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(9) Using a trigonal bipyramidal idealized geometry, with equatorial oxygens at 2.55 Å, and nitrogens at 2.65 Å, CNDO-2 charges of 0.25 (O) and 0.14 (N) lead to a reasonable[10] value of ca. 1 MHz for the quadrupolar coupling constant. This value could be somewhat higher if one were dealing with a sugar-bridged $Na^+ClO_4^-$ ion pair, bringing a perchlorate oxygen into van der Waals contact of the cation.
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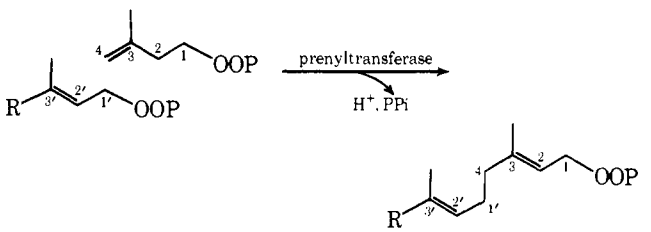
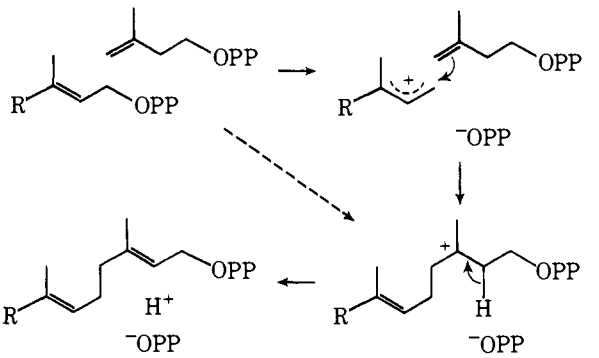
Prenyltransferase. The Mechanism of the Reaction[1]

Sir:

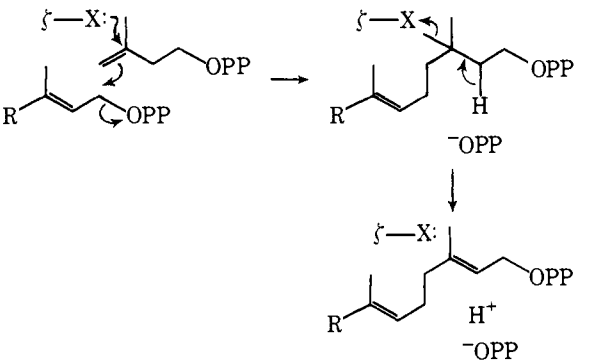
Prenyltransferase (EC 2.5.1.1) catalyzes the condensation between C_4 of isopentenyl pyrophosphate (**IPP**) and C_1' of an allylic pyrophosphate, giving the five-carbon homologue of the allylic pyrophosphate. This is the fundamental chain elongation



Scheme I. Ionization–Condensation–Elimination



Displacement–Elimination



reaction of terpene biosynthesis and leads to the formation of several important classes of natural products such as sterols, carotenoids, dolichols, and respiratory coenzymes. The mechanisms which have been proposed for prenyl transfer can be grouped into two broad categories (Scheme I): those in which condensation is initiated by heterolytic cleavage of the carbon–oxygen bond, with or without assistance from the double bond of isopentenyl pyrophosphate, yielding cationic intermediates (ionization–condensation–elimination),[2] and those in which condensation is initiated by attack of a nucleophilic group at the double bond of isopentenyl pyrophosphate with simultaneous formation of the C_1'–C_4 bond between the two substrates and rupture of the C_1'–oxygen bond (displacement–elimination).[2d,3] We reasoned that it would be possible to distinguish between the two mechanisms by replacing the R group in the allylic substrate by a trifluoromethyl group. The strong electron-withdrawing effect of a trifluoromethyl substituent ($\sigma^+ = 0.612$)[4] should retard the rate of

Table I. Effect of Methyl and Trifluoromethyl Groups

Reactant	T, °C	Solvent (% acetone–H_2O)	k	k_{CF_3/CH_3}
SN1				
E-3-Trifluoromethyl-2-buten-1-yl methanesulfonate (*E*-**1**-OMs)	20	92	5.5×10^{-8} [a-c]	1.8×10^{-6}
	20	50	1.55×10^{-6} [a,c]	
	60	50	$1.28 \pm 0.03 \times 10^{-4}$ [c]	
	70	50	$3.06 \pm 0.20 \times 10^{-4}$ [c]	
	80	50	$7.98 \pm 0.55 \times 10^{-4}$ [c]	
3-Methyl-2-buten-1-yl methanesulfonate (**4**-OMs)[d]	0	92	$3.82 \pm 0.11 \times 10^{-3}$ [c]	
	20	92	$2.99 \pm 0.12 \times 10^{-2}$ [c]	
SN2				
E-1-Chloro-4,4,4-trifluoro-2-butene (**5**)	20	100	68.8×10^{-5} [e,f]	11
E-1-Chloro-2-butene (**6**)	20	100	6.1×10^{-5} [e,g]	

[a] Extrapolated from higher temperatures. $\Delta H^{\ddagger} = 20.7$ kcal mol^{-1}, $\Delta S^{\ddagger} = -15$ eu. [b] Extrapolated 50% acetone–water, $m = 0.383$, $Y = 2.25$. [c] Units are s^{-1}. [d] $\Delta H^{\ddagger} = 15.8$ kcal mol^{-1}, $\Delta S^{\ddagger} = -12$ eu. [e] For displacement with I^-(KI), l. mol^{-1} s^{-1}. [f] E. T. McBee, R. D. Battershell, and H. P. Braendlin, *J. Am. Chem. Soc.*, **84**, 3157 (1962); J. A. Pegolotti and W. G. Young, *ibid.*, **83**, 3258 (1961). [g] L. F. Hatch and S. S. Nesbitt, *ibid.*, **73**, 358 (1951).

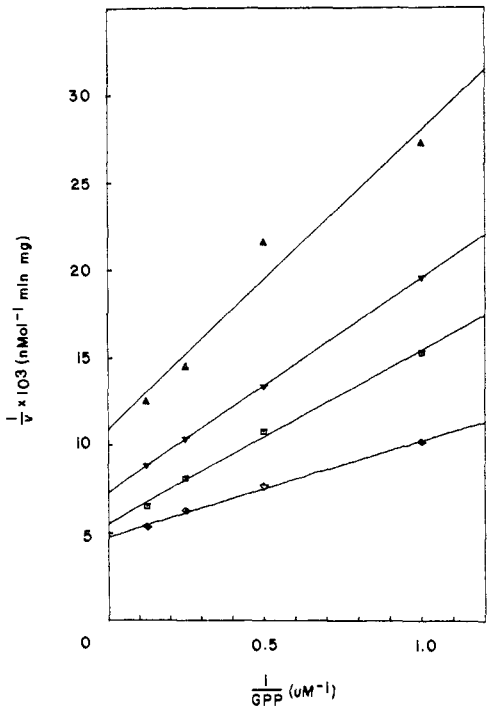
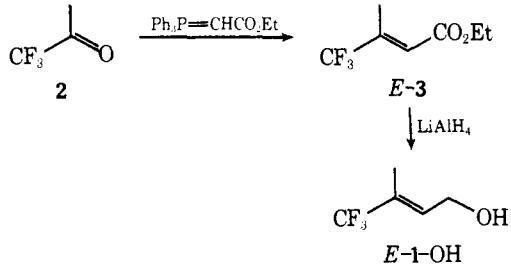


Figure 1. Inhibition of prenyltransferase by *E*-**1**-OPP. Incubations were at 37° in 200 μl of 10 mM potassium phosphate buffer, pH 7.0, 1 mM $MgCl_2$, 0.1 mM dithiothreitol, and 2 μM isopentenyl pyrophosphate with 69 ng of prenyltransferase (specific activity 250) and the indicated concentrations of geranyl pyrophosphate. Determinations were in duplicate. Concentration of *E*-**1**-OPP; ◇ none, □ 50 μM, ▽ 100 μM, △ 200 μM.

ionization of the allylic pyrophosphate while having little effect on the rate of a direct nucleophilic displacement at C_1'. In this communication, we present preliminary results with an allylic substrate analogue, *E*-trifluoromethyl-2-butenyl pyrophosphate (*E*-**1**-OPP).

E-3-Trifluoromethyl-2-buten-1-ol (*E*-**1**-OH) was prepared by the sequence of reactions shown below.



The Wittig condensation[5] gave a 97:3 mixture of *E*[6] and *Z*[7] isomers which were separated by spinning band distillation. The stereochemistry of the double bond was established by a nuclear Overhauser experiment in which irradiation of the protons at C_4 produced a 32 ± 3% enhancement of the olefinic proton in *Z*-**3** and no enhancement, 3 ± 3%, for the *E* isomer. Alcohol *E*-**1**-OH,[8] obtained by hydride reduction at 0°, was converted into methanesulfonate (*E*-**1**-OMs) and pyrophosphate[9,10] (*E*-**1**-OPP) derivatives for chemical and enzymatic studies.

The data in Table I illustrate the effect of replacing a methyl group by trifluoromethyl in typical SN1 (solvolysis in aqueous acetone) and SN2 (displacement by iodide in dry acetone) reactions for allylic systems. It is clear that the trifluoromethyl moiety severely retards heterolytic cleavage of the C_1–oxygen bond in *E*-**1**-OMs and mildly accelerates direct nucleophilic substitution at an allylic center.

When *E*-**1**-OPP is incubated[11] with isopentenyl pyrophosphate and prenyltransferase from porcine liver,[12] the rate of condensation is 5.1×10^{-4} nmol/(min mg).[13] In comparison, the rate observed with isopentenyl pyrophosphate and geranyl pyrophosphate is 7.4×10^2 nmol/(min mg)[14] under similar conditions. Assuming that the rate of the reaction varies linearly with the concentration of enzyme, *E*-**1**-OPP is *at least* 1.5×10^6 *times less reactive* than geranyl pyrophosphate.

Inefficient utilization of the trifluoromethyl analogue cannot be attributed to poor binding with prenyltransferase. The size of the trifluoromethyl group is similar to that of a methyl group,[15] and the enzyme catalyzes condensation between isopentenyl pyrophosphate and a variety of allylic pyrophosphates with different alkyl substituents at C_3'.[16] Also, kinetic studies summarized in Figure 1 show that *E*-**1**-OPP binds to the enzyme.[17]

We conclude that this condensation takes place by an ionization–condensation–elimination mechanism. The exact timing of condensation with regard to ionization cannot be firmly established although two observations suggest the possibility that condensation lags behind ionization. The trifluoromethyl moiety retards the rates for solvolysis and prenyl transfer to a similar extent, implying substantial delocalization of positive charge into the C_2'–C_3' double bond during ionization for both reactions. Also, we have recently shown that prenyltransferase catalyzes hydrolysis of its allylic substrate in the absence of isopentenyl pyrophosphate.[18] Experiments are underway in our laboratories which will further delineate the mechanism of prenyltransferase.

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