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Chemical Synthesis of Ficaprenyl α-D-Mannopyranosyl Phosphate†

Christopher D. Warren and Roger W. Jeanloz*

ABSTRACT: 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl phosphate (monoammonium salt, **2**), prepared by treatment of 1,2,3,4,6-penta-*O*-acetyl-α,β-D-mannopyranose with crystalline phosphoric acid, was deacetylated to give α-D-mannopyranosyl disodium phosphate which was characterized by its conversion into the dicyclohexylammonium and barium salts, and acetylated to give crystalline 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl dipotassium phosphate (**4**). This compound could also be obtained directly from **2** by cation exchange. After conversion of **4** into the pyridinium salt **5**, this substance was coupled with farnesol by the triisopropylbenzenesulfonyl chloride method. Deacetylation of the resulting product **8** gave the short-chain analog **9** of an isoprenoid "lipid intermediate." Ficaprenyl α-D-mannopyranosyl phosphate (**11**) was prepared by a similar procedure and found to be chromatographically indistinguishable from the mannolipid that is an active intermediate of mannan biosynthesis in *Micrococcus lysodeikticus*.

Lipid intermediates that contain a monophosphate or pyrophosphate bridge between a carbohydrate moiety and a polyisoprenoid alcohol residue are an important step in the biosynthesis of complex polysaccharides of the bacterial cell wall (Lennarz and Scher, 1972; Rothfield and Romeo, 1971). It is probable that monophosphate compounds are involved in the addition of single carbohydrate residues to existing polysaccharide chains, whereas pyrophosphate compounds are usually intermediates in the biosynthesis of the main chain (Scher and Lennarz, 1969). In the latter process, several monosaccharide residues are apparently linked in a specific sequence which is subsequently built into the growing polymer while it is still attached to the lipid (Robbins *et al.*, 1967; Kanegasaki and Wright, 1970; Wright, 1971). The active polyisoprenoid alcohol contains a terminal, unsaturated isoprene residue adjacent to the phosphate group. Therefore, the "lipid intermediates" contain the highly reactive allylic phosphate group, which differentiates them from the dolichol-containing monophosphate derivatives which have recently been isolated from some mammalian systems (Behrens and Leloir, 1970; Richards and Hemming, 1972). In the biosynthesis of the cell wall mannan of *Micrococcus lysodeikticus*, the participation of D-mannosyl undecaprenyl phosphate as a lipid intermediate is well established (Scher *et al.*, 1968; Scher and Lennarz, 1969; Lahav *et al.*, 1969) and a very similar compound, decaprenyl D-mannosyl phosphate, was formed by a cell-free, particulate enzyme system, isolated from *Mycobacterium tuberculosis* (Takayama and Goldman, 1970). It has not as yet been clearly demonstrated whether or not "lipid intermediates" containing the allylic phosphate group have any activity in biosynthetic systems obtained from higher organisms, although Jankowski and Chojnacki (1972) have shown that ficaprenyl phosphate can act as an acceptor for D-glucose from UDP-D-glucose in a microsomal system of rat liver.

In order to obtain unambiguous conformation of the structure established for a monophosphate diester active in bacterial polysaccharide biosynthesis, and to test such a compound for possible activity in mammalian systems, we have undertaken the synthesis of monophosphate diesters of D-mannose with farnesol, as a model compound, and with ficaprenol (which is closely similar to bacterial undecaprenol, having one extra double bond in the trans configuration), as a long-chain polyisoprenoid lipid.

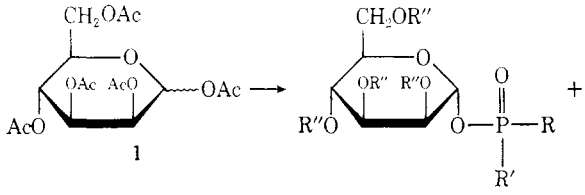
Results and Discussion

In earlier work, the preparation of crystalline 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl monoammonium phosphate and amorphous 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-

† From the Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114. *Received July 16, 1973.* This is publication No. 614 of the Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School at the Massachusetts General Hospital. This investigation was supported by Grants AM-03564 from the National Institute of Arthritis, Metabolism, and Digestive Diseases and AI-06692 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service. This is part III of the series, Lipid Intermediates of Complex Polysaccharide Biosynthesis; for parts I and II, see Warren and Jeanloz (1972) and Warren *et al.* (1973).

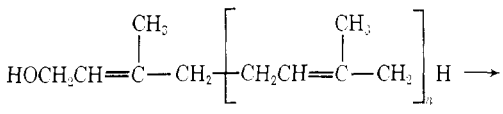
glucopyranosyl monoammonium phosphate has been described (Warren and Jeanloz, 1972; Warren *et al.*, 1973). Both compounds were successfully used in the synthesis of pyrophosphate "lipid intermediates." The advantages of a protected sugar phdsphate for phosphate diester synthesis are the solubilito of the reaction products in organic solvents and their mobility in silica gel thin-layer and column chromatography. In addition, the formation of carbohydrate phosphate diesters as by-products is avoided.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranysyl phosphate (**2**) was prepared by a modification of the MacDonald procedure (MacDonald, 1962, 1966), similar to the one previously described for D-galactose (Warren and Jeanloz, 1972). Since Hill and Ballou (1966) have shown that the yield of α-D-mannopyranosyl phosphate in the MacDonald procedure was the same whether or not a crystalline α-D anomer was used as starting material, syrupy penta-*O*-acetyl-α,β-D-mannopyranose (**1**) was prepared by the acetic anhydride–pyridine method

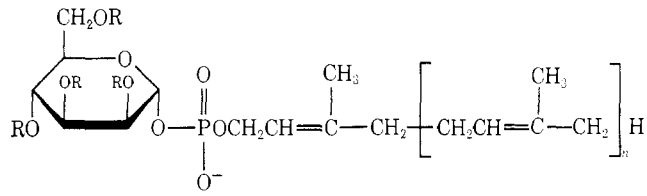


1

2, R = OH; R′ = ONH_4; R″ = Ac
3, R = R′ = O^-; R″ = H
4, R = R′ = OK; R″ = Ac
5, R = R′ = $ONHC_5H_5$; R″ = Ac



6, $n = 2$
7, $n = 10$



8, R = Ac; $n = 2$
9, R = H; $n = 2$
10, R = Ac; $n = 10$
11, R = H; $n = 10$

(Conchie and Levvy, 1963). The ammonium salt (**2**) was isolated as described for the D-galactose derivative (Warren and Jeanloz, 1972), except that the product could not be crystallized. After treatment with sodium methoxide, the precipitated α-D-mannopyranosyl disodium phosphate (**3**, sodium salt), too hygroscopic to be characterized, was readily converted into the dicyclohexylammonium salt (Hill and Ballou, 1966). For further characterization of the product as a pure α-D anomer, a portion of **3** was converted into the barium salt, which was compared with the compound prepared by Perchemlides *et al.* (1967). This method for preparing α-D-mannopyranosyl phosphate is very simple, since it involves no ion-exchange resin separations, and both unchanged starting material and inorganic phosphate are very easily separated from the final product. The yield based on compound **1** is 53% and this method can, therefore, be considered a useful variation of the usual procedure.

Crystalline **3** (disodium salt) was acetylated by the acetic anhydride–pyridine method, as there was no risk of cyclic phosphate formation. The resulting compound was converted in a 28% yield, based on **1**, into the crystalline dipotassium salt (**4**). Compound **4** was also obtained directly from the crude product arising from the phosphoric acid treatment of **1**, without intermediate deacetylation. The product was stirred for 2 days with a cation-exchange resin (potassium form) and then crystallized from methanol in a yield (49%) higher than that obtained by the first method. This is in contrast to the difficulties reported by previous workers in the purification of an acetylated mannopyranosyl phosphate (Cawley and Letters, 1971). The nmr spectrum of **4** in deuterium oxide showed only clear signals for the acetoxy protons, while the spectrum of the pyridinium salt **5** in [^{2}H]chloroform showed the signal from the anomeric proton as a poorly resolved doublet with a coupling constant having the expected value for a glycosyl phosphate; further resolution of this peak into a twin doublet was not observed but, from the results of other workers (Onodera and Hirano, 1966), the two pairs of peaks arising from an α-D-mannopyranosyl phosphate residue would be expected to be very close.

The most successful method previously used for the preparation of carbohydrate-containing monophosphate diesters is the condensation of a sugar phosphate with an alcohol (which can be another carbohydrate residue) in the presence of either dicyclohexylcarbodiimide[1] (DCC) or triisopropylbenzenesulfonyl chloride[1] (iPr_3PhsCl) (Lohrmann and Khorana, 1966). As described in the previous synthesis of P^1-ficaprenyl P^2-α-D-galactopyranosyl pyrophosphate (Warren and Jeanloz, 1972), farnesol was chosen as a short-chain model polyisoprenoid compound that contains a terminal allylic isoprene unit, and it was condensed with **5** in the presence of either DCC or iPr_3PhsCl in anhydrous pyridine. Thin-layer chromatography showed that DCC yields a complex mixture of products, whereas a single main product having the mobility expected for the acetylated phosphate diester **8** was obtained with 1 equiv of iPr_3PhsCl in a concentrated pyridine solution for 3 days. After the removal of derivatives of mannosyl phosphate, the crude reaction product was separated from unchanged farnesol and further purified by tlc to give 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl farnesyl phosphate (**8**) as the syrupy monopyridinium salt, which was characterized by its ir spectrum, optical rotation, and elementary analysis. Thin-layer chromatographic examination in chloroform–methanol (5:1) clearly showed the presence of two close-running substances corresponding to the isomers of farnesol (trans,trans and cis,trans). Deacetylation of **8** with methanolic sodium methoxide gave a very pure farnesyl α-D-mannopyranosyl phosphate (**9**), which was not subjected to further preparative chromatography after removal of the excess sodium methoxide. The sodium salt of **9** was obtained as a waxy solid, which was characterized by its ir spectrum, optical rotation, and elementary analysis. The nmr, recorded in [$^2H_5$]pyridine after conversion into the pyridinium salt, showed two strong, sharp signals arising from methyl protons adjacent to cis and trans carbon–carbon double bonds. A broad, unresolved peak resulting from the methylene protons in an unsaturated environment, and the absence of any signals arising from methyl or methylene protons in positions adjacent to saturated carbon–carbon bonds clearly differentiate this

[1] Abbreviations used are: dicyclohexylcarbodiimide, DCC; triisopropylbenzenesulfonyl chloride, iPr_3PhsCl.

spectrum from that obtained from the citronellol derivative (Warren and Jeanloz, 1973).

The preparation of ficaprenyl α-D-mannopyranosyl phosphate (**11**) from **5** in the presence of iPr_3PhsCl in anhydrous pyridine solution was based on that of the farnesol derivative. Ficaprenol (**7**) was obtained as a mixture of isomeric polyprenols with the C_{55} compound predominating, from an extract of *Ficus elastica* as previously described (Warren and Jeanloz, 1972; Warren *et al.*, 1973). However, a major by-product derived from ficaprenol was also formed, and in this respect the reaction was less efficient than the one based on farnesol. After the removal of derivatives of mannosyl phosphate, the crude product was purified as the sodium salt by column chromatography. This gave, in addition to unchanged ficaprenol, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl ficaprenyl phosphate (**10**) as a syrup, which was characterized by its ir spectrum, optical rotation, elementary analysis, and dilute acid hydrolysis in methanol solution, which gave 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate. Under comparable conditions, dolichyl α-D-mannopyranosyl phosphate (Warren and Jeanloz, 1973), gave dolichyl phosphate and free D-mannose, together with methyl mannosides. The results of the catalytic hydrogenolysis of **10** were also significantly different from those of the hydrogenolysis of citronellyl α-D-mannopyranosyl phosphate (Warren and Jeanloz, 1973) which served as a model compound for the dolichyl derivative. A single product, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate, was obtained when the reaction mixture was buffered to avoid the acid-catalyzed autohydrolysis which would otherwise result from the generation of an acidic medium during hydrogenation (Warren *et al.*, 1973). After deacetylation of **10** with sodium methoxide, the resulting compound was freed from minor contaminants by preparative thin-layer chromatography to give the sodium salt of ficaprenyl α-D-mannopyranosyl phosphate (**11**) as a waxy solid that readily formed gels when treated with solvents. Therefore, the pyridinium salt was prepared as a syrup, which gave clear solutions in organic solvents for optical rotation measurement. Compound **11** was also characterized by its ir spectrum, elementary analysis, and thin-layer chromatography in a variety of solvent systems with spray reagents specific for unsaturation, isoprenoid alcohol content, and phosphate ester content. This phosphate diester, although containing the highly reactive allylic phosphate group, did not possess the extreme instability that characterized the pyrophosphates previously synthesized (Warren and Jeanloz, 1972; Warren *et al.*, 1973). Ficaprenyl α-D-mannopyranosyl sodium phosphate (**11**) and dolichyl α-D-mannopyranosyl sodium phosphate (Warren and Jeanloz, 1973) did not cochromatograph on thin layers in two of the solvent systems which are most commonly used to identify lipid intermediates in biosynthetic systems.

Synthetic ficaprenyl α-D-mannopyranosyl phosphate (**11**) has been compared with the active lipid intermediate of mannan biosynthesis in *M. lysodeikticus* (Scher *et al.*, 1968; Scher and Lennarz, 1969; Lahav *et al.*, 1969). The two mannolipids were found to cochromatograph in three solvent systems on paper coated with silica gel, which shows that they have a very similar structure. However, it is also apparent that the chromatography does not distinguish between compounds having lipid moieties that differ only in minor details of double-bond stereochemistry or chain length, as ficaprenols (C_{50}–C_{60}) have three internal trans double bonds, whereas bacterial undecaprenol (C_{55}) has only two. Synthetic **11** has not been tested as yet for activity as a donor of D-mannose in a cell-free enzyme preparation from the same organism. Synthetic **11** has also been compared with synthetic dolichyl α-D-mannopyranosyl phosphate (Warren and Jeanloz, 1973) as a possible lipid intermediate in glycoprotein biosynthesis (Evans and Hemming, 1973). Preliminary results (F. W. Hemming, personal communication) indicate that the ficaprenol derivative has negligible activity, since it did not stimulate, to any great extent, the incorporation of D-[^{14}C]mannose into endogenous mannolipid when incubated with a pig-liver microsomal preparation and GDP-D-[^{14}C]mannose, whereas synthetic dolichyl α-D-mannopyranosyl phosphate did stimulate this incorporation.

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. Nuclear magnetic resonance (nmr) spectra were recorded at 60 MHz with a Varian A-60 spectrometer and with [$^2H_5$]pyridine ("Silanor P") or [^{2}H]chloroform ("Silanor C") as solvent, containing 1% tetramethylsilane as internal standard (MSD Isotopic Products, Montreal, Canada). The cation-exchange resin used was AG 50W-X8 (200–400 mesh, Bio-Rad Laboratories, Richmond, Calif. 94804). 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 C, H, and N microanalyses were performed by Dr. M. Manser, Zurich, Switzerland, and the P microanalyses by Galbraith Laboratories Inc., Knoxville, Tenn. 37921.

Chromatographies. Silica gel column chromatography was performed on silica gel 0.05–0.20 mm (70–325 mesh, E. Merck A.G., Darmstadt, Germany) used without pretreatment. Thin-layer chromatography (tlc) was performed on precoated plates of silica gel G (Merck) or Cellulose F (Merck); the plates supplied (20 × 20 cm) were cut to a length of 6 cm before use, and used without pretreatment. However, in experiments where one or more of the samples were applied to the plate in aqueous solution, all the other samples were treated with water (1–2 μl) before the plate was dried in a current of air prior to elution. Tlc refers to thin-layer chromatography on silica gel unless otherwise stated. Preparative tlc was carried out on precoated plc plates, silica gel F 254 (Merck). The spray reagent used, unless otherwise stated, was anisaldehyde–sulfuric acid–ethanol (1:1:18) (Dunphy *et al.*, 1966), and the plates were heated to 125°. The spray reagent used to detect unsaturation was 1% aqueous potassium permanganate in 2% aqueous sodium carbonate (Gigg and Gigg, 1966). The spray reagent of Dittmer and Lester (1964) was used to detect phosphate groups. Solvents A, B, and C for tlc were chloroform–methanol–water (60:25:4), (60:35:6), and (10:10:3), respectively. Solvent D was 2,6-dimethyl-4-heptanone–acetic acid–water (20:15:2) and solvent E was 2-propanol–15 M ammonium hydroxide–water (6:3:1). The R_F is calculated from measurement of the distance from the origin of the chromatogram to the point of maximum intensity of the spot after development.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl Ammonium Phosphate (**2**). Syrupy 1,2,3,4,6-penta-*O*-acetyl-α,β-D-mannopyranose was prepared from D-mannose with acetic anhydride–pyridine (Conchie and Levvy, 1963). Crystalline phosphoric

acid (1.2 g, Fluka AG, Buchs S.G., Switzerland) was quickly weighed into a round-bottomed flask (50 ml) and dried *in vacuo* overnight, over magnesium perchlorate. A solution of the syrupy pentaacetate **1** (1.0 g) in ether (20 ml) was added to the flask, and the ether was carefuliy evaporated under reduced pressure. A white precipitate was observed to form at this stage, presumably because of traces of pyridine remaining in D-mannose pentaacetate, but this did not interfere with the preparation of **2**. The flask was immersed in an oil bath at 65° and evacuated (oil pump), and the content was mixed by means of a magnetic stirrer. A vigorous evolution of acetic acid vapor took place, and after 2 hr the almost colorless syrupy mixture was dissolved in anhydrous tetrahydrofuran. The solution was cooled to −10°, and concentrated (58%) ammonium hydroxide (1.5 ml) was added rapidly with vigorous stirring until the pH of the solution reached *ca.* 6. 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 which, on examination by tlc, showed a main product having R_F 0.20 (solvent A) and R_F 0.40 (solvent B), a minor contaminant R_F 0.33 (solvent B), unchanged starting material, and another by-product (nonphosphorylated) running near the solvent front.

The crude product was dissolved in water and the solution extracted with chloroform (six times) to remove the nonphosphorylated contaminants. After addition of a small amount of pyridine, the aqueous solution was evaporated, and toluene was added to the residue and evaporated twice, to give 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl ammonium phosphate (**2**) as a syrup (0.92 g), pure on tlc (solvent B) except for a trace of material (R_F 0.33).

α-D-Mannopyranosyl Phosphate (**3**). *Dicyclohexylammonium Salt.* A solution of **2** (0.92 g) in methanol (25 ml) was treated with a solution of 1% sodium methoxide in methanol until no further precipitation took place, and cooled to 0° for several hours. The very hygroscopic precipitate (0.42 g, 53%), presumably the disodium salt of α-D-mannopyranosyl phosphate, was washed extensively with methanol and filtered off. A solution of the 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), the solution was evaporated, and toluene was added and evaporated three times to give a solid residue. The product was dissolved in a minimum of water, and ethanol was carefully added until an amorphous precipitate started to appear. The solution was then kept at room temperature and the gradual crystallization was completed after several hours by the addition of an excess of ethanol (0.36 g, 28% based on **1**). The crystalline dicyclohexylammonium salt contains two molecules of water of crystallization: mp 137–138.5°, $[\alpha]_D^{20}$ +26° (*c* 1.65, water), +27° (after correction for water content). *Anal.* Calcd for $C_{18}H_{39}N_2O_9P \cdot 2H_2O$: C, 43.73; H, 8.78; N, 5.67. Found: C, 43.80; H, 8.57; N, 5.53. Hill and Ballou (1966) obtained a product that had been purified by chromatography on ion-exchange Sephadex and showed $[\alpha]_D^{20}$ +28.7°. The yield was 75% based on D-mannose when syrupy 1,2,3,4,6-penta-*O*-acetyl-α,β-D-mannopyranose was used as starting material, and this yield was not increased when crystalline 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose was employed. We prepared this compound for characterization purposes and, therefore, no attempt was made to improve the yield.

Barium Salt. The disodium salt (0.1 g) obtained by de-O-acetylation of **2** was converted into the pyridinium form as described in the preparation of the dicyclohexylammonium salt. The combined aqueous solution and washings were treated with a cation-exchange resin (barium form). The mixture was stirred for 24 hr, and the resin was removed by filtration. Evaporation of the solution gave a syrupy residue which was triturated with ether. The resulting solid was washed, by decantation, with ether, then methanol, and dried *in vacuo* at room temperature over P_2O_5 to give the barium salt of **3** (0.1 g), $[\alpha]_D^{20}$ +34° (*c* 2.2, water); Posternak and Rosselet (1953) reported $[\alpha]_D^{25}$ +33.7°; Perchemlides *et al.* (1967) reported $[\alpha]_D^{25}$ +46.3° after extensive drying at an elevated temperature.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl Dipotassium Phosphate (**4**). (a) The precipitated disodium salt (0.4 g) obtained by de-O-acetylation of **2** was converted into the pyridinium form as described in the preparations of the dicyclohexylammonium and barium salts of **3**. The combined aqueous solution and washings from the resin were evaporated to dryness. After two additions and evaporations of toluene, the syrupy product was treated with pyridine (5 ml) and acetic anhydride (5 ml), and the mixture was stirred until a clear solution was obtained. After being kept at room temperature for 15 hr, the reaction mixture was treated with water (cooling if necessary) until no further heat evolved. After a further 2 hr at room temperature, the solution was evaporated, and traces of acetic acid and pyridine were removed by two additions and evaporations of toluene. The residue was dissolved in water (25 ml) and extracted three times with chloroform to remove traces of nonpolar contaminants. The aqueous solution was stirred for 48 hr with a large excess of a cation-exchange resin (potassium form). The resin was filtered off and the solution evaporated to give a solid product, which was recrystallized from a minimum of hot methanol to give **4** (0.36 g, 28% based on 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose), $[\alpha]_D^{20}$ +33.5° (*c* 1.0 methanol). *Anal.* Calcd for $C_{21}H_{31}K_2O_{13}P$: C, 33.33; H, 3.80; P, 6.14. Found: C, 33.33; H, 3.80; P, 5.65.

(b) A solution of **2** (0.73 g) in water (35 ml) was stirred with a large excess of a cation-exchange resin (potassium form) for 48 hr at room temperature. The resin was filtered off and washed with water, and the combined filtrate and washings were evaporated to give a syrupy residue. This product was dissolved in a minimum of methanol and the solution kept at room temperature. After several hours, the dipotassium salt **4** was obtained as white crystals (0.5 g, 49%), shown by optical rotation and tlc (R_F 0.40, solvent B) to be identical with the crystalline product obtained by method (a). Cawley and Letters (1971) prepared 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl disodium phosphate in 28% yield, by a method based on the one of Posternak and Rosselet (1953); however, their attempts to purify the product were unsuccessful.

In order to convert **4** into the pyridinium form **5** as used in further synthesis, the required quantity of **3** was dissolved in water and the solution passed through a column of a cation-exchange resin (pyridinium form). The resin was washed with water and the combined aqueous solutions evaporated in the presence of pyridine (10 ml). After three additions and evaporations of toluene, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate was obtained in the pyridinium form (**5**) as a syrup that was stored as a 1% solution in 1,2-dichloroethane until needed.

The nmr spectrum of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate was recorded as the dipotassium salt (**4**, 30 mg) in deuterium oxide (0.5 ml) and as the pyridinium

salt (**5**, 50 mg) in [^{2}H]chloroform (0.5 ml). The sample was dried thoroughly *in vacuo* over phosphorus pentaoxide, and then subjected to three additions and evaporations of the deuterated solvent. Finally, the sample was dissolved in the required volume of solvent, and the solution filtered into the nmr tube. The main peaks occurred at δ 2.04, 2.12, 2.15, 2.23 (Me protons of –OAc), 4.28, 5.29, 5.37, 5.40 (pyranose ring protons), and 5.57 ppm (unresolved composite peak, anomeric proton of α-D-mannopyranosyl phosphate) for the potassium salt in D_2O; for the pyridinium salt in [^{2}H]chloroform, δ 1.96, 2.03, 2.13 (the center peak consists of two superimposed signals, Me protons of –OAc), 4.19 (CH_2 protons of C-6 of the D-mannopyranosyl residue), 5.25, 5.33 (a composite peak with several superimposed signals, pyranose ring protons), 5.57 (a poorly resolved doublet, anomeric proton of α-D-mannopyranosyl phosphate, J_{H_1-P} *ca.* 7 Hz, no $J_{H_1-H_2}$ value could be measured), 7.32, 7.36, and 8.00 ppm (aromatic protons of the pyridinium residue). Onodera and Hirano (1966) gave δ 5.34–5.68 ppm for the anomeric proton of several α-D-mannopyranosyl phosphate derivatives, $J_{H_1-H_2}$ = *ca.* 1.0, J_{H_1-P} = 7–8.5 Hz. Paulsen and Thiem (1973) gave δ 5.65 ppm (quartet), $J_{H_1-H_2}$ = 1.5, J_{H_2-P} = 6.4 Hz, for the anomeric proton of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl dimethyl phosphate.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl Farnesyl Sodium Phosphate (**8**). A mixture of farnesol (**6**, 0.1 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate (pyridinium form, **5**, 0.2 g) with iPr_3PhsCl (0.12 g) was exhaustively dried by repeated additions and evaporations of toluene, then dissolved in dry pyridine (2.5 ml) and kept at room temperature for 72 hr. Examination by tlc showed the presence of a major product having R_F 0.66 (chloroform–methanol, 5:1) and R_F 0.75 (solvent A), as well as a considerable amount of unreacted farnesol (running near the solvent front in both solvents) and approximately equal proportions of **5** and of a compound with the expected mobility of peracetylated di-P^1,P^2-D-mannosyl pyrophosphate (R_F 0.26, solvent A). Methanol (5 ml) was added and the mixture kept at room temperature for a further 2 hr. The solvents were evaporated, the residue was dissolved in chloroform (20 ml), and the solution was extracted four times with water to remove **5**, the pyrophosphate derived from **5**, and any other minor by-products arising from **5**. The chloroform solution was evaporated, and the residue, together with a small proportion of water, was dissolved in methanol (20 ml) and extracted four times with hexane to remove unchanged farnesol (40 mg); more water was added when necessary, in order to obtain two phases. Examination of the methanol solution by tlc showed that it still contained some farnesol and, therefore, it was evaporated and the crude residue (0.22 g) was purified by preparative tlc in chloroform–methanol (5:1) on two 20 × 20 cm plates. After being dried in air, the bands containing the desired acetylated phosphate diester **8** and farnesol (**6**, the upper band) were located by spraying with potassium permanganate reagent an area (0.3-cm wide) on each side of the plate, 1–2 cm from the edge. Syrupy compound **8** (0.11 g) was extracted by stirring overnight with solvent C, and farnesol (**6**, 21 mg) was extracted with chloroform–methanol (5:1). Tlc indicated that **8** contained a contaminant (R_F 0.10, in chloroform–methanol, 5:1; R_F 0.16 in solvent A). Therefore, the preparative tlc was repeated with solvent A, the extraction from the plate being performed as just described, to give **8** (pyridinium form, 82 mg). The product was pure according to tlc in three solvent systems after being detected with the anisaldehyde, potassium permanganate, and phosphate specific spray reagents: R_F 0.43 and 0.56 in chloroform–methanol, 5:1 (corresponding to cis,trans and trans,trans isomers of farnesol); 0.8 (solvent A); and 0.64 (solvent D). The R_F values of pure **8** do not necessarily correspond to the mobilities of the product in the crude reaction mixture, as the other components of the mixture affect its migration. The product was a syrup: $[\alpha]_D^{20}$ +22° (*c* 1.20, chloroform); ir spectrum ν_{max}^{film} 2960 (CH_3, stretching), 2930 and 2855 (CH_2, stretching), 1745–1750 (C=O, acetyl), 1660 (C=C, stretching), 1440–1450 (–CH_2, –CH_3), 1375 (CCH_3), 1260 (C=O, stretching), 1220 (P=O), 1150 (CH_3CCH_3), 1050 (PO–, allylic), 870 cm^{-1} (–CH=C<). *Anal.* Calcd for $C_{34}H_{49}NO_{13}P \cdot H_2O$: C, 56.03; H, 7.07; N, 1.92; P, 4.25. Found: C, 56.29; H, 7.42; N, 1.73; P, 3.45.

Farnesyl α-D-Mannopyranosyl Phosphate (**9**). *Sodium Salt.* The peracetate **8** (40 mg) was treated with 1% sodium methoxide in methanol (1 ml). After 30 min at room temperature, tlc showed that all the starting material (R_F 0.80, solvent A) had been converted into a new product (R_F 0.15, solvent A). The solution was diluted to 5 ml and neutralized with a small amount of a cation-exchange resin (pyridinium form), the resin was filtered off, a large excess of a cation-exchange resin (sodium form) was added, and the mixture was stirred for 48 hr at room temperature. The resin was filtered off and washed well with methanol, and the combined filtrates were evaporated to dryness. The residue was triturated with hexane to give a waxy solid (31 mg) which was pure according to tlc in four solvent systems using three different spray reagents, as described in the preparation of **8**: R_F 0.15 (solvent A), 0.51 (solvent B), 0.21 (solvent D), and 0.70 (solvent E). Farnesyl α-D-mannopyranosyl sodium phosphate had mp 140–142°; $[\alpha]_D^{20}$ +27° (*c* 0.56, methanol); ir spectrum ν_{max}^{KBr} 3350 (OH), 2960 (CH_3, stretching), 2930 and 2855 (CH_2, stretching), 1660 (C=C, stretching), 1460 (–CH_2, –CH_3), 1375 (CCH_3), 1220 (P=O), 1160 (CH_3CCH_3), 1105 (CHOH), 985, 925, 880, and 805 cm^{-1} (–CH=C<). The sodium salt (18 mg) was converted into the pyridinium salt by a slow passage of the solution in methanol through a small column (0.5 × 2 cm) of a cation-exchange resin (pyridinium form). The resin was washed with methanol, and the combined eluates were evaporated. After three additions and evaporations of toluene, the sample was kept *in vacuo* over phosphorus pentaoxide for 20 hr. Dissolution of the dried sample in [$^2H_5$]pyridine (0.5 ml) and evaporation of the solvent was repeated twice, then a final solution was made in "Silanor P" (0.4 ml) and this solution was filtered into the nmr tube. The main peaks were at δ 1.6 (CH_3 protons of CH_3C=C, trans), 1.69 (CH_3 protons of CH_3C=C, cis), 2.08 [broad, CH_2 protons of $CH_2C(CH_3)$=C and CH_2CH=C], 3.6 (CH_2 protons of OCH_2C=C), 4.43 (broad, OH and CH protons of α-D-mannopyranosyl residue), 4.65 (HDO), 7.26, and 7.62 ppm (aromatic protons of pyridinium residue). *Anal.* Calcd for $C_{21}H_{35}NaO_9P \cdot 2H_2O$: C, 48.35; H, 7.55; P, 5.94. Found: C, 48.41; H, 7.24; P. 4.25.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl Ficaprenyl Phosphate (**10**). Ficaprenol (**7**), a mixture of mainly C_{50}, C_{55}, and C_{60} isomers, the C_{55} compound predominating, was prepared from an extract of *F. elastica* as described previously (Warren and Jeanloz, 1972; Warren *et al.*, 1973). A mixture of **7** (0.35 g) and **5** (0.2 g) with iPr_3PhsCl (0.12 g) was completely dehydrated by repeated additions and evaporations of toluene, then dissolved in dry pyridine (2.5 ml) and kept for 72 hr at room temperature. Examination of the mixture by tlc showed the presence of a major product (R_F 0.58, chloroform–methanol, 5:1; 0.7, solvent A) together with a considerable amount of a contaminant (R_F 0.38, chloroform–methanol, 5:1; 0.62, solvent A). A substance having the mobility of peracetylated

di-P^1,P^2-D-mannosyl pyrophosphate (R_F 0.26, solvent A), a small amount of **5**, and some unchanged **7** were also present. Methanol (10 ml) was added, and the mixture was kept at room temperature several hours longer. The solvents were evaporated, the residue was dissolved in chloroform (50 ml), and the solution was extracted four times with water. The chloroform solution was evaporated to give a crude product (0.5 g) that was dissolved in methanol containing a trace of chloroform, and the solution was stirred for 48 hr with a large excess of a cation-exchange resin (sodium form). The resin was filtered off and washed with methanol, and the combined filtrates were evaporated to dryness. The product was purified by dissolution in a minimum of chloroform and chromatography on a column of silica gel (25 g). Unreacted ficaprenol (**7**, 0.12 g) was eluted with chloroform, and examination by tlc in benzene–methanol (49:1) showed that it was identical with the starting material. The ficaprenols exhibited a diagnostic behavior when the chromatographic plate was carefully heated after being sprayed with the anisaldehyde reagent. The leading edge of the spot having R_F 0.30 turned green, while the central part was an intense blue color, and the rear part purple, these colorations presumably being derived from the individual isomers present in the ficaprenol mixture. This effect was reproducible; after strong heating, the whole spot had the violet-purple color characteristic of the reaction of polyprenols with this spray reagent.

Elution of the column was continued with chloroform–methanol (50:1), followed by chloroform–methanol (25:1). The desired product **10** was eluted with chloroform–methanol (10:1), and was obtained in seven 10-ml fractions. Further elution gave only contaminated material. The pure fractions (tlc) were combined and evaporated to give the sodium salt of **10** (86 mg) as a syrup, pure according to tlc in three solvent systems with spray reagents as described for the preparation of **8** (R_F 0.44, chloroform–methanol, 5:1; 0.7, solvent A; 0.62, solvent D): $[\alpha]_D^{20}$ +8.0° (*c* 1, chloroform); ir spectrum ν_{max}^{film} 2960 (CH_3, stretching), 2930 and 2855 (CH_2, stretching), 1745–1750 (C=O, acetyl), 1660 (C=C, stretching), 1440–1450 (–CH_2, –CH_3), 1375 (CCH_3), 1260 (C=O, stretching), 1225 (P=O), 1150 (CH_3CCH_3), 1045 (PO–, allylic), 1090, 980, 875, and 835 cm^{-1} (–CH=C<). *Anal.* Calcd for $C_{69}H_{108}NaO_{13}P \cdot H_2O$: C, 68.07; H, 9.16; P, 2.54. Found: C, 67.85; H, 9.19; P, 2.29.

Dilute Acid Hydrolysis of **10**. A mixture of **10** (1 mg) with 1 M hydrochloric acid and methanol (1:10, 0.2 ml) was treated with chloroform until a clear solution was obtained (*ca.* 2 drops) and kept for 2 min at 100°. Examination of the solution by tlc showed that all the starting material had been converted into: (a) a compound that cochromatographed with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate (R_F 0.40, solvent B) together with a small amount of material of R_F 0.33 (as in the preparation of compound **2**); (b) a trace of **7** (R_F 0.30, benzene–methanol, 49:1) together with at least five substances of higher chromatographic mobility, presumably rearrangement and decomposition products derived from ficaprenol. In order to ascertain that no D-mannose had been formed, the hydrolysate was evaporated under a stream of nitrogen, then dried *in vacuo* over potassium hydroxide. The residue was treated with 1% sodium methoxide in methanol, until a strongly basic solution was obtained. The mixture was kept at room temperature for 30 min, and then treated with a small amount of water to dissolve the precipitated material. Examination by tlc (solvent B) showed that no D-mannose (R_F 0.27) was present.

Hydrogenolysis of **10**. A mixture of compound **10** (1 mg) in chloroform–methanol (1:1) was treated with tributylamine (5 mg) and a small excess of glacial acetic acid, and hydrogenated at 1 atm pressure in the presence of a 5% platinum-on-charcoal catalyst (Matheson Coleman and Bell, E. Rutherford, N. J. 07073) for 4 hr. Examination of the solution revealed the presence of only one product, having the mobility of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate, and of no starting or reduced starting material.

Ficaprenyl α-D-Mannopyranosyl Phosphate (**11**). (a) Compound **10** (77 mg) was dissolved in chloroform (2 ml) and treated with 1% sodium methoxide in methanol (1 ml). The solution was kept at room temperature for 30 min, when tlc showed that the starting material (R_F 0.70, solvent A) had been converted into a new product (R_F 0.25). The solution was concentrated to *ca.* 1 ml and applied to a preparative tlc plate (20 cm × 16 cm), which was developed in solvent A. The band containing **11** was located and extracted as previously described in the preparation of **8**. Compound **11** was not soluble in chloroform–methanol mixtures or in chloroform alone. Therefore, it was dissolved in chloroform–methanol–hexane (*ca.* 2:1:2) and, after filtration, the solution was evaporated to give the sodium salt of **11**, a waxy solid (27 mg) showing no definite melting point, pure according to tlc in four solvent systems with the anisaldehyde, potassium permanganate, and phosphate specific spray reagents: R_F 0.25, solvent A; 0.62, solvent B; 0.5, solvent D; and 0.7, solvent E; ir spectrum ν_{max}^{KBr} 3250 (OH), 2960 (CH_3, stretching), 2930 and 2855 (CH_2, stretching), 1660 (C=C, stretching), 1450 (–CH_2, –CH_3), 1375 (CCH_3), 1225 (P=O), 1145 (CH_3CCH_3), 1100 (CHOH), 1045 (PO–, allylic), 1075, 1000, 980, 920, and 880 cm^{-1} (–CH=CH<). *Anal.* Calcd for $C_{61}H_{100}NaO_9P \cdot H_2O$: C, 69.77; H, 9.71; P, 2.95. Found: C, 70.06; H, 9.06; P, 2.35.

(b) Compound **10** (22 mg) was dissolved in chloroform (0.7 ml) and treated with 1% sodium methoxide in methanol (0.4 ml). The solution was kept at room temperature for 30 min, and then neutralized with a cation-exchange resin (pyridinium form). The resin was filtered off, and the solution was evaporated to give the pyridinium salt of **11** as a syrup (19 mg), pure according to tlc in solvents D and E, and showing a trace of a contaminant, running just ahead of the main spot, in solvents A and B; $[\alpha]_D^{20}$ +17° (*c* 1.1, chloroform–methanol, 5:1). The product (19 mg) was stirred with a large excess of cation-exchange resin (sodium form), in a mixture of chloroform and methanol, for 48 hr at room temperature. Removal of the resin and evaporation gave **11** as the sodium salt (18 mg), a waxy solid which formed an insoluble gel when treated with chloroform–methanol (5:1).

Chromatographic Comparison of Ficaprenol and Dolichol Derivatives. Synthetic ficaprenyl α-D-mannopyranosyl phosphate was compared with synthetic dolichyl α-D-mannopyranosyl phosphate (Warren and Jeanloz, 1973) by tlc in two solvent systems. In solvent A the R_F values for the ficaprenol and dolichol compounds were 0.25 and 0.54, respectively. In solvent D, the R_F values were 0.50 and 0.67, respectively. When the two compounds were applied to the plate on the same spot, they did not cochromatograph.

Chromatographic Comparison of **11** *with Mannosyl Undecaprenyl Phosphate*.[2] D-[^{14}C]Mannosyl undecaprenyl phosphate, obtained from the mannan synthesizing system of *M. lysodeikticus* (Lahav *et al.*, 1969), was compared with compound **10** by chromatography on Whatmann SG 81 paper, treated with 2% EDTA. The spots were detected by autoradiography, or by staining with Rhodamine (Skipski and

[2] This experiment was performed by Dr. C. J. Waechter.

Barclay, 1969) and the two compounds were found to co-chromatograph in three solvent systems: in chloroform–methanol–water (65:25:4), R_F 0.41 (ficaprenyl phosphate; R_F 0.51); in 2,6-dimethyl-4-heptanone–acetic acid–water (20:15:2), R_F 0.57 (ficaprenyl phosphate: R_F 0.68); and in chloroform–methanol–58% ammonium hydroxide (36:13:3), R_F 0.30 (ficaprenyl phosphate: R_F 0.18). Synthetic ficaprenyl α-D-mannopyranosyl phosphate (**11**) was also homogeneous according to tlc on silica gel H (Merck) in chloroform–methanol–water (65:25:4) with the Rhodamine and anisaldehyde spray reagents.

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