213. *Synthesis of Ribitol 1,5-Diphosphate and of a Polymeric Ribitol Phosphodiester.*

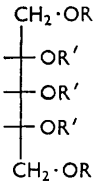
By D. A. Applegarth, J. G. Buchanan, and J. Baddiley.

Ribitol 1,5-diphosphate, a degradation product of ribitol teichoic acids, has been synthesised by phosphorylation of 2,3,4-tri-*O*-benzoyl-ribitol. Polymeric ribitol phosphodiesters of the type found in teichoic acids have been synthesised by the action of diphenyl phosphorochloridate on 2,3-*O*-isopropylidene-D-ribitol 4- and 5-phosphates followed by removal of protecting groups. The hydrolysis of this product, which contains mainly 1,4-linkages and has an average chain length of about 5·5 units, is described. In alkali, the polymer gives a small amount of anhydroribitol phosphate, a property which is shared with some ribitol teichoic acids.

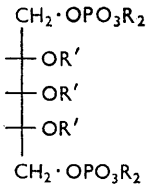
Teichoic acids are polymeric ribitol or glycerol phosphodiesters found in or beneath the walls of a number of Gram-positive bacteria.[1,2] They are usually composed of a straight chain of polyol units joined by phosphodiester linkages. Specific sugars are attached as glycosides and normally D-alanine is attached to polyol or sugar by ester linkages. Acid hydrolysis of the ribitol teichoic acids from bacteria such as *Bacillus subtilis* [3] yields small amounts of isomeric ribitol diphosphates together with other major products. The presence of a ribitol diphosphate amongst these products provides strong support for the structure assigned to this polymer, showing that ribitol residues are joined directly through phosphodiester linkages. This distinguishes a structure of the teichoic acid type from those of the capsular substances of certain species of *Pneumococcus*, in which the phosphodiester linkage is between ribitol and a sugar, *e.g.*, D-galactose.[4-6]

An authentic sample of a ribitol diphosphate was required for chromatographic comparison. During the course of the work a sample of ribitol 1,5-diphosphate was isolated from the teichoic acid from the walls of *Lactobacillus arabinosus* [7] by Dr. A. R. Archibald and it is the synthesis of this isomer that is described here.

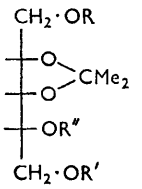
[1] Armstrong, Baddiley, Buchanan, Carss, and Greenberg, *J.*, 1958, 4344.
[2] Baddiley, *J. Roy. Inst. Chem.*, 1962, 366.
[3] Armstrong, Baddiley, and Buchanan, *Biochem. J.*, 1960, **76**, 610.
[4] Rebers and Heidelberger, *J. Amer. Chem. Soc.*, 1959, **81**, 2415.
[5] Roberts, Buchanan, and Baddiley, *Biochem. J.*, 1963, **88**, 1, and unpublished work.
[6] Rao, Buchanan, and Baddiley, unpublished work.
[7] Archibald, Buchanan, and Baddiley, *Biochem. J.*, 1961, **81**, 124, and unpublished work.



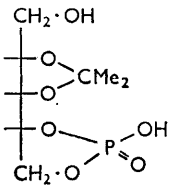
(I: R = R′ = H)
(II: R = CPh_3, R′ = H)
(III: R = CPh_3, R′ = COPh)
(IV: R = H, R′ = COPh)
(VIII: R = CPh_3, R′ = CH_2Ph)
(IX: R = H, R′ = CH_2Ph)



(V: R = CH_2Ph, R′ = COPh)
(VI: R = H, R′ = COPh)
(VII: R = R′ = H)



(X: R = R′ = COPh, R″ = H)
(XI: R = R′ = COPh, R″ = PO_3Ph_2)
(XIII: R = R″ = H, R′ = PO_3H_2)
(XIV: R = R′ = H, R″ = PO_3H_2)



(XII)

Ribitol (I) was converted into the 1,5-dityrityl ether (II) by Bien and Ginsburg's method [8] and treated directly with benzoyl chloride to give the tribenzoate (III). When the trityl groups were removed with hot 80% acetic acid, the resulting tribenzoate could not be tritylated and migration of benzoyl groups had clearly occurred. The required tribenzoate (IV) was obtained by reaction of the ether (III) with ice-cold hydrogen bromide in acetic acid; [9] its structure was proved by tritylation to the ether (III), and it could be rearranged by heating it in 80% acetic acid or by chromatography on neutral alumina to the isomeric compound of undetermined structure.

Phosphorylation of the tribenzoate (IV) with dibenzyl phosphorochloridate [10] gave a syrupy neutral ester (V), which was hydrogenolysed over palladised charcoal, and the diphosphate (VI) separated from inorganic phosphate by means of its tri-*n*-octylammonium salt. Benzoyl groups were removed with aqueous ammonia, and ribitol 1,5-diphosphate (VII) was isolated as its lithium salt after purification by paper chromatography. It reacted with 2 mol. of sodium periodate and no formaldehyde was produced; it was chromatographically identical with the product from the degradation of the ribitol teichoic acid of *L. arabinosus*.[7]

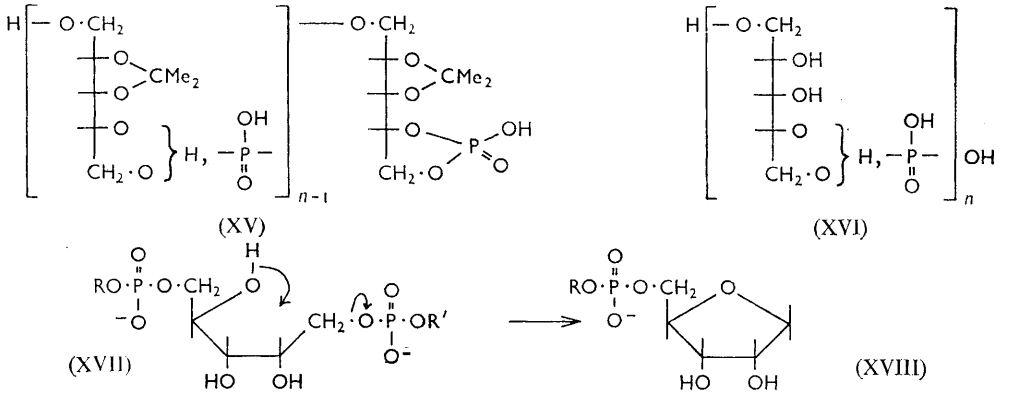
Ribitol 1,5-diphosphate was also prepared by phosphorylation of 2,3,4-tri-*O*-benzylribitol (IX). The separation of the product from inorganic phosphate was more difficult than in the other synthesis, and in the experimental section only the preparation of the crystalline ditrityl ether (VIII) and its detritylation are described.

Teichoic acids show immunological properties,[11–19] and their specificity appears to depend mainly on the nature of the sugar residues and the configuration of the glycosidic linkages to the polyol. However, with a glycerol phosphate polymer [20] from *Streptococci* in group A, the precipitin reaction is inhibited by synthetic polyglycerol phosphate [21]

[8] Bien and Ginsburg, *J.*, 1958, 3189.
[9] Helferich, *Adv. Carbohydrate Chem.*, 1948, **3**, 79.
[10] Atherton, Openshaw, and Todd, *J.*, 1945, 382.
[11] Baddiley and Davison, *J. Gen. Microbiol.*, 1961, **24**, 295.
[12] Haukenes, Ellwood, Baddiley, and Oeding, *Biochim. Biophys. Acta*, 1961, **53**, 425.
[13] Sanderson, Juergens, and Strominger, *Biochem. Biophys. Res. Commun.*, 1961, **5**, 472.
[14] Wicken, Elliott, and Baddiley, *J. Gen. Microbiol.*, 1963, **30**, 111.
[15] Morse, *J. Exper. Med.*, 1962, **116**, 229.
[16] Elliott, *Nature*, 1963, **200**, 1184.
[17] Sharpe, Davison, and Baddiley, *J. Gen. Microbiol.*, 1964, **34**, 333.
[18] Davison, Baddiley, Hofstad, Losnegard, and Oeding, *Nature*, 1964, **202**, 872.
[19] Davison and Baddiley, *Nature*, 1964, **202**, 874.
[20] McCarty, *J. Exp. Med.*, 1959, **109**, 361.
[21] Michelson, *J.*, 1959, 1371.

and a sugar residue is probably not involved. For this reason it was of interest to prepare a polyribitol phosphate.

1,5-Di-*O*-benzoyl-2,3-*O*-isopropylidene-D-ribitol (X) [22] was treated with diphenyl phosphorochloridate [23] in pyridine to give the crystalline neutral ester (XI). This resisted catalytic hydrogenolysis in the presence of sodium acetate, a method used previously [24] to hydrogenolyse phenyl groups without causing hydrolysis of an *O*-isopropylidene group by liberated acid. Conversion of the ester (XI) into the cyclic phosphate (XII) by treatment with aqueous ammonia in dioxan was then attempted; [25] direct treatment of the cyclic phosphate with diphenyl phosphorochloridate by Michelson's procedure [21] should then give polymeric material. This proved difficult and, when methanolic ammonia was used, followed by aqueous ammonia, the cyclic phosphate was contaminated with a methyl ester. Consequently, this crude product was hydrolysed with aqueous barium hydroxide to give a mixture of monoesters (XIII) and (XIV) isolated as their barium salts. The tri-*n*-octylammonium salts of this mixture were treated with diphenyl phosphorochloridate to give, first the cyclic phosphate (XII), and then the polymer containing mixed 1,4 and 1,5 linkages



(XV) (XVI)

(XVII) (XVIII)

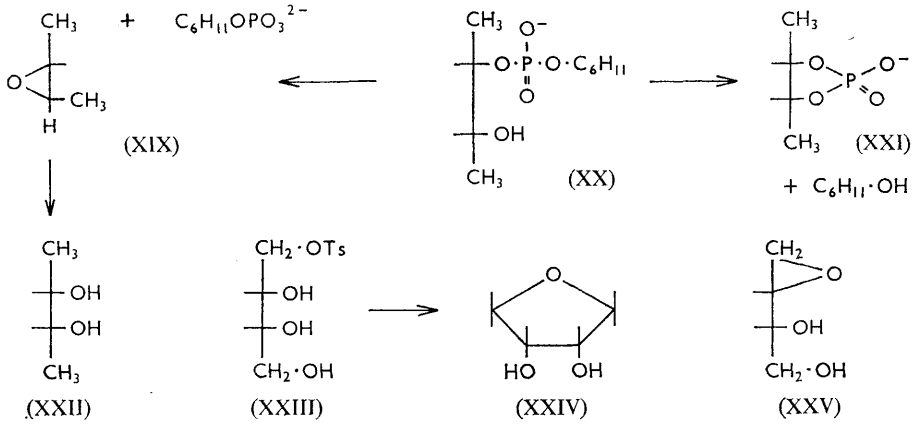
(XV). Hydrolysis with dilute acid removed the isopropylidene groups and opened the terminal cyclic phosphate group to give the polymer (XVI), which was purified by dialysis and isolated as its lithium salt.

The average chain length was determined by treatment with a phosphomonoesterase, and by measurement of the formaldehyde produced on oxidation of the polymer with periodate. The figures were in good agreement and indicate chain lengths of 5·6 and 5·3, respectively. The amount of periodate consumed was also measured in order to estimate the relative number of 1,4 and 1,5 linkages in the polymer. A polymer of n units containing only 1,5-linkages would react with $(2n + 1)/n$ mol. of periodate for each phosphate group, whereas for a 1,4 linked polymer the figure would be $(n + 1)/n$. When n is 5, these become 2·2 mol. and 1·2 mol. The value 1·5 mol. found experimentally indicates that the polymer contains mainly 1,4 linkages.

When ribitol teichoic acid preparations are hydrolysed with alkali and the products dephosphorylated enzymically, traces of 1,4-anhydroribitol are formed.[26] With the teichoic acid from *L. arabinosus* a greater amount is formed, and crystalline 1,4-anhydroribitol has been isolated from this source and characterised.[7] It seems likely that the larger amount in this case is a consequence of the number of glucoside-free ribitol units in this teichoic acid. When ribitol, its mono- or di-phosphate were treated with alkali under the conditions used

[22] Sargent, Buchanan, and Baddiley, *J.*, 1962, 2184.
[23] Brigl and Müller, *Ber.*, 1939, **72**, 2121.
[24] Baddiley, Buchanan, and Sanderson, *J.*, 1958, 3107.
[25] Cf. Kilgour and Ballou, *J. Amer. Chem. Soc.*, 1958, **80**, 3956.
[26] Armstrong, Baddiley, and Buchanan, *Biochem. J.*, 1961, **80**, 254.

for the hydrolysis of teichoic acids and the resulting products examined chromatographically after dephosphorylation, no 1,4-anhydroribitol was detected. When the synthetic polymer was thus treated, chromatography before treatment with phosphates indicated the formation of a trace of 1,4-anhydroribitol; after dephosphorylation of the product, an appreciable amount of anhydroribitol was detected together with ribitol. In a separate experiment, the products after hydrolysis with alkali were subjected to paper chromatography, and the areas corresponding to mono- and di-phosphates cut out separately. Treatment of the monophosphate area with phosphatase yielded both ribitol and anhydroribitol, while similar treatment of the diphosphates gave mainly ribitol together with a trace of anhydroribitol. This suggests that anhydroribitol is produced by cleavage of a phosphodiester linkage as in (XVII) ⟶ (XVIII), and that this reaction takes place together with the normal cyclic phosphate sequence.[27] Recently, Brown and Usher [28] have summarised the evidence for a second pathway in the hydrolysis of certain phosphodiesters bearing a neighbouring hydroxyl group; isotopic, stereochemical and kinetic evidence has been presented for the formation of a 3-membered epoxide ring as an intermediate. For example, cyclohexyl *erythro*-3-hydroxy-2-butyl hydrogen phosphate (XX) in N-sodium hydroxide yields not only the product of hydrolysis of the cyclic phosphate



(XXI) but also *meso*-butane-2,3-diol (XXII) by way of the epoxide (XIX). It may be argued that, in the ribitol case also, a vicinal epoxide would be involved. Hartman and Barker [29] have shown, however, that treatment of D-erythritol 1-toluene-*p*-sulphonate (XXIII) with alkali yields 1,4-anhydroerythritol (XXIV), but that the epoxide (XXV) is probably not an intermediate. Treatment of ribitol 1(5)-phosphate with 2N-hydrochloric acid is known to yield 1,4-anhydroribitol with no free ribitol.[30] In this case, also, there is no reason to believe that a vicinal epoxide is an intermediate. Further work on optically active model compounds containing ribitol is necessary to confirm these ideas.

Experimental

Evaporations were carried out under reduced pressure. Infrared spectra were determined by using potassium bromide discs. Analytical paper chromatography of phosphoric esters and polyols was carried out with Whatman No. 4 paper, which had been washed with 2N-acetic acid and then with water; propan-1-ol–aqueous ammonia (*d* 0·88)–water (6 : 3 : 1 by volume) was used for irrigation. Phosphates were detected with acid molybdate [31] and α-glycols with sodium

[27] Brown, in " Advances in Organic Chemistry," Ed. Raphael, Taylor, and Wynberg, Interscience, New York, 1963, pp. 81—83.
[28] Brown and Usher, *Proc. Chem. Soc.*, 1963, 309.
[29] Hartman and Barker, *J. Org. Chem.*, 1963, **28**, 1004.
[30] Baddiley, Buchanan, and Carss, *J.*, 1957, 4058.
[31] Hanes and Isherwood, *Nature*, 1949, **164**, 1107.

periodate and Schiff's reagent.[32] Whatman No. 3 paper was used for preparative purposes. Trityl ethers were examined chromatographically by using Wickberg's dimethyl sulphoxide–isopropyl ether system,[33] and were detected by a perchloric acid spray.[34]

2,3,4-*Tri*-O-*benzoyl*-1,5-*di*-O-*tritylribitol* (III).—Ribitol (1·88 g.) was dissolved in pyridine (25 ml.), trityl chloride (7·54 g., 2·1 mol.) added and the solution kept at room temperature for 96 hr. Benzoyl chloride (5·2 ml., 5 mol.) was added and after a further 48 hr. at room temperature the product was isolated by the addition of a little ice and pouring the solution into ice-water. The *benzoate* was recrystallised from ethanol and had m. p. 161° (yield 10·7 g., 87%) (Found: C, 80·6; H, 5·8. $C_{64}H_{52}O_8$ requires C, 81·0; H, 5·55%).

2,3,4-*Tri*-O-*benzoylribitol* (IV).—The above trityl ether (1 g.) was dissolved in alcohol-free chloroform (10 ml.) and acetic acid (8·5 ml.) added. The resulting solution was concentrated to 8 ml. below 35°. To this was added a solution containing acetyl bromide (0·23 ml.), water (0·06 ml.) and acetic acid (2 ml.), the whole at 5°. The trityl bromide was filtered off immediately through sintered glass, and the filtrate passed directly into ice–water which was then extracted with chloroform. The chloroform extract was treated in orthodox fashion to give the *ribitol tribenzoate* (0·5 g., 80%), m. p. 102°, from ether–light petroleum (b. p. 40—60°) (Found: C, 67·8; H, 5·5. $C_{26}H_{24}O_8$ requires C, 67·3; H, 5·2%). The infrared spectrum showed a strong singlet at 1718 cm.$^{-1}$ (aromatic ester C = O).

The benzoate (0·05 g.), when treated with trityl chloride (0·1 g.) in pyridine (1 ml.) for 5 days at room temperature, yielded the dityrityl ether (0·07 g., 75%), m. p. 159°, undepressed in admixture with the ether (III) above, m. p. 161°. The infrared spectra were identical.

Tri-O-*benzoylribitol*.—The dityrityl ether (III) (0·5 g.) was dissolved in hot acetic acid (40 ml.), water (10 ml.) added and the solution heated at 100° for 45 min. The product was isolated by extraction with chloroform, and triturated with light petroleum (b. p. 60—80°) to dissolve triphenylmethanol. The residue crystallised from ether–light petroleum (b. p. 40—60°) giving the *tribenzoate* (0·12 g., 60%), m. p. 167° (Found: C, 67·9; H, 5·25. $C_{26}H_{24}O_8$ requires C, 67·3; H, 5·2). The infrared spectrum showed a doublet at 1724 cm.$^{-1}$ (aromatic ester C=O) and 1704 cm.$^{-1}$ (bonded C=O).

The same tribenzoate was obtained from 2,3,4-tri-*O*-benzoylribitol by treatment with 80% acetic acid at 100° for 2 hr. or by chromatography on neutral alumina.

2,3,4-*Tri*-O-*benzoylribitol* 1,5-*Diphosphate*.—2,3,4-Tri-*O*-benzoylribitol (0·51 g.) was dissolved in anhydrous pyridine and dibenzyl phosphorochloridate[10] (2·3 g.) in carbon tetrachloride (4 ml.) added below 0°. The solution was kept in an ice–water bath, which reached room temperature overnight. After 20 hr. the product was isolated by extraction with chloroform, then dissolved in ethanol (30 ml.) and hydrogenated over palladised charcoal (0·3 g.). Paper chromatography showed the presence of a phosphate with R_F 0·69, together with inorganic phosphate. Tri-n-octylamine (1·3 ml.) was added and the solution was evaporated to dryness; the residue was dissolved in benzene (75 ml.) and shaken with water (4 × 40 ml.). The benzene layer was evaporated to dryness and excess of tri-n-octylamine removed by light petroleum (b. p. 60—80°). The residue was dissolved in 95% ethanol (10 ml.) and allowed to pass slowly through Dowex 50 (H^+ form, 60 ml.) resin made up in ethanol. The acidic eluate was neutralised with cyclohexylamine to pH 8 and evaporated to dryness. The product was triturated with ether to yield a *solid* (0·5 g., 50%), m. p. 166—168° (Found: C, 56·3; H, 7·6; P, 6·7. $C_{44}H_{65}N_3O_{14}P_2$ requires C, 56·3; H, 7·2; P, 6·6%).

Ribitol 1,5-*Diphosphate*.—The above cyclohexylammonium salt (0·15 g.) was dissolved in aqueous ammonia (*d* 0·88, 20 ml.) and kept for 48 hr. After removal of ammonia under reduced pressure the solution was freeze-dried and the whole product subjected to paper chromatography as a band (75 cm.) on Whatman No. 3 paper. The phosphate with R_F 0·11 was eluted with water and the eluate passed through Dowex 50 resin (Li form, 10 ml.). The residue, after freeze-drying, was purified by precipitation from an aqueous solution (0·5 ml.) with ethanol (10 ml.) to give a *solid* (39 mg., 80%) (Found: P, 18·6. $C_5H_{10}O_{11}P_2Li_4$ requires P, 18·5%). It reacted with 1·9 mol. of sodium periodate[35] and no formaldehyde was produced.[36]

2,3,4-*Tri*-O-*benzyl*-1,5-*di*-O-*tritylribitol*.—1,5-Di-*O*-tritylribitol[8] (3·0 g.) was dissolved in dry

[32] Baddiley, Buchanan, Handschumacher, and Prescott, *J.*, 1956, 2818.
[33] Wickberg, *Acta Chem. Scand.*, 1958, **12**, 516.
[34] Applegarth and Buchanan, *J.*, 1960, 4706.
[35] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.
[36] Hanahan and Olley, *J. Biol. Chem.*, 1958, **231**, 813.

benzene (17 ml.). After addition of dry dioxan (8 ml.), powdered potassium hydroxide (15 g.) and redistilled benzyl chloride (11 ml.) the mixture was heated under reflux with vigorous stirring and protection from moisture for 4·5 hr. An excess of acetic acid was added followed by benzene (35 ml.). The benzene layer was separated, washed with aqueous sodium hydrogen carbonate solution and water, and then dried over anhydrous potassium carbonate. Evaporation at $110°/10^{-3}$ mm. removed most of the benzyl chloride; last traces were removed by treatment with pyridine (25 ml.) at room temperature for 24 hr. The solution was poured into water and the *benzyl ether* was isolated by extraction with chloroform. The product (4·2 g., 85%) crystallised from ether–light petroleum (b. p. 60—80°) and had m. p. 106° (Found: C, 84·7; H, 6·4. $C_{64}H_{58}O_5$ requires C, 84·8; H, 6·4%).

2,3,4-*Tri*-O-*benzylribitol*.—The above trityl ether (0·44 g.) in acetic acid (25 ml.) and water (4 ml.) was heated at 100° for 2·5 hr. The solution was evaporated to dryness and the product in benzene was chromatographed on Grade 0 alumina. Chloroform–ethanol (19 : 1) eluted the *benzyl ether* (0·2 g., 98%) as a syrup (Found: C, 74·2; H, 7·6. $C_{26}H_{30}O_5$ requires C, 73·9; H, 7·2%). It did not yield a crystalline acetate.

1,5-*Di*-O-*benzoyl*-2,3-O-*isopropylidene*-D-*ribitol* 4-(*Diphenyl phosphate*).—1,5-Di-*O*-benzoyl-2,3-*O*-isopropylidene-D-ribitol [22] (0·8 g.) in dry pyridine (15 ml.) was cooled to 0° and diphenyl phosphorochloridate [23] (1·0 ml., 1·8 mol.) was added. After 10 min. at 0° the reaction was allowed to reach room temperature overnight. After 20 hr. a little ice was added and after 20 min. the solution ws poured on to ice and the product isolated by extraction with chloroform. The *ester* (0·7 g., 70%), m. p. 83°, $[\alpha]_D^{18°}$ −19·5° (*c* 4·0 in $CHCl_3$), crystallised from light petroleum (b. p. 60—80°) (Found: C, 64·6; H, 5·3; P, 5·1. $C_{34}H_{33}O_{10}P$ requires C, 64·6; H, 5·2; P, 4·9%).

2,3-O-*Isopropylidene*-D-*ribitol* 4- *and* 5-*Phosphates*.—The neutral ester described above (0·95 g.) was dissolved in methanol saturated with ammonia at 0° (30 ml.), and the solution kept at room temperature overnight. After 20 hr. aqueous ammonia (*d* 0·88) (300 ml.) was added and the solution was kept for 8 hr. The mixture was heated under reflux for 10 min., the ammonia removed *in vacuo* and the remaining solution freeze-dried. The residue was triturated with ether to remove benzamide and phenol, and then heated in 0·1M-barium hydroxide solution (40 ml.) at 100° for 2 hr. Solid carbon dioxide was added to the cooled solution and barium carbonate removed by centrifugation. The supernatant liquid was concentrated to 5 ml. and centrifuged again, and the clear solution added to acetone (150 ml.). The *barium salt* (0·6 g., 85%) was collected at the centrifuge (Found: P, 7·35. $C_8H_{15}O_8PBa$ requires P, 7·6%). Hydrolysis in 0·1N-hydrochloric acid at 18° for 3 hr. produced only ribitol monophosphates identified by paper chromatography.

Poly(*ribitol Phosphate*).—The above barium salt (1 g.) was dissolved in water (10 ml.) and ethanol (90 ml.) added. Tri-n-octylamine (1 ml.) was added and the solution passed through a column of Dowex 50 (tri-n-octylammonium form, 30 ml.) resin previously made up in ethanol. To the eluate was added tri-n-octylamine (1 ml.) and the column was washed with ethanol (200 ml.). The combined eluates were evaporated and dried (P_2O_5). The salt was dissolved in dioxan (12 ml.) and diphenyl phosphorochloridate (0·7 ml.) and tri-n-butylamine (1·2 ml.) were added. After 1·5 hr. paper chromatography showed the presence of diphenyl hydrogen phosphate and 2,3-*O*-isopropylidene-D-ribitol 4,5-(hydrogen phosphate). After a further 1·5 hr. diphenyl phosphorochloridate (0·7 ml.) and tri-n-butylamine (1·2 ml.) in dioxan (2 ml.) were added and the mixture was kept for a further 4 hr. Water (1 ml.) was added, the solution was kept at 0° overnight and then evaporated to dryness. The residue was treated with ether and extracted with aqueous ammonia (*d* 0·88, 3 × 50 ml.). The combined aqueous extracts were extracted with ether (50 ml.) and then freed from ammonia by evaporation under reduced pressure. 2N-Hydrochloric acid was added to give an acid concentration of 0·1N and, after 3 hr. at room temperature, the pH was adjusted to 7 with lithium hydroxide solution. The solution was then concentrated to 25 ml. and ethanol (270 ml.) and acetone (90 ml.) were added. After 3 hr. at 0° the resulting solid (0·3 g., 40%) was removed by centrifugation. The product was dialysed in water (70 ml.) against distilled water (2 l.) for 3 hr. and again (2·5 l. for 17 hr.) at 5°. The product (0·08 g., 10%) had R_F 0·00—0·18 (Found: P, 12·8. $C_5H_{10}O_7PLi$, H_2O requires P, 13·0%).

Determination of Average Chain Length of the Polymer.—(i) *By phosphomonoesterase*. The above lithium salt (2·5 mg.) in water (10 ml.) was treated with calf intestinal phosphomonoesterase (Sigma Chemical Co., St. Louis, Mo., U.S.A.; 1·5 mg.) at 37°. At timed intervals, the

ratio of total phosphate : inorganic phosphate was measured on a portion.[37] After 30 min. this reached the value 5·6 and was constant for 2 hr. thereafter.

(ii) *By periodate oxidation.* The polymer was allowed to react with sodium periodate, formaldehyde being determined by Hanahan and Olley's method;[36] periodate uptake was determined spectrophotometrically,[35] and both were related to the total phosphorus content.[37] The ratio of periodate reduced : total P was 1·5 : 1 and of total P : formaldehyde was 5·3 : 1.

Hydrolysis of the Polymer in Alkali.—(*a*) The polymer (6 mg.) was dissolved in N-sodium hydroxide (0·5 ml.) and heated in a sealed tube at 100° for 3 hr. The solution was passed through Dowex 50 (NH_4^+ form) resin, the column washed with water, and the combined eluate evaporated to dryness. The residue was dissolved in water (0·2 ml.). To half of the solution was added ammonium carbonate (10—15 mg.) and phosphomonoesterase (as above, 1 mg.) and the mixture incubated under toluene at 37° for 24 hr. The solutions were examined by paper chromatography. Before enzyme treatment, the main products were ribitol mono- and di-phosphates, together with a trace of anhydroribitol. The enzyme caused formation of much more anhydroribitol together with ribitol and inorganic phosphate.

(*b*) The polymer was hydrolysed with alkali as above and the products converted to ammonium salts. The mixture was chromatographed as a band on Whatman No. 3 paper and the mono- and di-esters were separately eluted from appropriate areas. These were each dephosphorylated enzymically and the products examined chromatographically. The mono-ester band yielded both anhydroribitol and ribitol. The diester band gave ribitol together with what may have been a trace of anhydroribitol.

Hydrolysis of Ribitol Mono- and Di-phosphates in Alkali.—When treated exactly as above no anhydroribitol was produced from D-ribitol 2-, 3-, or 5-phosphates or from ribitol 1,5-diphosphate.

We thank Professor D. Ginsburg for a sample of 1,5-di-*O*-tritylribitol. This work was carried out during the tenure (by D. A. A.) of a D.S.I.R. Studentship and a George Angus Studentship. Financial assistance from the Nuffield Foundation is gratefully acknowledged.

DEPARTMENT OF ORGANIC CHEMISTRY, THE UNIVERSITY,
NEWCASTLE UPON TYNE. [*Received, April 23rd,* 1964.]

[37] Chen, Toribara, and Warner, *Analyt. Chem.*, 1956, **28**, 1756.