Chemical Synthesis of Pyrophosphodiesters of Carbohydrates and Isoprenoid Alcohols. Lipid Intermediates of Bacterial Cell Wall and Antigenic Polysaccharide Biosynthesis†

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ABSTRACT: The pyrophosphodiesters of monosaccharides and isoprenoid alcohols are intermediates in the biosynthesis of bacterial cell wall components. They have been chemically synthesized as a proof of structure and for greater availability as follows. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl phosphate (**1**) was prepared as the crystalline ammonium salt, by treatment of β-D-galactose pentaacetate with crystalline phosphoric acid or by acetylation of α-D-galactopyranosyl phosphate. Farnesyl monophosphate was converted into the diphenyl pyrophosphate and condensed with **1** to give a fully acetylated pyrophosphodiester of farnesol and D-galactose (**4**), which was deacetylated, giving a short-chain analog of the lipid intermediate. Ficaprenyl phosphate (**5**) was prepared by phosphorylation of a mixture (containing mainly C_{55}) of ficaprenols with *o*-phenylene phosphochloridate to give the *o*-hydroxyphenyl phosphate, which was treated with lead tetraacetate, and then with potassium hydroxide in methanol–dioxane. The diphenyl pyrophosphate obtained from **5** was condensed with **1** to give a fully acetylated pyrophosphodiester of ficaprenol and D-galactose, which was deacetylated with sodium methoxide in methanol to give the synthetic lipid intermediate.

Lipid intermediates have been well characterized as key compounds in the biosynthesis of the bacterial lipopolysaccharide "O antigen" (Wright *et al.*, 1967; Osborn and Yuan Tze-Yuen, 1968; Wright, 1971; Nikaido and Nikaido, 1971) and peptidoglycan (Anderson *et al.*, 1967; Higashi *et al.*, 1967, 1970). More recently, their participation has been demonstrated in the biosynthesis of a capsular polysaccharide (Troy *et al.*, 1971), mannan (Scher *et al.*, 1968; Scher and Lennarz, 1969; Lahav *et al.*, 1969), mannolipids in mycobacteria (Takayama and Goldman, 1970), and probably teichoic acids (Douglas and Baddiley, 1968; Brooks and Baddiley, 1969a,b; Stow *et al.*, 1971; Watkinson *et al.*, 1971). From this work, lipid intermediates have been shown to contain a pyrophosphate, or, less commonly, a monophosphate linkage between sugar and lipid moieties, the latter moiety being identified as a long-chain isoprenoid alcohol containing (in bacteria) 55 carbon atoms. There is increasing evidence for the involvement of similar substances in the biosynthesis of yeast mannan (Tanner, 1969; Tanner *et al.*, 1971; Sentandreu and Lampen, 1971), mammalian glycolipids, and glycoproteins (for example, Tetas *et al.*, 1970; Behrens and Leloir, 1970; DeLuca *et al.*, 1970; Alam *et al.*, 1971; Richards *et al.*, 1971; Molnar *et al.*, 1971; Behrens *et al.*, 1971).

In the biosynthesis of the polysaccharide chain, the lipid phosphate is continually regenerated, and therefore the lipid intermediate is present in only very small quantities. In addition, it is chemically very reactive, owing to the presence of allylic phosphate as well as glycosyl phosphate linkages. This instability compounds the difficulty of isolation of lipid intermediates from natural sources, and evidence for their participation in a biosynthetic process is often indirect.

Chemical synthesis of this class of compounds could provide unambiguous evidence for the participation of such a substance in a biosynthetic process, and, additionally, would make available relatively large amounts of material for further biological experiments, for the study of the physical and chemical properties of phosphodiesters of this type, and for the evaluation of the importance of the lipid structure in the biosynthetic process. The present paper describes the synthesis of farnesyl α-D-galactopyranosyl pyrophosphate as a model compound and of ficaprenyl α-D-galactopyranosyl pyrophosphate, a biosynthetically active lipid intermediate.

Results and Discussion

In order to obtain compounds which could be tested in various biological systems, we have attempted to develop a synthesis in which both carbohydrate and lipid moieties could be easily varied, and have concentrated, initially, on the substances containing the more widely occurring pyrophosphate and α-D-glycosidic linkages. A specific and unambiguous synthesis was based on the phosphorylation of the sugar and lipid moieties and their efficient coupling into a pyrophosphodiester, each stage being carried out in a nonpolar medium—unlike the related synthesis of nucleotide anhydrides—with a minimum of purification at the end of the process. Owing to the highly unstable nature of the final compound, full characterization was performed on the relatively stable acetylated intermediate. Initially, D-galactose was chosen since this sugar is readily available and is part of the phospholipid intermediates in the Salmonella O antigen (Wright *et al.*, 1967; Osborn and Yuan Tze-Yuen, 1968) and capsular polysaccharide (Troy *et al.*, 1971) biosynthetic systems. The product resulting from the phosphorylation of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose by a modification of the method of

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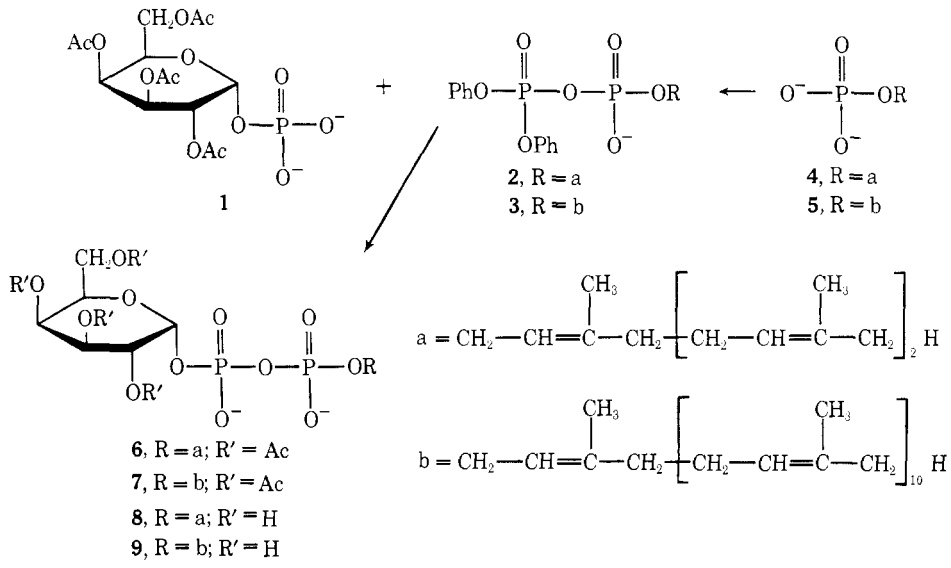
SCHEME I



MacDonald (1962, 1968) showed the presence, before purification, of unchanged starting material and some tetraacetate, presumably formed by the presence of traces of water in the reaction mixture. The fully acetylated α-D-galactopyranosyl phosphate (**1**) (Scheme I) was obtained, as the crystalline monoammonium salt, in a yield varying from 42 to 24% according to the quality of the crystalline phosphoric acid, and it was characterized by infrared (ir) and nuclear magnetic resonance (nmr) spectra and by elementary analysis. The assignment of the α-D-configuration was based on the method of preparation (MacDonald, 1966), the high positive rotation, and the conversion into the known α-D-galactopyranosyl dicyclohexylammonium phosphate (MacDonald, 1962; Putman and Hassid, 1957). Alternatively, deacetylation, with sodium methoxide in methanol, of the crude phosphorylation product gave the disodium salt as a hygroscopic solid, which was converted into the same dicyclohexylammonium salt in 40% yield (based on β-D-galactose pentaacetate). Acetylation with acetic anhydride and tetraethylammonium acetate (Rammler *et al.*, 1963), followed by treatment with cation-exchange resin (ammonium form), gave **1** in a pure state. When the acetylation was performed in the presence of pyridine as base, the experimental procedure was simpler, but a contaminated product was obtained. Thus, the tetraethylammonium acetate method can be used to provide a protected crystalline salt of a sugar phosphate suitable for further synthesis in situations where a purified tetra-*O*-acetyl derivative cannot be obtained directly. Finally, in order to obtain solubility in organic solvents, **1** was converted into the bis(tributylammonium) salt by treatment with a cation-exchange resin (pyridinium form) followed by treatment with tributylamine.

Farnesol was chosen for the preparation of a model compound. Phosphorylation was performed by a modification of a method, previously described by Popjak *et al.* (1962), involving treatment with trichloroacetonitrile and bis(triethylammonium) phosphate in acetonitrile. Separation from farnesyl pyrophosphate, and purification were achieved at a later stage of the synthesis (**2**).

Two methods for the synthesis of the pyrophosphate linkage were available. The one using a morpholidate intermediate (Moffatt and Khorana, 1961; Roseman *et al.*, 1961) was first attempted. Farnesyl phosphate was converted into the morpholidate by treatment with morpholine and dicyclohexylcarbodiimide, and the product, purified by chromatography, was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl phosphate (**1**) in anhydrous pyridine to give a small amount of material having the expected mobility of a pyrophosphate on thin-layer chromatography (tlc). It was shown later that this product cochromatographed with the desired pyrophosphodiester **6**. However, a considerable amount of hydrolysis took place to give the starting phosphate **4** despite rigorous efforts to exclude all moisture from the reaction vessel. In the modification that uses dimethyl sulfoxide instead of pyridine (Kochetkov *et al.*, 1969), hydrolysis did not occur, but the morpholidate was so unreactive that most of it was left unchanged after several weeks. Therefore, farnesyl bis(tributylammonium) phosphate was treated according to Michelson (1964) with diphenyl phosphochloridate to give the diphenyl pyrophosphate **2**. When the diphenyl pyrophosphate was treated with morpholine in 1,1-dichloroethanol, a product with the same mobility on tlc as the farnesyl phosphoromorpholidate was obtained as expected. In the past, pyridine has proved to be the most effective medium for displacement of the diphenyl phosphate anion by an attacking nucleophile, *e.g.*, α-D-glucopyranosyl phosphate (Michelson, 1964). However, when the diphenyl pyrophosphate **2** was treated with anhydrous pyridine, it immediately decomposed to give several products, as observed on tlc, probably *via* solvolysis and formation of an allylic carbonium ion. Therefore, a concentrated solution of **2** in 1,1-dichloroethanol was treated with a twofold excess of **1** [in the bis(tributylammonium)-form] in the presence of 1 equiv of pyridine (based on the proportion of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl phosphate). After 15 hr, the starting material had completely disappeared with formation of a single product (**6**) having the expected mobility on tlc. After removal of the excess of **1** and of diphenylphosphate, the pyrophosphodiester of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose and farnesol was characterized by elementary analysis, estimation of acid-labile phosphate and galactose content, ir and nmr spectra, and hydrolysis by dilute acid and base. Deacetylation of **6** to give a short-chain analog of the lipid intermediate **8** was performed with dilute ammonium hydroxide or with sodium methoxide

in methanol, with only a trace of cleavage of the pyrophosphate linkage. An attempt to perform the condensation with α-D-galactopyranosyl phosphate was unsuccessful, many products being formed. This is not surprising in view of the lability of such compounds as **8**.

Ficaprenol-eleven (together with a small proportion of other homologs) was chosen as the long-chain isoprenoid alcohol for the synthesis of a biologically active compound because of its close similarity to the bacterial lipid (Wright *et al.*, 1967; Lahav *et al.*, 1969; Christenson *et al.*, 1969) and because of its availability in large amounts (Stone *et al.*, 1967). The published isolation procedure (Stone *et al.*, 1967) was modified so that silica gel column chromatography could be used in the later stages of the purification, instead of preparative thin-layer chromatography. The final separation of the C_{55} alcohol from smaller quantities of lower and higher homologs by preparative, reversed-phase, tlc was not performed. Several groups of workers have identified small amounts of C_{45}, C_{50}, or C_{60} isomers occurring with the main C_{55} component of the active, bacterial lipid (Higashi *et al.*, 1967; Wright, 1971; Troy *et al.*, 1971). Phosphorylation of ficaprenol by the method used for farnesol was unsatisfactory because of the insolubility of the material in acetonitrile. However, the method used by Khwaja *et al.* (1970) for the phosphorylation of allylic alcohols proved to be applicable, with some modifications, to ficaprenol. This was treated, in *p*-dioxane solution, with an equivalent amount of *o*-phenylene phosphochloridate in the presence of 2,6-dimethylpyridine. The resulting triester was immediately hydrolyzed with aqueous 2,6-dimethylpyridine to give a syrupy *o*-hydroxyphenyl phosphate of ficaprenol. Since treatment with periodic acid gave a complex mixture of products, oxidation was performed with lead tetraacetate in *p*-dioxane. The resulting product, not isolated, was directly treated with dilute methanolic potassium hydroxide to yield the crude dipotassium salt of ficaprenyl phosphate (**5**). Purification was easily achieved at this stage by preparative tlc, but was found to be unnecessary for the next stage of the synthesis. The pure potassium, sodium, and barium salts of ficaprenyl phosphate could not be obtained in crystalline form.

The potassium salt was converted into the bis(tributylammonium) form, and the diphenyl pyrophosphate **3** and pyrophosphate **7** were obtained as described for the farnesol derivative. However, it was found more convenient to perform the column chromatographic purification after formation of the pyrophosphate **7** rather than at the diphenyl pyrophosphate **3** stage. This gave the pure, noncrystalline tetra-*O*-acetyl pyrophosphodiester of ficaprenol and galactose **7**, of which the structure, like that of the farnesol derivative **6**, was confirmed by analytical, spectral, and hydrolysis data. Dilute acid hydrolysis gave mainly 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl phosphate, and various rearrangement and decomposition products of the polyprenol (see Wright *et al.*, 1967; Troy *et al.*, 1971). Dilute alkaline hydrolysis gave mainly ficaprenyl phosphate and α-D-galactopyranosyl 1,2-monophosphate, identified (Troy *et al.*, 1971) by comparison to the product of alkaline hydrolysis of UDP-galactose (Paladini and Leloir, 1952).

Deacetylation of **7** was best performed with a dilute solution of sodium methoxide in methanol, and it gave a product containing only small quantities of hydrolysis products resulting from the alkaline scission of the pyrophosphate bond. The lipid intermediate **9** was finally purified by preparative tlc and stored in chloroform solution at −15°, under which conditions it was reasonably stable. Compound **9** has been shown to be an active intermediate in the biosynthesis of Salmonella O antigen. Employing a particulate fraction from a mutant form of *Salmonella anatum* A_1 deficient in the enzyme UDP-glucose 4-epimerase, the incorporation of [^{14}C]rhamnose from TDP-[^{14}C]rhamnose was found to be dependent on the presence of synthetic galactosyl ficaprenyl pyrophosphate, and the kinetics of the stimulated incorporation were measured. A further incubation was carried out in the presence of GDP-mannose, and the nature of the O antigenic polysaccharide product investigated by acid hydrolysis, the oligosaccharide fragments being identified by paper chromatography and paper electrophoresis. The results of these experiments showed that the biosynthetic system was functioning normally under these conditions (A. Wright, S. Kanegasaki, and C. D. Warren, in preparation).

Experimental Section

General Methods. Melting points were determined on a Mettler FP2 hot stage equipped with microscope, and correspond to "corrected" melting point. Optical rotations were determined in 1-dm semimicro tubes with a Perkin-Elmer Model 141 polarimeter. Infrared spectra were recorded with a Perkin-Elmer spectrophotometer, Model 237. Absorbancy at 820 nm was measured with a Zeiss spectrophotometer, Model PMQ 11. Nmr spectra were recorded with a Varian A-60 spectrometer, with deuterium oxide as solvent and sodium 3-(trimethylsilyl)propanesulfonate as internal standard. The cation-exchange resin used was AG 50WX8 (200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.). In all cases, the amount of resin used was in at least a twofold excess over the necessary quantity to obtain complete ion exchange. Evaporations were carried out under reduced pressure, with an outside bath temperature kept below 30°. The microanalyses were performed by Dr. M. Manser, Zurich, Switzerland. Acid-labile phosphate was estimated by the method of Chen *et al.* (1956).

Chromatogrphies. Silica gel column chromatography was performed on silica gel 0.05–0.2 mm (70–325 mesh, E. Merck A. G., Darmstadt, Germany), or on silica gel Davison, grade 950 (60–200 mesh, Davison Chemical, Baltimore, Md.). Both materials were used without pretreatment. Alumina column chromatography was performed on aluminum oxide Fluka type 507C (Fluka AG, Buchs S.G., Switzerland), which was treated with 6% water before use to give Brockmann activity 3. Tlc was performed on precoated plates (6 cm long) of silica gel G (Merck) or cellulose F (Merck), the plates being cut to size before use. Preparative tlc was carried out on precoated preparative tlc plates of silica gel F 254 (Merck). Reversed-phase tlc was carried out on kieselguhr F (Merck), the plates being impregnated with a solution of 5% liquid paraffin in hexane. The spray reagent used, unless otherwise stated, was anisaldehyde–sulfuric acid–ethanol (1:1:18), and the plates were heated to 125°. Solvent A for tlc was chloroform–methanol–water (60:25:4). R_F refers to tlc on silica gel, unless otherwise stated.

Gas–liquid partition chromatography (glc) was performed on a Perkin-Elmer Model 900, equipped with a flame-ionization detector, on a column of stainless steel (150 × 0.3 cm) packed with 0.1% OV-17 on No. GLC 110, lot 4463 (120–140 mesh) (Supelco, Inc., Bellefonte, Pa.) with the per(trimethylsilyl) ether of *myo*-inositol as reference compound.

2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl Phosphate (**1**). (a) 1,2,3,4,6-Penta-*O*-acetyl-β-D-galactopyranose (1.0 g) was placed in the upper part of a Thunberg oxidation tube, with

TABLE I: Nuclear Magnetic Resonance Spectra of Farnesol Phosphate (**4**), P¹-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl P²-Farnesyl Pyrophosphate (**6**), and 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl Phosphate (**1**).[a]

Compound **4** δ (ppm)	Compound **6** δ (ppm)	Compound **1** δ (ppm)
1.55 CH_3 trans	1.53 CH_3 trans	
1.63 CH_3 cis	1.60 CH_3 cis	
	1.95	
	1.99	
2.0 $(CH_2)_4$	2.04 ($(CH_3)_4$ acetoxy and $(CH_2)_4$)	2.02
	2.06	2.07
	2.09	2.10 ($(CH_3)_4$ acetoxy)
		2.20
	4.14 Pyranose ring H	4.14 Pyranose ring H
	4.25 CH_2O (C-6 of pyranose)	4.24 CH_2O (C-6 of pyranose)
	4.67 HDO	4.64 HDO
5.12 (=$CH)_2$		
	5.3 Anomeric H	5.3 Anomeric H, 2 doublets ($J_{H_1-H_2}$ = 3 cps, J_{H_1-P} = 7 cps)[b]
5.4 =CH		

[a] Only well-resolved peaks are shown. [b] The values for *J* are in agreement with the assignment of the α-D configuration (Perchemlides *et al.*, 1967).

crystalline phosphoric acid (1.2 g, Fluka, dried overnight at room temperature *in vacuo* over magnesium perchlorate before use) in the bottom part. The tube was evacuated (oil pump) and the phosphoric acid fused at 60°. The β-D-galactose pentaacetate was mixed into the melt, and a vigorous evolution of acetic acid vapor took place. After 2 hr at 60°, most of the gas evolution had ceased, and the brown, fused residue was dissolved in anhydrous tetrahydrofuran. The solution was cooled to −10°, and concentrated (58%) ammonium hydroxide (1.5–2 ml) was added rapidly with vigorous stirring until the pH of the solution reached 6–7. The precipitate of ammonium phosphate was filtered off and washed with tetrahydrofuran at room temperature. The combined filtrate and washings were evaporated to give a syrupy residue. Examination by tlc in chloroform–methanol (10:1) showed a main compound having R_F 0.15 and other compounds having R_F 0.7 and 0.9. Alternatively, in solvent A, the relative mobilities corresponded to R_F 0.25, 0.85, and 0.95, respectively. Since a byproduct of this reaction may be 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose resulting from the presence of a small quantity of water in the crystalline phosphoric acid, a chromatographic standard was synthesized from benzyl β-D-galactopyranoside. Acetylation with acetic anhydride and pyridine gave syrupy benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (R_F 0.2 in 1:1 ether–hexane, and 0.9 in ether), which was hydrogenated at 1.4 atm in glacial acetic acid over a 10% palladium-on-charcoal catalyst. The resulting 2,3,4,6-tetra-*O*-acetyl-D-galactose showed R_F 0.7 (chloroform–methanol, 10:1) and 0.85 (solvent A). The other material, having the highest mobility in tlc, corresponded to the starting material.

The crude product was dissolved in water, and the solution was extracted with chloroform (six times) to remove the nonphosphorylated contaminants. After addition of a small amount of pyridine, the solution was evaporated and toluene was added to the residue and evaporated twice to give crude 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl ammonium hydrogen phosphate (**1**, 0.88 g). Trituration with acetone at room temperature gave the crystalline salt, the yield varying from 0.47 g (42%) to 0.27 g (24%) (presumably according to the quality of the phosphoric acid, which had a strong tendency to deteriorate during use). The compound was recrystallized from ether–methanol as small plates: mp 164–165°; $[\alpha]_D^{20}$ +105° (*c* 1, water); ir spectrum ν_{max}^{KBr} 1750, 1730 (C=O), and 1250 cm^{-1} (broad, P=O). The nmr spectrum of the derived disodium salt (after passage of an aqueous solution through a cation-exchange resin in the sodium form) is shown in Table I. The $J_{H_1-H_2}$ and J_{H_1-P} values are consistent with an α-D-configuration assignment for the phosphate group (see Perchemlides *et al.*, 1967). *Anal.* Calcd for $C_{14}H_{21}NO_{13}P$: C, 37.77; H, 5.43; N, 3.15; P, 6.96. Found: C, 37.78; H, 5.80; N, 3.14; P, 7.25.

(b) The chloroform extract of the crude phosphorylation product (0.9 g) described in the preceding paragraph was evaporated, and the residue was dissolved in dry methanol and treated with 0.1% sodium methoxide in methanol until there was no further precipitation. The very hygroscopic precipitate (0.16 g), presumably the disodium salt of α-D-galactopyranosyl phosphate, was filtered off. A solution of this product in water was passed through a column of cation-exchange resin (pyridinium form), and the resin was washed with water. The combined solutions were concentrated in the presence of pyridine to a small volume. After addition of an excess of cyclohexylamine (1 g) in pyridine, the solution was evaporated, and toluene was added and evaporated three times to give a syrup which crystallized on addition of ethanol. The resulting α-D-galactopyranosyl dicyclohexylammonium phosphate (0.47 g, 40%) was recrystallized from ethanol, hygroscopic needles: mp 141–143°, $[\alpha]_D^{20}$ +71° (*c* 1.47, water); the compound was dried over phosphorus pentoxide *in vacuo* for 24 hr at 75° before determination of the optical rotation. *Anal.* Calcd for $C_{18}H_{39}N_2O_9P$: C, 47.20; H, 8.58; N, 6.12. Found: C, 46.92; H, 8.81; N, 5.82. Putman and Hassid (1957) reported for a compound which was not a hydrate and was dried in air at room temperature: mp 147–153°, $[\alpha]_D^{26}$ +78.5° (*c* 2.5, water). MacDonald (1962) obtained a hygroscopic product, $[\alpha]_D^{20}$ +78.5° (*c* 2, water) which was a hemihydrate. Deacetylation of the crystalline ammonium salt of **1** (0.1 g), under the same conditions as just described, gave α-D-galactopyranosyl di(cyclohexylammonium) phosphate (56 mg, 53%), having identical properties as the compound just described.

A solution of the di(cyclohexylammonium) salt (0.1 g) in water was passed through a column of cation-exchange resin (pyridinium form). The eluate and washings were evaporated, and the residual syrup was treated with tetraethylammonium acetate tetrahydrate (1.0 g). After repeated addition and evaporation of pyridine, and then toluene, the last traces of water were removed by drying *in vacuo* over phosphorus pentoxide at room temperature. The resulting gum was dissolved in acetic anhydride (2 ml), and the mixture kept overnight at room temperature. Unless the glycosyl phosphate is converted into the noncrystalline pyridinium form, a clear solution cannot be achieved at this stage. The reaction mixture was treated with pyridine (5 ml) and water (5 ml), and the mixture was kept for another 2 hr at room temperature before evaporation. The residual pyridine was removed by

repeated addition and evaporation of toluene. After a final addition of water, the solution was cooled to 0°, filtered, and passed through a column of cation-exchange resin (hydrogen form). The eluate and washings were evaporated in the presence of pyridine, and after two additions and evaporations of toluene, an aqueous solution of the product was stirred overnight with cation-exchange resin (ammonium form). Evaporation of the solution and washings gave a syrup which crystallized on treatment with acetone to yield 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl phosphate (**1**, ammonium salt, 55 mg, 59%). Examination of this material by tlc showed that it was very pure (R_F 0.25, solvent A) and, after one crystallization from ether–methanol, plates were obtained, mp 165–166°. There was no depression of melting point with the material obtained by method a.

For comparison purposes, α-D-galactopyranosyl di(cyclohexylammonium) phosphate was converted into the pyridinium form, as just described, and after evaporation of the solution, the syrup was directly acetylated with a mixture of pyridine (2 ml) and acetic anhydride (1 ml) for 15 hr at room temperature. After treatment with water (2 ml) for 2 hr at room temperature, the reaction mixture was evaporated. Toluene was added and evaporated three times. The residue was dissolved in water and the solution stirred with cation-exchange resin (ammonium form) for 15 hr. Examination of the product by tlc (solvent A) showed the presence of a major by product (R_F 0.3), as well as the expected material (R_F 0.25), and smaller amounts of other contaminants. No crystalline material could be obtained.

Farnesyl Phosphate (**4**). Attempts to prepare this compound as described by Popjak *et al.* (1962) gave very variable and generally poor yields of crystalline cyclohexylammonium monophosphate. Therefore, farnesol (Aldrich Chemical Co., 0.66 g) was dissolved in acetonitrile (50 ml) and trichloroacetonitrile (1.8 ml) and bis(triethylammonium) phosphate (2.1 g) were added. The mixture was stirred until all solid had dissolved, to give a pale yellow solution which was kept for 1.5 hr at room temperature. Tlc showed that the reaction mixture contained mainly unchanged farnesol (R_F 1.0, solvent A), farnesyl monophosphate (R_F 0.3), and a trace of pyrophosphate (R_F 0.1). A longer reaction time increased the proportion of pyrophosphate. The reaction mixture was poured into ether (300 ml) and extracted twice with 10% aqueous pyridine (100 ml). The ether layer containing a large amount of unreacted farnesol was washed with 0.1 M hydrochloric acid and saturated aqueous potassium chloride, and dried with magnesium sulfate. After evaporation, the residue was rephosphorylated by the method just described, and the whole process repeated, so that altogether the farnesol was subjected to three phosphorylations. The combined aqueous extracts were evaporated to dryness, the residue was dissolved in methanol (60 ml), and the solution was treated with ether (750 ml). After several hours, the clear supernatant was decanted from a thick, syrupy precipitate and evaporated to give the syrupy pyridinium salt of monophosphate (**4**, 1.23 g). It contained a small amount of pyrophosphate and inorganic material, both of which being readily eliminated at the next stage.

For characterization purposes, a crystalline product was obtained as follows. After the reaction mixture had been poured into ether, it was extracted twice with aqueous 0.1 M ammonium hydroxide (100 ml). The combined extracts were evaporated to about 10 ml and treated with cyclohexylamine (2 ml). After evaporation, and three additions and evaporations of toluene, the residue was dissolved in methanol (20 ml) and treated with ether (250 ml). The solution was filtered and evaporated, and the residue was dissolved in a minimum of water and kept at 0° to give the crystalline monocyclohexylammonium salt of farnesyl phosphate (0.18 g). Recrystallization from ether–methanol gave small needles: mp 146–149°; ir spectrum: ν_{max}^{KBr} 1175 (P–O–C), 1250 (P=O), and 1675 cm^{-1} (C=C). *Anal.* Calcd for $C_{21}H_{40}NO_4P$: C, 62.81; H, 10.64; N, 3.50; P, 7.71. Found: C, 62.83; H, 9.98; N, 3.82; P, 6.13. For the determination of the nmr spectrum (Table I), the compound was converted into the sodium salt, by passage through a cation-exchange resin in the sodium form.

P^1-Diphenyl P^2-Farnesyl Pyrophosphate (**2**). To a solution of farnesyl monophosphate (1.23 g, crude pyridinium salt), in pyridine (25 ml) was added tributylamine (0.7 g). The solution was evaporated. After three additions and evaporations of toluene, the residue was dissolved in a small volume (*ca.* 10 ml) of dichloroethane, and tributylamine (0.6 g) and diphenyl phosphochloridate (0.6 g) were added. After 2 hr at room temperature, tlc showed that the reaction mixture contained a single, main product having R_F 0.8 (chloroform–methanol, 5:1), and some slow-moving contaminants. After most of the chloroform had been evaporated, the mixture was applied directly to a column of silica gel (Davison, 400 g) and eluted first with chloroform (500 ml), and then with chloroform–methanol (40:1). The fractions (200 ml) were analyzed by tlc, and those containing the pure product of R_F 0.8 were combined and evaporated. After three additions and evaporations of toluene, the diphenyl pyrophosphate (**2**) was obtained as a syrup, together with a small proportion of tributylamine.

P^1-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl) P^2-Farnesyl Pyrophosphate (**6**). P^1-Diphenyl P^2-farnesyl pyrophosphate (**2**), obtained from 0.66 g of farnesol, was dissolved in 1,2-dichloroethane (30 ml). 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl phosphate (**1**, ammonium salt, 0.25 g) was dissolved in water (5 ml) and passed through a column of cation-exchange resin (pyridinium form). The eluate and washings from the resin were collected and evaporated in the presence of pyridine. After three additions and evaporations of toluene, the residue was dissolved in 1,2-dichloroethane (30 ml) and treated with tributylamine (0.1 g).

A part of the solution of **1** (10 ml) was mixed with a part of the solution of **2** (5 ml) and, after evaporation, the syrupy mixture was dissolved in 1,2-dichloroethane (1 ml) containing pyridine (32 mg) (it is important to avoid an excess of pyridine). The reaction was followed by tlc in 2,6-dimethyl-4-heptanone–acetic acid–water (20:15:2) which showed that **2** (R_F 0.75) and **1** (R_F 0.25) were gradually converted into a single product (R_F 0.5), the process being complete in 15–20 hr. After this time the reaction mixture was diluted with a large volume of chloroform and extracted three times with water (10 ml) to remove the excess of **1**. The chloroform solution was dried with magnesium sulfate and evaporated. The syrupy residue was dissolved in methanol (50 ml) and water (10 ml) and stirred with a cation-exchange resin (sodium form) overnight. The solution and washings from the resin were evaporated, and the residue was triturated with hexane to give a solid (75 mg), which was filtered off and washed with hexane. The presence of diphenyl phosphate, an expected byproduct in the pyrophosphate-forming reaction, was indicated by tlc in 2,6-dimethyl-4-heptanone–acetic acid–water (20:15:2) as a pink spot (R_F 0.6). Most of the diphenyl phosphate was removed by crystallization from an ether solution containing a trace of methanol, kept overnight at 0°. The diphenyl phosphate was filtered off as needles, mp 187°.

Evaporation of the filtrate gave a crystalline product still contaminated by traces of diphenyl phosphate, indicated by the presence of a uv-fluorescent spot on silica gel GF-254 (Merck) plates (R_F 0.6 in the solvent just described). In solvent A, the diphenyl phosphate cochromatographed with product **6** (R_F 0.45). Investigation of the solid product under the microscope showed **6** as microcrystalline masses with isolated needles of diphenyl phosphate. The ir spectrum (KBr disk) showed a weak absorption at 1495 cm^{-1} (aromatic) and the nmr spectrum a signal at δ 7.26 and 7.3 ppm (aromatic protons). The contaminant was finally removed by dissolution in anhydrous ether and filtration, and repeating this procedure. The filtrate was evaporated, the residue dissolved in a small volume of water, and the solution lyophilized to give a solid (**6**, 42 mg): mp 173–174°; $[\alpha]_D^{20}$ +44° (*c* 1.05, in methanol); ir spectrum ν_{max}^{KBr} 925 (P–O–P), 1250 (P=O, broad), 1750 (C=O, acetyl), and 3450 cm^{-1} (OH, broad); nmr spectrum signals due to the tetra-*O*-acetyl-α-D-galactopyranosyl and farnesyl residues (Table I). *Anal.* Calcd for $C_{29}H_{44}Na_2O_{16}P_2 \cdot H_2O$: C, 44.96; H, 5.99. Found: C, 44.81; H, 5.49. After drying over phosphorous pentoxide *in vacuo* for 48 hr at room temperature, the ir spectrum of **6** showed no more an absorption at ν_{max}^{KBr} 3450 cm^{-1} (OH), but it was amorphous and without a definite melting point (150–170°). *Anal.* Calcd for $C_{29}H_{44}Na_2O_{16}P_2$: C, 46.03; H, 5.87. Found: C, 46.03; H, 5.92.

Estimation of Galactose Content of **6**. Compound **6** (200 μg) was methanolyzed with 0.5 M hydrogen chloride in methanol overnight, at reflux temperature. The resulting methyl D-galactoside (mixture of α and β anomers) was determined by glc, after per(trimethylsilyl)ation. Calcd: 23.8%. Found: 19.4%.

Estimation of Acid-Labile Phosphate Groups of **6**. Reagent C (Chen *et al.*, 1956) consisting of ascorbic acid (500 mg) in water (10 ml) was mixed, immediately before use, with ammonium molybdate (125 mg) in water (10 ml). Standard solutions of α-D-galactopyranosyl phosphate, di(cyclohexylammonium) salt, containing (a) 3 mg (0.8 mg of PO_4), (b) 6 mg (1.61 mg of PO_4), (c) 8 mg (2.15 mg of PO_4), and (d) 12 mg (3.22 mg of PO_4), in 100 ml of water were prepared. The sample consisted of 0.2 ml of a solution of **6** (6 mg) in water (50 ml). Aliquots of this sample and of the standard solution were treated with 2 M sulfuric acid (0.17 ml) for 2 hr at 100°. Water was added to each sample to bring the volume to exactly 1 ml, and then reagent C (0.47 ml) was added. The mixtures were kept for 2.5 hr at 37°, and the absorption at 820 nm was measured in a quartz cell. *Anal.* Calcd: 2.89 mg/100 ml, for 2 moles of acid-labile phosphate. Found: 2.91 mg/100 ml.

Acid Hydrolysis of **6**. A solution of compound **6** in methanol (9 mg) and 1 M hydrochloric acid (1 ml) was heated for 5 min at reflux. Tlc, in solvent A, indicated complete hydrolysis of the compound, giving products having the mobility of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl phosphate (R_F 0.25), and of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose (R_F 0.85), as well as various rearrangement and decomposition products of farnesol (R_F 1.0); tlc in ether–hexane (3:1) showed that these latter products were mainly fast-moving hydrocarbon substances, with a trace of farnesol (R_F 0.2) or a substance isomeric with farnesol.

Alkaline Hydrolysis of **6**. A solution of compound **6** in 0.1 M sodium hydroxide in methanol was kept at room temperature for 15 min. Examination by tlc, in 2,6-dimethyl-4-heptanone–acetic acid–water (20:15:2) showed that complete hydrolysis had occurred, giving products having the mobility of farnesyl monophosphate (R_F 0.7), and of galactose or galactosyl phosphate (R_F 0.0, for identification of this product as α-D-galactopyranosyl 1,2-monophosphate, see characterization of compound **7**).

P^1-α-D-Galactopyranosyl P^2-Farnesyl Pyrophosphate (**8**). (a) A solution of the acetylated derivative **6** (25 mg) in 0.4 M aqueous ammonium hydroxide (5 ml) was kept for 24 hr at room temperature. Examination by tlc (solvent A) revealed that all the starting material (R_F 0.45) had been converted into a product having R_F 0.1, a trace of farnesyl phosphate (R_F 0.3) and a small proportion of carbohydrate material (R_F 0.0), both produced by hydrolysis of the pyrophosphate linkage. The reaction mixture was treated with a small excess of acetic acid, and then evaporated in the presence of pyridine. The residue was dissolved in dry pyridine (1 ml), and the solution was cooled to −15° and kept overnight at this temperature. The precipitated ammonium acetate together with carbohydrate material (R_F 0.0) was filtered off, and evaporation of the filtrate gave **8** as a syrup (10 mg, sodium salt).

(b) The acetylated derivative **6** (25 mg) was treated with 0.13% sodium methoxide in methanol (2 ml). After 15 min, tlc (solvent A) showed that deacetylation was complete, and cation-exchange resin (pyridinium form) was added. After 1 hr, the resin was filtered off and washed with methanol. Evaporation of the filtrate gave **8** as a syrup (15 mg, pyridinium salt). Apart from a trace of material having the mobility of farnesyl phosphate, **8** was obtained in homogeneous state, as shown by tlc in solvent A (R_F 0.1), and after three developments in solvent A, R_F 0.25. A similar spot was observed with sprays specific for unsaturated compounds [1% (v/v) potassium permanganate in 2% (w/v) sodium carbonate (Gigg and Gigg, 1966)] and for phosphate ester content (Dittmer and Lester, 1964).

Ficaprenyl Phosphate (**5**). The initial extraction and purification of ficaprenols from *Ficus elastica* was carried out according to Stone *et al.* (1967), with the following modification. The chromatography of the lipid extract on alumina (Brockman activity 3) and the separation of ficaprenols from other lipids, also on a column of alumina, were monitored by tlc (49:1 benzene–methanol) and only the fractions containing the required material (R_F 0.3) were kept. Thus, 400 g of leaf material gave a crude lipid (6.1 g) which, after alkaline saponification and rechromatography, gave a crude mixture of ficaprenols (0.77 g). The purification of the material by preparative thin-layer chromatography was replaced by a chromatography on a column of silica gel (Merck, 50 g) in hexane–ether (4:1) as solvent. The main fractions were combined (0.73 g) and showed, on tlc, the presence of only a minor, nonprimary alcoholic contaminant (R_F 0.2) which was readily separated at the phosphorylation stage. Comparison by tlc with pure ficaprenol-eleven showed the same mobility (R_F 0.3,49:1, benzene–methanol), whereas solanesol (C_{45}, R_F 0.25) and farnesol (C_{15}, R_F 0.2) were less mobile. The mixture of ficaprenols was also investigated by reversed-phase partition tlc according to Stone *et al.* (1967). In order to achieve a good separation, it was necessary to impregnate the kieselguhr plates with liquid paraffin in hexane for several hours, and preferably overnight. By comparison to standards of ficaprenol-eleven (C_{55}) and solanesol (C_{45}), it was apparent that the main isomer contained in the mixture was C_{55}, accompanied by a lesser amount of C_{60}, and a small proportion of C_{45} and C_{50}. The mobilities observed were lower than those reported, since the kieselguhr plates had a thickness of 0.25 mm instead of 0.20 mm: C_{45}, R_F 0.48; C_{50}, R_F 0.36; C_{55}, R_F 0.25; and C_{60}, R_F 0.18.

Ficaprenol (77 mg, *ca.* 0.1 mmole) in *p*-dioxane (0.6 ml) containing 2,6-dimethylpyridine (11 mg) was stirred at 0° and treated with *o*-phenylene phosphochloridate (20 mg), prepared by the method of Khwaja *et al.* (1970), in *p*-dioxane (0.2 ml). The temperature was allowed to rise to 20° and, after 10 min, the 2,6-dimethylpyridinium hydrochloride was filtered off and washed with *p*-dioxane (1 ml). The filtrate was treated with 2,6-dimethylpyridine (11 mg) and water (20 μl) and, after 5 min, the *p*-dioxane was evaporated. Small amounts of 2,6-dimethylpyridinium hydrochloride were removed by dissolution of the residue in chloroform and filtration. Evaporation gave the *o*-hydroxyphenyl phosphate as a syrup (R_F 0.7, solvent A). The product was dissolved in *p*-dioxane (5 ml) and treated with lead tetraacetate (Alfa Inorganics, 0.1 g). The dark brown mixture was stirred for 30 min, and then treated with 0.1 M potassium hydroxide in methanol until the pH reached 10 and a brown precipitate had formed. After 30 min, a slight excess of glacial acetic acid was added. Evaporation of the solvents, followed by three additions and evaporations of toluene, gave a solid brown residue, which was extracted three times with methanol to remove the inorganic material and a red-colored organic by-product and leave the crude potassium salt of ficaprenyl phosphate (**5**) as a brown syrup (90 mg). Examination of the product by tlc showed a main spot having R_F 0.5 (solvent A) and by-products having R_F 0.65, 0.7, and *ca.* 1.0. A sample of this product (50 mg) was purified by preparative tlc in solvent A. The band containing the required material was revealed by spraying the edges of the plate with 1% potassium permanganate in 2% sodium carbonate. After removal from the plate, the silica gel was extracted three times with methanol–chloroform (3:1). Evaporation gave **5** as a syrup (35 mg), R_F 0.5 (solvent A); ir spectrum: ν_{max}^{film} 1020 (allylic C–O), 1100 (trans C=C), 1380 (C–H), 1460 (C–H), and 1660 cm^{-1} (C=C stretching). This product was dissolved in acetone–1,2-dichloroethane and the solution was stirred overnight with a cation-exchange resin (pyridinium form). The resin was filtered off and washed with methanol, and the combined filtrate and washings were evaporated to give the pyridinium form of **5**. This was dissolved in methanol and treated with a cation-exchange resin (barium form) to give the barium salt of **5**, also a syrup. *Anal.* Calcd for $C_{55}H_{89}Ba_{0.5}O_4P$: C, 72.26; H, 9.83; for $C_{60}H_{97}Ba_{0.5}O_4P$: C, 73.40; H, 10.00. Found: C, 73.47; H, 10.77.

P^1-Diphenyl P^2-Ficaprenyl Pyrophosphate (**3**). Crude ficaprenyl phosphate (**5**, 90 mg, potassium salt) was converted into the pyridinium form, as just described, and treated with tributylamine (25 mg) in methanol (5 ml). The solution was evaporated and, after three additions and evaporations of toluene, the tributylammonium form of **3** was treated, in 1,2-dichloroethane (2 ml) with tributylamine (20 mg) and diphenyl phosphochloridate (20 mg). After 2 hr, tlc in chloroform–methanol (5:1) showed the formation of the diphenyl pyrophosphate **3** (R_F 0.8). The reaction mixture was treated with methanol (0.5 ml) and kept for 30 min at room temperature. Evaporation, followed by three additions and evaporations of toluene, gave **3** as a syrup.

P^1-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl) P^2-Ficaprenyl Pyrophosphate (**7**). 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl phosphate (**1**, 0.25 g, ammonium salt) was converted into the tributylammonium form and dissolved in 1,2-dichloroethane (30 ml) as described for the preparation of **6**. The solution (2 ml) was added to **3** (from 77 mg of ficaprenol) and, after the solvent had been evaporated, the residue was dissolved in 1,2-dichloroethane (0.2 ml) containing pyridine (0.6 mg). Formation of the pyrophosphate **7** was followed by removal of a small sample from the reaction mixture, dilution with chloroform, extraction three times with water to remove the excess of galactosyl phosphate (**1**), and tlc (solvent A) which showed a new major product having R_F 0.65. After 20 hr the reaction was completed, and the reaction mixture was diluted with chloroform (25 ml) and extracted three times with water. The chloroform solution was dried with magnesium sulfate and evaporated to yield a residue which was chromatographed on silica gel (Merck, 8.4 g). Elution with chloroform–methanol (40:1) gave material having R_F 1.0 (solvent A), and a prolonged elution with chloroform–methanol (20:1) removed a substance of R_F 0.8. When tlc showed that no further elution was taking place, the eluent was changed to chloroform–methanol (10:1) and 5-ml fractions were collected. Tlc showed that a small amount of material having R_F 0.7 was being slowly removed, while elution of the product having R_F 0.65 was beginning. Chloroform–methanol (3:1) eluted most of **7**, and pure fractions were combined and evaporated to give a syrup (30–34 mg).

As an alternative to column chromatography, preparative tlc in solvent A was used. The chloroform solution of the reaction products, after removal of the excess of **1** and drying with magnesium sulfate, was concentrated to a small volume and applied to a 25 × 25 cm plate. After development, the position of the required band was identified, and the product was extracted, as described in the preparation of **5**. The yield (24 mg) of **7** (tributylammonium form) was inferior to that of the column method, but the material was very pure and suitable for characterization: $[\alpha]_D^{20}$ +17° (*c* 0.6, chloroform); ir spectrum ν_{max}^{film} 925 cm (P–O–P), 1250 (P=O, broad), 1380 (C–H), 1460 (C–H), and 1750 cm^{-1} (C=O, acetyl). Estimation of the galactose content was made by glc after methanolysis of **7** (376 μg), as described for the characterization of **6**. *Anal.* Calcd for 1 mole of galactose: 11.0%. Found: 13.5%. Estimation of acid-labile phosphate was performed with a standard of α-D-galactopyranosyl phosphate, as described for the characterization of **6**. *Anal.* Calcd for 2 moles of labile phosphate: 2.17 mg/100 ml. Found: 2.20 mg/100 ml. *Anal.* Calcd for $C_{93}H_{168}N_2O_{16}P_2$: C, 68.37; H, 10.40; for $C_{98}H_{176}N_2O_{16}P_2$: C, 69.20; H, 10.46. Found: C, 69.39; H, 10.84.

Acid Hydrolysis of Compound **7**. A sample (0.5 mg) was treated with 10 mM hydrochloric acid (0.1 ml) for 10 min at 100°. Examination by tlc (solvent A) showed the disappearance of the starting material to give a substance having the mobility of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl phosphate. The liberated lipids and decomposition products were extracted into hexane, and tlc of the extract in benzene–methanol (49:1) showed that it contained only a trace of material (R_F 0.3) having the mobility of ficaprenol. The main products had R_F 0.4, 0.8, and 0.9.

Alkaline Hydrolysis of Compound **7**. A sample (1 mg) was treated with 2 M ammonium hydroxide (0.2 ml) for 1 hr at 37°, when tlc (solvent A) showed the disappearance of the starting material and the formation of a product having the mobility of ficaprenyl phosphate (R_F 0.5). After evaporation, the product was divided into two parts. The first part was treated with 10 mM hydrochloric acid (0.1 ml) for 10 min at 100°, and then evaporated. UDP-galactose (Sigma, 1 mg) was treated in exactly the same way, and the products of both reactions were compared by tlc (cellulose) in ethanol–ammonium acetate (7:3). The spots were detected with the silver nitrate reagent after periodic acid treatment (Troy *et al.*, 1971). This showed that both compounds initially gave a product having the mobility of α-D-galactopyranosyl 1,2-

monophosphate (R_F 0.42) which gave, after dilute acid hydrolysis, a product of R_F 0.14 (presumably D-galactose 2-phosphate); this mobility is slightly different from that of α-D-galactopyranosyl phosphate (R_F 0.07).

P^1-(α-D-Galactopyranosyl) P^2-Ficaprenyl Pyrophosphate (**9**). The acetylated **7** (24 mg, tributylammonium form) was stirred in methanol with a cation-exchange resin (pyridinium form) for 1 hr. Evaporation, followed by three additions and evaporations of toluene, gave the pyridinium salt, which was treated with 0.13% sodium methoxide in methanol (3 ml). The deacetylation was followed by tlc (solvent A) and was complete in 20–25 min to give a product having R_F 0.2 (*cf.* mobility of starting material R_F 0.65). The reaction mixture was treated with a cation-exchange resin (pyridinium form) for 1 hr, and the resin was filtered off and washed with methanol–chloroform (3:1). The combined filtrate and washings were evaporated to give the deacetylated **9** (pyridinium form), as a syrup (17 mg) which contained traces of hydrolysis products. The yield of synthetic lipid intermediate was 14–19.5% based on ficaprenol (depending on the method of chromatography of **7**). Final purification was achieved by preparative tlc (solvent A). The product (10 mg) was applied to a 10 × 25 cm plate in concentrated methanol solution. After development, the required band was located and the product extracted,[1] as for the preparation of **5**, to give pure **9** (2.5 mg, pyridinium form) as a syrup. This was shown to be homogeneous by tlc (solvent A) with spray reagents specific for unsaturated compounds and phosphate ester content (see **8**). The ir spectrum showed: ν_{max}^{film} 925 (P–O–P), 1020 (C–O allylic), 1100 (C=C, trans), 1250 (P=O), 1380 (C–H), 1460 (C–H), and 1495 cm^{-1} (C–C, aromatic).

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References

Alam, S. S., Barr, R. M., Richards, J. B., and Hemming, F. W. (1971), *Biochem. J. 121*, 19P.

Anderson, J. S., Matsuhashi, M., Haskin, M. A., and Strominger, J. L. (1967), *J. Biol. Chem. 242*, 3180.

Behrens, N. H., and Leloir, L. F. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 153.

Behrens, N. H., Parodi, A. J., and Leloir, L. F. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 2857.

Behrens, N. H., Parodi, A. J., Leloir, L. F., and Krisman, C. R. (1971), *Arch. Biochem. Biophys. 143*, 375.

Brooks, D., and Baddiley, J. (1969a), *Biochem. J. 113*, 635.

Brooks, D., and Baddiley, J. (1969b), *Biochem. J. 115*, 307.

Chen, P. S., Toribara, T. Y., and Warner, H. (1956), *Anal. Chem. 28*, 1756.

Christenson, J. G., Gross, S. K., and Robbins, P. W. (1969), *J. Biol. Chem. 244*, 5436.

DeLuca, L., Rosso, G., and Wolf, G. (1970), *Biochem. Biophys. Res. Commun. 41*, 615.

Dittmer, J. C., and Lester, R. L. (1964), *J. Lipid Res. 5*, 126.

Douglas, L. J., and Baddiley, J. (1968), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 1*, 114.

Gigg, J., and Gigg, R. H. (1966), *J. Chem. Soc. C*, 82.

Higashi, Y., Strominger, J. L., and Sweeley, C. C. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1878.

Higashi, Y., Strominger, J. L., and Sweeley, C. C. (1970), *J. Biol. Chem. 245*, 3697.

Khwaja, T. A., Reese, C. B., and Stewart, J. C. M. (1970), *J. Chem. Soc. C*, 2092.

Kochetkov, N. K., Budovsky, E. I., Shibaev, V. M., and Lebedeva, K. S. (1969), *Izv. Akad. Nauk SSSR, Ser. Khim.*, 897.

Lahav, M., Chiu, T. H., and Lennarz, W. J. (1969), *J. Biol. Chem. 244*, 5890.

MacDonald, D. L. (1962), *J. Org. Chem. 27*, 1107.

MacDonald, D. L. (1966) *Carbohyd. Res. 3*, 117.

MacDonald, D. L. (1968), *Carbohyd. Res. 6*, 376.

Michelson, A. M. (1964), *Biochim. Biophys. Acta 91*, 1.

Moffatt, J. G., and Khorana, H. G. (1961), *J. Amer. Chem. Soc. 83*, 649.

Molnar, J., Chao, H., and Ikehara, Y. (1971), *Biochim. Biophys. Acta 239*, 401.

Nikaido, K., and Nikaido, H. (1971), *J. Biol. Chem. 246*, 3912.

Osborn, M. J., and Yuan Tze-Yuen, R. (1968), *J. Biol. Chem. 243*, 5145.

Paladini, A. C., and Leloir, L. F. (1952), *Biochem. J. 51*, 426.

Perchemlides, P., Osawa, T., Davidson, E. A., and Jeanloz, R. W. (1967), *Carbohyd. Res. 3*, 463.

Popjak, G., Cornforth, J. W., Cornforth, R. H., Ryhage, R., and Goodman, D. S. (1962), *J. Biol. Chem. 237*, 56.

Putman, E. W., and Hassid, W. Z. (1957), *J. Amer. Chem. Soc. 79*, 5057.

Rammler, D. H., Lapidot, Y., and Khorana, H. G. (1963), *J. Amer. Chem. Soc. 85*, 1989.

Richards, J. B., Evans, P. J., and Hemming, F. W. (1971), *Biochem. J. 124*, 957.

Roseman, S., Distler, J. J., Moffatt, J. G., and Khorana, H. G. (1961), *J. Amer. Chem. Soc. 83*, 659.

Scher, M., and Lennarz, W. J. (1969), *J. Biol. Chem. 244*, 2777.

Scher, M., Lennarz, W. J., and Sweeley, C. C. (1968), *Proc. Nat. Acad. Sci. U. S. 59*, 1313.

Sentandreu, R., and Lampen, J. O. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 14*, 109.

Stone, K. J., Wellburn, A. R., Hemming, F. W., and Pennock, J. F. (1967), *Biochem. J. 102*, 325.

Stow, M., Starkey, B. J., Hancock, I. C., and Baddiley, J. (1971), *Nature (London) 229*, 56.

Takayama, K., and Goldman, D. S. (1970), *J. Biol. Chem. 245*, 6251.

Tanner, W. (1969), *Biochem. Biophys. Res. Commun. 35*, 144.

Tanner, W., Jung, P., and Behrens, N. H. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 16*, 245.

Tetas, M., Chao, H., and Molnar, J. (1970), *Arch. Biochem. Biophys. 138*, 135.

Troy, F. A., Frerman, F. E., and Heath, E. C. (1971), *J. Biol. Chem. 246*, 118.

Warren, C. D., and Jeanloz, R. W. (1971), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 30*, 1174 Abs.

Watkinson, R. J., Hussey, H., and Baddiley, J. (1971), *Nature (London) 229*, 57.

Wright, A. (1971), *J. Bacteriol. 105*, 927.

Wright, A., Dankert, M., Fennessey, P., and Robbins, P. W. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1798.

[1] The recovery of material from preparative tlc plates would be improved by the inclusion of water in the extraction medium (F. W. Hemming, private communication).