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THE MECHANISM OF THE ACID-CATALYZED DECOMPOSITION OF THE FARNESYL PHOSPHATES

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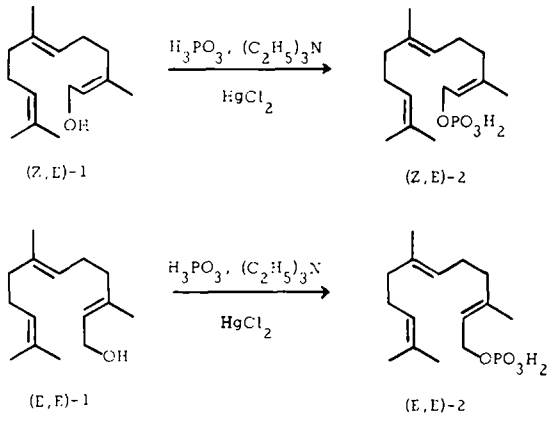
Abstract—The rates and (in some cases) products of the acid-catalyzed decomposition of (Z,E)- and (E,E)-farnesyl phosphate, (Z,E)- and (E,E) - 1,1 - dideutereofarnesyl phosphate, (Z)- and (E) - 6,7,10,11 - tetrahydrofarnesyl phosphate, and t-butyl phosphate have been studied in an attempt to determine whether (Z,E)-farnesyl phosphate ionizes with intramolecular assistance from the C-6/C-7 double bond or via an unassisted process leading to a simple allylic cation. Data in support of both possibilities are adduced, but it is concluded, primarily on the basis of the secondary deuterium kinetic isotope effects, that the ionization involves little, if any, assistance from the double bond.

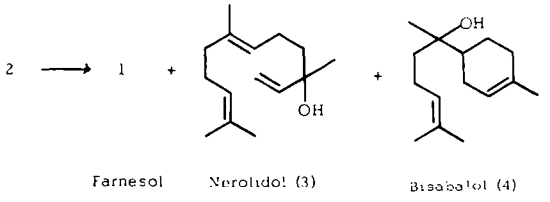
Work previously reported from this laboratory has dealt with the acid-catalyzed decomposition of farnesol (**1**),[1] the long-range goal of these studies being to develop polyfunctional compounds that are capable of catalyzing the cyclization of farnesol in fashions comparable to those observed in nature. In living systems, however, the starting material is farnesyl pyrophosphate rather than farnesol. Therefore, to investigate a system more closely resembling the natural substrate the behavior of farnesyl phosphate (**2**) in acid solutions has been investigated. Implicit in the working hypotheses for one of the anticipated polyfunctional catalysts is the idea that the phosphate group is expelled as the result of intramolecular attack by a double bond in the farnesyl chain. The objective of the present work was to determine whether this occurs to any extent in aqueous solution under the conditions of simple catalysis or whether the phosphate group leaves *before* intramolecular carbon–carbon bond formation has developed to any significant extent.



Synthesis of (Z,E)- and (E,E)-farnesyl phosphates (**2**). Although farnesyl phosphate has been synthesized,[2] the reported yield is only 10% and to achieve even this requires considerable finesse. Other methods, therefore, were investigated, including Tener's procedure[3] using β-cyanoethyl phosphate and dicyclohexylcarbodiimide, Dimroth's procedure[4] using di - β - cyanoethylphosphocloridate, Moffat and Khorana's procedure[5] using tetra - *p* - nitrophenylpyrophosphate reagent, and Obata and Mukaiyama's procedure[6] involving the action of phosphorus acid, triethylamine, and mercuric chloride on farnesol. Only the last of these three methods produced farnesyl phosphate in useful amount, providing the material in 40% yield (corrected for recovered farnesol) after certain modifications in the procedure were made. By means of this method, pure samples of (Z,E)- and (E,E)-farnesol (obtained by fractional distillation of commercial farnesol which contains *ca.* 68% of the (E,E)-isomer and 28% of the (Z,E)-isomer) were converted to (E,E)-farnesyl phosphate [(E,E)-2] and (Z,E)-farnesyl phosphate [(Z,E)-2].

Comparison of the product mixtures from the formolysis of (Z,E)- and (E,E)-farnesyl phosphate. The (Z,E)- and (E,E)-isomers of farnesol and farnesyl phosphate were treated under previously-described conditions[1] (10 min at 0° in anhydrous formic acid), and the product mixtures were hydrolyzed and assayed to give the results recorded in Table 1.

The two most striking differences between the farnesols and the corresponding phosphates are (a) the formation of significant amounts of diols and triols (assumed to be monocyclic compounds[1]) from the farnesols, in contrast to their absence when the farnesyl phosphates are used and (b) the formation of more cyclic compound from (Z,E)-farnesol than from (E,E)-farnesyl phosphate. The formation of more cyclic product from (Z,E)-farnesol than from (E,E)-farnesol, noted earlier,[1] is also observed for the farnesyl phosphates.



Composition of the product mixtures from the hydrolysis of (Z,E)- and (E,E)-farnesyl phosphates. The (Z,E)- and (E,E)-farnesyl phosphates were treated with aqueous buffers at 50°, and the compositions of the product mixtures were assayed to give the results recorded in Table 2. The dehydrogenation procedure for assessing the product composition[1] was not used in this case because of the large amount of acyclic material in the product. It has been observed[1] that acyclic material yields

Table 1. Products of formolysis of (Z,E)- and (E,E)-farnesols and farnesyl phosphates

	Initial reaction products							*Dehydrogenation products*		
	Alkenes, %	Bisabalol (4), %	Nerolidol (3), %	(Z,E)-Farnesol [(Z,E)-1], %	(E,E)-Farnesol [(E,E)-1], %	Diol, %	Triol, %	Fragmentation Products, %	Monocyclic Products, %	Bicyclic Products, %
(Z,E)-Farnesol	19	42	0	2	4	26	7	8	88	4
(Z,E)-Farnesyl Phosphate	9	77	1	6	7	0	0	5	95	0
(E,E)-Farnesol	12	31	0	2	11	22	22	25	73	2
(E,E)-Farnesyl Phosphate	6	43	2	6	43	0	0	35	59	6

Table 2. Products of hydrolysis of (Z,E)- and (E,E)-farnesyl phosphate

	pH	Alkenes, %	Bisabalol (4), %	Nerolidol (3), %	(Z,E)-Farnesol [(Z,E) – 1], %	(E,E)-Farnesol [(E,E)-1], %
(Z,E)-Farnesyl Phosphate	2	27	42	26	5	1
(with glycinate buffer)	3	23	45	25	5	2
(Z,E)-Farnesyl Phosphate	4	14	51	29	6	1
(with acetate buffer)	5	10	49	33	6	3
(E,E)-Farnesyl Phosphate	2	6	2	71	0	17
(with glycinate buffer)	3	9	4	70	0	17
(E,E)-Farnesyl Phosphate	4	5	3	75	0	17
(with acetate buffer)	5	2	0	83	0	15

fragmentation products along with small amounts of monocyclic and bicyclic products upon dehydrogenation. With larger amounts of acyclic material this complication becomes increasingly serious and tends to obscure the composition of the product mixture. The conditions that induce the hydrolysis of the farnesyl phosphates have relatively little effect on the farnesols themselves, indicating that the product mixtures shown in Table 2 are essentially those produced directly from the farnesyl phosphates.

The data in Table 2 show that the composition of the product mixtures from (Z,E)- and (E,E)-farnesyl phosphate does not vary greatly between pH 2 and pH 5, the greatest difference being in the amount of alkene formed from the (Z,E)-isomer. Of significance is (a) the difference in the amount of bisabalol (**4**) formed from the two isomers, the (Z,E)-compound producing **4** in 42–51% yield and the (E,E)-compound in negligible amount and (b) the formation of nerolidol (**3**) and (E,E)-farnesol in significant amounts from (E,E)-farnesyl phosphate, in contrast to the results obtained in anhydrous formic acid (Table 1). The preferential formation of cyclic products from (Z,E)-farnesol and (Z,E)-farnesyl phosphate is a consequence of the favorable geometry between the C-1 carbon and the C-6/C-7 double bond.

Determination of the dissociation constants of (Z,E)- and (E,E)-farnesyl phosphate. To study the kinetics of the acid-catalyzed decomposition of the farnesyl phosphates over the pH range of 2–6 it is necessary to know their dissociation constants. Because the half-life of farnesyl phosphate in solutions of pH 2–3 (i.e. near pK_1) is less than ten minutes at 50°, it was necessary to measure the pK values at several lower temperatures and then extrapolate the data to higher temperatures.

The dissociation constants were determined by measuring the pH of known mixtures of bis(tetramethylammonium) farnesyl phosphate and hydrochloric acid and treating the data by the methods of Albert and Serjeant.[7] The values are recorded in Table 3. Also measured were the dissociation constant of t-butyl phosphate which was used as a reference compound in the study of the kinetics of the hydrolysis (see next section).

It was necessary to determine the dissociation constants in solutions which were *ca.* 0.01 M in farnesyl phosphate, because for this type of measurement the negative logarithm of the concentration should be smaller than the pK being measured.[7] At this concentration, however, micelle formation tends to interfere with the

Table 3. Dissociation constants of (Z,E)-farnesyl phosphate, (E,E)-farnesyl phosphate, and t-butyl phosphate

Compound	Temp. (°C)	pK_1	pK_2
(Z,E)-Farnesyl phosphate	8.80	2.46 ± 0.04	6.77 ± 0.09
[(Z,E)-2]	13.40	2.65 ± 0.03	6.91 ± 0.05
	18.53	2.69 ± 0.04	6.90 ± 0.06
	23.80	2.81 ± 0.04	6.94 ± 0.06
(E,E)-Farnesyl phosphate	8.80		7.11 ± 0.05
[(E,E)-2]	13.40	3.15 ± 0.11	7.04 ± 0.03
	18.53	3.02 ± 0.11	7.02 ± 0.04
	23.80	3.11 ± 0.09	7.04 ± 0.05
(with micelle	8.80	3.61 ± 0.05	
formation)	13.40	3.54 ± 0.03	
	18.53	3.47 ± 0.04	
	23.80	3.37 ± 0.08	
t-Butyl phosphate	8.80	2.44 ± 0.03	8.08 ± 0.02
	13.40	2.46 ± 0.03	8.07 ± 0.01
	18.53	2.44 ± 0.04	8.06 ± 0.02
	23.80	2.59 ± 0.06	8.06 ± 0.01

measurements. Thus, in determining pK_1 for (E,E)-**2** it was found that the initially-measured values were *ca.* 0.5 pK units higher than those measured after the solution had been allowed to remain in the pH cell for several minutes.[8] The latter values are reasonably close to those measured for 0.001 M solutions of (E,E)-**2**, so it was concluded that they are the correct ones, uncomplicated by micelle formation. Since the phenomena that occurred with the (E,E)-isomer were not observed with the (Z,E)-isomer, it can be concluded that either (a) micelles were present under all circumstances of the measurements or (b) micelles were not present under any circumstances of the measurements. That the latter is probably true is suggested by the similarity of pK_1 values for (Z,E)-**2** and t-butyl phosphate.

Employing the method of Harned and Embree,[9] the pK data were extrapolated to 50°. The accuracy of the extrapolated values must be accorded some skepticism, however, because of the possible complication of micelle formation. For comparison with data in the literature, the dissociation constants for t-butyl phosphate were calculated at 25° and found to be $pK_1 = 2.61$ and $pK_2 = 8.06$. These differ somewhat from those reported by Lapidot *et al.*[10] ($pK_1 = 2.76$ and $pK_2 = 7.43$), a difference that, at least in part, may be ascribed to the inclusion of activity corrections for the values that are reported in the present paper.

Kinetics of the acid-catalyzed decompositon of (Z,E)- and (E,E)-farnesyl phosphate. The kinetic data were obtained in an automated apparatus[11] in which the rate of appearance of inorganic phosphate was measured.[12] The acid-catalyzed decompositions were carried out in aqueous buffer systems at 50° and a total ionic strength of 0.200 M. Among the buffer systems used were glycine-glycine hydrochloride, tetramethylammonium formate-formic acid, tetramethylammonium acetate-acetic acid, and bis(tetramethylammonium) maleate-tetramethylammonium maleate. All but one of these buffer systems were shown to be without effect on the rate of the reaction. The glycine-glycine hydrochloride buffer, however, slowed the reaction of both the (Z,E)- and (E,E)-isomers, reducing the reaction rate of the (E,E)-isomer by as much as 40%.

In the thought that the reduction in rate observed in the glycine-glycine hydrochloride buffer might be the result of ion-pairing between glycine and farnesyl phosphate, the effect of a series of ω-amino acids on the rate of acid-catalyzed decomposition was tested to see if such compounds fulfill the role of a bifunctional catalyst. However, the rate of decomposition remained unchanged in the presence of β-alanine, γ-aminobutyric acid and ϵ-aminocaproic acid buffer systems. The only substances, in addition to glycine, that were found to have a perceptible effect on the rate of the acid-catalyzed decomposition of the farnesyl phosphates are the α- and β-cycloamyloses, the larger effect being observed with the β-cycloamylose which reduces the rate by a factor of *ca.* 4-fold. This is in contrast to the effect of the cycloamyloses on the rate of hydrolysis of pyrophosphates, where a considerable enhancement has been reported.[13]

The problem of micelle formation, discussed in connection with the pK measurements, was also of concern in the kinetic measurements. To estimate the critical micelle concentration under the conditions of the acid-catalyzed decomposition, the rates of reactions at several initial concentrations of farnesyl phosphate were measured. These data, recorded in Table 4, show a significant reduction in rate for (E,E)-farnesyl phosphate at concentrations of *ca.* 2 mM[14] but a constant rate for (Z,E)-farnesyl phosphate over the concentration range of 0.4–2.3 mM. From this it is concluded that the critical micelle concentration for (E,E)-**2** is *ca.* 1.7 mM and that for (Z,E)-**2** is either below 0.4 mM or above 2.3 mM. For reasons already indicated, the latter is thought to be the more likely.

Table 4. Rate of hydrolysis of (Z,E)- and (E,E)-farnesyl phosphate at 50° as a function of initial concentration

Compound	Initial Conc. (mM)	pH	$k_\psi \times 10^4$
(Z,E)-Farnesyl phosphate	0.400	3.41	2.18 ± 0.03
	0.605	3.41	2.33 ± 0.02
	0.815	3.41	2.39 ± 0.02
	0.985	3.39	2.56 ± 0.02
	1.69	3.41	2.32 ± 0.02
	2.06	3.42	2.68 ± 0.04
	2.32	3.42	2.40 ± 0.02
(E,E)-Farnesyl phosphate	0.397	3.37	1.59 ± 0.01
	0.630	3.37	1.66 ± 0.01
	0.840	3.38	1.629 ± 0.007
	1.04	3.38	1.56 ± 0.01
	1.21	3.38	1.698 ± 0.004
	1.67	3.38	1.364 ± 0.006
	1.95	3.38	1.14 ± 0.01
	2.45	3.38	1.00 ± 0.01

The pH–log rate profiles for the acid-catalyzed decomposition of (Z,E)- and (E,E)-farnesyl phosphate resemble those of t-butyl phosphate,[10] benzyl phosphate,[15] sugar phosphates,[16–18] and allylic phosphates.[19,20] Unlike the pH–log rate profiles of phosphate esters such as methyl phosphate,[21] there is no maximum at *ca.* pH 4 but, instead, a continuous increase in rate with increasing hydrogen ion concentration. This can be interpreted in terms of a process in which the conjugate acid of the phosphate ($AH_2^\oplus$), the neutral phosphate (AH), and the monoanion of the phosphate ($A^\ominus$) all are susceptible to acid-catalized decomposition; i.e.

$$k_\psi = k_{AH_2^\oplus}[AH_2^\oplus] + k_{AH}[AH] + k_{A^\ominus}[A^\ominus].$$

To obtain values for each of the individual rate constants it is necessary to known the dissociation constants for $AH_2^\oplus$, AH and $A^\ominus$. Although those for AH and $A^\ominus$ have been measured (i.e. pK_1 and pK_2 of the phosphate esters), the dissociation constant of the conjugate acid $AH_2^\oplus$ can only be estimated. Expressing the concentrations of the phosphate species in terms of mole fractions, the following expression can be set up in which $X_{A^\ominus}$ is the mole fraction of the anion and $K_{AH_2^\oplus}$ and K_{AH} are the dissociation constants of the conjugate acid and the neutral species, respectively;

$$k_\psi = \left(\frac{k_{AH_2^\oplus}[H^\oplus]^2}{K_{AH_2^\oplus}K_{AH}} + \frac{k_{AH}[H^\oplus]}{K_{AH}} + k_{AH}\right) X_{A^\ominus}.$$

From a least squares fit of the observed rates to a parabola defined by the plot of $k\psi/X_{A^\ominus}$ vs $[H^\oplus]$, the following rate

constants are obtained:

	$k_{AH_2^{\oplus}}/K_{AH_2^{\oplus}}$	k_{AH}	$k_{A^{\ominus}}$
(Z,E)-Farnesyl phosphate	0.33 ± 0.02	$3.4 \pm 0.2 \times 10^{-4}$	$8 \pm 4 \times 10^{-6}$
(E,E)-Farnesyl phosphate	0.096 ± 0.001	$4.0 \pm 0.2 \times 10^{-4}$	$1 \pm 5 \times 10^{-7}$

The pK spacing of the dissociation constants of inorganic oxyacids is generally 4–5 pH units. Thus, if we estimate $K_{AH_2^{\oplus}}$ to be 10–100, $k_{AH_2^{\oplus}}$ acquires values of *ca.* 1–30, indicating thah the conjugate acid species ($AH_2^{\oplus}$) rea ts very much more rapidly than the neutral species (AH) or the anion species ($A^{\ominus}$). This is commensurate with the idea that a carbonium ion intermediate is involved in the reaction.

Measurements of hydrolysis rates were also carried out on the (Z)- and (E)-isomers of 6,7,10,11-tetrahydrofarnesyl phosphates, yielding the following comparative rate data: (E,E)-farnesyl phosphate—1.00; (Z,E)-farnesyl phosphate—1.19; (E)-6,7,10,11-tetrahydrofarnesyl phosphate—0.55; (Z)-6,7,10,11-tetrahydrofarnesyl phosphate —0.60. These data indicate that there is some rate enhancement in the farnesyl phosphates, possibly arising from participation by the C-6/C-7 double bond. The smallness of the enhancement, however, and the fact that the (Z,E)-isomer is only slightly more reactive than the (E,E)-isomer suggest that the degree of participation is small or nonexistent.

The product analysis of the acid-catalyzed decomposition of the farnesyl phosphates indicates that the *cis*-isomer forms considerably more cyclic product than the *trans*-isomer and that inversion of configuration around the C-2/C-3 double bond does not occur to any significant extent. The kinetic analysis indicates that the reaction is subject to specific hydrogen ion catalysis and is not significantly affected by buffer species. These observations can be accommodated either by (a) a mechanism involving the assisted (by the C-6/C-7 double bond) loss of a phosphate moiety to form a bridged carbonium ion which subsequently reacts with water to form farnesol, nerolidol, and bisabalol or (b) a mechanism involving the unassisted loss of a phosphate moiety (from the conjugate acid, the neutral phosphate, or the monoanion) to form a configurationally stable allylic cation[22] which reacts intermolecularly with water to form farnesol and nerolidol or intramolecularly with the C-6/C-7 double bond to form bisabalol (Fig. 1).

α-Deuterium isotope effect on the acid catalyzed decomposition of farnesyl phosphate. The ethyl esters of (Z,E)- and (E,E)-farnesic acid were synthesized by the method of Popjak *et al.*[23] and reduced with lithium ethoxyaluminum deuteride by the method of Davidson *et al.*[24] The resultant (Z,E)- and (E,E)-dideuteriofarnesols were converted to the phosphates. The rates of acid-catalyzed decomposition of these compounds were measured and compared with those of the proteo analogs, giving k_H/k_D values (per deuterium) of 1.21 ± 0.04 for the (Z,E)-isomer and 1.17 ± 0.03 for the (E,E)-isomer.

The magnitude of the secondary deuterium isotope effect has been used as a probe for neighboring group participation in nucleophilic displacement reactions.[25] In S_N1 processes in which no neighboring group participation occurs the k_H/k_D values are in the range of 1.15 per deuterium atom when the deuterium is at the α-carbon. To the extent that a neighboring group participates via bond formation at the face opposite to

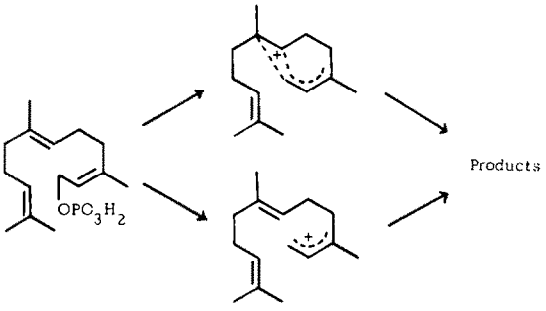
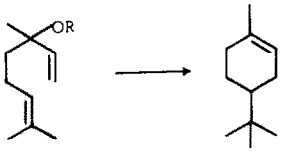


Fig. 1. Assisted and unassisted pathways for the conversion of farnesyl phosphate to the farnesyl cation.

that of the leaving group, the k_H/k_D ratio is reduced, approaching 1.00 in the extreme case. For example, the k_H/k_D values for the solvolyses of 4 - methoxy - 1 - pentyl *p* - bromobenzenesulfonate (methoxyl the neighboring group)[26] and 2 - (2 - cyclopentenyl)ethyl *p* - nitrobenzenesulfonate (double bond the neighboring group)[27] are 1.01 ± 0.02 and 1.06 ± 0.01, respectively, indicating that both the methoxyl and the double bond are participating as neighboring groups (the methoxyl being the more effective of the two). Clearly, the values observed for the (Z,E)- and (E,E)-farnesyl phosphates provide no evidence for an assisted process in which any significant bond formation between the C-6/C-7 double bond and the C-1 position occurs in the transition state.[28]

CONCLUSION

The experiments described in this paper have been directed to the question of whether the aqueous acid-catalyzed decomposition of (Z,E)-farnesyl phosphate occurs via intramolecular assistance from the C-6/C-7 double bond or via a classical allylic carbonium ion whose formation is unassisted. In support of the assisted process are (a) the product compositions, which show a considerably greater amount of cyclic product from the



5a (R = H) 6

5b (R = PO_3H_2)

5c (R = O_2N-C$_6$H$_4$-CO)

(Z,E)-isomer than from the (E,E)-isomer and (b) the formation of optically active α-terpineol (**6**) from optically active linalool (**5a**),[30] linaloyl phosphate (**5b**),[31] and linaloyl *p*-nitrobenzoate (**5c**).[32] In support of an unassisted process, however, is the α-deuterium isotope effect. Although an unequivocal decision is not possible, it

is the conclusion of the authors that the isotope data provide the most compelling evidence and that the reaction is best viewed as an essentially unassisted process.

EXPERIMENTAL[33]

Synthesis experiments

Separation of (Z,E)- and (E,E)-farnesol. A 1 lb sample of commerical farnesol (Givaudan Corp., Delawanne, N.J.) was distilled in a Nester–Faust annular spinning band column using a special flask[11] to accommodate this amount of material. With a reflux ratio of 150:1 and a boil-up rate of 30 drops/min, the material was fractionated into 100 g of (Z,E)-farnesol and 250 g of (E,E)-farnesol of greater than 99% purity and possessing NMR spectra identical with those reported.[34]

Cyclohexylammonium (Z,E)-farnesyl phosphate [(Z,E)-2]. A 3-necked 1 l. flask was fitted with a paddle stirrer and a sealed bearing in the center neck, a N_2 inlet in one of the side necks and a 2-necked Claisen adapter in the other side neck. A 300° thermometer was placed in the soln from the vertical neck of the Claisen adapter, and the side neck was fitted with a condenser topped with a N_2 outlet leading to a bubbler. To the flask was added, with stirring, 23.10 g (0.280 mol) of anhyd phosphorus acid, 214 ml (1.12 mol) of dry tri-n-propyl amine, and 300 ml of dry dimethylformamide. The flask was immersed in an oil bath and heated to 140°. Two 125 ml addition funnels fitted with large-bore stopcocks were prepared, one containing 50 g (0.224 mol) of (Z,E)-farnesol, and the other 78 g (0.280 mol) of mercuric chloride dissolved in 100 ml of dry dimethylformamide. The addition funnel containing the mercuric chloride soln was attached to the top of the addition funnel containing the farnesol and these piggybacked addition funnels were placed in the neck of the flask fitted with N_2 inlet (the N_2 inlet was attached to the top of the upper funnel). The farnesol was added quickly to the flask. The lower addition funnel stopcock was closed, and the mercuric chloride soln in the upper addition funnel was drained into the lower addition funnel. The mercuric chloride soln was then quickly added to the reaction vessel with vigorous stirring. Refluxing resulted, and the mixture turned black. As soon as the mixture cleared (*ca.* 5 min) the oil bath was removed, and the flask was cooled in an ice bath. Metallic Hg was removed by filtration, and the filtrate was added to a mixture of 1 l. of 0.4 M KOH and 1 l. of ether in a separatory funnel. After shaking, the separated ether layer was extracted with three 500 ml portions of 0.4 M KOH. The combined aqueous phase was extracted with three 500 ml portions of ether, and the ether layer was saved for later recovery. The aqueous phase was evaporated on a rotary evaporator (caution! foaming). The gummy residue was taken up in water and passed through a 1000 ml column of Rexyn-101 (40–100 mesh) in the ammonium form. No K ion was present in the effluent. The effluent was combined with 200 ml of cyclohexylamine and was slowly concentrated in a 5 l. flask by means of a rotary evaporator (caution! foaming). The ppt that formed was redissolved by addition of 30 ml of cyclohexylamine, and evaporation was continued. This procedure was repeated twice. The final soln of about 1.5 l. was cleared by the addition of 20 ml cyclohexylamine. The monocyclohexylammonium salt of farnesyl phosphate was precipated by adjusting to pH 5 with glacial AcOH.

The solid was removed by filtration and washed twice with 50 ml water. The filter cake was digested on a steam bath in 100 ml water. After cooling, the mixture was filtered and dried to give 19–20 g (22%) of a white solid; m.p. 172–4°. This material was recrystallized from water to give (Z,E)-2 as colorless plates: m.p. 137–8°; (KBr) 3000–2400 (PO–H, C–H, N–H) 1620 (C=C), 1190 (P=O, H-bonded), and 1030 cm^{-1} (P–O–C); NMR (D_2O, sodium salt), δ 5.57 (t, 1, J = 6.0 Hz, =C(2)H$_2$), 5.21 (bs, 2, =C(6 and 10)H), 4.41 (t, 2, J = 6.0 Hz, CH$_2$O), 2.20–2.06 (m, 8, CH$_2$) and 1.83–1.66 ppm (m, 12, CH$_3$). (Found: C, 62.40; H, 10.05; N, 3.56; P, 7.74. Calc. for $C_{21}H_{40}NO_4P$: C, 62.83; H, 10.04; N, 3.49; P, 7.71%). Alkaline phosphatase hydrolysis gave only (Z,E)-farnesol.

Cyclohexylammonium (E,E)-farnesyl phosphate [(E,E)-2]. Following the procedure described for the (Z,E)-isomer, (E,E)-2 was obtained as fluffy, white crystals: m.p. 174–5°, IR (KBr), 3000–2400 (PO–H, C–H, N–H), 1620 (C=C), 1190 (P=O, H-bonded), and 1030 cm^{-1} (P–O–C). The Na salt was prepared by adding 2 equivs of carbonate-free NaOH to a weighed sample and evaporating to dryness: NMR (D_2O) δ 5.57 (t, 1, J = 6.5 Hz, =C(2)H, 5.24 (bs, 2=C(6 and 10)H), 4.41 (t, 2, J=6.5 Hz, CH$_2$O), 2.13 (m, 8, CH$_2$), 1.79–1.69 ppm (m, 12, CH$_3$). (Found: C, 62.73; H, 10.02; N, 3.51; P, 7.58. Calc. for $C_{21}H_{40}NO_4P$: C, 62.82; H, 10.04; N, 3.49; P, 7.71%). Alkaline phosphatase hydrolysis gave only (E,E)-farnesol.

Bis(tetramethylammonium) farnesyl phosphate was prepared by dissolving 4.04 g (10.0 mmol) of the (Z,E)- or (E,E)-isomer of cyclohexylammonium farnesyl phosphate in CO_2-free water containing 20 mmol of tetramethylammonium hydroxide. The soln was evaporated to dryness, redissolved in CO_2-free water, and diluted to 500 ml to give a 20 mM soln that was used in the determination of the pK values and the kinetics of acid-catalyzed decomposition.

Cyclohexylammonium (Z,E) - 1,1 - dideuteriofarnesyl phosphate. A mixture of 34.5 mmol of lithium aluminum deuteride in 115 ml of tetrahydrofuran containing 0.30 g (6.9 mmol) of abs. EtOH was slowly added, with stirring, to 3.25 g (11.5 mmol) of ethyl (Z,E)-farnesate[23] in 50 ml of tetrahydrofuran. The mixture was stirred at room temp. for 1 hr and then worked up to give 3.25 g of product which was phosphorylated by the procedure described above, yielding 0.21 g of cyclohexylammonium (Z,E) - 1,1 - dideuteriofarnesyl phosphate as colorless crystals: m.p. 133–134°; IR (KBr) 3000–2400 (P–O–H, C–H, and N–H), C–D obscured by peak already present, 1630 (C=C), 1160 (P=O, H-bonded), 1070 (CD$_2$) and 1030 (P–O–C); NMR (D_2O, Na salt), δ 5.40 (s, 1, =C(3)H, 5.10 (bs, 2, =C(6 and 10)H), 2.20–1.90 (m, 8, CH$_2$) and 1.80–1.50 ppm (m, 12, CH$_3$). (Found: C, 62.25; H+D, 9.93; N, 3.55; P, 7.65. Calc. for $C_{24}H_{38}D_2NO_4P$: C, 62.50; H + D, 10.49; N, 3.47; P, 7.60%).

Cyclohexylammonium (E,E) - 1,1 - dideuteriofarnesyl phosphate. In the fashion described above a 5.0 g sample of ethyl (E,E)-farnesate[23] was converted to 0.60 g of cyclohexylammonium (E,E) - 1,1 - dideuteriofarnesyl phosphate, obtained as colorless crystals: m.p. 174–175°; IR (KBr) 3000–2400 (P–O–H, C–H, and N–H), C–D at 2200 obscured by a peak already present, 1630 (C=C), 1190 (P=O, H-bonded), 1070 (CD$_2$) and 1020 cm^{-1} (P–O–C); NMR (D_2O, Na salt), δ 5.40 (s, 1, =C(3)H), 5.07 (bs, 2, =C(6 and 10)H), 2.16–1.83 (m, 8, CH$_2$) and 1.75–1.50 ppm (m, 12, CH$_3$). (Found: C, 62.52; H+D, 9.88; N, 3.53. Calc. for $C_{21}H_{38}D_2NO_4P$: C, 62.50; H + D, 10.49; N, 3.47; P, 7.68%).

Cyclohexylammonium (Z,E) - 6,7,10,11 - tetrahydrofarnesyl phosphate. A mixture of 73.4 mmol of LAH in 127 ml of THF containing 0.64 g (14.7 mmol) of abs. EtOH[24] was slowly added, with stirring at room temp., to 7.00 g (24.4 mmol) of ethyl 3,7,11 - trimethyl - (Z) - 2 - dodecenoate[23] in 175 ml of THF. The mixture was stirred for 1 hr and then worked up to give a product that was shown by GC to contain 95% of the tetrahydro alcohol and 3% of the hexahydro alcohol. Phosphorylation by the procedure described above gave 1.24 g of ammonium (Z,E) - 6,7,10,11 - tetrahydrofarnesyl phosphate as colorless crystals after recrystallization from water: m.p. 142–143°; IR (KBr) 3000–2400 (P–O–H, C–H, N–H), 1630 (C=C), 1190 (P=O, H-bonded) and 1015 cm^{-1} (P–O–C); NMR (D_2O, Na salt), δ 5.52 (t, 1, J = 6.0 Hz, =CH), 4.40 (t, 2, J=6.0 Hz, CH$_2$O), 2.41–2.00 (m, 2, CH), 1.85 (s, 3, CH$_3$ at C-3), 1.32 (s, 12, CH$_2$) and 1.13–0.83 ppm (m, 9, CH$_3$). Alkaline phosphatase hydrolysis gave only (Z,E) - 6,7,10,11 - tetrahydrofarnesol. (Found: C, 62.19; H, 10.93; N, 3.52; P, 7.69. Calc. for $C_{21}H_{44}NO_4P$: C, 62.19; H, 10.97; N, 3.45; P, 7.64%).

Cyclohexylammonium (E,E) - 6,7,10,11 - tetrahydrofarnesyl phosphate. In the fashion described above, a 10.0 g sample of ethyl 3,7,11 - trimethyl - (E) - 2 - dodecenoate[23] was converted with LAH and EtOH[24] to a mixture containing 89% of the tetrahydro alcohol and 11% of the hexahydro alcohol and this, in turn, converted to 1.90 g of cyclohexylammonium (E,E) - 6,7,10,11 - tetrahydrofarnesyl phosphate, obtained after recrystallization from water as a colorless solid: m.p. 167–170°; IR (KBr) 3000–2400 (P–O–H, C–H, and N–H), 1640 (C=C), 1160 (P=O, H-bonded) and 1030 cm^{-1} (P–O–C); NMR (D_2O, sodium salt), δ 5.50 (t, 1, J = 6.5 Hz, =CH), 4.24 (t, 2, J = 6.5 Hz, CH$_2$O), 2.30–1.10 (m, 2, CH), 1.78 (s, 3, CH$_3$ on C(3)), 1.33 (bs, 12, CH$_2$) and 1.12–0.85 ppm

(m, 9, CH_3). Alkaline phosphatase hydrolysis of this material gave only (E,E) - 6,7,10,11 - tetrahydrofarnesol. (Found: C, 62.06; H, 10.86; N, 3.52; P, 7.82. Calc. for $C_{21}H_{44}NO_4P$: C, 62.19; H, 10.97; N, 3.45; P, 7.64%).

Bis(cyclohexylammonium) t-butyl phosphate. Following the literature procedure[10] bis(cyclohexylammonium) t-butyl phosphate was obtained as colorless crystals; m.p. 191–193° (lit.[10] 191–193°). A phosphate analysis of the product showed it to contain 1.1% inorganic phosphate, 8.8% organic phosphate (Calc. 8.9%), and an apparent formula weight of 351.3 (Calc. 352.45).

Product studies

Formolysis of farnesols and farnesyl phosphates. A 1 mmol sample of farnesol or cyclohexylammonium farnesyl phosphate was added, along with 40 mg of 1,2-dimethylnaphthalene (as an internal standard), to a 10 ml flask. The flask was stoppered, cooled in an ice bath, treated with 10 ml of 100% formic acid (cooled to 0°), and the contents stirred at 0° for 10 min. The contents of the flask were then poured into 40 ml of 30% NaOH, 50 ml of ice, and 50 ml of EtOH. The mixture was allowed to stand overnight and was then extracted 3 times with 50 ml portions of ether. Evaporation of the combined ether extract left a liquid that was analyzed by GC (oven temp. 200°) to yield the data shown in Table 1. The detector response factors that were used were as follows: hydrocarbon (1.04), nerolidol (1.12), bisabalol (1.16), (Z,E)-farnesol (1.00), (E,E)-farnesol (1.00), diol (1.37), triol (1.57), 1,2 - dimethylnaphthalene (1.00). The dehydrogenation of the product was carried out with 0.20 g of 10% palladium on charcoal as previously described[1] to give the data shown in Table 1. The detector response factors that were used were as follows: fragmentation products (0.80), monocyclic compounds (1.12), bicyclic compounds (1.12), 1,2 - dimethylnaphthalene (1.00).

Hydrolysis of farnesyl phosphates. To a 1 l. volumetric flask containing 900 ml of the appropriate buffer (at a concentration such that dilution to 1 l. would give the desired pH and an ionic strength of 0.200 M) equilibrated at 50°, a solution of 20 mM of bis(tetramethylammonium) farnesyl phosphate in *ca.* 50 ml of water, also equilibrated at 50°, was added. The solution was made up to 1 l., and the reaction was allowed to proceed for the desired length of time. It was then added to a mixture of 165 ml of 50% sodium hydroxide and 250 ml of EtOH, allowed to stand overnight, and worked up and assayed as described above to give the results shown in Table 2.

pK Measurements. Solutions of known HCl concentration were prepared by transferring measured volumes of standardized 0.1 M HCl into 100 ml volumetric flasks and diluting with CO_2-free water. Flasks containing the HCl soln and the bis(tetramethylammonium) farnesyl phosphate solutions (see above) or bis(tetramethylammonium) t-butyl phosphate were equilibrated at the temperature of the pK measurement, rapidly mixed by shaking in a vortex mixer, and then immediately passed through a constant flow pH cell[11] submerged in the constant temp. bath. For each pK determination the effluent from the pH cell was analyzed by treating a 1 ml sample with 2 M HCl and heating to 100° for 20 min. To this was added 1 ml of 2 M NaOH, and the sample was diluted to 50 ml and analyzed for inorganic phosphate. The value that was obtained was taken as the concentration of the phosphate ester in the original soln. The pK_1 values were calculated by the method of Albert and Serjeant[7] using the expression.

$$pK_1 = pH + \log \frac{[ROPO_3H_2] - [H^{\oplus}]}{[ROPO_3H^{\ominus}] + [H^{\oplus}]} - \log y_{\pm}$$

where $[ROPO_3H_2]$ is the stoichiometric concentration of the fully protonated phosphate ester and is equal to $[Cl^{\ominus}] - [P_{tot}]$; $[Cl^{\ominus}]$ is equal to 0.5 [HCl] where [HCl] is the concentration of the HCl soln before dilution. The stoichiometric phosphate ester monoanion concentrations, $[ROPO_3H^{\ominus}]$, is equal to $[P_{tot}] - [ROPO_3H_2]$. The hydrogen ion in the solution is $[H^{\oplus}]$. The activity coefficient, $\log y_{\pm}$, was calculated from the Davies equation.[35] Similarly, pK_2 was calculated using the expression

$$pK_2 = pH + \log \frac{[ROPO_3H^{\ominus}]}{[ROPO_3^{\ominus}]} - 3 \log y_{\pm}$$

where $[ROPO_3H^{\ominus}]$ is equal to the chloride concentration $[Cl^{\ominus}]$ which is 0.5 [HCl], the concentration of the HCl soln used. The concentration of the dianionic phosphate ester $[ROPO_3^{\ominus}]$ is equal to $[P_{tot}] - [POPO_3H^{\ominus}]$. The $\log k_{\pm}$ value was calculated from the Davies equation,[35] and $\{H^{\oplus}\}$ was used for $[H^{\oplus}]$ in the ionic strength calculation since it causes less than 1% error in the activity coefficient. The pK values, measured at several temperatures, are recorded in Table 3.

Kinetic measurements

Farnesyl phosphates. Flasks containing the buffer soln and the bis(cyclohexylammonium) farnesyl phosphate soln (see above) were kept in a constant temp. bath until they had reached thermal equilibrium with the bath. A 5 ml sample of the phosphate soln was then added to the appropriate amount of buffer soln and diluted to 100 ml. The composition of the buffer was adjusted to establish the desired pH after dilution and a total ionic strength of 0.200 (by the addition of the appropriate amount of tetramethylammonium chloride). Tetramethylammonium ion was used as the cation for all the carboxylic acid buffers. The flask was allowed to remain in the constant temp. bath, and aliquots of the mixture were periodically analyzed for inorganic phosphate by means of an automatic analyzer.[11] The concentration of unchanged farnesyl phosphate (FP) was determined from the optical density reading using the following equations:

$$\frac{(O.D._x) - (O.D._{blank})}{(O.D._{std}) - (O.D._{blank})} = \frac{[P_x]}{[P_{std}]}$$

$$\ln [FP] = \ln \{([P_T] - [P_o]) - ([P_x] - [P_o])\}$$

where $(O.D._x)$ and $(O.D._{std})$ are the absorbances read for the unknown and the standard, respectively; $[P_x]$, $[P_{std}]$, $[P_T]$ and P_o are unknown, standard, final and initial inorganic phosphate concentration, respectively. Using a linear least squares program written for the Hewlett–Packard 9100A calculator,[36] the slope of the line of the plot of ln [FP] vs time was determined, and the negative of the slope was taken as the rate constant recorded in Table 4.

t-Butyl phosphate. The disodium salt of t-butyl phosphate was prepared by dissolving 3.55 g (10 mmol) of bis(tetramethylammonium) t-butyl phosphate in 18.5 ml of 1.08 M CO_2-free water and evaporating to dryness. The residue was dissolved in 500 ml of CO_2-free water, and samples of this soln were subjected to acid-catalyzed hydrolysis as described for the farnesyl phosphates with the exception that sodium ion was used as the counter ion in buffers.

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