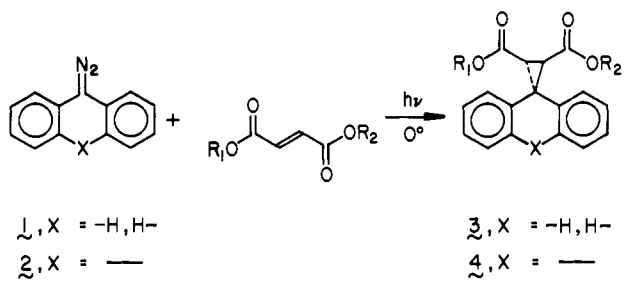


Figure 2. Products of diaryldiazomethane photoinduced cyclopropanation.

Asymmetric Induction in Diarylcarbene Cyclopropanations

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The mechanism of addition of carbenes to olefins is a subject of historical and current interest.[2] These mechanisms range from a concerted one involving a symmetric transition state (path A of Figure 1) to a stepwise one involving either prior complex formation or diradical formation (path B of Figure 1). We have developed a direct method for distinguishing between synchronous and asynchronous mechanisms based upon a geometric relationship between the asymmetric induction produced when a reacting partner bears one or two chiral prosthetic groups.[3] We now report that application of this method to cyclopropanation of fumarate esters by (triplet) fluorenylidene and diphenylmethylene implicates a nonconcerted mechanism involving a reversible first step.

The substrates in our reactions were dimethyl, methyl 1-bornyl, and di-1-bornyl fumarate. Our principle of cooperativity[3a] requires that the asymmetric ratio produced by the bischiral fumarate be the square of the monochiral ratio for the synchronous pathway (path A). When 0.031 M acetonitrile solutions of each fumarate containing either diazofluorene or diazodiphenylmethane maintained at 0 °C were irradiated using 0.1 M K_2CrO_4 filtered Hanovia light, diarylcyclopropane dicarboxylates were formed in 20–35% yield (see Figure 2), as well as unidentified polar products. The 1-bornyl esters were hydrolyzed in aqueous HCl and reesterified with diazomethane to yield the known dimethyl 3,3-diarylcyclopropane-1,2-dicarboxylates.[4] The enantiomeric ratios determined by NMR using the chiral shift reagent tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium(III) are reported in Table I. With both diaryl carbene precursors, the asymmetric induction produced with two chiral groups was nearly the same as that produced with one. Experimentally identical asymmetric yields were obtained when the fumarate concentration was reduced by a factor of 20. In a control experiment, an unirradiated solution of diazofluorene and di-1-bornyl

Table I. Asymmetric Ratios

cyclopropane-dicarboxylate	diazofluorene	diazodiphenyl-methane
dimethyl	1.00 ± 0.05	1.00 ± 0.04
methyl 1-bornyl	1.26 ± 0.06	1.49 ± 0.07
di-1-bornyl (exptl)	1.30 ± 0.05	1.56 ± 0.07
di-1-bornyl (predicted)	1.59 ± 0.10	2.43 ± 0.14
di-1-bornyl (unirrad)	1.00 ± 0.05	

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(2) (a) Eisenthal, K. B.; Moss, R. A.; Turro, N. J. *Science (Washington, D.C.)* **1984**, *225*, 1439. (b) Tomioka, H.; Hayashi, N.; Izawa, Y.; Liu, M. T. H. *J. Am. Chem. Soc.* **1984**, *106*, 454. (c) Tomioka, H.; Ohno, K.; Izawa, Y.; Moss, R. A.; Munjal, R. C. *Tetrahedron Lett.* **1984**, 5415. (d) See also: Warner, P. M. *Ibid.* **1984**, 4211.

(3) (a) Tolbert, L. M.; Ali, M. B. *J. Am. Chem. Soc.* **1981**, *103*, 2104. (b) Tolbert, L. M.; Ali, M. B. *Ibid.* **1982**, *104*, 1742. (c) Tolbert, L. M.; Ali, M. B. *Ibid.* **1984**, *106*, 3804.

(4) (a) *trans*-2,3-Dicarbomethoxyspiro[cyclopropane-1,9′-fluorene]: ^1H NMR ($CDCl_3$) δ 7.26–8.00 (m, 8 H), 3.60 (s, 6 H), 3.43 (s, 2 H); mp (uncorr) 137.0–139.0 °C (lit.[4b] mp 135.5–136 °C). Dimethyl *trans*-3,3-diphenyl-1,2-cyclopropanedicarboxylate: ^1H NMR ($CDCl_3$) δ 7.13–7.50 (m, 10 H), 3.43 (s, 6 H), 3.23 (s, 2 H); mp (uncorr) 179.5–181.0 °C (lit.[4c] mp 174–174.5 °C). (b) Braun, A. M.; Cassidy, H. G.; Schulz, R. C.; Tanaka, H. *Makromol. Chem.* **1971**, *146*, 195. (c) Jones, W. M. *J. Am. Chem. Soc.* **1959**, *81*, 3776.

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