was that of Sahyun et al.[21] to which 0.6% acid hydrolyzed casein was added. Growth of the test organisms was read as turbidity in the Klett-Summerson photoelectric colorimeter. The results are expressed as the micromolar concentration required to produce 50% growth inhibition (IC_{50}) of the strain under the conditions used. Where sufficient drug was available, concentrations up to 1000 μg/ml were tested; when this was not possible the highest concentration used was 100 μg/ml.

In addition to antibacterial activity, tests were made for the reversal of the drug inhibition by PABA in the case of *E. coli*, folic acid with *S. faecium* and *L. casei*, and folinic acid with *P. cerevisiae*. The reversal was indicated as positive when addition of either 10 μg/ml of PABA or ten times the amounts of folic or folinic acids normally used produced at least a fourfold decrease in activity of the compound.

Strains of *S. faecium*, *L. casei*, and *P. cerevisiae* were made resistant to CGT by serial transfer in increasing amounts of the drug.[5] The most highly resistant strains obtained with each organism were used in the tests reported here. From these data the fold increase in resistance (FIR) of the CGT_R strains compared to the respective sensitive strains for each compound has been calculated (IC_{50} R/IC_{50} S). Thus, for most compounds ten quantitative and seven qualitative (reversal) parameters have been determined.

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Inhibition of Squalene Synthetase by Farnesyl Pyrophosphate Analogues[1]

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The pyrophosphates of the following farnesol analogues have been synthesized: 2-methylfarnesol; 7,11-dimethyl-3-ethyl-2,6,10-dodecatrien-1-ol; 3-demethylfarnesol; 4-methylthiofarnesol; 7,11-dimethyl-3-iodo-2,6,10-dodecatrien-1-ol; 7,11-dimethyl-2-iodo-2,6,10-dodecatrien-1-ol; 7,11-dimethyldodeca-6,10-dien-2-yn-1-ol; phytol; 3,7,11-trimethyl-2-dodecen-1-ol; 3,7,11-trimethyldodecan-1-ol; and geraniol. The double bonds in all the above compounds were in the *E* configuration, except phytol, which was a 7:3 mixture of 2*E* and 2*Z* isomers. Each of the pyrophosphates inhibits the incorporation of labeled farnesyl pyrophosphate into squalene by a yeast enzyme preparation. Free alcohols and monophosphates are inactive. The analogues, listed in order of decreasing inhibitory strength, are, by kinetic analysis, competitive or mixed inhibitors. Irreversible inhibition is not observed. The results suggest that binding to the enzyme is primarily mediated by the pyrophosphate moiety assisted by relatively nonspecific lipophilic interactions. Decreasing the chain length and saturating double bonds severely reduces binding, while substitution at the 2, 3, and 4 positions, and lengthening of the chain, is well tolerated.

The incidence of atherosclerotic disorders, a major cause of death in the United States and other industrial societies, is closely correlated with occurrence of elevated plasma cholesterol levels.[2] Methods for effective reduction of plasma cholesterol levels are therefore of high interest, even though the role of this sterol in the etiology of the disease is not clearly defined.[2,3] In theory, cholesterol concentration can be reduced by decreasing the amount of the sterol, by enhancing its metabolism and elimination, and by decreasing its rate of biosynthesis. Cholesterol synthesis, however, is subject to feedback regulation,[4] so that decreases in cholesterol levels tend to be compensated for by increased biosynthesis. Removal of sterols in rats due to cholestyramine feeding, for example, causes a 200–300% increase in hepatic cholesterol synthesis.[5] The

Table I. Physical Properties of Analogues

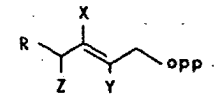
Compd	Phosphorus analysis (% of theory)	GC retention time (min) of free alcohol isomers 2Z	2E	Temp, °C	GC retention time (min) of isomers of ethyl ester precursor 2Z	2E	Temp, °C
1	101	13.13	15.03	150	18.0	17.0	150
2	114	17.40	20.29	150	17.0	21.0	150
3	106	9.26	10.05	150			
4	93	18.75	20.36	150	18.8	20.8	150
5	100		20.93	175			
6	93		21.94	175			
7	103		24.94	175		8.5	175
8	102	4.39	4.91	125			
9	107	8.06	9.19	150	7.5	11.1	150
10	95	13.58	15.56	175			
11	91	15.03		150			
12	93	7.01		150	6.11		150

lesterol levels, therefore, are likely to include inhibition of its biosynthesis as a vital component.

The normal rate-controlling step in the biosynthesis of cholesterol is the formation of mevalonic acid from 3-hydroxy-3-methylglutaryl coenzyme A.[4] Mevalonic acid, however, is the precursor of all isoprenyl derivatives, including in animals coenzyme Q, heme A, and the dolichols. The first biosynthetic step which leads exclusively to sterols, the condensation of two farnesyl pyrophosphates (1) to give squalene, is a possible site of secondary regulatory mechanisms.[6] Inhibition of cholesterol biosynthesis at this stage is therefore attractive, not only because nonsteroidal pathways will be minimally affected but also because degradative processes exist for removal of farnesyl pyrophosphate.[7] The synthesis of squalene from farnesyl pyrophosphate involves an isolable intermediate, presqualene pyrophosphate.[8] The entire synthetic sequence is catalyzed by squalene synthetase, a complex, membrane-bound enzyme.[9] As part of our current efforts to define the mechanism and active site topology of this enzyme,[10,11] and to develop effective inhibitors of it, we now describe a study of structure–activity relationships which govern inhibition of the enzyme by substrate analogues.

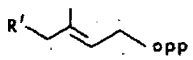
Synthesis. Pyrophosphates 1–12 were obtained from the corresponding farnesol analogues by pyrophosphorylation with di(triethylammonium) phosphate and trichloroacetonitrile.[12,33] The formation of pyrophosphates was verified by quantitative phosphorus analysis (Table I),[13] although traces of mono- or polyphosphate contaminants were apparent in some cases. The integrity of the phosphorylated structures was confirmed by regeneration of the starting alcohols on treatment with bacterial alkaline phosphatase.[14]

Geraniol and phytol, the precursors of 8 and 10, respectively, were purchased commercially. The farnesol analogues required in the synthesis of 3, 5, 6, and 11 were prepared by literature procedures.[15,16] Hexahydrofarnesol (12 alcohol) was obtained by catalytic hydrogenation of ethyl farnesoate, followed by $LiAlH_4$ reduction of the ester function. The precursor of 9, 6,7,10,11-tetrahydrofarnesol, was made by analogous reduction of ethyl 3,7,11-trimethyl-2(*E*)-dodecenoate.[17] A 1:1 mixture of 2*E* and 2*Z* isomers of ethyl 2-methylfarnesoate (13) was obtained by Wadsworth–Emmons condensation[18] of geranyl acetone with diethyl 1-carboethoxyethyl phosphonate.[19] The 2*E* isomer, isolated by spinning-band distillation, was reduced with $LiAlH_4$ to 2-methylfarnesol, the precursor of 2. Similar condensation of 7,11-dimethyl-6(*E*),10-dodecadien-3-one (14) and triethyl phosphonoacetate yielded, as a 6:4 mixture of 2*E* and 2*Z* isomers, the 3-ethyl analogue of ethyl farnesoate (15). Spinning-band distillation and



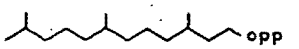
	X	Y	Z
1	Me	H	H
2	Me	Me	H
3	H	H	H
4	Et	H	H
5	I	H	H
6	H	I	H
7	Me	H	MeS

R = geranyl = $(CH_3)_2C{=}CHCH_2CH_2(CH_3)C{=}CHCH_2-$



	R'
8	$(CH_3)_2C{=}CHCH_2-$
9	$(CH_3)_2CHCH_2CH_2CH_2CH(CH_3)CH_2CH_2-$
10	$(CH_3)_2CHCH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2CH_2-$

11 

12 

prepared from geranyl bromide and ethyl 3-ketopentanoate by base-promoted condensation, ester hydrolysis, and decarboxylation. Finally, 4-methylthiofarnesol (7 alcohol) was obtained by reduction of the corresponding ethyl ester 17. Condensation of 3-methylthiogeranyl acetone (16) and triethyl phosphonoacetate furnished 17, essentially as the pure 2*E* isomer. The required geranyl acetone derivative 16 was made from the sodium salt of 1-methylthio-2-propanone[20] and geranyl bromide.

Analysis by GC showed that the farnesol analogue precursors of all the pyrophosphates except 10 were essentially pure all-*E* isomers, contaminated in no instance with more than 4% of the 2*Z* isomer. Analogue 10 was an exception, commercial phytol consisting of a 7:3 mixture of 2*E* and 2*Z* isomers, respectively. Stereochemical assignments have been made in the literature for the farnesol analogues except the precursors of 2, 4, and 7. As has been noted,[21] and extensively confirmed in our work (Table I), the all-*E* isomers of prenyl alcohols and esters have higher retention times (R_t) on GC than isomers with one or more double bonds in the *Z* configuration. The assignment of stereochemistry to analogues 2, 4, and 7, suggested by their

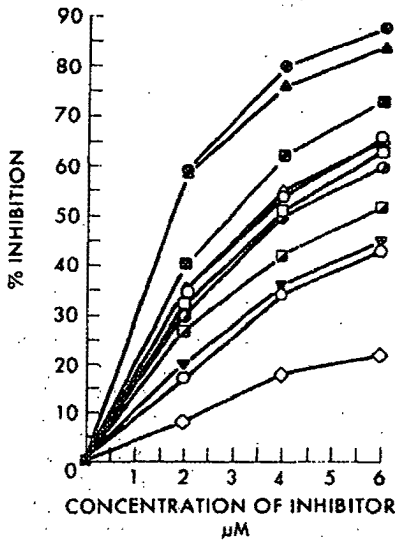


Figure 1. Relative inhibition of squalene synthetase by farnesyl pyrophosphate analogues. The incorporation of [1-^{3}H]farnesyl pyrophosphate (2.5 μM) into squalene in the presence of 2, 4, and 6 μM concentrations of inhibitor is plotted as a percent of the incorporation observed with no inhibitor present. Inhibitor: 2 (●), 4 (▲), 3 (■), 7 (○), 5 (△), 6, (□), 11 (◑), 10 (◪), 9 (▼), 12 (○), and 8 (◇).

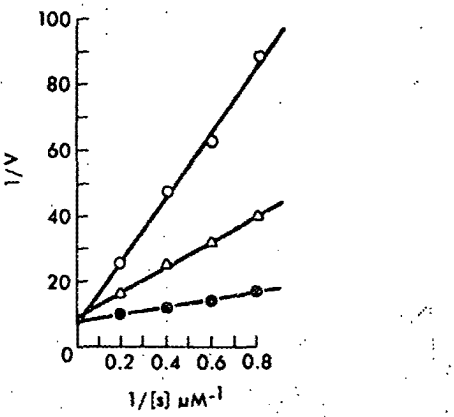


Figure 2. Lineweaver–Burk graphic analysis of the inhibition of squalene synthetase by 2-methylfarnesyl pyrophosphate (2). [S] is the concentration of [1-^{3}H]farnesyl pyrophosphate; initial velocity (V) is expressed in nanomoles of squalene formed per minute per milligram of protein. Concentration of inhibitor (2): 0 (●), 1 (△), and 3 μM (○).

verified. The 2*E* and 2*Z* isomers of 13 were distinguished by correlating the NMR shifts of the C-2 and C-3 methyl groups in each isomer with those of the corresponding methyls in methyl 2,3-dimethyl-2-butenoate.[22] In the 2*E* isomer, the 2-methyl is at 1.87 ppm and the 3-methyl at 2.00 ppm, while the corresponding peaks in the 2*Z* isomer are at 1.81 and 1.79 ppm. In the model compound the 2-methyl and the *trans*-3-methyl appear at 1.81 ppm, while the *cis*-3-methyl is at 2.00 ppm.[22] Unambiguous identification of the stereoisomers of 4 alcohol, however, could not be made by NMR correlation with model structures.[23] This difficulty was overcome by NMR studies using the shift reagent Eu(fod)$_3$.[24] A plot was made of the shift of the 3-ethyl protons in each isomer as a function of increasing shift reagent to compound ratio. A large difference was observed in the shifts of the two isomers. At a reagent to analogue ratio of 0.5, for example, the ethyl methylene group in one isomer had shifted by 0.42 ppm and in the other by 0.75 ppm. The isomer in which the 3-ethyl was more responsive to the shift reagent was assigned 2*E* stereochemistry,[25] in agreement with the GC data. The assignment of 2*E* stereochemistry to 7 alcohol was established by desulfurization with Raney nickel of the single isomer of 17 which was obtained synthetically. Ethyl 2(*E*)-farnesoate was shown by GC to be a major product, while the other isomer was absent.

Biological Results. The relative inhibition of squalene synthetase by analogues 2–12 has been measured, while kinetic and inhibitor–enzyme preincubation studies have been used to characterize the type of inhibition observed. An insoluble preparation of squalene synthetase from yeast was employed in this work, since the enzyme from this source is contaminated with much lower levels of interfering phosphatases.[26]

The relative potencies of the synthetic analogues as inhibitors were determined by measuring the decrease in enzymatic incorporation of [1-^{3}H]farnesyl pyrophosphate into squalene caused by fixed concentrations of the analogues. The results of these studies are presented in incubations with no inhibitors, vs. the concentration of the inhibitor. The strongest inhibitors are 2 and 4, while geranyl pyrophosphate (8) is the weakest. Phytyl pyrophosphate (1) is slightly more potent than shown, since no adjustment has been made for the presence of the presumably less active 2*Z* isomer. A limited selection of the alcohol and monophosphate precursors of pyrophosphates 2–12 has also been evaluated as inhibitors. None of these gave significant inhibition when present in concentrations up to 20 μM.

The inhibition of squalene synthetase by pyrophosphorylated analogues has been subjected to kinetic analysis. Double reciprocal plots of initial reaction velocities vs. substrate concentration, at fixed inhibitor concentrations, have been made.[27] Kinetic constants have not been extracted from the data because the relatively crude enzyme system used is not amenable to such treatment. The farnesyl pyrophosphate K_m value, for example, varied slightly from one enzyme preparation to another. Nevertheless, our accumulated data indicate that 2 has an apparent K_i value of approximately 0.5 μM. Furthermore, the inhibition patterns clearly establish that each of the analogues is a competitive, or at least partially competitive (mixed), inhibitor.[27] Compound 2, for example, gives the competitive inhibition pattern in Figure 2, while 3 is shown to be a mixed inhibitor in Figure 3. Patterns similar to one or the other of these plots were obtained for all of the analogues. The results are summarized in Table II. The conclusion that the analogues compete with the substrate for a berth at the active site is well substantiated.

The kinetic mechanism of the enzyme, involving a probable covalent intermediate,[9] suggested that substrate analogues might cause irreversible inhibition. This would be the consequence if an analogue were accepted as a substrate, forming the covalent complex, but was unable to complete the synthetic sequence. Compounds 2, 5–7, and 11, in particular, were prepared with such a possibility in mind. To determine if irreversible inhibition was occurring, the analogues were preincubated for up to 5 h at ambient temperature with the enzyme and all assay components except the substrate. Normal bioassay was then initiated by addition of [1-^{3}H]-1. Irreversible binding is characterized by time-dependent increases in observed inhibition.[28] The time-dependent *decrease* in inhibition actually observed (Figure 4), however, is incompatible with

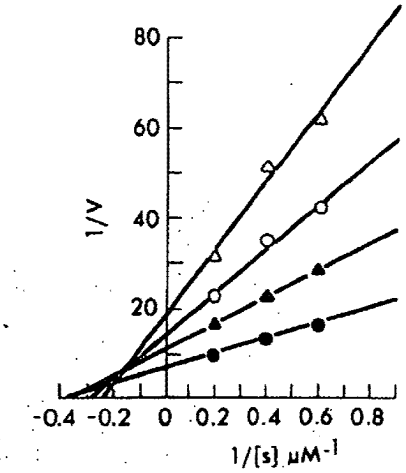


Figure 3. Lineweaver–Burk graphic analysis of the inhibition of squalene synthetase by 3-demethylfarnesyl pyrophosphate (3). [S] is the concentration of [1-^{3}H]farnesyl pyrophosphate; initial velocity (V) is expressed in nanomoles of squalene formed per minute per milligram of protein. Concentration of inhibitor (3): 0 (●), 2 (▲), 4 (○), and 6 μM (△).

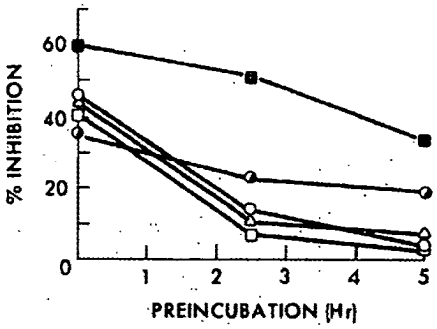


Figure 4. Effect of incubating inhibitors (4 μM) with squalene synthetase prior to addition of [1-^{3}H]farnesyl pyrophosphate (5 μM) and normal bioassay. Preincubation was at ambient temperature with the full assay system, excluding the substrate. Inhibitor: 3 (■), 5 (△), 6 (□), 7 (○), and 11 (◑).

decrease in inhibition with time can be explained by slow enzymic and chemical degradation of the inhibitor.

Conclusions

The binding of farnesyl pyrophosphate and its analogues to squalene synthetase is strongly dependent on the pyrophosphate moiety and, to a lesser extent, on relatively nonspecific lipophilic interactions of the hydrocarbon chain. This conclusion derives from the impotence of free alcohol and monophosphate analogues as inhibitors, coupled with the appreciable inhibition exhibited by structures with fairly gross alterations in the carbon framework. Substitution at C-2, C-3, and C-4 (analogues 2, 4, and 7), for example, is well tolerated. Saturation of the double bonds (9, 12) and shortening of the hydrocarbon chain (8), on the other hand, significantly reduce binding. Increasing the chain length appears to enhance binding, since 10 is a better inhibitor than 9, particularly when allowance is made for the fact that the data on 10 are based on a mixture containing 30% of the presumably less active 2Z isomer. The pairing of strong pyrophosphate binding with relatively nonspecific attachment of the hydrocarbon chain is reminiscent of the forces involved in binding of geranyl pyrophosphate to prenyl transferase[29] and of prenyl pyrophosphates to the polypeptide antibiotic bacitracin.[30]

The competitive nature of the inhibition indicates that the structure–activity relationships observed reflect the selectivity of the active site. A sharp contrast exists, however, between the relatively loose requirements for binding and the catalytic demands of the enzyme. We have subsequently found that 2 and 3 are only cosubstrates for the enzyme,[10,11] while 4, 7, and 9 are not acceptable as substrates at all.[31] These results exclude all the analogues in this paper, except possibly 5, as first substrates for the enzyme. The lack of irreversible inhibition is thus rationalized, since formation of an enzyme–substrate covalent complex requires that an analogue be catalytically acceptable as a first substrate. The search for suitable structures, consistent with the criteria now available, is continuing in our laboratories.

Experimental Section

Geraniol and phytol were purchased from Aldrich, NADPH from Sigma, bacterial alkaline phosphatase from Worthington

Table II. Classification of Inhibitors

2, competitive	6, competitive	10, competitive
3, mixed	7, competitive	11, mixed
4, competitive	8, (competitive)[b]	12, competitive
5, competitive	9, competitive[a]	

[a] The lines in the Lineweaver–Burk plot curved upward at values of 1/[S] greater than 0.6 μM^{-1}. [b] The lines due to this weak inhibitor did not differ sufficiently in slope for unambiguous assignment.

and cake baker's yeast from a local bakery. Eastman (No. 1381) or Analtech silica gel precoated plates were used for TLC analysis, while Merck silica gel 60 (70–230 mesh) was used for column chromatography after deactivation with 10% water. A Varian Model 2100 GC instrument with flame ionization detectors, nitrogen carrier gas (18 ml/min), and 6 ft × 2 mm i.d. glass columns packed with 3% OV-225 on 100–120 mesh Varoport 30 was used for all analytical gas chromatography. Infrared spectra were obtained as thin films on a Perkin-Elmer Model 337, while a Karl Zeiss PMQ11 spectrophotometer was used to measure optical densities. NMR spectra were taken on a Varian A-60 as approximately 25% v/v deuteriochloroform solutions. Shifts are reported in parts per million downfield from Me_4Si. The NMR shift reagent Eu(fod)$_3$ was obtained from Willow Brook Laboratories. Mass spectra were measured on an AEI MS-9 adapted to a chemical ionization mode (isobutane gas). Curve fitting was accomplished by linear regression analysis, using either a Hewlett-Packard Model 9100 calculator (program no. 70803) or the PROPHET system sponsored by NIH for chemical–biological information handling. Microanalyses were performed by the Berkeley Microanalytical Laboratory.

Preparation of Enzyme.[9] Baker's yeast (200 g) was suspended in 200 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 5 mM $MgCl_2$, 1 mM sodium EDTA, and 10 mM 2-mercaptoethanol. The suspension was sonicated in 100-ml aliquots with a Branson W-185D sonifier (90-W output). Each aliquot was sonicated with ice cooling for two 5-min periods. The crude mixture was centrifuged at 0 °C and 7000g for 10 min. The supernatant was centrifuged at 73000g for 45 min. The fraction of the resulting high-speed supernatant which precipitated between 30 and 55% of ammonium sulfate saturation was in turn isolated by centrifugation at 12000g for 10 min. The pellet, resuspended in 15 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 20 mM 2-mercaptoethanol, was dialyzed against 1100 ml of the same buffer mixture. The resulting enzyme preparation was divided into small aliquots which were separately stored in a freezer until individually used. Protein concentrations were assayed by the procedure of Sutherland.[32]

Inhibition Bioassay. The incorporation of [1-^{3}H]farnesyl pyrophosphate into squalene in the presence of inhibitors was assayed in standard 1-ml incubation mixtures, each containing (concentration units): [1-^{3}H]farnesyl pyrophosphate (6 mCi/mmol), 1–10 μM; $MgCl_2$, 10 mM; NADPH, 1.6 mM; NH_4OH, 0.8

1.34 mg/ml; and potassium phosphate buffer (pH 7.5), 50 mM. The inhibitor concentration varied between 0 and 6 μM. Each standard incubation was initiated by addition of the enzyme to a mixture of the other components prewarmed to 37 °C. After 2 min at 37 °C in a reciprocating bath, the incubation was terminated by addition of ethanol (2 ml). The aqueous mixture was extracted with two 1.5-ml and two 3.0-ml aliquots of 30–60 °C petroleum ether, each aliquot being added directly to a disposable 0.6 × 5 cm column of 10% water deactivated silica gel. The minicolumn effluent was directly collected in a scintillation vial. The amount of radioactivity in the vial was determined on a Packard Tri-Carb Model 3375 liquid scintillation spectrometer after addition of New England Nuclear Aquasol (10 ml). Control experiments demonstrated that squalene was cleanly eluted under these conditions, whereas polar products like farnesol remained on the columns. Each bioassay data point was measured in at least two independent experiments. With a given enzyme preparation, equivalent data points were essentially superimposable, rarely differing by more than 5%.

Inhibitor Preincubations. The complete standard incubation mixture, excluding the substrate but including the enzyme, was incubated with 4 μM concentrations of inhibitor for 2.5 or 5 h at ambient temperature. Control incubations were treated identically, except no inhibitor was added. The mixtures were prewarmed to 37 °C at the end of the preincubation period, [1-^{3}H]farnesyl pyrophosphate (5 μM concentration) was added, and the assay was completed as described.

Synthesis of Pyrophosphates.[12] Di(triethylammonium) phosphate[33] (450 mg, 1.5 mmol) in 30 ml of dry CH_3CN was added over 4 h at ambient temperature to a stirred solution of the farnesol analogue (0.5 mmol) and trichloroacetonitrile (650 mg, 4.5 mmol) in 5 ml of CH_3CN. The mixture was stirred 24 h and concentrated on a rotary evaporator. The yellow residue was transferred to a centrifuge tube with 10 ml of acetone and concentrated NH_4OH (0.5–1 ml) was added. The precipitated ammonium salts, isolated by centrifugation, were washed twice by resuspension in 5-ml aliquots of acetone containing 0.01 N NH_4OH. Purification was accomplished by one of the following.

Procedure A. A solution of the resulting white solid in 5 ml of 0.01 N NH_4OH was added to a stirred slurry of 100 g of prewashed Amberlite XAD-2 resin[33] in 100 ml of 0.01 N NH_4OH. The resin, collected on a sintered funnel after stirring overnight, was washed with the same solvent (5 × 60 ml). The organic phosphates were eluted from the resin with methanol containing a few drops of NH_4OH (5 × 60 ml). Solvent removal (rotary evaporator below 50 °C) gave a solid which was transferred to a centrifuge tube with 2–3 ml of methanol. The pyrophosphates, contaminated with traces of monophosphates, were precipitated by addition of 4–8 ml of acetone to which a few drops of NH_4OH had been added. Repetition of the precipitation sequence gave essentially pure pyrophosphates (by TLC). These were dried under high vacuum.

Procedure B.[34] The crude product obtained from 150 mg of starting alcohol was dissolved in a minimal amount of 1-propanol–ammonia–water (9:4:1) and was added to a 2.5 × 18 cm column containing 40 g of silica gel. The silica gel had been preconditioned by stirring in 300 ml of 1:1 concentrated HCl–water for 2 h, standing overnight, decantation of the aqueous layer, washing with water (6 × 300 ml), and drying at 150 °C for 48 h. The column was prewashed and eluted with a 9:4:1 1-propanol–NH_4OH–H_2O mixture (flow rate 1.5 ml/min), collecting 3-ml fractions. For analogue 9, fractions 1–15 contained no phosphates, 15–23 the monophosphate, and 32–42 the pyrophosphate. The combined pyrophosphate fractions were carefully concentrated to 2 ml on a rotary evaporator (below 25 °C), lyophylized, and dried under high vacuum.

The pyrophosphates obtained by either procedure, in 10–30% yield, were virtually free of mono- or polyphosphates, as judged by TLC on silica gel plates using 1-propanol–NH_4OH–H_2O (6:3:1) as developing solvent. Monophosphates appear as blue spots and pyrophosphates as purple spots on visualization with Rosenberg's reagent.[35] The pyrophosphates were stored as solutions in 0.01 N NH_4OH in a deep freeze.

Alkaline Phosphatase Hydrolysis. The organic pyrophosphates were hydrolyzed with bacterial alkaline phosphatase as described by Goodman and Popjak.[14] The recovered alcohols were compared with authentic samples by TLC and GC. In each case the recovered alcohol was essentially identical with the corresponding starting material.

Ethyl 2,3,7,11-Tetramethyl-2(*E*),6(*E*),10-dodecatrienoate (13). Sodium hydride (3.62 g of 57% oil suspension, 86 mmol), washed with petroleum ether, was stirred in 400 ml of dry THF (nitrogen atmosphere). Diethyl 1-carboethoxyethyl phosphonate[19] (20.78 g, 87 mmol) in 100 ml of THF was added at 0 °C over 30 min. The mixture was stirred at 30–40 °C until hydrogen evolution ceased (1 h). After cooling to 0 °C, 16.7 g (86 mmol) of geranylacetone in 100 ml THF was added and the mixture stirred 24 h at 60 °C. Water (300 ml) was added, the layers were separated, the aqueous layer was extracted with petroleum ether, and the combined organic layers were washed with water and finally dried over $MgSO_4$. Solvent removal (rotary evaporator) and distillation through a short Vigreux column gave 4.88 g of recovered geranylacetone and 14.67 g (84% adjusted yield) of a 1:1 mixture of 2(*E*)- and 2(*Z*)-13 [bp 110 °C (0.15 mm)]. Removal of the lower boiling 2*Z* isomer by spinning-band distillation, filtration of the pot residue through basic alumina (grade II, 20 g, petroleum ether solvent), solvent removal, and bulb-to-bulb distillation gave 2.5 g of isomerically pure 13: IR 1730, 1660 cm^{-1}; NMR 1.28 (t, J = 7 Hz, 3 H, ethoxy CH_3), 1.60 and 1.66 (singlets, 9 H, allyl CH_3), 1.87 (s, 3 H, 2-CH_3), 1.76–2.23 (m, 11 H, allyl CH_2, 3-CH_3), 4.18 (q, J = 7 Hz, 2 H, OCH_2), and 5.15 ppm (br s, 2 H, vinyl H); CIMS m/e 279 (MH^+). Anal. ($C_{18}H_{30}O_2$) C, H.

2,3,7,11-Tetramethyl-2(*E*),6(*E*),10-dodecatrien-1-ol (2-Methylfarnesol, 2 Alcohol). A solution of 13 (1.5 g, 5.39 mmol) in 15 ml of dry ether was injected into a stirred suspension of $LiAlH_4$ (256 mg, 6.7 mmol) in 20 ml of ether at 0 °C. The mixture was stirred 1.5 h at ambient temperature. Water (0.486 ml, 27 mmol) was carefully added at 0 °C. The resulting slurry was stirred 5 min and filtered through a sintered glass funnel (washing with ether). Solvent removal and bulb-to-bulb distillation gave 1.1 g (86%) of 2 alcohol: a single isomer by GC; IR 3300, 1680 cm^{-1}; NMR 1.61 and 1.69 (s, 12 H, allyl CH_3), 1.74 (s, 3 H, 2-CH_3), 1.83 (s, 1 H, D_2O exchange, OH), 1.93–2.2 (m, 8 H, allyl CH_2), 4.12 (s, 2 H, CH_2O) and 5.61 ppm (br s, 2 H, vinyl H); CIMS m/e 237 (MH^+). Anal. ($C_{16}H_{28}O$) C, H.

7,11-Dimethyl-6(*E*),10-dodecadien-3-one (14). Ethyl 3-ketopentanoate (30 g, 0.205 mol) was added dropwise at 42–48 °C (under nitrogen) to a stirred solution of sodium ethoxide prepared from 4.72 g of sodium and 150 ml of anhydrous ethanol. The resulting solution, cooled to ambient temperature, was added with stirring to 64 g (0.295 mol) of geranyl bromide in 40 ml of ethanol at −7 °C. The mixture was stirred 30 min at −4 °C and 3 h at ambient temperature. Solvent removal (rotary evaporator) gave a yellow oil which was hydrolyzed by stirring in 100 ml of 13% aqueous sodium hydroxide for 2 h at 80 °C. The oil layer, combined with a benzene extract of the aqueous layer, was washed with water, dried over $MgSO_4$, and freed of solvent, yielding 39.36 g of oil. Fractional distillation through a short Vigreux column gave 18.0 g (42%) of 14: bp 64 °C (0.05 mm); IR 1725, 1680 cm^{-1}; NMR 1.03 (t, J = 7.5 Hz, 3 H, ethyl CH_3), 1.59 and 1.70 (singlets, 9 H, 3 allyl CH_3), 2.5 (q, J = 7.5 Hz, 2 H, ethyl CH_2), 2.0–2.7 (m, 8 H, allyl CH_2), 5.12 ppm (br s, 2 H, vinyl H); CIMS m/e 209 (MH^+). Anal. ($C_{14}H_{24}O$) C, H.

Ethyl 3-Ethyl-7,11-dimethyl-2(*E*),6(*E*),10-dodecatrienoate (15). Reaction of 14 (17.58 g, 85 mmol) with the sodium salt from 21 g (93 mmol) of triethyl phosphonoacetate, essentially as described in the preparation of 13, gave 19 g of a 6:4 mixture of the 2*E* and 2*Z* isomers, respectively, of 15. Spinning-band distillation and purification as before yielded 3.5 g of pure (by GC) 2(*E*)-15: IR 1725 cm^{-1}; NMR 1.05 (t, J = 7.5 Hz, 3 H, ethyl CH_3), 1.26 (t, J = 7 Hz, 3 H, ethoxy CH_3), 1.6–1.68 (3 s, 9 H, allyl CH_3), 1.93–2.33 (m, 8 H, allyl CH_2), 2.65 (q, J = 7.5 Hz, 2 H, ethyl CH_2), 4.17 (q, J = 7 Hz, 2 H, ethoxy CH_2), 5.15 (br s, 2 H, vinyl H), and 5.67 ppm (s, 1 H, conjugated vinyl H); CIMS m/e 279 (MH^+). Anal. ($C_{18}H_{30}O_2$) C, H.

3-Ethyl-7,11-dimethyl-2(*E*),6(*E*),10-dodecatrien-1-ol (4 Alcohol). Sodium bis(2-methoxyethoxy)aluminum hydride in benzene (Realco Vitride, 3.58 M, 0.691 ml, 2.47 mmol) was added under nitrogen at 0 °C to a stirred solution of 15 (627 mg, 2.25 mmol) in 5 ml of dry ether. The mixture was allowed to warm to ambient temperature and was stirred 2.5 h. Addition of water at 0 °C until precipitation occurred, filtration, solvent removal,

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