explains why this exchange was not observed earlier.[17,18] The possibility of an oxygen exchange was also investigated by other authors in the vanadium(V) hydroperoxide system,[19] but no evidence of it was found. However, in this case the peroxo species cannot have the cyclic side-bonded structure and hence the oxygen exchange would have to take place by a different route.

The understanding of the reaction implied by the findings reported herein will certainly require further experimental work; however, the unveiling of this fairly easy oxygen exchange of hydrogen peroxide calls for caution in interpreting studies both in chemistry and in biochemistry.

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Farnesylpyrophosphate Synthetase. A Stepwise Mechanism for the 1'-4 Condensation Reaction[1,2]

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Farnesylpyrophosphate synthetase (EC 2.5.1.1) is a key enzyme in the biosynthetic pathways for several important classes of terpenes, including sterols, dolichols, and possibly ubiquinones.[3,4] It catalyzes the 1'-4 condensation[5] between isopentenyl-PP (**1**-PP) and dimethylallyl-PP (**2**-PP) or geranyl-PP (**3**-PP), reactions typical of the sequential five-carbon polymerizations that constitute the major building steps in terpene metabolism. While conducting experiments with farnesylpyrophosphate synthetase that established the electrophilic nature of the condensation reactions,[3,6,7] we became interested in the timing of the changes in covalent bonding that occur during the (a) ionization, (b) condensation, and (c) elimination phases of the reaction (Scheme I).

It is commonly assumed that the topology of the substrates in the E·S complex is optimal for coupling of **1**-PP with the hydrocarbon moieties of **2**-PP or **3**-PP.[3] In such an orientation, the π electrons of the C(3)-C(4) double bond in **1**-PP are, coincidentally, in a suitable position to assist with heterolysis of the carbon-oxygen bond during catalysis. Several studies have shown, however, that the extent to which π electrons participate during ionization depends on the demand for stabilization by the developing electron-deficient center[8] and the availability of the electrons in the neighboring group,[9-13] even when the two groups are properly positioned. In addition, two phenomena are associated with participation by π electrons. First, a threshold exists for participation by the neighboring group, and below this threshold, anchimeric assistance of the ionization step is not observed.[8] Second, above the threshold, the electron-deficient center is subtantially *less* sensitive to substituent effects than a corresponding system where participation cannot occur.[8] In this communication we report the results of a linear free energy study with farnesylpyrophosphate synthetase which indicates that cleavage of the carbon-oxygen bond in **3**-PP is a discrete step,

Scheme I. Reorganization of Covalent Bonds during 1'-4 Condensation

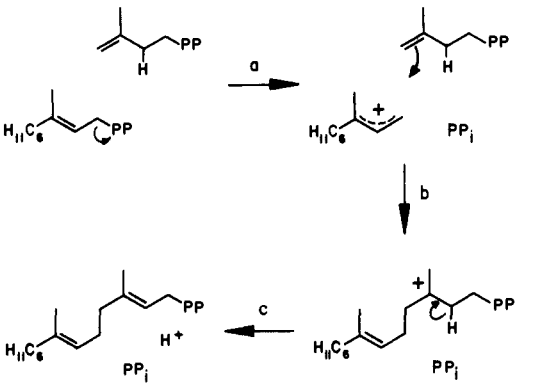

Table I. Kinetic Constants[a] for 1'-4 Condensation of 1-PP with **3**-PP, **5**-PP, **6**-PP, and **7**-PP

allylic substrate	$V/V^{3\text{-PP}}$	$k_s/k_s^{3\text{-PP}}$	$K_M^{1\text{-PP}}$, μM
3-PP	1	1	0.45 ± 0.05
5-PP	1.75×10^{-2}	3.72×10^{-4}	0.35 ± 0.1
6-PP	1.90×10^{-6}	4.03×10^{-8}	0.56 ± 0.3
7-PP	3.62×10^{-7}	7.70×10^{-9}	0.20 ± 0.1

[a] Measured at 37 °C in 10 mM Pipes, 1 mM $MgCl_2$, 10 mM β-mercaptoethanol, 0.1 μM NaN_3, 0.1% BSA, pH 7.0.

Table II. First-Order Rate Constants for Solvolysis of **3**-Ms, **5**-Ms, **6**-Ms, and **7**-Ms[a]

reactant	T, °C	acetone/H_2O (v/v), %	k, s^{-1}	$k/k^{3\text{-Ms}}$
3-Ms	0	90	$1.46 \pm 0.18 \times 10^{-3}$	
	25	90	$2.57 \pm 0.14 \times 10^{-2}$	
	60	90	0.74[b]	1
5-Ms	60	90	$5.68 \pm 0.29 \times 10^{-4}$	7.7×10^{-4}
6-Ms	60	40	$7.31 \pm 0.39 \times 10^{-4}$	
	60	50	$2.90 \pm 0.07 \times 10^{-4}$	
	60	90	1.60×10^{-6} [c]	2.2×10^{-6}
7-Ms	60	40	$1.19 \pm 0.32 \times 10^{-4}$	
	60	50	$4.78 \pm 0.45 \times 10^{-4}$	
	60	90	2.95×10^{-7} [d]	4.0×10^{-7}

[a] Measured by the conductance method and analyzed by curve fitting with the nonlinear least-squares procedure of Powell and MacDonald: Powell, D. R.; MacDonald, J. R. *Comput. J.* **1977**, *15*, 148-158. [b] Extrapolated from lower temperatures, $\Delta H^{\ddagger} = 18.6$ kcal/mol, $\Delta S^{\ddagger} = -3.5$ eu. [c] Extrapolated from 40% and 50% acetone/H_2O using the mY correlation of Winstein and Grunwald; $m = 0.69$: Fainberg, A. H.; Winstein, S. *J. Am. Chem. Soc.* **1956**, *78*, 2770-2777. [d] Extrapolated from 40% and 50% acetone/H_2O; $m = 0.68$.

[†] National Institutes of Health Research Career Development Awardee, 1975-1980 (HL-00084).
[‡] National Science Foundation undergraduate research participant.
(1) Support of this research by the National Institutes of Health, GM-21328 and RR-07092, is gratefully acknowledged.
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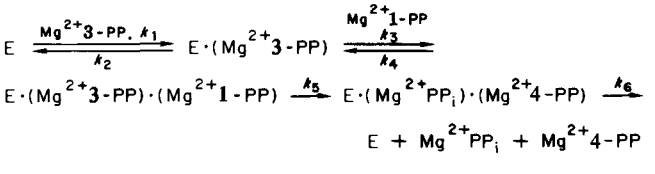
Scheme II. Binding Mechanism for 1′–4 Condensation



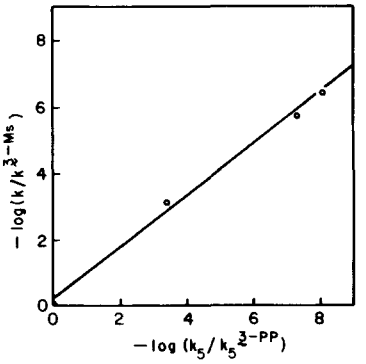


Figure 1. A Hammett plot of the ratio of rate constants for the catalytic step in 1′–4 condensation between **1-PP** and allylic substrates **3-PP**, **5-PP**, **6-PP**, and **7-PP** ($k_5/k_5^{3\text{-PP}}$) and the ratio of rate constants for solvolysis of **3-Ms**, **5-Ms**, **6-Ms**, and **7-Ms** ($k/k^{3\text{-Ms}}$).

yielding a geranyl cation–PP_i ion pair, and that this reactive species subsequently alkylates the double bond in **1-PP**.

Three analogues of **3-PP**—3-(fluoromethyl)-7-methyl-2,6-octadienyl pyrophosphate (3′-fluorogeranyl-PP, **5-PP**), 3-(difluoromethyl)-7-methyl-2,6-octadienyl pyrophosphate (3′,3′-difluorogeranyl-PP, **6-PP**), and 3-(trifluoromethyl)-7-methyl-2,6-octadienyl pyrophosphate (3′,3′,3′-trifluorogeranyl-PP, **7-PP**)—were prepared from the corresponding alcohols.[14,15] Maximum velocities (V) were measured by the acid lability method[16] using crystalline enzyme from avian liver,[17] specific activity 1.5 units/mg. Saturating concentrations of [1-^{14}C]isopentenyl-PP (20 μM, 56 μCi/μmol) and allylic substrate (400 μM) were used. Each run was accompanied by a blank determination with only the allylic pyrophosphate omitted from the buffer. The production of acid labile counts was linear up to ca. 20% conversion, and V (see Table I) was calculated from initial velocities in the linear region of the curve.[18] Michaelis constants (K_M) were also evaluated for **1-PP** with **3-PP** and the fluorinated analogues. The values are all similar and indicate that the replacement of **3-PP** in the active site by the analogues does not significantly perturb binding of **1-PP** to the enzyme, although the V's for **3-PP** and **7-PP** span a range almost of 10^8!

The effect of the fluorinated substituents on the rates for ionization of geranyl derivatives was evaluated in a model reaction where participation by π electrons is not possible by measuring the rate constants for solvolysis of methanesulfonates **3-Ms**, **5-Ms**, **6-Ms**, and **7-Ms** in acetone–water.[7] The values listed in Table II cover a range of more than 10^6. Two lines of evidence indicate that the cationic mechanism for solvolysis of **3-Ms** does not change as the system is destabilized by fluorine. A plot of log $k/k^{3\text{-Ms}}$ against σ_R[19,20] is linear ($R^2 = 0.995$), and the sensitivities of the less reactive analogues (**6-Ms** and **7-Ms**) to changes in the composition of the solvent are in the range expected for limiting solvolysis reactions according to Winstein-Grunwald m values.[6,21,22]

Farnesylpyrophosphate synthetase catalyzes the 1′–4 condensation reaction by the ordered, sequential mechanism shown in Scheme II,[23] and at steady state the maximum velocity is given by eq 1.[24] Furthermore, the rate constant for the catalytic step

$$V = k_5k_6E_t/(k_5 + k_6) \quad (1)$$

(k_5) is 47 times slower than the rate-limiting step k_6.[23] Assuming that the fluorinated farnesyl derivatives produced from **5-PP**, **6-PP**, and **7-PP** are released at least as rapidly as **4-PP** (i.e., $k_6^{\text{analogues}} \geq k_6^{4\text{-PP}}$), the decrease in V we observed for the fluorinated substrates must result from a situation where k_5 is significantly less than k_6, in which case $V = k_5E_t$. On the basis of this assumption, values for $k_5/k_5^{3\text{-PP}}$ were calculated and are listed in Table I.

A Hammett plot of $k_5/k_5^{3\text{-PP}}$ vs. $k/k^{3\text{-Ms}}$ (see Figure 1) is particularly informative. Since the rate ratios for 1′–4 condensation and solvolysis correlate linearly ($R^2 = 0.993$) over the entire range of reactivities, we have not traversed a threshold for π participation, even with the less reactive analogues. Also, the slope of the plot (0.77) demonstrates that 1′–4 condensation is slightly *more* sensitive to the electron-withdrawing groups at C(3) in the geranyl analogues than is the model solvolysis reaction. If π participation were important for the enzymatic reaction, one would expect the slope in Figure 1 would be considerably greater than unity.[8,25] We conclude, therefore, that (a) ionization and (b) condensation are not concerted. In view of the spatial limitations expected in the active site, we further conclude that an allylic cation–PP_i ion pair is the reactive species which alkylates **1-PP**. Experiments are now under way to determine if the changes in covalent bonding that occur during the condensation and elimination phases are stepwise or concerted.

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Farnesylpyrophosphate Synthetase. Evidence for a Rigid Geranyl Cation–Pyrophosphate Anion Pair[1,2]

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The terpene biosynthetic pathway is unique among major metabolic pathways in that carbon–carbon bonds are formed by electrophilic attack on a π-electron functional group. This general biosynthetic strategy was first deduced from the remarkable parallel that exists between the structures of natural isoprenoids and products of putative carbocationic precursors.[3,4] More re-

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(2) Abbreviations used in this communication are farnesyl-PP, farnesyl pyrophosphate; 2-fluoroisopentenyl-PP, 2-fluoroisopentenyl pyrophosphate; GC/MS, gas chromatography/mass spectroscopy; geranyl-PP, geranyl pyrophosphate; isopentenyl-PP, isopentenyl pyrophosphate; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); PP_i, inorganic pyrophosphate.
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