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# Radiolabeled Allylic Isoprenoid Pyrophosphates: Synthesis, Purification, and Determination of Specific Activity[1]

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A new procedure for the preparation of [1-$^3$H]allylic isoprenoid pyrophosphates is reported. Tritium is introduced into dimethylallyl alcohol, geraniol, and farnesol by oxidation to the corresponding aldehydes followed by reduction with sodium [1-$^3$H]borohydride. The alcohols are phosphorylated by conversion to allylic halides and treatment of the halides with tris-tetra-*n*-butylammonium hydrogen pyrophosphate. The pyrophosphates are purified by chromatography on cellulose. Specific activities are determined from the corresponding naphthoate esters whose concentrations can be measured accurately by ultraviolet spectroscopy. Alternatively, the specific activity of tritium is determined for the naphthoate esters prepared with [$^{14}$C]naphthoic acid of known specific activity by measurement of $^3$H/$^{14}$C ratios. The methods described should prove advantageous over existing procedures for radiochemical preparations of this class of metabolites. 

Allylic isoprenoid pyrophosphates are key intermediates in biosynthetic pathways leading to several important classes of metabolites, including sterols, carotenoids, dolichols, and ubiquinones (*1–3*). Although these important compounds are used in numerous biological studies (*4–11*), they are difficult to prepare and purify, and none are available commercially. The situation is exacerbated when radiolabeled materials are required (*12–18*). The allylic intermediates must be synthesized by inefficient routes or prepared biosynthetically from commercially available precursors such as mevalonic acid or isopentenyl pyrophosphate. Purification of these materials is labor intensive and usually yields pyrophosphates of variable purity. In addition, with few exceptions (*8, 10, 11*), specific activities of the pyrophosphates are not determined accurately.

We recently reported improved procedures for synthesis and purification of primary allylic pyrophosphates by displacement of activated allylic derivatives with inorganic pyrophosphate (*19*). Since many of our mechanistic studies with the enzymes of the isoprene pathway require radiolabeled substrates of high purity and known specific activity, we have modified our pyrophosphorylation to this end. We now report procedures for the synthesis, purification, and determina-

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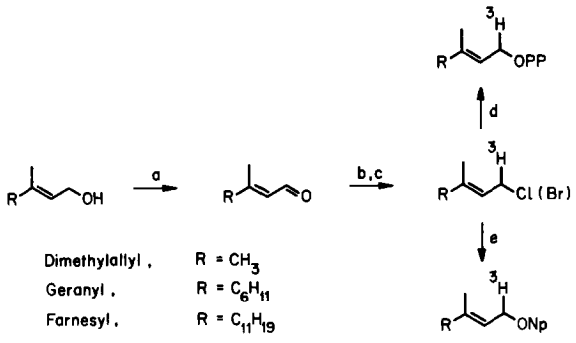


SCHEME 1. Synthesis of [1-$^3$H]dimethylallyl, [1-$^3$H]geranyl, and [1-$^3$H]farnesyl pyrophosphate. (a) $MnO_2$. (b) $NaB^3H_4$. (c) *N*-Chlorosuccinimide, $Me_2S$ ($PBr_3$, R = $CH_3$). (d) ($n$-$Bu_4N)_3HP_2O_7$. (e) 2-Naphthoic acid, dicyclohexylcarbodiimide, 4-(*N*,*N*-dimethylamino)pyridine · HCl.

tion of specific activity for derivatives of dimethylallyl-PP,[3] geranyl-PP, and farnesyl-PP (*3*).

## RESULTS AND DISCUSSION

The general procedures for synthesis of allylic pyrophosphates are summarized in Scheme 1. Tritium is introduced by the well-established oxidation–reduction sequence using a stirred suspension of manganese dioxide in pentane to convert the allylic alcohols to the corresponding aldehydes, followed by reduction with sodium [$^3$H]borohydride in methanol (*4, 13, 15, 16, 20, 21*). The radiolabeled alcohols can be used immediately in the pyrophosphorylation step without purification or can be stored at −20°C for several weeks prior to use. [1-$^3$H]Geraniol and [1-$^3$H]farnesol were converted to the corresponding chlorides and then treated with tris-tetra-*n*-butylammonium pyrophosphate as previously described for unlabeled material (*19*). A different procedure was used for halogenation of dimethylallyl alcohol because of the volatility of the $C_5$ chloride. Treatment of the alcohol with phosphorus tribromide in pentane yields a solution containing dimethylallyl bromide that is used directly in the phosphorylation reaction (*22*). The isoprenoid pyrophosphates were purified by chromatography as previously described (*19*).

The sequence of reactions used to convert the alcohols to pyrophosphates is simple to perform experimentally and gives material in which greater than 95% of the radioactivity comigrates with authentic samples of unlabeled pyrophosphates on a cellulose thin-layer system. Although the radiochemical yield of dimethylallyl pyrophosphate is only 5% from dimethylallyl aldehyde, the experimental manipulations are easy to perform and the desired product is the only phosphorylated radioactive species produced in the reaction. The overall radiochemical yields of geranyl pyrophosphate (18%) and farnesyl pyrophosphate (21%) were substantially higher.

[3] Abbreviation used: PP, pyrophosphate.

We typically perform the [$^3$H]borohydride reductions with an excess of isoprenoid aldehyde to ensure maximal utilization of the radioisotope, and finish the reaction with sodium borohydride. Thus, it is important to have a reliable procedure for establishing the specific activity of the products. The alcohols themselves are poor candidates for a direct measurement because it is difficult to establish their concentrations in dilute solution accurately by techniques which minimize contamination other than scintillation spectrometry. We decided that naphthoate esters would be suitable derivatives for this purpose. The compounds are not volatile, can be readily purified by TLC or HPLC, and contain an intense chromophore which can be used to establish the concentrations of dilute solutions of radiolabeled material.

Unlabeled naphthoate esters were prepared for use as chromatographic and uv standards. A small portion of each radioactive isoprene alcohol was efficiently converted to the corresponding ester using a modified version of the Steglich procedure (*23, 24*). 2-[$^{14}$C]Napthoic acid (1.28 $\mu$Ci/$\mu$mol) was used in the esterification to provide an independent measure of the tritium specific activity by $^3$H/$^{14}$C double isotope counting. The esters were purified by thin-layer chromatography and specific activities were calculated using concentrations determined from uv measurements and $^3$H/$^{14}$C isotope ratios. The results are shown in Table 1. As a check on the accuracy of the method, excellent agreement was found between the specific activities of 2-[$^{14}$C]naphthoic acid determined from weighed samples and those calculated from $^{14}$C dpm in samples of $^3$H/$^{14}$C-labeled material whose concentrations were determined spectrophotometrically. In addition, a sample of [1-$^3$H]farnesyl naphthoate was prepared from [1-$^3$H]alcohol obtained by hydrolysis of [1-$^3$H]farnesyl pyrophosphate with *Escherichia coli* alkaline phosphatase. The specific activity of this material, as determined by spectrophotometry, agrees with values determined by the double isotope procedure. These results demonstrate that concentrations from uv measurements can be used to calculate the specific activities of [1-$^3$H]dimethylallyl, [1-$^3$H]geranyl, and [1-$^3$H]farnesyl naphthoates. In light of previous reports concerning the stability of allylic pyrophosphates (*5, 9, 13*), it should be noted that [1-$^3$H]farnesyl pyrophosphate was stored for 2 months before performing the final specific activity experiments.

The procedures we describe provide allylic isoprenoid pyrophosphates of high chemical and radiochemical purity. By using the naphthoate moiety as a handle to

TABLE 1

ANALYSIS OF NAPHTHOATE ESTERS

| | | | $^3$H | | | $^{14}$C | | |
|---|---|---|---|---|---|---|---|---|
| R | $^3$H/$^{14}$C | sp act[a] ($\mu$Ci/$\mu$mol) | Concentration[b] ($\mu$M) | $^3$H (dpm/$\mu$l) | sp act ($\mu$Ci/$\mu$mol) | Concentration[b] ($\mu$M) | $^{14}$C (dpm/$\mu$l) | sp act ($\mu$Ci/$\mu$mol) |
| $CH_3$ | 17.9 ± 0.7 | 23.0 | | | | 196 ± 5 | 564 ± 10 | 1.31 |
| $C_6H_{11}$ | 25.9 ± 0.4 | 33.1 | | | | 442 ± 42 | 1243 ± 83 | 1.29 |
| $C_{11}H_{19}$ | 24.9 ± 0.6 | 31.8 | 346 ± 14 | 4050 ± 20 | 32.6 | 346 ± 14 | 996 ± 50 | 1.30 |

[a] Based on $^{14}$C sp act = 1.28 $\mu$Ci/$\mu$mol.
[b] Measured by uv.

determine the concentration of dilute solutions, the specific activities of the materials can be determined with high accuracy. This is a general strategy for determination of specific activity which should be useful for any alcohol, especially those that are volatile or difficult to purify. The overall yields for synthesis of radioactive materials were lower than those obtained for unlabeled pyrophosphates (*19*) because of technical limitations imposed by scale and the precautions required to guard against contamination. However, except for the dimethylallyl system, the overall radiochemical yields of pure product exceed what is possible by other procedures.

## EXPERIMENTAL

2-Bromonaphthalene, 2-naphthoic acid, 3-methyl-2-butene-1-ol, geraniol, farnesol, *N*,*N*′-dicyclohexylcarbodiimide, 4-dimethylaminopyridine, and phosphorus tribromide were purchased from Aldrich Chemical Company. Magnesium was purchased from Fisher Scientific Company. Disodium dihydrogen pyrophosphate and *E. coli* alkaline phosphatase (Type III) were purchased from Sigma Chemical Company, and lysine hydrochloride was purchased from J.T. Baker Chemical Company. Sodium borohydride was purchased from Alfa Products. Silica gel 60 F 254 plates (EM reagents), 0.25 mm thick, were used for all thin-layer chromatographies. Radioactivity was measured with a Packard Tri-Carb 4530 liquid scintillation spectrometer and either commercial Instafluor or Instagel cocktails. A Guilford 2600 spectrophotometer was employed for uv measurements. Methanol was purified as described by Burfield and Smithers (*25*). Anhydrous tetrahydrofuran was obtained by distillation from sodium benzophenone. Tetrahydrofuran used for chromatography was purified by passage through alumina prior to distillation. Tris-tetra-*n*-butylammonium hydrogen pyrophosphate was dried by evaporation of 2 vol of dry acetonitrile at room temperature under vacuum immediately prior to use. All other reagents were prepared as previously described (*19*).

*2-[$^{14}C$]Naphthoic acid.* A flask which contained 0.73 g (30 mmol) of magnesium turnings was flame-dried under a nitrogen atmosphere before addition of 2 ml of dry tetrahydrofuran. To the magnetically stirred suspension was added 0.5 g (2.4 mmol) of 2-bromonaphthalene. After 1 h, a 1-ml aliquot of the Grignard solution was transferred by syringe to a 1 × 9-cm reaction vessel to which an ampoule containing 1 mCi (51 mCi/mmol) of carbon-$^{14}$C dioxide (New England Nuclear) had been fused. A small magnet was placed above the breakseal in the side arm. The solution was cooled to −50° and the seal was broken by rapidly moving the stir bar with a second external magnet. After 15 min, the cold bath was removed and the carbon dioxide ampoule was briefly heated to flush any remaining gas into the reaction vessel. The reaction was allowed to continue for an additional 1 h. The contents were rinsed into a 25-ml conical test tube with 2 ml of ethyl acetate. The solution was washed with 2 ml of 6 N hydrochloric acid and extracted with 1 ml of 1 N potassium hydroxide. The basic extract was washed with two 1-ml portions of hexanes and acidified to pH 2 with 6 N hydrochloric acid. The resulting

aqueous solution was extracted with three 1-ml portions of ethyl acetate and solvent was evaporated. The residue was purified by preparative thin-layer chromatography using a 70 : 23 : 7 (v/v/v) mixture of hexanes, ethyl acetate, and isopropanol as the eluent. 2-[$^{14}$C]Naphthoic acid was extracted, filtered, and dried. The resulting solid was dissolved in 1 ml of ethyl acetate and radioactivity was determined by liquid scintillation. The radiochemical yield was 0.29 mCi (24%). To this solution was added 17.2 mg (0.1 mmol) of 2-naphthoic acid and the sample was dried at reduced pressure. Three samples were weighed on a microbalance and counted by liquid scintillation spectrometry. The specific activity was determined to be 1.28 ± 0.01 $\mu$Ci $\mu$mol$^{-1}$.

*3-[1-$^3$H]Methyl-2-buten-1-ol.* To an oven-dried 5-ml reaction vial was added 31 mg (0.37 mmol) of the dimethylallyl aldehyde. A solution containing 25 mCi (350 mCi/mmol, 71 $\mu$mol) of sodium [$^3$H]borohydride (Amersham) in 0.5 ml of methanol was added directly to the reaction vial and the resulting solution was diluted to 1 ml with methanol. After 5 h, 13.7 mg (0.36 mmol) of sodium borohydride was added, and the reaction was allowed to continue for 12 h before addition of 200 mg of sodium bicarbonate and 0.5 ml water. The mixture was extracted with 2 ml of a 1 : 1 (v/v) mixture of pentane and diethyl ether followed by four 1-ml portions of pentane. The combined extracts were passed through a cone of magnesium sulfate and concentrated to $\frac{1}{2}$ vol by evaporation under a gentle stream of nitrogen. The solution was analyzed for [1-$^3$H]dimethylallyl alcohol by thin-layer chromatography using an 8 : 2 (v/v) mixture of hexanes and ethyl acetate as the eluent. The radiochemical recovery was 4 mCi (16%). The solution of [1-$^3$H]dimethylallyl alcohol was stored at −20°C for less than 2 weeks before pyrophosphorylation.

*[1-$^3$H]Geraniol.* To a 5-ml oven-dried reaction vial was added 50 mg (0.33 mmol) of geranial in 0.1 ml of methanol. To this magnetically stirred solution was added 25 mCi (350 mCi/mmol, 71 $\mu$mol) of sodium [$^3$H]borohydride (Amersham) in 1.5 ml of methanol. After 4.5 h, 12 mg (0.32 mmol)) of sodium borohydride was added and the reaction was continued overnight. One milliliter of saturated sodium chloride solution was added, the mixture was extracted with three 1-ml portions of pentane, and the combined extracts were dried by passage through a small cone of magnesium sulfate. Solvent was removed with a gentle stream of nitrogen and 0.6 ml of dichloromethane was added to the residue. This solution was stored at −20°C prior to phosphorylation. The radioactivity recovery of 21 mCi (84%) was in excess of the theoretical value based on the specific activity of the geranyl unit in [$^3$H,$^{14}$C]geranyl naphthoate. The nature of the excess radioactivity is unknown and was not traced in any of the subsequent reactions. [1-$^3$H]Geraniol was detected by thin-layer chromatography.

*[1-$^3$H]Farnesol.* To an oven-dried 2-ml reaction vial was added 22 mg (0.1 mmol) of farnesal, followed by 8 mCi (350 mCi/mmol, 23 $\mu$mol) of sodium [$^3$H]borohydride (Amersham) in 0.3 ml of methanol. The vial was capped and allowed to stand 4 h before the addition of 4 mg (0.1 mmol) of sodium borohydride in 0.1 ml of methanol. After an additional 1 h, 1 ml of saturated sodium chloride was added and the workup was performed as for [1-$^3$H]geraniol. The radiochemical recovery (55%) was 4.4 mCi. The presence of [1-$^3$H]farnesol was verified by thin-layer chromatography. As in the case for [1-$^3$H]geraniol, the recovered radio-

activity was in excess of the theoretical value based on the specific activity of the naphthoate.

*[1-$^3$H]Dimethylallyl pyrophosphate.* A solution of [1-$^3$H]dimethylallyl alcohol (0.31 mCi) in 0.5 ml of pentane was added to a 1-ml reaction vial. The vial was cooled to 0°C in an ice bath and 2 μl (0.013 mmol) of freshly distilled phosphorous tribromide was added. After 15 min, 0.1 ml of methanol was added and the resulting suspension was stirred at 0°C for 10 min before the methanol layer was removed by syringe. A solution of tris-tetra-*n*-butylammonium hydrogen pyrophosphate (50 mg, 0.55 mmol) in 1 ml of acetonitrile was added and the layers were mixed by rapid magnetic stirring. After 24 h solvent was removed by rotary evaporation. To the resultant oil was added 1 ml of a solution of 25 mM ammonium bicarbonate containing 2% isopropanol. The resulting solution was applied to a 0.5 × 12-cm column of Dowex AG 50W-X8 ion exchange resin (ammonium form) and slowly eluted with the same buffer. After lyophilization, the solid was dissolved in 1 ml of 0.1 M ammonium bicarbonate and treated with 3 ml of 1 : 1 (v/v) acetonitrile and isopropanol. The supernatant was removed and the procedure was repeated. The combined extracts were filtered, concentrated by rotary evaporation, and applied to a 2 × 11-cm column of CF11 cellulose. The column was eluted with a 3.5 : 3.5 : 3 (v/v/v) mixture of acetonitrile, isopropanol, and 50 mM ammonium bicarbonate. A total of forty 6-ml fractions were collected and 1-μl aliquots were assayed for radioactivity. The active fractions were pooled, concentrated by rotary evaporation, and dried by lyophilization. The residue was dissolved in 1 ml of 25 mM ammonium bicarbonate, pH 7.2, and analyzed by thin-layer chromatography on cellulose. The recovered radioactivity was 54.5 μCi (5% yield based on 50 μmol of dimethylallyl aldehyde). The material was >95% radiochemically pure as judged by comparison with an authentic sample on cellulose thin-layer chromatography. The pyrophosphate was stored at −20°C.

*[1-$^3$H]Geranyl pyrophosphate.* In a dried 2-ml reaction vial were combined, with magnetic stirring under nitrogen, 6 mg (0.045 mmol) of *N*-chlorosuccinimide and 0.1 ml of dichloromethane. The solution was cooled to −20°C and 5 μl (4 mg, 0.06 mmol) of dimethyl sulfide was added. [1-$^3$H]Geraniol (0.87 mCi) in 0.4 ml of dichloromethane were added by syringe. Within 15 min, the cold mixture became homogeneous. After 0.5 h, the ice bath was removed and 1 ml of pentane was added. The mixture was transferred to a 25-ml conical test tube and diluted with 2 ml of pentane. Saturated sodium chloride, 0.5 ml, was added and the resulting solution was agitated on a Vortex mixer until the organic layer became clear. The pentane layer was removed and briefly dried over magnesium sulfate. After filtration, the solvent was removed with a gentle stream of nitrogen and the residual oil was treated with 0.1 g (0.11 mmol) of tris-tetra-*n*-butylammonium hydrogen pyrophosphate in 0.4 ml of dry acetonitrile. The reaction vial was purged with nitrogen and tightly capped. The solution was stirred for 24 h. Solvent was removed by rotary evaporation and the residue was dissolved in 2 ml of 25 mM ammonium bicarbonate containing 2% isopropanol. The resulting solution was applied to a 0.7 × 8.3-cm column of Dowex AG 50W-X8 ion exchange resin (ammonium form) and the column was eluted with 2 column vol of the same buffer. After lyophilization, the residue was dissolved in 0.75 ml of 0.1 M ammo-

nium bicarbonate and treated with 3 ml of a 1 : 1 (v/v) mixture of acetonitrile and isopropanol. The supernatant was removed and the procedure was repeated. The combined extracts were filtered and concentrated to 2 ml by rotary evaporation before chromatography on a 2 × 14-cm CF11 cellulose flash column. The column was eluted as previously described for geranyl pyrophosphate (*19*) or with an 8 : 2 (v/v) mixture of tetrahydrofuran and 50 mM ammonium bicarbonate. Typically fifty 5-ml fractions were collected and 1-μl aliquots were assayed by liquid scintillation spectrometry. The radioactive fractions were combined, concentrated by rotary evaporation, and dried by lyophilization. The residue was dissolved in 1 ml of 25 mM ammonium bicarbonate, pH 7.2, and analyzed by thin-layer cellulose chromatography and liquid scintillation spectrometry. Greater than 96% of the radioactivity comigrated with an authentic sample of geranyl pyrophosphate. A total of 191 μCi (5.8 μmol, sp act 33.1, 18% based on 30 μmol of geranial) was recovered. The solution was stored at −20°C.

*[1-$^3$H]Farnesyl pyrophosphate.* The procedure was similar to that described for [1-$^3$H]geranyl pyrophosphate. A solution of [1-$^3$H]farnesol (1.39 mCi) in 0.4 ml of dichloromethane was added by syringe to a suspension of 5.3 mg (0.04 mmol) of *N*-chlorosuccinimide and 3.5 μl (2.8 mg, 0.045 mmol) of dimethyl sulfide in 0.2 ml of dichloromethane at −20°C. After 30 min, the reaction was worked up as described for geranyl pyrophosphate. The residual oil was then treated with a solution of 90 mg (0.1 mmol) of tris-tetra-*n*-butylammonium hydrogen pyrophosphate in 0.3 ml of acetonitrile. The vial was purged with nitrogen for 5 min, tightly capped, and then stirred for 18 h. The material was worked up as described for [1-$^3$H]geranyl pyrophosphate. The residue was purified by flash chromatography on cellulose and eluted with an 85 : 15 (v/v) mixture of tetrahydrofuran and 0.1 M ammonium bicarbonate. A total of twenty-five 4-ml fractions were collected and treated as previously described. The radioactive product was dissolved in 0.5 ml of 25 mM ammonium bicarbonate, pH 7.2, and analyzed by thin-layer cellulose chromatography and liquid scintillation spectrometry. Greater than 97% of the radioactivity comigrated with authentic material. A total of 221 μCi (6.9 μmol, sp act 31.8, 21% based on 33 μmol of farnesal) was recovered. The solution was stored at −20°C.

*Synthesis of $^3$H/$^{14}$C-labeled naphthoate esters.* In a 1-ml serum vial were combined 0.34 mg (1.95 μmol) of 2-[$^{14}$C]naphthoic acid (sp act 1.28 μCi/μmol) and 0.1 ml of a solution of dichloromethane 40 mM in both 4-dimethylaminopyridine and 4-dimethylaminopyridine hydrochloride. To this solution was added 25 μl of a 0.16 M solution of *N,N'*-dicyclohexylcarbodiimide in dichloromethane. After 1 h, a solution of the appropriate [1-$^3$H]alcohol (0.1 to 0.3 μCi) was added. The vial was tightly capped and the mixture stirred for 18–24 h. The reactions were monitored by thin-layer chromatography with 1 : 1 hexanes : dichloromethane as the eluent. The initial solution contains two uv active materials, $R_f$ at 0.3 and 0.1. After addition of the [1-$^3$H]alcohols, a new product with a higher $R_f$ formed. An appropriate amount of [1-$^3$H]alcohol was added to ensure the complete consumption of naphthoic acid ($R_f$ 0.10). The reaction mixture were evaporated to dryness with a gentle stream of nitrogen and the residue was extracted with two 1-ml portions of hexanes. The combined extracts were concentrated and chromato-

graphed on 20 × 20-cm thin-layer plates. The plates were developed at least three times with a 1 : 1 (v/v) mixture of hexanes and dichloromethane. Occasionally, residual methanol from the borohydride reductions was carried along into the esterification reactions. Authentic methyl naphthoate was synthesized to demonstrate that the material was separated from isoprene naphthoates during chromatography. The isoprene naphthoate was extracted from the silica gel with dichloromethane and solvent was removed at reduced pressure. The resulting material was then dissolved in 1 ml of HPLC grade acetonitrile and samples were removed for analysis by uv and liquid scintillation spectrometry. All dual-labeled counting was performed on multiple samples in 10 ml of Instafluor in borosilicate scintillation vials. Ultraviolet determinations were performed in HPLC grade acetonitrile.

Authentic samples of the three isoprenoid naphthoates were prepared from commercially available alcohols as analytical standards.

*Dimethylallyl naphthoate.* $^1$H NMR ($CDCl_3$): δ 8.6 (s, 1 H), 8.06 (m, 1 H), 7.95 (m, 1 H), 7.85 (d, 2 H), 7.55 (m, 2 H), 5.52 (br t, $J$ = 9 Hz, 1 H), 4.87 (d, $J$ = 9 Hz, 2 H), 1.8 (s, 3 H); uv ($\lambda_{max}$, acetonitrile) 236 nm (ε, 6.2 × $10^4$); $R_f$ 0.4 (1 : 1 dichloromethane : hexanes).

*Geranyl naphthoate.* $^1$H NMR ($CDCl_3$): δ 8.6 (s, 1 H), 8.06 (m, 1 H), 7.95 (m, 1 H), 7.85 (d, 2 H), 7.55 (m, 2 H), 5.5 (br t, $J$ = 9 Hz, 1 H), 5.1 (m, 1 H), 4.88 (d, $J$ = 9 Hz, 2 H), 2.1 (m, 4 H), 178 (s, 3 H), 1.58 (s, 3 H), 1.55 (s, 3 H); uv ($\lambda_{max}$, acetonitrile) 236 nm (ε, 5.9 × $10^4$); $R_f$ 0.33 (1 : 1 dichloromethane : hexanes).

*Farnesyl naphthoate.* $^1$H NMR ($CDCl_3$): δ 8.6 (s, 1 H), 8.06 (m, 1 H), 7.95 (m, 1 H), 7.85 (d, 2 H), 7.55 (m, 2 H), 5.51 (br t, $J$ = 9 Hz, 1 H), 5.1 (m, 2 H), 4.92 (d, $J$ = 9 Hz, 2 H), 2.12–2.0 (m, 8 H), 1.8 (s, 3 H), 1.65 (s, 3 H), 1.58 (s, 6 H); uv ($\lambda_{max}$, acetonitrile) 236 nm (ε, 5.9 × $10^4$); $R_f$ 0.3 (1 : 1 dichloromethane : hexanes).

*Conversion of [l-$^3$H]farnesyl pyrophosphate to [l-$^3$H]farnesyl naphthoate.* To each of two 1-ml reaction vials was added 0.35 μmol of [1-$^3$H]farnesyl pyrophosphate in 0.1 ml of 25 mM ammonium bicarbonate. To each was added 0.1 ml of 0.2 M lysine buffer, pH 10.5, and 0.2 ml of water. *E. coli* alkaline phosphatase (60 μg) was added and the resulting mixture was incubated at 37°C for 4.5 h. Samples (3 μl) were removed periodically and extracted with petroleum ether (bp 90–120°C). When production of hydrocarbon-soluble radioactivity stopped, the reaction mixture was extracted with two 2.5-ml portions of dichloromethane. The combined extracts were dried over sodium sulfate and filtered. The filtrate was concentrated to <0.5 ml and transferred to a 1-ml reaction vial. To this solution was added 0.3 mg (1.7 μmol) of naphthoic acid and 30 μl of a 0.1 M dichloromethane solution which contained both 4-dimethylaminopyridine and 4-dimethylaminopyridine hydrochloride. Thirty microliters of a 0.21 M solution of dicyclohexylcarbodiimide in dichloromethane was added and after 16 h, the reaction was worked up as previously described.

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