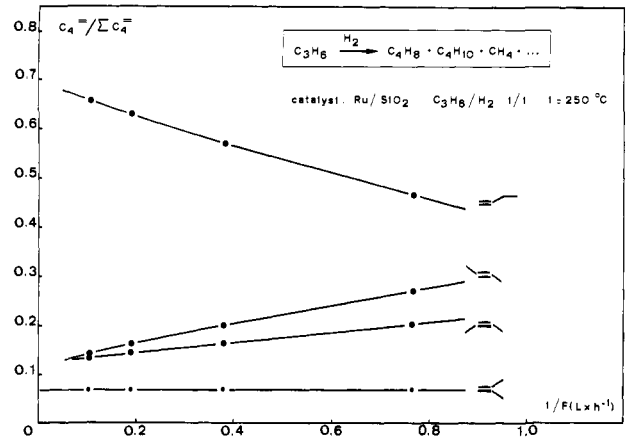

Figure 3. Product distribution in the C_4 olefinic compounds as a function of contact time (1/flow rate) in $C_3H_6 + H_2$ reaction (p = 1 atm) (catalyst 150 mg).

Table I. Isobutene/*n*-Butene Ratios (at 0% Conversion) in CO + H_2 Reaction and $C_3H_6 + H_2$ Reaction with Fe/SiO_2, Ru/SiO_2, and Os/SiO_2 Catalysts

		isobutene/*n*-butene ratios (1 atm)	
catalyst	temp, °C	$CO/H_2 = 1/1$	$C_3H_6/H_2 = 1/1$
Fe/SiO_2	250	0.10 ± 0.01	0.09 ± 0.01
	300	0.13 ± 0.01	0.15 ± 0.01
Ru/SiO_2	250	0.06 ± 0.01[a]	0.08 ± 0.01[b]
Os/SiO_2	250	0.05 ± 0.02	0.08 ± 0.01

[a] The same value has been obtained with the CO/H_2 ratios of 1.5/1, 1/1, and 1/2. [b] The same value has been obtained with the C_3H_6/H_2 ratios of 2/1, 1/1, and 1/2.

noted (1-butene → 2-butene) (Figures 2 and 3).

For a given catalyst and a given temperature, the iso/*n* ratios of C_4 olefinic hydrocarbons at zero conversion are the same for $CO + H_2$ reaction and $C_3H_6 + H_2$ reaction (within exprimental error) (Table I).

One should also notice that both reactions exhibit a high initial selectivity for α olefins. There are some differences in the cis-trans ratio of 2-butenes and in the initial selectivity for α olefins between Figures 2 and 3. These differences may be due to the presence of CO in the syn gas reaction that competes with the olefin for coordination to the surface and modifies the overall kinetics of double-bond migration and cis-trans isomerization. Apparently, the presence of CO does not modify significantly the kinetic parameters that govern the formation of branched olefin.

It appears from the above results (carbon number distribution and initial selectivity) that the mechanism of C–C bond formation is the same in syn gas reaction and olefin homologation on Fe-, Ru-, and Os/SiO_2 catalysts. The C–C bond formation does not require the presence of molecular CO in agreement with Pettit and co-workers,[1] Biloen,[6] Bell,[7] and others.[8] Our results, along with those of Pettit[1] indicate the same mechanism of C–C bond formation starting from $CO + H_2$, $CH_2N_2 + H_2$, or $C_nH_{2n} + H_2$. Besides we show here that this mode of C–C bond formation involves the addition of a C_1 fragment to a C_n fragment. These results do not demonstrate whether the mechanism for chain growth involves an insertion of a carbene to a metal alkyl or the coupling of a carbene to an olefin. It also does not enlighten on the mechanisms of formation of oxygenated compounds where the support may play an important role.

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Registry No. Ethene, 74-85-1; propene, 115-07-1; 1-butene, 106-98-9; 2-methyl-1-propene, 115-11-7; 3,3-dimethyl-1-butene, 558-37-2; (*Z*)-2-butene, 590-18-1; iron, 7439-89-6; ruthenium, 7440-18-8; osmium, 7440-04-2; rhodium, 7440-16-6; carbon monoxide, 630-08-0.

Enzymatic Cyclization of [1-^{18}O]Geranyl Pyrophosphate to *l-endo*-Fenchol

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The recent discovery that enzyme systems isolated from sage (*Salvia officinalis*) and from tansy (*Tanacetum vulgare*) will catalyze the conversion of geranyl pyrophosphate (**1**) to the *d* and *l* enantiomers, respectively, of 2-bornyl pyrophosphate (**2**)[2] has already led to several critical insights into the mechanism of terpenoid cyclization reactions.[3] The facts that neither enzyme requires redox cofactors and that, in the absence of competing phosphatases and pyrophosphatases, both geranyl pyrophosphate and its tertiary allylic isomer, linalyl pyrophosphate (**3**), are strongly preferred as substrates over the corresponding cis allylic pyrophosphate, neryl pyrophosphate, are fully consistent with the simple stereochemical model illustrated in Scheme I. The latter model, based on the generation of a series of ion-paired intermediates, is completely supported by the results of numerous model studies of terpenoid cyclizations.[4] On the basis of this model, we have recently investigated the role of the pyrophosphate moiety in the formation of bornyl pyrophosphate and have reported the unexpected observation that the original pyrophosphate ester oxygen of geranyl pyrophosphate is the exclusive source of the corresponding pyrophosphate ester oxygen of the *d*- or *l*-bornyl pyrophosphate product.[5] The lack of detectable positional isotope exchange implies a remarkably tight restriction on the motion of the transiently generated pyrophosphate anion during the cyclization process. We now report the extension of our studies to the enzymatic formation of *l-endo*-fenchol (**4**) and present results that further illustrate the generality of our proposed stereochemical model.

Cell-free extracts of fennel (*Foeniculum vulgare*) have previously been shown to contain a cyclase that catalyzes the conversion of geranyl pyrophosphate to *l-endo*-fenchol (**4**).[6] In spite of numerous efforts to obtain a phosphatase-free preparation of this cyclase, these and all other attempts to detect the corre-

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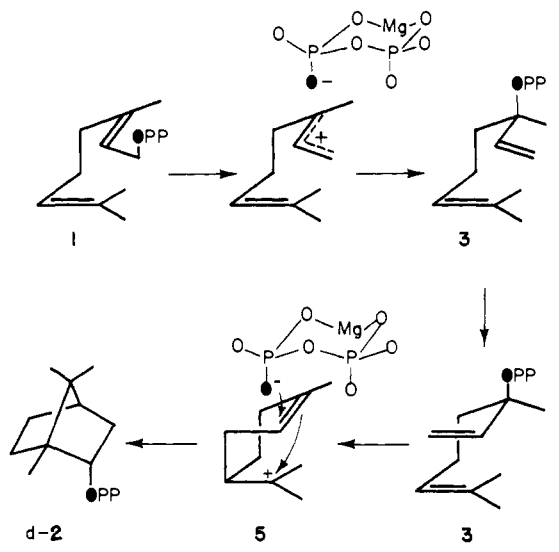
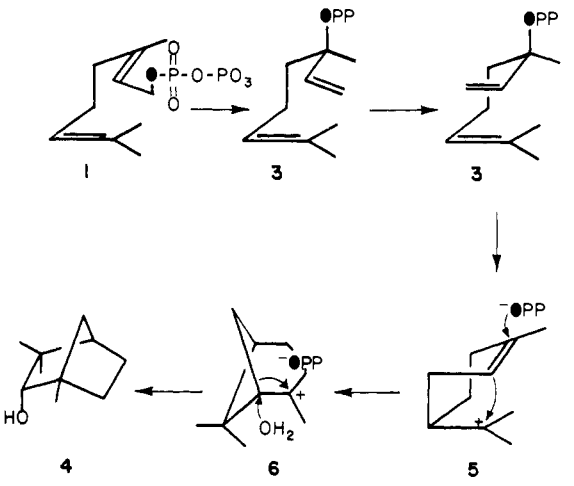
Scheme I



Scheme II



sponding *l-endo*-fenchyl pyrophosphate ester were without avail. The reasons for this failure become apparent, however, when the proposed stereochemical cyclization model is applied to the formation of *l-endo*-fenchol. As illustrated in Scheme II, cyclization of the initially generated (4*R*)-α-terpinyl cation (**5**) is expected to yield *exo*-2-pinanyl pyrophosphate (**6**) or the corresponding ion-paired intermediate. Formation of the fenchyl skeleton from **6** requires a 1,2-shift of the C-7 bridging carbon atom with concomitant displacement of the pyrophosphate moiety. It is evident that the endo face of the resulting 2-fenchyl cation is completely inaccessible to the pyrophosphate anion, which is now situated anti to the cationic center as a result of the Wagner–Meerwein rearrangement. Instead, capture of the fenchyl cation by water should lead directly to the observed *l-endo*-fenchyl product. We have now tested these ideas by incubation of [1-^{18}O]geranyl pyrophosphate with a cell-free preparation of fenchyl synthetase followed by analysis of the ^{18}O content of the resulting *l-endo*-fenchol.

For preparative-scale experiments with *l-endo*-fenchol cyclase, 105 000g supernatants from whole-leaf homogenates of fennel were prepared as previously described,[6] treated with XAD-4 polystyrene resin to remove endogeneous terpenoids,[7] and then dialyzed before use against 5 mM barbital sodium acetate, pH 6.95, containing 10% glycerol, 1.0 mM dithiothreitol, 2.0 mM sodium ascorbate, and 1.0 mM sodium molybdate (as a phosphatase inhibitor). Aliquots (1.0 mL, containing ca. 1 mg of protein) were incubated in screw-capped vials at 30 °C for 3 h after the addition of 1 mM $MnCl_2$ and 1.5×10^{-5} M [1-^{18}O,8,9-^{14}C]geranyl pyrophosphate (68.2 ± 4.4% ^{18}O atom, 3.55×10^4 dpm/μmol.[5] At the end of the incubation period, fenchol was extracted from the NaCl-saturated reaction mixture with redistilled pentane (2 × 1 mL), and the pooled, concentrated extract was benzoylated.[8]

A total of 3.88 μmol of [1-^{18}O,8,9-^{14}C]geranyl pyrophosphate was processed over a period of several months by a series of such incubations, resulting in the eventual isolation of ca 0.11 μmol of crude fenchyl benzoate. After preliminary purification by preparative thin-layer chromatography, the fenchyl benzoate was subjected to repeated purification by HPLC (μPorasil; 0.4% Et_2O/pentane, v/v; $k' = 2.7$), resulting in the recovery of 2.7 μg (10.3 nmol) of *l-endo*-fenchyl benzoate (1.27×10^4 dpm/μmol).[9] Analysis of the parent (*m/e* 258) and P + 2 (*m/e* 260) ions by GC–mass spectrometry[10] and comparison with the corresponding peak intensities of an unlabeled reference sample of *l-endo*-fenchyl benzoate recorded under identical conditions established that the enzymatically derived fenchyl benzoate contained 0 ± 1% ^{18}O atom.

The complete *absence* of ^{18}O in the fenchol product contrasts with the complete *retention* of ^{18}O in the *d*- and *l*-bornyl products. These divergent results are nonetheless fully consistent with the unified stereochemical model summarized in Schemes I and II. Since the minimum ^{18}O content compatible with recapture of a fully scrambled pyrophosphate moiety, corrected for dilution by endogeneous, unlabeled material, would have been 4% (68.2% × (1.27/3.55)/6), these results establish unambiguously that the alcohol oxygen atom of *l-endo*-fenchol is not derived from the pyrophosphate moiety of the geranyl pyrophosphate precursor.[11] Recognizing that all phosphatases that have been examined to data have been shown to hydrolyze phosphate esters with P–O bond cleavage,[12] fenchyl pyrophosphate is thus excluded as an intermediate in the formation of *l-endo*-fenchol.

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(9) To determine the specific activity of the purified fenchyl benzoate, the sample was first dissolved in 1.00 mL of 0.4% Et_2O/pentane, v/v. Several aliquots (10–40 μL) of this solution were analyzed by HPLC (254-nm UV detector) under the conditions described in the text, and the fenchyl benzoate concentration was calculated by comparison of the resulting peak heights with those of a series of fenchyl benzoate standards of known concentration. Control experiments showed this method to be reproducible within ±5%. A 0.30-mL aliquot (3.1 nmol) of the same solution counted by liquid scintillation spectrometry (23X, 100 min each) showed 53.4 ± 0.2 dpm ^{14}C (61.2% efficiency, quench corrected). After subtraction of 15.3 ± 0.5 dpm due to background, the measured activity corresponded to 38.1 ± 0.6 dpm.

(10) GC–MS analyses were performed on a tandem Hewlett-Packard 5840A-5985B System using a 25-m fused-silica capillary column coated with OV-1 and run isothermally at 185 °C. Spectra were obtained at 20 eV with an accelerating voltage of 2200 V. Each gas chromatographic trace was scanned at 0.03-min intervals. In a typical run, at least four scans bracketing the retection time for fenchyl benzoate, 3.86 ± 0.06 min, were selected, the peak intensities for *m/e* 258 and 260 were measured and corrected for background contribution, and the ratio 260/(258 + 260) was calculated. As a control, the ratio (258 + 260)/105 (base peak) was also calculated and compared with the corresponding ratio determined on unlabeled reference fenchyl benzoate. Finally, each complete GC–MS analysis was repeated several times with consistent results.

(11) A referee has suggested that the absence of excess ^{18}O in the recovered fenchol might be due to in situ equilibration with fenchone catalyzed by the crude enzyme preparation and exchange of the latter substrate with water. This explanation is ruled out on two grounds: (1) the enzyme preparation does not contain the requisite redox coenzymes, having been treated with XAD-4 resin and subjected to dialysis before each incubation; (2) ketones exchange oxygen with water at a significant rate only under acidic or basic catalysis or at elevated temperatures. For example, acetone does not show detectible exchange after 24 h at 25 °C (Cohn, M.; Urey, H. C. *J. Am. Chem. Soc.* **1938**, *60*, 679. See also: Hutchinson, C. R.; Sherman, M. M.; Vederas, J. C.; Nakashima, T. T. *Ibid.* **1981**, *103*, 5953).

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