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Farnesyl Pyrophosphate Synthetase

MECHANISTIC STUDIES OF THE 1′-4 COUPLING REACTION WITH 2-FLUOROGERANYL PYROPHOSPHATE*

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The mechanism of the 1′-4 coupling reaction between isopentenyl pyrophosphate and geranyl pyrophosphate catalyzed by farnesyl pyrophosphate synthetase from porcine liver was studied with the allylic substrate analogue 2-fluorogeranyl pyrophosphate. 2-Fluorogeranyl pyrophosphate is an alternate substrate for the enzyme, yielding 6-fluorofarnesyl pyrophosphate upon condensation with isopentenyl pyrophosphate. The Michaelis constant for the fluoroanalogue, K_m = 1.1 μM, is similar to that measured for geranyl pyrophosphate, K_m = 0.7 μM. However, the rate of condensation with the fluoroanalogue was only 8.4 × 10^{-4} that of the normal reaction. A similar rate depression (4.4 × 10^{-3}) was found for solvolysis of geranyl methanesulfonate and the corresponding 2-fluoro derivative, reactions known to proceed via cationic intermediates. In contrast, displacement of chlorine from geranyl chloride and 2-fluorogeranyl chloride by cyanide showed a small (2-fold) rate enhancement for the fluoro compound. Finally, 2-fluorogeranyl pyrophosphate is a competitive inhibitor against geranyl pyrophosphate. These data are interpreted in terms of an ionization-condensation-elimination mechanism for the 1′-4 coupling reaction.

The mechanism of the 1′-4 condensation (1) between isopentenyl pyrophosphate and an allylic pyrophosphate in the terpene biosynthetic pathway has been the subject of speculation for over 20 years (2). The earliest proposals envisioned carbon-carbon bond formation between C(4) of isopentenyl pyrophosphate and C(1) of the allylic substrate via cationic intermediates (see Scheme 1) (3–6). About 10 years ago, another mechanism which involved displacement of the pyrophosphate moiety from C(1) of the allylic substrate with simultaneous bonding to C(4) of isopentenyl pyrophosphate was introduced (6–8). Outlined in its broadest context, the new mechanism could, but did not necessarily, involve carbonium ion intermediates (6) and was thought to be more compatible with the stereochemistry of the 1′-4 condensation. After examining the data that had been published, we decided that it was not possible to distinguish between ionization and nucleophilic displacement mechanisms. Although the stereochemistry of the 1′-4 condensation is compatible with that expected of a displacement reaction, it is quite possible that the stereospecificity found for the reaction is dictated by the topology of the enzyme·substrate complex.

We reasoned that it would be possible to differentiate between displacement and ionization mechanisms by selectively substituting hydrogen with fluorine in the allylic substrate. The powerful electron-withdrawing effect of fluorine (9) should retard ionization of the allylic pyrophosphate, while having little influence on the rate of a direct nucleophilic displacement. Recently, we reported that (*E*)- and (*Z*)-3-trifluoromethyl-2-buten-1-yl pyrophosphate (analogues for dimethylallyl pyrophosphate) were about 10^7 times less reactive than the normal substrate during the 1′-4 condensation (10). However, some ambiguities remained. Inhibition studies with the C_5 analogues against geranyl pyrophosphate and isopentenyl pyrophosphate showed "mixed linear" patterns with fairly large inhibition constants, K_i values ≃ 50 μM. These data suggest that the trifluoromethyl analogues bind to both the allylic and isopentenyl sites. Thus, the observed retardation for the rate of the enzymatic reaction cannot be simply related to a reduction in the rate of the catalytic step. Also, the condensation of isopentenyl pyrophosphate with the fluoro analogues was too slow to permit products to be isolated and fully characterized.

We decided that these problems could be alleviated by constructing a substrate analogue which was specific for the allylic site and whose reactivity was intermediate between those of the normal substrates and the trifluoromethyl analogues. 2-Fluorogeranyl pyrophosphate proved to be an ideal choice for studying the 1′-4 condensation catalyzed by farnesyl pyrophosphate synthetase from porcine liver (11).

EXPERIMENTAL PROCEDURES

General

Boiling and melting points are uncorrected. ^{1}H nmr spectra were recorded on Varian A-60, EM-390, or XL-100-15 spectrometers using tetramethylsilane or sodium 4,4-dimethyl-4-silapentane sulfonate as internal standards. ^{19}F nmr spectra were obtained on a Varian EM-390 spectrometer using trichlorofluoromethane as an internal standard or trifluoroacetic acid (−77 ppm relative to trichlorofluoromethane) as an internal standard unless otherwise indicated. Analytical gas chromatography was carried out on a Varian Model 1200 gas chromatograph with a flame ionization detector using an open tubular column (500 feet × 0.03 inch) coated with Carbowax 20 M. Microanalyses were performed by Chemalytics, Inc. Low resolution mass spectra were obtained on an LKB Model 9000S mass spectrometer. High resolution mass spectra were performed by the Department of Biochemistry at Michigan State University. Infrared spectra were recorded on a Beckman Acculab 3 Spectrometer.

Materials

Porcine liver farnesyl pyrophosphate synthetase was obtained from Professor H. C. Rilling (12). [1-^{14}C]Isopentenyl pyrophosphate purchased from Amersham/Searle was used directly or diluted to a

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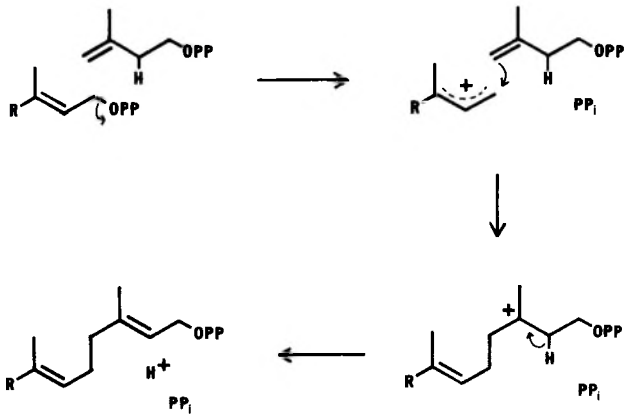


SCHEME. 1. Ionization-condensation-elimination mechanism for 1′-4 coupling.

specific activity of 10 μCi/μmol with synthetic material. Geranyl pyrophosphate was prepared from the corresponding alcohol (Givaudan) according to the method of Cramer (13, 14) and purified by ion exchange chromatography.

Synthesis of Analogues

See miniprint supplement.[1]

Assays

All incubations with prenyltransferase were run at 37°C in a buffer consisting of 10 mM potassium phosphate, 1 mM magnesium chloride, 0.1 mM dithiothreitol, and 1 μM sodium azide, at pH 7.4. The acid lability assay (15) was used to determine the extent of the reaction. Radioactivity was measured by liquid scintillation spectrometry in 10 ml of 0.4% Omnifluor (New England Nuclear) in toluene.

Studies with Farnesyl Pyrophosphate Synthetase

Kinetics—Incubations were carried out for 10 min in the standard buffer (200 μl total volume) at 37°C. All determinations were in duplicate. Double reciprocal plots are computer-drawn (Hewlett Packard model 9810 A calculator-plotter) "least squares" lines determined from four concentrations of variable substrate. The error range for K_i values and K_m values represent the average of the maximum and minimum values based on the standard deviation of the slopes and intercepts used in the calculations. The kinetic data are presented in Table I.

Products—Thirty milliliters of the standard buffer which contained 3.0 mg (8.3 μmol) of 2-fluorogeranyl pyrophosphate and 1.1 mg (4.1 μmol, 75 nCi/μmol) of [1-^{14}C]isopentenyl pyrophosphate were incubated with enzyme at 37°C. The progress of the reaction was followed by subjecting 10-μl portions of the mixture to the acid lability assay. The aqueous layer was also monitored after a thorough extraction with hexane. After 48 h, approximately 66% of isopentenyl-PP had been consumed as judged by production of acid-labile material. No further condensation was seen at longer reaction times although the enzyme was still active. The sample was freeze-dried, the residue was dissolved in 4 ml of saturated ammonium sulfate, and the resulting solution was stirred overnight with 4 ml of isobutyl alcohol. The aqueous layer was repeatedly extracted with isobutyl alcohol until the alcohol extract was free of radioactivity. The combined isobutyl alcohol fractions were made basic with a drop of concentrated ammonium hydroxide and blown dry at 37°C under nitrogen. The residue was dissolved in 10 ml of 0.1 M lysine containing 5 mg of calf mucosa alkaline phosphatase (Sigma) and incubated at 47°C for 48 h. The aqueous solution was extracted with hexane, and the combined layers were dried over sodium sulfate. Solvent was reduced to approximately 100 μl at 37°C under nitrogen. The remaining oil was dissolved in 1 ml of dry ether, and three drops of pyridine and one drop of benzoyl chloride were added. After standing overnight, the solution was extracted twice with 2 N HCl, twice with saturated sodium bicarbonate, and once with water. The ether layer was dried over magnesium sulfate and solvent was removed with a stream of dry nitrogen. The residue was purified by high pressure liquid chromatography on a Porasil A column (1% tetrahydrofuran in hexane) and the radioactive portions of the eluate were recombined. The material was analyzed by glc-mass spectrometry (3 feet × ⅛ inch OV-17) and gave two peaks. In a temperature-programmed run, the first compound eluted at 138°C and had a retention volume and mass spectrum identical to those of 2-fluorogeranyl benzoate. The second component eluted at 180°C, and its mass spectrum is shown in Fig. 1.

Stereochemistry—2*R*-[1-^{14}C, 2-^{3}H]Isopentenyl pyrophosphate (0.43 μM; ^{14}C, 10μCi/μmol; ^{3}H, 205 μCi/μmol) and 2*S*-[1-^{14}C, 2-^{3}H]-isopentenyl pyrophosphate (0.37 μM; ^{14}C, 10 μCi/μmol; ^{3}H, 205 μCi/μmol) were incubated in separate experiments with no added allylic pyrophosphate, geranyl pyrophosphate (50 μM), and 2-fluorogeranyl pyrophosphate (50 μM) using the standard buffer (200 μl total volume). Incubations with 2-fluorogeranyl pyrophosphate and the blanks (22 h) contained 35 μg of enzyme (specific activity ~ 200). Incubations with geranyl pyrophosphate (30 min) contained 1.3 μg of enzyme. The results are presented in Table II.

Solvolysis Studies

Ionization—Kinetic measurements for geranyl and 2-fluorogeranyl methanesulfonate were carried out and analyzed using published procedures (9). The results are shown in Table III.

Displacement—Rates of the displacement reactions were measured by following the incorporation of [^{14}C]cyanide into hexane-soluble products. A standard solution of potassium cyanide was prepared as follows. Four hundred milligrams (1.5 mmol) of 18-crown-6 (Parrish) were placed in a 250-ml round bottom flask equipped with a magnetic stirrer. To this was added 1 g (15 mmol) of potassium cyanide and 100 ml of dry acetonitrile. The solution was stirred for 3 h and filtered to remove insoluble matter. For a typical run, 2.5 ml of standard solution were placed in a 5-ml round bottom flask along with 1.25 μCi of potassium [^{14}C]cyanide (New England Nuclear), and the flask was equilibrated at 25°C. Then, 2.5 μl of a 187 mg/ml solution of geranyl chloride (16) (0.468 mg, 2.7 μmol) or 3 μl of a 171 mg/ml solution of 2-fluorogeranyl chloride (0.514 mg, 2.7 μmol) were introduced. The reaction mixture was sampled at various times as follows. Fifty microliters of the reaction mixture were pipetted into approximately 1 ml of H_2O, and 1 ml of hexane was layered over the aqueous phase. After 15 s of agitation, water was removed and the hexane layer was washed with two additional 1-ml portions of water. A portion (0.5 ml) of the hexane layer was counted as described in the assay for farnesyl pyrophosphate synthetase. Pseudo-first order rate constants are listed in Table III.

TLC analysis (95:5 hexane:ethyl acetate on TLC-7GF) of the products from samples used for the kinetic measurements showed that the radioactivity co-migrated with authentic samples of 2-fluorogeranyl cyanide (R_F 0.35), *Z*-3-fluoro-4,8-dimethyl-2,7-nonadiene nitrile (R_F 0.42), and geranyl cyanide (R_F 0.54).

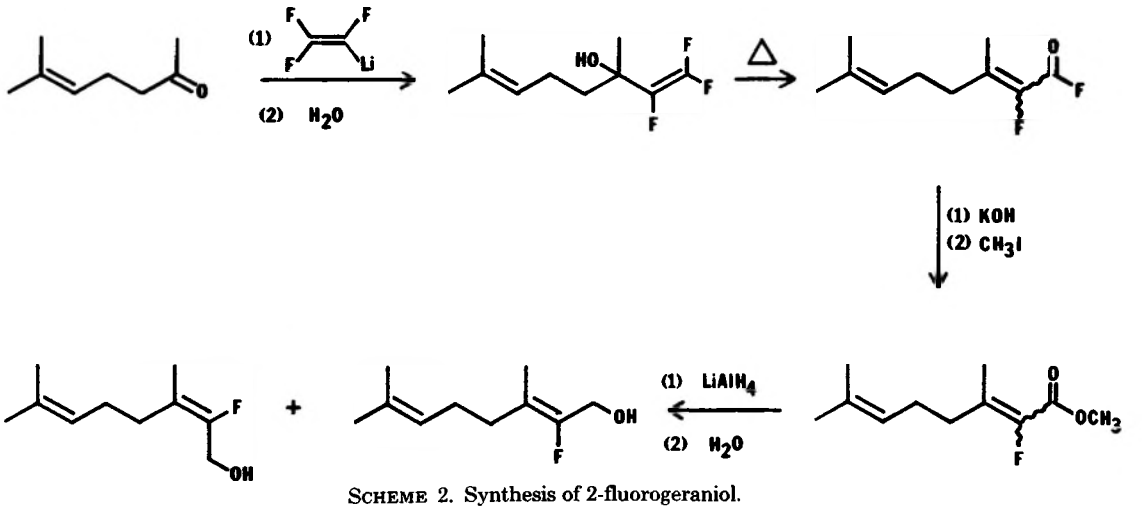
RESULTS

Synthesis of 2-Fluorogeranyl Pyrophosphate

2-Fluorogeranyl pyrophosphate was prepared from 6-methyl-5-heptene-2-one by the sequence of reactions shown in Scheme 2. A 35:65 mixture of (*E*)- and (*Z*)-2-fluoro-3,7-dimethyl-2,6-octadienoyl fluoride was obtained by the procedure of Drakesmith and co-workers (17). The acid fluorides were hydrolyzed to the corresponding acids which were in turn converted to a mixture of methyl esters. Reduction with lithium aluminum hydride afforded a 35:65 mixture of 2-fluoronerol and 2-fluorogeraniol (18). The alcohols were easily separated by medium pressure chromatography on silica gel, and 2-fluorogeraniol was pyrophosphorylated in low yield using the procedure developed by Cramer (13, 14).

The stereochemistry of the double bond at C(2) was determined from chemical shift data (see Table IV). For the acid fluorides, carboxylic acids, and methyl esters, the methyl groups at C(3) for the *Z* isomers are deshielded by slightly more than 0.2 ppm when compared with those of the *E* compounds (19). We also found that the four-bond coupling

[1] Portions of this paper are presented in miniprint at the end of this paper. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78M-263, cite author(s), and include a check or money order for $1.30 per set of photocopies.



SCHEME 2. Synthesis of 2-fluorogeraniol.

TABLE I

Kinetic constants for porcine liver farnesyl pyrophosphate synthetase

Substrate concentrations were varied between 0.125 and 1.0 μM for condensation between isopentenyl-PP and geranyl-PP and between 1.0 and 8.0 μM for condensation between isopentenyl-PP and 2-fluorogeranyl-PP. For inhibition studies, the concentration of isopentenyl-PP was fixed at 0.5 μM and geranyl-PP was varied between 0.125 and 1.0 μM at concentrations of 0, 2.5, 5, and 10 μM for 2-fluorogeranyl-PP.

Substrates	Inhibitor	V_{max}	$K_m^{isopentenyl\ PP}$	$K_m^{allylic}$	K_i
		nmol mg⁻¹ min⁻¹	*μM*	*μM*	*μM*
Isopentenyl-PP, geranyl-PP		370 ± 30	0.5 ± 0.1	0.71 ± 0.06	
Isopentenyl-PP, 2-fluorogeranyl-PP		0.31 ± 0.62	0.35 ± 0.36	1.1 ± 0.2	
Isopentenyl-PP, geranyl-PP	2-Fluorogeranyl-PP				2.4 ± 0.5

TABLE II

1'-4 Condensation with 2(R)- and 2(S)-isopentenyl pyrophosphate

The product was decomposed as described for the acid lability assay and hexane-soluble material was counted. Counting efficiency was measured by the internal standard method.

Substrate		^3H	^{14}C
Homoallylic	Allylic		
		dpm	
2(*R*)-[1-^{14}C, 2-^{3}H]isopentenyl-PP	Geranyl-PP	14	1716
	2-Fluorogeranyl-PP	24	1731
2(*S*)-[1-^{14}C, 2-^{3}H]isopentenyl-PP	Geranyl-PP	11,979	1712
	2-Fluorogeranyl-PP	12,253	1824

TABLE III

Rate constants for solvolysis and displacement

Solvolyses of the methanesulfonates were carried out in 90% acetone:water. Pseudo-first order rate constants for the displacement reaction were measured in dry acetonitrile.

Reactant	*T*	*k*
		s^{-1}
Geranyl methanesulfonate	0°C	$(1.74 \pm 0.07) \times 10^{-3}$
	25	$(2.47 \pm 0.03) \times 10^{-2}$
	60	5.2×10^{-1}
2-Fluorogeranyl methanesulfonate	60	$(2.29 \pm 0.03) \times 10^{-3}$
Geranyl chloride	25	$(1.86 \pm 0.3) \times 10^{-4}$
2-Fluorogeranyl chloride	25	$(2.95 \pm 0.5) \times 10^{-4}$

constants ($^4J_{^1H-^{19}F}$) for the fluorine at C(2) and the hydrogens of the C(3) methyl group follow a regular pattern with $E(^4J_{^1H-^{19}F}) > Z(^4J_{^1H-^{19}F})$. Overlapping peaks obscured the coupling constants for 2-fluoronerol and 2-fluorogeraniol, but the pattern re-emerged when the alcohols were converted to benzoate esters, although the difference between the four-bond couplings had diminished. Our assignments were confirmed with 2-fluoro-3-methyl-2-buten-1-ol. In deuteriochloroform, both methyl groups appear at 1.69 ppm. Upon addition of Eu(fod)$_3$,[2] one methyl moves to lower field (2.76 ppm/eq of Eu(fod)$_3$) significantly faster than the other (1.93 ppm/eq of Eu(fod)$_3$) and the faster moving methyl has a smaller hydrogen-fluorine coupling constant ($^4J_{^1H-^{19}F}$ = 3.0 Hz *versus* $^4J_{^1H-^{19}F}$ = 3.3 Hz). Since the methyl group at C(3) which is *cis* to the hydroxyl moiety at C(1) will be deshielded to a greater extent upon addition of Eu(fod)$_3$ (20), it follows that the larger four-bond ^1H-^{19}F coupling occurs when the methyl group and fluorine are *cis* on the C(2)-C(3) double bond.

Product of the 1'-4 Coupling with 2-Fluorogeranyl Pyrophosphate

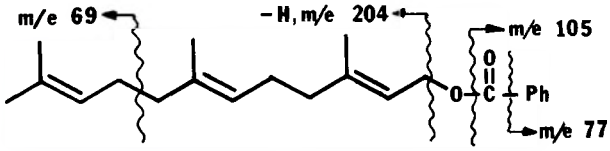
[1-^{14}C]Isopentenyl pyrophosphate and 2 eq of 2-fluorogeranyl pyrophosphate were incubated with farnesyl pyrophosphate synthetase from porcine liver. Pyrophosphates were cleaved with alkaline phosphatase, and the resulting mixture was extracted with hexane. The hexane-soluble material was treated with benzoyl chloride and pyridine. Purification by high pressure liquid chromatography gave a fraction which contained 37% of the expected counts based on consumption of 1 mol of isopentenyl pyrophosphate per mol of 2-fluorogeranyl pyrophosphate.

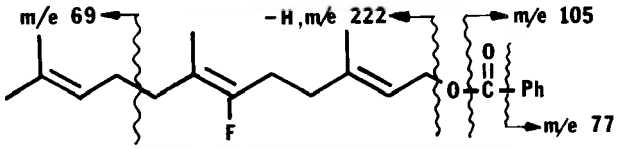
GLC-mass spectrometry data showed that the radioactive material was a C_{15} fluorine containing terpene. The product eluted as a single, sharp peak on an OV-17 column (3 feet × ⅛ inch). The retention volume of the radioactive product was identical to that of farnesyl benzoate and considerably later than the benzoate derivative obtained from unconsumed 2-fluorogeraniol. Unfortunately, the benzoate of 6-fluorofarnesol did not give a molecular ion at *m/e* 344. This is consistent with the observation that the molecular ion for farnesyl ben-

[2] The abbreviations used are: Eu(fod)$_3$, 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctanedionatoeuropium (III); isopentenyl-PP, isopentenyl pyrophosphate; geranyl-PP, geranyl pyrophosphate; 2-fluorogeranyl-PP, 2-fluorogeranyl pyrophosphate.

zoate at *m/e* 326 is very weak. However, if one compares the mass spectra of farnesyl benzoate and the radioactive product and allows for the shift of 18 mass units for those peaks containing fluorine, the similarity is striking (see Fig. 1). For both compounds, the base peak is *m/e* 69. This is typically one of the major fragment ions for polyisoprenoids, representing the C_5H_9 isoprene moiety. The benzoate moieties in both compounds also gave typical peaks at *m/e* 77 and 105.

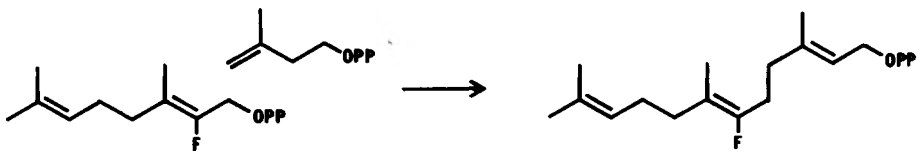
There are three major peaks above *m/e* 150 in the mass spectra of both compounds. Those seen for farnesyl benzoate at *m/e* 161, 189, and 204 are shifted by 18 mass units in the radioactive product to 179, 207, and 222, respectively. Presum-





ably, the peaks at *m/e* 204 and 222 represent the farnesyl moieties minus a hydrogen atom. This assignment is reinforced by the peak at *m/e* 122 seen for the fluorinated material (and to a smaller extent for farnesyl benzoate) which could represent the benzoate moiety plus a hydrogen. The peaks at *m/e* 189 and 207 represent loss of a methyl group from the farnesyl residues while those at 161 and 179 presumably result from loss of a methyl group plus ethylene.

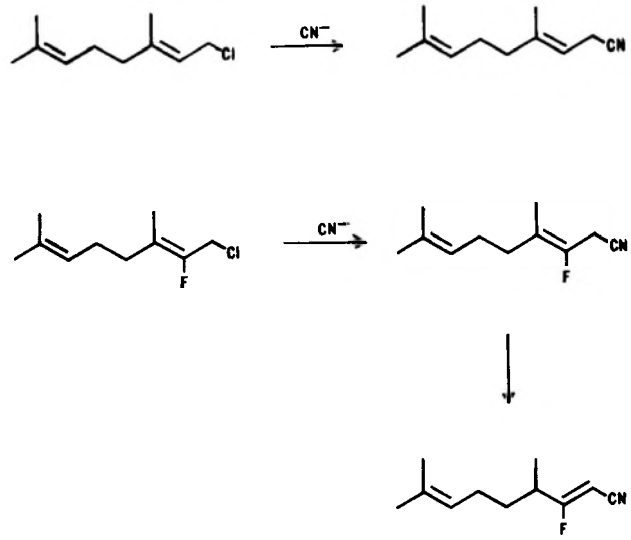
The GLC-mass spectrometry data clearly show that the radioactive material is a C_{15} terpene benzoate with a fluorine in the isoprene moiety. Since we used farnesyl pyrophosphate synthetase that had been purified to homogeneity, 2-fluorogeranyl pyrophosphate as an alternate allylic substrate, and [1-^{14}C]isopentenyl pyrophosphate for the incubation, there is little doubt that the hydrogen moiety is a 6-fluorofarnesyl residue. The only question that remains is the stereochemistry of the C(2)-C(3) double bond. This point was resolved by incubating 2-fluorogeranyl pyrophosphate with (*R*)- and (*S*)-[1-^{14}C, 2-^{3}H]isopentenyl pyrophosphate and determining residual ^{3}H in the acid-labile product. The data listed in Table II show that the 2-(*R*)-tritium was cleanly removed, and the 2-(*S*)-tritium was retained during the 1′-4 condensation. Cornforth and co-workers (21) previously showed that the 2-(*R*)-tritium is lost exclusively when *E,E*-farnesyl pyrophosphate is formed during the normal reaction catalyzed by farnesyl pyrophosphate synthetase from rat liver and yeast. Thus, we conclude that the product of the condensation between isopentenyl pyrophosphate and 2-fluorogeranyl pyrophosphate is *E,Z*-6-fluorofarnesyl pyrophosphate, as shown above.



Kinetic Studies

Solvolysis and Displacement—The effect of replacing the hydrogen at C(2) on the reactivity of the geranyl system toward ionization and displacement was determined in suitable model reactions. Solvolysis of methanesulfonate derivatives in aqueous acetone was chosen as a typical reaction in which heterolysis of the C(1)-oxygen bond is rate-limiting. In 90% acetone:water, geranyl methanesulfonate and 2-fluorogeranyl methanesulfonate showed excellent first order kinetic behavior over four half-lives (>90% reaction). Rate constants for the two compounds are listed in Table III. Since the 2-fluoro derivative was significantly less reactive than geranyl methanesulfonate, it was not possible to measure rates at the same temperature, and the rate constant at 60°C for the parent compound was extrapolated (10) from the values obtained at 0° and 25°C. Replacement of the hydrogen at C(2) by a fluorine depressed the rate of solvolysis by 4.4×10^{-3}.

Pseudo-first order rate constants for the displacement of chloride by cyanide were measured at 25°C in dry acetonitrile using a 14-fold molar excess of potassium cyanide·18-crown-6 complex. In this case, the 2-fluoro analogue was twice as reactive as geranyl chloride. The expected products of a displacement by cyanide, geranyl cyanide, and 2-fluorogeranyl cyanide were found. In addition, 2-fluorogeranyl chloride gave



27% of (*Z*)-3-fluoro-4,8-dimethyl-2,7-nonadiene nitrile, presumably formed by isomerization of the β,γ-unsaturated nitrile to its α,β-isomer in the presence of the cyanide anion. Under the conditions of the displacement reaction, a sample of pure 2-fluorogeranyl cyanide gave a 20:60 mixture of α,β and β,γ isomers after 38 h. The position of the C(2)-C(3) double bond was deduced from nmr and uv spectra (see miniprint supplement) and the assignment of stereochemistry was based on the magnitude of the coupling constant between the hydrogen at C(2) and the fluorine at C(3) (22).

1′-4 Condensation—Michaelis constants for the 1′-4 condensation of isopentenyl pyrophosphate with geranyl pyrophosphate and 2-fluorogeranyl pyrophosphate are listed in Table I. Plots of $1/v$ *versus* the reciprocal of isopentenyl and allylic pyrophosphate concentrations gave a series of parallel lines similar to those reported by Reed and Rilling (15) for the avian liver enzyme. Values of $K_m^{\text{geranyl-PP}}$ and

TABLE IV

Chemical shifts and ^1H-^{19}F coupling constants for the methyl group at C(3)

Compound	δ	$^4J_{^1H-^{19}F}$
	ppm	*Hz*
(*E*)-2-Fluoro-3,7-dimethyl-2,6-octadienoyl fluoride	1.97	4.5
(*Z*)-2-Fluoro-3,7-dimethyl-2,6-octadienoyl fluoride	2.19	3.6
(*E*)-2-Fluoro-3,7-dimethyl-2,6-octadienoic acid	1.87	4.5
(*Z*)-2-Fluoro-3,7-dimethyl-2,6-octadienoic acid	2.12	3.3
Methyl (*E*)-2-fluoro-3,7-dimethyl-2,6-octadienoate	1.86	4.5
Methyl (*Z*)-2-fluoro-3,7-dimethyl-2,6-octadienoate	2.07	3.4
2-Fluoronerol	1.67	—[a]
2-Fluorogeraniol	1.68	—[a]
2-Fluoroneryl benzoate	1.75	3.4
2-Fluorogeranyl benzoate	1.77	3.0

[a] Obscured by overlapping peaks.

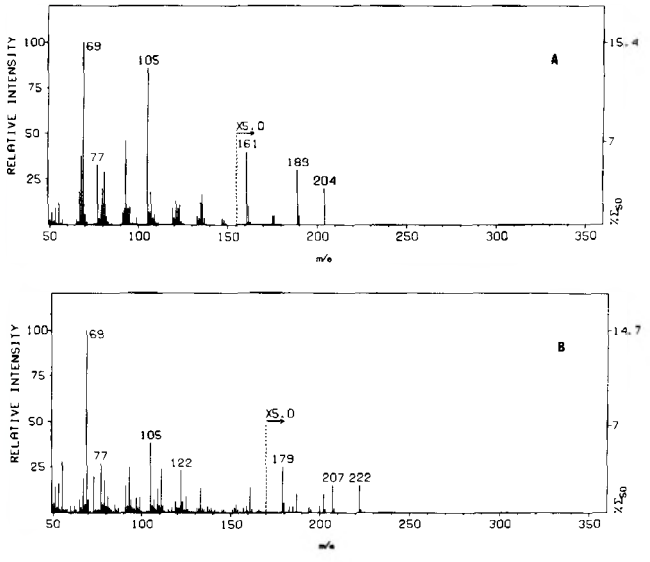


FIG. 1. Mass spectra of farnesyl benzoate (*A*) and 6-fluorofarnesyl benzoate (*B*).

$K_m^{2\text{-fluorogeranyl-PP}}$ were determined from the replots of the $1/v$ intercepts *versus* the concentration of the allylic substrate. Initial velocities were determined from single point measurements in the linear portion of the velocity *versus* time curve.

Inhibition studies showed that 2-fluorogeranyl pyrophosphate is a competitive inhibitor against geranyl pyrophosphate (see Fig. 2). The value for $K_i^{2\text{-fluorogeranyl-PP}}$ was obtained from the appropriate slope and intercept replots, and is listed in Table I.

DISCUSSION

Substitution of the hydrogen at C(2) in geranyl pyrophosphate by fluorine apparently does not alter the binding properties of the substrate appreciably. The Michaelis constants for the natural substrate and the fluorine analogue are similar, and 2-fluorogeranyl pyrophosphate is a competitive inhibitor against geranyl pyrophosphate. In addition, the fluoro derivative participates in the 1′-4 condensation reaction with isopentenyl pyrophosphate to yield 6-fluorofarnesyl pyrophosphate. Thus, the regiochemistry and the stereochemistry of the normal condensation is preserved when 2-fluorogeranyl pyrophosphate serves as an alternate substrate.

However, V_{max} for the 1′-4 coupling is reduced by 8.4×10^{-4} when the vinyl hydrogen at C(2) is replaced by fluorine. This dramatic reduction in V_{max} when binding properties have not been appreciably perturbed is best interpreted as a reduction in the rate of the catalytic step. The data shown in Tables I and III clearly demonstrate that reduction in the rates of the 1′-4 condensation with geranyl pyrophosphate and the 2-fluoro analogue parallel those for solvolysis of the methanesulfonates. Thus, we conclude that the 1′-4 coupling reaction involves ionization of the allylic substrate. The small difference of a factor of 5 in the retardations of the solvolytic and enzymatic reactions is not surprising in view of the obvious difference in local environments during ionization, the difference in leaving groups, and the observation that the 2-fluoro analogue may bind slightly less tightly than geranyl pyrophosphate as evidenced by the Michaelis constants for the two compounds. In contrast, fluorine is slightly accelerating relative to hydrogen for displacement of chloride by cyanide in the geranyl system.

Other experiments also point toward an ionization-condensation-elimination mechanism. For example, as we previously mentioned, *E*- and *Z*-trifluoromethyldimethylallyl derivatives show parallel kinetic behavior for 1′-4 coupling and solvolysis with even more dramatic rate retardations than found for the geranyl analogue (10). In addition, we recently reported that farnesyl pyrophosphate synthetase hydrolyzes its allylic substrates when incubated with inorganic pyrophosphate in the absence of isopentenyl pyrophosphate (23). Since the reaction proceeds with cleavage of the C(1)-oxygen bond and inversion of configuration, it can best be explained as an aborted 1′-4 coupling in which a molecule of water occupies the hydrocarbon portion of the isopentenyl site and captures the allylic cation at C(1) following ionization.

Several years ago, Popják and coworkers (24) studied several analogues of dimethylallyl pyrophosphate, including 2-

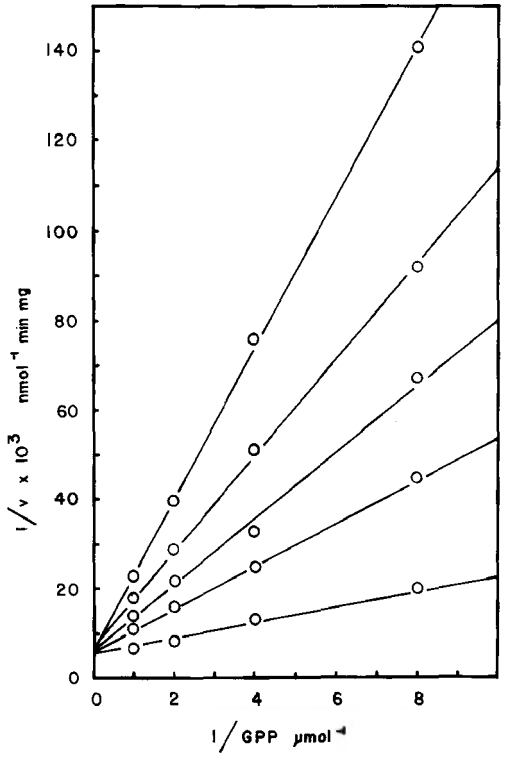


FIG. 2. Inhibition of farnesyl pyrophosphate synthetase with 2-fluorogeranyl pyrophosphate. Concentrations of 2-fluorogeranyl pyrophosphate: none, 2.5, 5, 10, and 20 μM.

propenyl pyrophosphate and *E*- and *Z*-2-butenyl pyrophosphate. While farnesyl pyrophosphate synthetase accepted analogues with increased bulk in the C(3) region as alternate substrates, the desmethyl analogues did not participate in the 1′-4 coupling reaction. The lack of reactivity in the enzymatic reaction can be attributed to poor binding or a low rate for ionization, or both. The latter possibility is particularly attractive since the reactivity of allylic derivatives toward ionization is critically dependent on the nature of the substituents attached to the allyl moiety. For example, replacing both methyl groups at C(3) by hydrogen reduces the reactivity of dimethylallyl derivatives by more than 10^5 (25). Under the conditions of the experiment, products from allylic pyrophosphates with rate depressions of more than 10^2 relative to the dimethylallyl system may have been missed.

The question of timing of the ionization, condensation, and elimination steps has not been resolved. However, the parallel between rate retardations seen for solvolysis and 1′-4 coupling with the trifluoromethyl analogues (10) and 2-fluorogeranyl pyrophosphate suggests substantial delocalization of positive charge into the allyl moiety during the enzyme catalyzed reaction. Thus, it is quite possible that ionization and condensation are discrete steps. Such a proposal does not conflict with the observation that C(1′) is inverted in the coupled product. Although the barrier to rotation about the C(1′)-C(2′) bond in 3,3-dialkyl-substituted allylic cations is not known, estimates place a minimum value at approximately 28 kcal/mol (26). This is too high for bond rotation to occur during the short lifetime of the highly reactive allylic cation. Thus, if ionization occurs from an orientation in which C(4) is positioned at the backside of C(1′), inversion at C(1′) would result unless the entire allylic cation flipped in the active site (2). This is a rather unlikely occurrence in view of the rigidity of the entire allyl moiety.

In summary, 2-fluorogeranyl pyrophosphate reacts with isopentenyl pyrophosphate in the presence of prenyltransferase to yield a C_{15} fluorine-containing homologue. The substrate analogue binds specifically to the allylic site, and kinetic behavior suggests that the binding is almost as tight as that of the natural substrate. Finally, replacing the C(2) hydrogen in the geranyl system by fluorine retards the rate of solvolysis, a reaction known to proceed through a carbonium ion intermediate, and V_{max} of the prenyltransfer reaction by similar amounts. Thus, we conclude that the head-to-tail coupling reaction catalyzed by prenyltransferase proceeds by an ionization-condensation-elimination mechanism.

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MINIPRINT SUPPLEMENT

Farnesyl Pyrophosphate Synthetase. Mechanistic Studies of the 1'-4 Coupling Reaction with 2-Fluorogeranyl Pyrophosphate

C. Dale Poulter, J. Craig Argyle, and Eugene A. Mash

E and Z-2-Fluoro-3,7-dimethyl-2,6-octadienoyl fluoride. Trifluoroethylene (4.1 g, 0.050 mol, PCR) was distilled into a flask containing 120 mL of anhydrous ether which had been cooled to -100° (methanol/N_2 slush). A solution of n-butyl lithium in hexane (31 mL of 1.6 M solution, 0.050 mol) was mixed with 30 mL of ether, cooled to -78° and added dropwise to the stirred reaction solution. The solution was maintained at -100° for one hour and then allowed to warm to -78°. A solution of 6-methyl-5-hepten-2-one, (Givaudan, 6.3 g, 0.050 mol), in 30 mL of ether was cooled to -78° and added dropwise. The solution was allowed to attain room temperature over two hr. Upon addition of 20 mL of water and 20 mL of 6N HCl a white precipitate formed which redissolved on stirring. The organic layer was separated, dried over calcium sulfate, and filtered. Solvent was removed on a rotary evaporator. The remaining oil was slowly heated to 125°C. Hydrogen fluoride vigorously evolved, and the oil darkened. When the evolution of gas ceased, the residue was neutralized with aqueous sodium bicarbonate. Ether (30 mL) was added, the two phases were separated, and the aqueous phase was extracted with ether. The combined ether phase was washed with water and dried over calcium sulfate. Ether was removed on a rotary evaporator to give 4.9 g (52%) of a light yellow oil. A small portion of the material was bulb-to-bulb distilled and the two major components, E- and Z-2-fluoro-3,7-dimethyl-2,6-octadienoyl fluoride, were separated by glpc on a 12' x 1/4" Carbowax 20 M column at 110°. The minor isomer (35%) was E-2-fluoro-3,7-dimethyl-2,6-octadienoyl fluoride, ir (neat) 3050(w), 2990, 2940, 2880, 1818(s), 1660, 1457, 1387, 1297(s), 1248, 1243, 1182, 1110, 1048, 925, 845, and 770 cm^{-1}; ^1H nmr ($CDCl_3$) 1.59 and 1.68 (6, two s, C(7) methyls), 1.97 (3, d, $^5J_{H_3-^{19}F}$ = 4.5 Hz, C(3) methyl), 2.20 (2, m, C(5) protons), 2.55 (2, m, C(4) protons), and 5.10 ppm (1, m, C(6) proton); ^{19}F nmr ($CDCl_3$, 1% CF_3COOH) 21.4 (1, d, $^3J_{1'-^{19}F}$ = 27 Hz, F on C(1')), and -126.2 ppm (1, d further split, F on C(2)). The major isomer (65%) was the Z acid fluoride; ir (neat) 3050(w), 2985, 2940, 2880, 1818(s), 1660, 1457, 1390, 1310(s), 1225(s), 1120, 1045, 930, and 840 cm^{-1}; ^1H nmr ($CDCl_3$) 1.59 and 1.66 (6, two s, C(7) methyls), 2.19 (3, d, $^5J_{H_3-^{19}F}$ = 3.6 Hz, C(3) methyl), 2.32 (4, m, C(4) and C(5) protons), and 5.13 ppm (1, m, C(6) proton); ^{19}F nmr ($CDCl_3$, 1% CF_3COOH) 22.0 (1, d, $^3J_{1'-^{19}F}$ = 27 Hz, F on C(1')) and -129.5 ppm (1, d, q, F on C(2)).

E and Z-2-Fluoro-3,7-dimethyl-2,6-octadienoate. Acid fluoride from the previous experiment (4.8 g, 25 mmol) was dissolved in 30 mL of ether and the resulting solution was stirred vigorously overnight with 100 mL of 2N sodium hydroxide. The layers were separated, and the aqueous phase was extracted with ether. The aqueous layer was acidified with 2N hydrochloric acid and extracted twice with 50 mL of ether. The combined ether layers were dried over calcium sulfate, and solvent was removed at reduced pressure to give 4.5 g (96%) of crude E and Z acids.

A solution of the acids (4.5 g, 24 mmol) and 30 mg of 18-crown-6 in 100 mL of benzene was allowed to stir at reflux over 1.95 g (24 mmol) of potassium carbonate for 4 hr. As the carbonate disappeared, a gelatinous precipitate formed. Benzene was removed on a rotary evaporator, and 100 mL of dry acetonitrile was added. Methyl iodide (0.37 g, 26 mmol) and an additional 30 mg of 18-crown-6 were added. The resulting suspension was stirred at reflux for 8 hr. The gelatinous precipitate disappeared, and a fine precipitate of potassium iodide formed. Solvent was removed on a rotary evaporator, and 100 mL of water added to the residue. The mixture was extracted twice with 75 mL portions of ether (it was necessary to add a small amount of saturated sodium chloride solution to break up the emulsion which formed). The combined ether layers were washed in succession with 20 mL of saturated sodium bicarbonate and 30 mL of water. The ether layer was dried over magnesium sulfate and filtered through Silica gel. Upon removing the ether on a rotary evaporator, 4.0 g of an oil were obtained. Analysis by glpc on Carbowax 20M indicated two major products (65% of the volatile material) in a ratio of 35:65. The two products were separated by preparative glpc for analysis. The less abundant component was methyl E-2-fluoro-3,7-dimethyl-2,6-octadienoate; ir (neat) 3058(w), 2970, 2930, 2870, 1730(s), 1666, 1447, 1384, 1308(s), 1254, 1181, 1141, 1128, 1070, and 785 cm^{-1}; ^1H nmr ($CDCl_3$) 1.62 and 1.68 (6, two s, C(7) methyls), 1.86 (3, d, $^5J_{H_3-^{19}F}$ = 4.5 Hz, E(3) methyl), 2.16 (2, m, C(5) protons), 2.54 (2, m, C(4) protons), 3.82 (3, s, $-OCH_3$), and 5.11 ppm (1, m, C(6) proton); mass spectrum (70eV) m/e (rel. intensity) 200(1), 180(7), 169(1), 165(1), 148(2), 144(4), 143(2), 142(2), 139(3), 132(23), 100(8), 70(6), 69(100), 68(2), 67(4), 59(5), 53(5), 41(55), 39(7); exact mass for $C_{11}H_{17}FO_2$, 200.12123; found, 200.11996.

The more abundant component was the Z isomer; ir (neat) 3060(w), 3038(w), 2975, 2940, 2875, 1732(s), 1669, 1447, 1388, 1322(s), 1248, 1182, 1140, 1118, 1090 and 790 cm^{-1}; ^1H nmr ($CDCl_3$) 1.60 and 1.67 (6, two s, C(7) methyls) 2.07 (3, d, $^5J_{H_3-^{19}F}$ = 3.4 Hz, C(3) methyl), 2.22 (4, m, C(4) and C(5) protons), 3.79 (3, s, $-OCH_3$), and 5.08 ppm (1, m, C(6) protons); mass spectrum (70eV) m/e (rel. intensity) 200(0.2), 180(4), 169(1), 148(0.5), 139(2), 132(13), 100(5), 70(5), 69(100), 68(2), 67(3), 59(3), 53(4), 41(46), 39(7); exact mass for $C_{11}H_{17}FO_2$, 200.12123; found 200.12034.

A minor (5%) component was thought to arise from an acid catalyzed isomerization of the C(6)-C(7) double bond during the rearrangement to the acyl fluoride and was identified as methyl 2-fluoro-3,7-dimethyl-2,7-octadienoate, ir (neat) 3090, 3030(w), 2970, 2950, 2885, 1731(s), 1662, 1448, 1387, 1321(s), 1245, 1181, 1135, 1098, 905 and 788 cm^{-1}; ^1H nmr ($CDCl_3$), 1.58 (2, m, C(5) protons), 1.72 (3, s, C(7) methyl), 2.08 (3, d, $^5J_{H_3-^{19}F}$ = 3.1 Hz, C(3) methyl), 2.16 (4, m, C(4) and C(6) protons), 3.80 (3, s, $-OCH_3$), and 4.68 ppm (2, m, C(8) protons); mass spectrum (70eV) m/e (rel. intensity) 200(2), 194(1), 185(55), 180(11), 144(71), 132(32), 129(42), 121(19), 113(21), 112(14), 109(24), 108(12), 100(12), 96(12), 85(15), 83(11), 81(14), 69(98), 66(22), 59(37), 55(26), 54(21), 41(100), 39(37).

Z- and E-2-Fluoro-3,7-dimethyl-2,6-octadien-1-ol (2-Fluorogeraniol and 2-Fluoronerol). A solution of 3.90 g (19.5 mmol) of a mixture of methyl E- and Z-2-fluoro-3,7-dimethyl-2,6-octadienoate in 50 mL of ether was added to a slurry of 0.23 g (60 mmol) of lithium aluminum hydride (Alfa Ventron) in 50 mL of ether. The stirred mixture was heated at reflux for 40 hr. Excess hydride was destroyed by the dropwise addition with vigorous stirring of saturated ammonium chloride. The ether layer was decanted off, and the inorganic salts were washed twice with 10 mL of ether. The combined ether layers were washed with water, dried over magnesium sulfate, and filtered. Solvent was removed on a rotary evaporator to give a crude yield of 3.1 g. This material was then placed on a 4' x 1" column packed with 0.032-0.063 mm silica gel (ICN) and eluted with 8% ethyl acetate/benzene. The E isomer (2-fluoronerol) eluted between 915 and 1115 mL. After solvent was removed, the residue was distilled (15u, bath 80°) to give 1.0 g (30%) of a colorless oil; ir (neat) 3350(b), 3040(w), 2980, 2940, 2880, 1708(w), 1455, 1385, 1275, 1184, 1150, 1122, 1095, and 1025 cm^{-1}; ^1H nmr ($CDCl_3$) 1.61 (3, s, C(7) methyl), 1.67 (6, broad s, C(3) and C(7) methyls), 2.11 (5, m, C(4), C(5), and -OH protons), 4.22 (2, d, $^3J_{H_1-^{19}F}$ = 22.8 Hz, C(1) protons), and 5.12 ppm (1, m, C(6) proton); mass spectrum (70eV) m/e (rel. intensity) 172(2), 154(1), 152(1), 141(2), 111(11), 70(12), 69(100), 68(6), 67(5), 55(18), 53(16), 41(88), 39(24).

Anal. Calcd. for $C_{10}H_{17}FO$: C, 69.73; H, 9.95. Found: C, 69.77; H, 9.69.

The Z isomer (2-fluorogeraniol) eluted between 1235 and 1555 mL. After molecular distillation, 1.4 g (42%) of a colorless oil was obtained; bp 74.5 - 75.0° (0.4 mm/Hg); ir (neat) 3350(b), 3040(w), 2980, 2940, 2870, 1705(w), 1460, 1385, 1280, 1170, 1125, 1100 and 1025 cm^{-1}; ^1H nmr ($CDCl_3$) 1.62 (3, s, C(7) methyl), 1.68 (6, broad s, C(3) and C(7) methyls), 2.11 (5, m, C(4) and C(5) and -OH protons), 4.22 (2, d, $^3J_{H_1-^{19}F}$ = 22.8 Hz, C(1) protons), and 5.12 ppm (1, m, C(6) proton); mass spectrum (70eV) m/e (rel. intensity) 172(2), 154(1), 152(1), 141(2), 111(13), 70(14), 69(100), 68(6), 67(6), 57(14), 55(20), 53(30), 43(16), 41(74), 39(15).

Anal. Calcd. for $C_{10}H_{17}FO$: C, 69.73; H, 9.95. Found: C, 69.63; H, 10.04.

Z-2-Fluoro-3,7-Dimethyl-2,6-Octadien-1-yl Benzoate (2-Fluorogeranyl Benzoate). To a dry, 5 mL flask were added 24 mg (0.15 mmol) of 2-fluorogeraniol, 1 mL of dry pyridine and 42 mg (0.30 mmol) of benzoyl chloride. The flask was sealed with a rubber septum and heated at 50°C for 2 hr. White crystals formed when the solution cooled. The contents were mixed with 5 mL of ether. The ether solution was decanted off of the white precipitate which formed. The solvent and excess pyridine were removed on a rotary evaporator. The remaining oil was dissolved in 10 mL of ether and extracted in succession with 10 mL portions of 2N HCl, saturated sodium bicarbonate (twice) and water. After drying over magnesium sulfate, the ether was removed to give 34 mg (83%) of oil; ^1H nmr ($CDCl_3$) 1.57 and 1.64 (6, two s, C(7) methyls), 1.77 (3, d, $^5J_{H_3-^{19}F}$ < 3.0 Hz, C(3) methyl), 2.12 (4, m, C(4) and C(5) protons), 4.92 (2, d, $^3J_{H_1-^{19}F}$ = 22 Hz, C(1) protons), 5.08 (1, m, C(6) proton), 7.47 (3, m, m and p protons), 8.03 ppm (2, m, o protons); mass spectrum (70eV) m/e (rel. intensity), 276 (0.1), 207(10), 154(16), 139(5), 134(5), 125(4), 111(23), 110(11), 106(8), 105(100), 98(7), 93(4), 86(27), 85(7), 78(5), 70(5), 69(96), 68(18), 67(6), 53(10), 51(18), 50(5), 41(60).

E-2-Fluoro-3,7-Dimethyl-2,6-Octadien-1-yl Benzoate (2-Fluoroneryl Benzoate). Following the procedure described for the Z isomer, 36 mg, (88%) of crude product was obtained; ^1H nmr ($CDCl_3$) 1.57 and 1.64 (6, two s, C(7) methyls), 1.75 (3, d, $^5J_{H_3-^{19}F}$ = 3.4 Hz, C(3) methyl), 2.12 (4, m, C(4) and C(5) protons), 4.93 (2, d, $^3J_{H_1-^{19}F}$ = 22 Hz, C(1) protons), 5.08 (1, m, C(6) proton), 7.49 (3, m, m and p protons), 8.05 ppm (2, d, d, o protons); mass spectrum (70eV) m/e (rel. intensity), 276 (0.1), 207(8), 154(15), 139(7), 134(4), 125(4), 111(27), 110(11), 106(8), 105(100), 98(8), 93(5), 86(19), 85(6), 77(41), 70(41), 69(74), 68(17), 67(6), 53(9), 51(15), 50(4), 41(44).

Z-2-Fluoro-3,7-dimethyl-2,6-octadien-1-yl Pyrophosphate (2-Fluorogeranyl Pyrophosphate). 2-Fluorogeraniol, (0.10 g, 0.58 mmol) and freshly distilled trichloroacetonitrile (0.51 g, 3.5 mmol) were combined in a dry, nitrogen-flushed flask. Di(triethylamine) phosphate (1.19 g, 4.0 mol) was added to a 50 mL of freshly distilled acetonitrile. The solution was added to the alcohol over a period of 2 hr. After 20 hr 50 mL of methanol-water (90:10) containing 1 mL of concentrated ammonium hydroxide was added. The mixture was placed on a 1 x 20 cm Dowex 1-X8 formate column that had been previously washed with 50 mL of methanol containing 1 mL concentrated ammonium hydroxide. The column was developed with a 300 mL linear gradient (0 to 0.42 N) of ammonium formate in methanol-water (90:10). Ammonium molybdate was used to visualize the pyrophosphate when spotted on paper. The fractions containing pyrophosphate were combined, and solvent was removed on a rotary evaporator at 35°C. Absolute ethanol containing a few drops of concentrated ammonium hydroxide was added to azeotrope off residual water. Ammonium formate was removed by sublimation (35°, 4u for 15 hr), yielding 22 mg of a white powder. TLC was performed on the residue using silica gel H buffered plated $CHCl_3$, MeOH, H_2O:5,5,1) followed by visualization with a dilute solution of potassium permanganate in aqueous methanol (R_f's for mono- and pyrophosphate were 0.80 and 0.55, respectively).

E-3,7-Dimethyl-2,6-octadien-1-yl Methanesulfonate (Geranyl Methanesulfonate). A solution of 1 mL of anhydrous pentane, 1 mL of anhydrous benzene, and 15 mg (0.0974 mmol) of geraniol was allowed to stir in an ice-methanol bath. To this mixture was added 7 µL (0.0104 g, 0.090 mmol) of methanesulfonyl chloride. After 10 min, 25 µL (0.0182 g, 0.180 mmol) of dry triethylamine was added. This addition was accompanied by formation of a white precipitate. After 20 min, 0.5 mL of dry pentane was added to the reaction mixture. The resulting suspension was rapidly filtered through a pasteur pipette plugged with base-washed glass wool. The clear solution was collected in a small, dry test tube where the volume was reduced to 0.2 mL by evaporation of pentane using dry nitrogen. The solution was kept covered and frozen in a Dry Ice-acetone bath until used in kinetic runs; ^1H nmr (CCl_4, -35°C), 1.59 (3, s, C(7) methyl), 1.65 (3, s, C(7), methyl), 1.77 (3, s, C(3) methyl), 2.08 (4, m, C(4) and C(5) protons), 2.92 (3, s, mesylate methyl), 4.68 (2, d, $^3J_{H_1-H}$ = 7.5 Hz, C(1) protons), 5.02 (1, br s, H at C(6)), and 5.39 ppm (1, t, H at C(2)).

Z-2-Fluoro-3,7-dimethyl-2,6-octadien-1-yl Methanesulfonate (2-Fluorogeranyl Methanesulfonate). A procedure analogous to that for geraniol was employed for the synthesis of 2-fluorogeranyl methanesulfonate. The amount of 2-fluorogeraniol used was 15 mg (0.0872 mmol), and the amounts of other components were unchanged; ^1H nmr (CCl_4) 1.60 (3, s, C(7) methyl), 1.67 (3, s, C(7) methyl), 1.75 (3, d, $^5J_{H_3-^{19}F}$ = 3 Hz, C(3) methyl), 2.13 (4, m, C(4) and C(5) protons), 2.92 (3, s, mesylate methyl), 4.76 (2, d, $^3J_{H_1-^{19}F}$ = 22.8 Hz, C(1) protons), and 5.05 ppm (1, m, C(6) proton).

E-1-Chloro-3,7-Dimethyl-2,6-octadiene (Geranyl Chloride). Geranyl chloride was prepared according to the procedure of Calzada and Hooz[11] and purified by bulb-to-bulb distillation. Nmr and ir spectra of the pure compound were consistent with published spectra.

Z-1-Chloro-2-fluoro-3,7-dimethyl-2,6-octadiene (2-Fluorogeranyl Chloride). A procedure analogous to that used in the preparation of geranyl chloride was employed for the synthesis of 2-fluorogeranyl chloride. 2-Fluorogeraniol (113 mg, 0.66 mmol) gave 93.3 mg (75%) of 2-fluorogeranyl chloride after GLC purification on a 6' x 1/4" 10% Carbowax 20 M column at 150°; ir (neat) 2965, 2920, 2855, 1685(m), 1660(w), 1450, 1435, 1380, 1370, 1325, 1285, 1248, 1215, 1200, 1165, 1140, 1110, 1080, 888, 870, 830 and 720 cm^{-1}; ^1H nmr (CCl_4) 1.60 (3, s, methyl at C(7)), 1.66 (3, s, methyl at C(7)), 1.70 (3, br s, methyl at C(3)), 2.12 (4, m, protons on C(4) and C(5)), 4.07 (2, d, protons on C(2), $^3J_{H_1-^{19}F}$ = 22 Hz), and 5.01 ppm (1, m, proton on C(6)); ^{19}F nmr (CCl_4, 3% CF_3CO_2H external standard) -116.22 ppm (1, $^3J_{H_1-^{19}F}$ = 22.6 Hz with protons at C(1), $J_{H_1-^{19}F}$ = 2 Hz with protons on C(3) methyl); mass spectrum (80eV) m/e (rel. intensity) 154(2), 136(2), 134(7), 131(4), 130(2), 129(13), 128(4), 111(6), 109(2), 99(7), 86(9), 85(7), 79(3), 77(3), 70(11), 69(100), 68(7), 67(9), 65(4), 59(5), 55(3), 53(11), 51(5), 43(5), 42(5), 41(95), 40(5), 39(18).

Anal. Calcd. for $C_{10}H_{16}ClF$: C, 62.99; H, 8.46. Found: C, 62.98 H, 8.80.

E-4,8-Dimethyl-3,7-nonadiene Nitrile (Geranyl Cyanide). A solution of 2 mL of dry acetonitrile, 172 mg (1 mmol) of geranyl chloride, 670 mg (10 mmol) of dry, powdered potassium cyanide, and a catalytic amount of purified 18-crown-6 was stirred overnight. The reaction mixture was diluted with 20 mL of distilled water, and the aqueous phase was extracted twice with 10 mL of ether. The ether phases were combined and dried. Solvent was removed by rotary evaporation. GLC analysis gave a single component (10% Carbowax 20M, 170°), which was collected and analyzed; ir (neat) 2960, 2910, 2840, 2245, 1705(vw), 1655(w), 1440, 1410, 1370, 1103, 915, and 790 cm^{-1}; ^1H nmr (CCl_4) 1.66 and 1.72 (3, s, and 6, s, respectively, methyls on C(4) and C(8)), 2.05 (4, m, protons on C(5) and C(6)), 2.97 (2, d, protons on C(2), $^3J_{H_2-H}$ = 6.9 Hz), 5.05 (1, m, partially obscured), and 5.17 ppm (1, broad triplet, partially obscured, $^3J_{H_3-H}$ = 6.9 Hz); mass spectrum (80eV) m/e (rel. intensity) 163(4), 162(1), 148(8), 123(19), 120(15), 119(9), 95(9), 91(5), 79(8), 77(7), 70(27), 69(100), 68(26), 67(25), 66(5), 65(7), 55(7), 53(25), 52(5), 51(8).

Anal. Calcd. for $C_{11}H_{17}N$: C, 80.92; H, 10.50. Found: C, 80.72; H, 10.67.

Z-3-Fluoro-4,8-dimethyl-3,7-nonadiene Nitrile (2-Fluorogeranyl Cyanide). A procedure analogous to that used in the preparation of geranyl cyanide was used to synthesize 2-fluorogeranyl cyanide. 2-Fluorogeranyl chloride (93 mg, 0.49 mmol) was dissolved in approximately 1.5 mL of dry acetonitrile containing a catalytic amount of 18-crown-6. This solution was stirred over 500 mg (7.68 mmol) of potassium cyanide overnight and the reaction worked up as described for geranyl cyanide. GLC analysis on 10% Carbowax indicated the formation of two principal products which were collected and analyzed. One product was identified as 2-fluorogeranyl cyanide; ir (neat) 2965, 2925, 2855, 2255, 1700, 1655(w), 1445, 1405, 1372, 1312, 1228, 1150, 1110, 1083, 980, 912, 885, 864, 828, 765, and 736 cm^{-1}; ^1H nmr (CCl_4) 1.58 (3, s), 1.64 (3, partially obscured doublet) 1.66 (3,s), 2.06 (4, m, H at C(5) and C(6)), 3.19 (2, d, H at C(1), $^3J_{H_1-^{19}F}$ = 18.0 Hz), and 4.98 ppm (1, m, H at C(7)); ^{19}F nmr (CCl_4, 3% CF_3CO_2H, external standard), -109.42 ppm (1, t of q, $^3J_{H_1-^{19}F}$ = 18.6 Hz with protons at C(2), $^4J_{H_4-^{19}F}$ = 2 Hz with protons on C(4) methyl); mass spectrum (80eV) m/e (rel. intensity) 161(1), 155(3), 154(28), 153(9), 152(6), 121(4), 120(7), 119(5), 70(9), 69(100), 67(6), 53(7), 41(94), 39(14), 32(6); chemical ionization (methane) m + 1 = 182.

Anal. Calcd. for $C_{11}H_{16}FN$: C, 72.89; H, 8.90. Found: C, 72.69; H, 9.11.

The second product of the reaction was identified as Z-3-fluoro-4,8-dimethyl-2,7-nonadiene nitrile; ir (CCl_4) 3070, 2965, 2920, 2847, 2227(s), 1665(s), 1454, 1380, 1320, 1160, and 1115 cm^{-1}; uv (acetonitrile) λ_{max} 198.5 nm (ϵ_{max} 8 x 10^3); ^1H nmr (CCl_4) 1.14 (3, d, $^3J_{H_8-H}$ = 7.2 Hz, C(4) methyl), 1.57 (3, s, C(8) methyl), 1.66 (3, d, $^5J_{H_6-^1H}$ = 2 Hz, C(8) methyl), 1.75-2.15 (4, m, C(5) and C(6) protons), 2.15-2.62 (1, m, C(4) proton), 4.65 (1, d, $^3J_{H_1-^{19}F}$ = 32.4 Hz, proton on C(2)), and 4.95 ppm (1, partially resolved triplet, proton on C(7)); ^{19}F nmr (CCl_4, 3% CF_3CO_2H, external standard) -82.09 ppm (1, dd, $^3J_{H_1-^{19}F}$ = 20.5 Hz with proton on C(4), $^2J_{H_1-^{19}F}$ = 32.6 with proton on C(2)); mass spectrum (80eV) m/e (rel. intensity) 181(3), 180(4), 166(9), 126(4), 121(10), 112(15), 99(9), 98(8), 93(6), 83(13), 82(9), 81(8), 79(8), 73(8), 71(6), 70(15), 69(51), 67(16), 59(16), 57(33), 56(22), 55(60), 53(60), 53(9), 51(6), 43(33), 42(20), 41(100), 39(24); chemical ionization (methane) m + 1 = 182.