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IMPROVED METHODS FOR THE ENZYMIC PREPARATION AND CHROMATOGRAPHY OF OCTULOSE PHOSPHATES*

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ABSTRACT

High-yield methods are presented for the synthesis of D-*glycero*-D-*altro*-octulose 8-phosphate and 1,8-diphosphate, and D-*glycero*-*ido*-octulose 1,8-diphosphate and 8-phosphate. Formate ion-exchange and DEAE-Sephadex A-25 borate column chromatography were used for the isolation of these compounds. A new phenylboronate-containing solvent for p.c. and t.l.c. of sugar phosphates is described. The ^{13}C-n.m.r. assignments of the major contributing structure of D-*glycero*-D-*altro*-octulose 8-phosphate in aqueous solution (pH 7.0) are presented.

INTRODUCTION

The phosphoric esters of D-*glycero*-D-*ido*-octulose (D-*g*-D-*ido*-oct, **1**) and D-*glycero*-D-*altro*-octulose (D-*g*-D-*alt*-oct, **2**), shown as their acyclic forms, occur in animal tissues[1–3] and higher plants[4]; D-*glycero*-D-*manno*-octulose (D-*g*-D-*man*-oct), D-*glycero*-L-*galacto*-octulose (D-*g*-L-*gal*-oct), and L-*glycero*-L-*galacto*-octulose (L-*g*-L-*gal*-oct) have also been isolated from various plant sources[5–8]. The phosphoric esters of **1** and **2** are postulated to have an intermediary role in the pentose phosphate pathway of glucose metabolism in liver[9,10]. Although our main effort has been directed towards elucidation of the mechanistic and quantitative aspects of this metabolic process, we have also sought to improve the methods for the synthesis of these sugars, to characterise octulose phosphates in terms of their structures[11] and biological activity[10], as well as developing preparative and analytical chromatographic procedures.

Methods for the synthesis of octulose diphosphates have been described[12,13] and, like the earliest reported procedure[14], were based on the condensation of dihydroxyacetone phosphate (DHAP) with appropriate pentose 5-phosphates. Although the yields of these octuloses were often good, their purity was not always satisfactory, possibly because of minor contaminating enzymic impurities present in the commercial aldolase (EC 2.2.1.1) preparations used in their synthesis. We

*Dedicated to Professor Karl Decker on the occasion of his 60th birthday.

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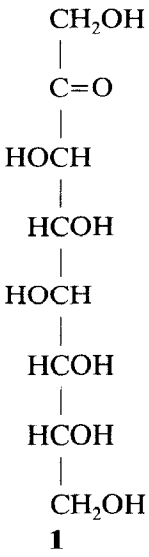

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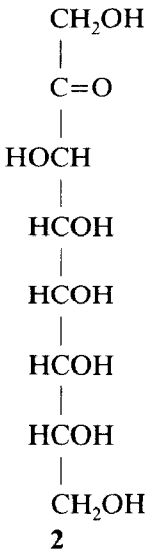

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now report satisfactory methods for the synthesis and purification of octulose diphosphates.

Improved methods for the preparation of D-*g*-D-*ido*-oct 8-P and its *altro* epimer *via* transketolase(EC 4.1.2.13)-mediated reactions involving β-hydroxypyruvate with the appropriate aldohexose 6-phosphates are also described which, in contrast to earlier methods[15], gave high yields and used only small amounts of yeast transketolase.

RESULTS AND DISCUSSION

Synthesis of octulose diphosphates. — D-*g*-D-*alt*-Oct 1,8-P_2 was synthesised from D-ribose 5-phosphate (Rib 5-P) and D-fructose 1,6-diphosphate (Fru 1,6-P_2) in the presence of aldolase and triosephosphate isomerase (EC 5.3.1.1). After purification by ion-exchange chromatography, the yield (80%) was close to that reported[13]. The product was free from Fru 1,6-P_2 as measured by the specific enzymic procedure[16]. Analysis of the total and organic phosphorus of the D-*g*-D-*alt*-oct 1,8-P_2 showed that only ~2% was not accounted for by the DHAP and Rib 5-P released when it was cleaved in the aldolase assay[13]. ^{13}C-N.m.r. analysis of D-*g*-D-*alt*-oct 1,8-P_2 showed that traces of formate were present in spite of exhaustive ether extraction of the fractions from column chromatography. Formate could be completely removed by p.c. using the GW3 solvent[17]. This two-step chromatographic purification gave an overall yield of 70–75% of D-*g*-D-*alt*-oct 1,8-P_2.

Enzymic analysis showed that <10% of the Fru 1,6-P_2 remained after 3 h of incubation at 25°. The formation of a side-product was consistently observed during the synthesis of D-*g*-D-*alt*-oct 1,8-P_2. This unidentified compound was eluted from the formate column immediately following D-*g*-D-*alt*-oct 1,8-P_2, gave a pink–orange

colour (λ_{max} at 510 nm) in the cysteine–sulphuric acid reaction[13], and released DHAP when treated with aldolase. These properties are consistent with a heptulose diphosphate[18].

The synthesis of D-*g*-D-*ido*-oct 1,8-P_2 followed the same procedure as that described for the *altro* epimer, except that D-arabinose 5-phosphate (Ara 5-P) was used and the incubation time was 18–20 h. The yield was 60–65%, and a side-product similar to that encountered during the synthesis of D-*g*-D-*alt*-oct 1,8-P_2 was formed and was eluted from the formate column just after D-*g*-D-*ido*-oct 1,8-P_2. However, the resolution was not complete and the yield of D-*g*-D-*ido*-oct 1,8-P_2 was therefore decreased.

Attempts to purify D-*g*-D-*ido*-oct 1,8-P_2 in one step, using a borate exchange procedure on DEAE-Sephadex, were only partly successful. Although the putative heptulose-diphosphate contaminant was removed by this procedure, the product was still contaminated with Fru 1,6-P_2. A further potential problem concerned the contamination of some commercial preparations of Ara 5-P with up to 1% of Rib 5-P. In view of the greater activity of Rib 5-P with muscle aldolase[13], a mixture consisting of mostly the *ido*-isomer, with a small amount of D-*g*-D-*alt*-oct 1,8-P_2, was generated but they could be separated by p.c. using the GW3 solvent containing 2% of phenylboronic acid (see below). The two-stage purification involving formate ion-exchange chromatography and p.c. gave 50–55% of a 96–97% pure product.

Fru 1,6-P_2 has been used[14,19] as a source of triose phosphate in aldolase-mediated syntheses, but the yields were low unless triosephosphate isomerase was included in the incubation mixture. This latter enzyme presumably maintained a low concentration of glyceraldehyde 3-phosphate (Gra 3-P), since resynthesis of Fru 1,6-P_2 was sufficiently slow to permit the condensation of DHAP with other aldehydes such as pentose 5-phosphate. Laing *et al.*[20] have described the synthesis of [1-^{32}P]sedoheptulose 1,7-diphosphate (Seh 1,7-P_2) from erythrose 4-phosphate (Ery 4-P) and [1-^{32}P]-Fru 1,6-P_2. Using a similar method, [1-^{32}P]-D-*g*-D-*ido*-1,8-P_2 of high specific radioactivity was prepared by incubating [1-^{32}P]-Fru 1,6-P_2 with Ara 5-P in the presence of aldolase and triosephosphate isomerase. Chromatographic and enzymic analysis of the product revealed no contamination by Fru 1,6-P_2. An alternative method involved the phosphorylation of octulose 8-phosphate with [1-^{32}P]-ATP catalysed by 6-phosphofructokinase (EC 2.7.1.11; rabbit-muscle enzyme). However, the reaction rate was considerably lower with D-*g*-D-*ido*-oct 8-P than with Fru 6-P or Seh 7-P.

Synthesis of octulose monophosphates. — The synthesis of D-*g*-D-*ido*-oct 8-P, based on the method of Datta and Racker[15], was improved by using 3 mol of β-hydroxypyruvate per mol of Glc 6-P and gave yields of >60%. The reaction

$$\text{Glc 6-P} + \beta\text{-hydroxypyruvate} \rightarrow \text{D-}g\text{-D-}ido\text{-oct 8-P} + HCO_3$$

does not proceed to completion in spite of the fact that CO_2 is formed. D-*g*-D-*ido*-Oct 8-P was obtained free from Glc 6-P by boiling to stop the reaction followed by

a second incubation (30°) for 2 h with a 2-mol excess of ATP over the remaining Glc 6-P, 20 U of glucosephosphate isomerase (EC 5.3.1.9) and 10 U of 6-phosphofructokinase. Formate ion-exchange chromatography then yielded D-*g*-D-*ido*-oct 8-P free from contamination by other sugar monophosphates. When [6-^{14}C]-Glc 6-P was the substrate, 20% of the ^{14}C was recovered in Fru 1,6-P_2, 60% in D-*g*-D-*ido*-oct 8-P, and 20% in dephosphorylated products. Spinach transketolase[21] was also used for the synthesis of D-*g*-D-*ido*-oct 8-P and gave good yields with only a short reaction period[22]. However, the preparation of the enzyme was time-consuming and significant losses of spinach transketolase activity were experienced upon prolonged storage. An alternative method for the preparation of D-*g*-D-*ido*-oct 8-P involved selective acid hydrolysis of the 1,8-diphosphates[2]. However, the method was capricious, with yields varying between 10 and 40%.

The transketolase-catalysed reaction of D-allose 6-phosphate (All 6-P) with 3 mol of β-hydroxypyruvate gave 85% of D-*g*-D-*alt*-oct 8-P. In contrast to the synthesis of D-*g*-D-*ido*-oct 8-P, the rate of formation of D-*g*-D-*alt*-oct 8-P was rapid, presumably because All 5-P is a better analogue of Rib 5-P (the most active aldose 5-phosphate for transketolase) than Glc 6-P. This method was superior, in terms of yield and purity, to that involving acid hydrolysis of D-*g*-D-*alt*-oct 1,8-P_2.

In continuing the studies of the structures of octulose phosphates[11], the ^{13}C-n.m.r. spectrum of D-*g*-D-*alt*-oct 8-P in aqueous solution was obtained. The β-furanose constituted 71% of the equilibrium mixture at pH 7.0 and 25° together with the α-furanose (16.5%) and pyranose structures (12.5%). Due to overlapping signals, only the resonances of the β-furanose structure could be assigned as follows: δ 63.3 (C-1), 102.4 (C-2), 76.7 (C-3), 75.6 (C-4), 81.4 (C-5), 72.0 (C-6), 72.0 (C-7), 66.0 ($^2J_{POC}$ 4.4 Hz, C-8). The observed $^2J_{POC}$ coupling is in the expected range. Overlapping signals from C-6 and C-7 prevented observation of $^3J_{P,C}$. The behaviour of D-*g*-D-*alt*-oct 8-P in aqueous solution is similar to that of Seh 7-P and Fru 6-P; consequently, it should be a good substrate for enzymes [transketolase, transaldolase (EC 2.2.1.2), and possibly 6-phosphofructokinase] which act on Fru 6-P and Seh 7-P.

Chromatography of octulose phosphates. — Of the many anion-exchange chromatographic procedures applied to sugar phosphates, the system[23] using Dowex 1 ($HCOO^-$) resin and formic acid–ammonium formate was selected, and gave good resolution and excellent reproducibility. In addition, the removal of ammonia and formic acid was relatively easy and could be carried out, if necessary, at low temperatures to minimise losses of the less-stable sugar phosphates. Relatively small columns (1.5 × 30 cm) did not always afford the desired resolution even if shallow gradients were used. Also, an increase in the volume of the eluting solvent resulted in unacceptably long runs as well as peak-broadening. The latter features presented particular problems in the analysis of metabolite mixtures derived from liver cells or chloroplasts.

Longer columns (1.5 × 90 cm) gave satisfactory results and were simple to operate for both analytical and preparative purposes. A typical elution profile is

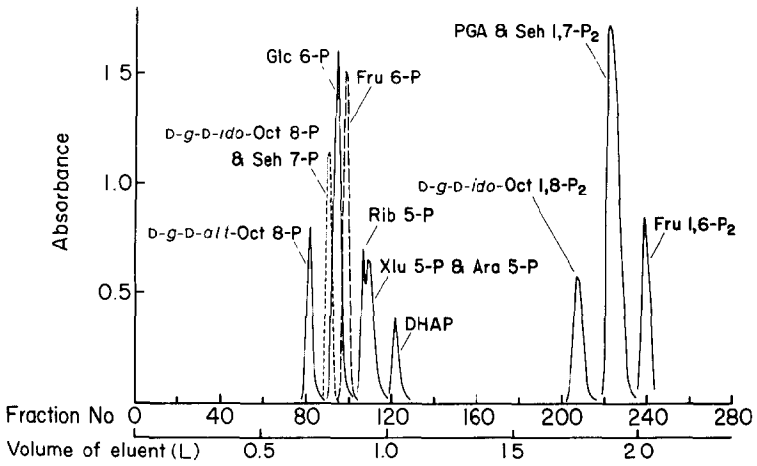

Fig. 1. Separation, by formate ion-exchange chromatography, of octulose phosphates from intermediates of the pentose phosphate pathway and of the path of carbon in photosynthesis (10 μmol of each sugar phosphate was loaded onto a 1.5 × 90-cm column; see Experimental for details).

TABLE I

THE RELATIVE MOBILITY (R_{P_i}) IN p.c.[a] OF PHOSPHORYLATED SUGAR INTERMEDIATES OF THE PENTOSE PATHWAY AND THE PATH OF CARBON REACTIONS IN PHOTOSYNTHESIS

Compound	*Mobility relative to that of inorganic phosphate* (R_{P_i})	
	GW3[b]	*GW3–PBA*[b]
3-Phosphoglyceric acid	0.80	0.81
Xylulose 5-P	0.68	0.68
Arabinose 5-P	0.64	0.64
Ribose	0.63	0.93
Ribulose 1,5-P_2	0.42	0.43
Glucose 6-P	0.38	0.39
Fructose 6-P	0.47	0.47
Fructose 1,6-P_2	0.35	0.35
Allose 6-P	0.46	0.46
Altrose 6-P	0.41	0.41
Sedoheptulose 7-P	0.40	0.50
Sedoheptulose 1,7-P_2	0.29	0.29
D-*g*-D-*ido*-Oct 8-P	0.41	1.20[c]
D-*g*-D-*ido*-Oct 1,8-P_2	0.29	0.76
D-*g*-D-*alt*-Oct 8-P	0.43	0.43
D-*g*-D-*alt*-Oct 1,8-P_2	0.26	0.26

[a]See Experimental. [b]GW3 solvent: 1-butanol–1-propanol–acetone–80% (w/v) formic acid–30% (w/v) trichloroacetic acid (40:20:25:25:15); GW3–PBA contains 2% of phenylboronic acid. [c]Elongated "spot".

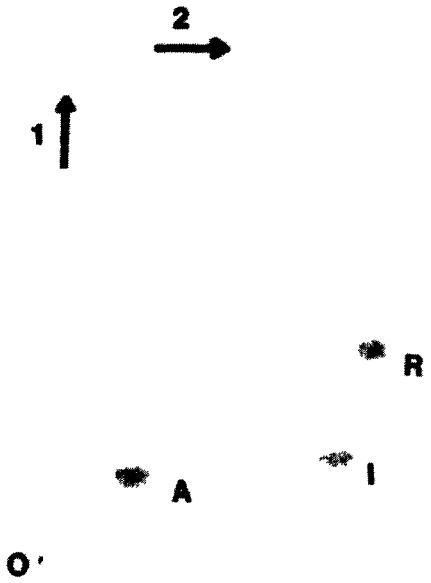

Fig. 2. Two-dimensional t.l.c. of Rib 5-P (R), D-*g*-D-*alt*-oct 1,8-P_2 (A), and D-*g*-D-*ido*-oct 1,8-P_2 (I), using the GW3 solvent[17] and then the GW3–2% PBA solvent. The chromatogram was developed twice in each direction, using the ascending mode. Sugar phosphates were visualised with the molybdate reagent[30] The chromatogram was loaded with 0.05 μmol of R and 0.015 μmol of A and I. O' marks the origin.

shown in Fig. 1. The separations compare favourably with those reported by Heldt *et al.*[24] who used capillary ion-exchange columns. The larger columns were preferred because the separations were more reproducible than those obtained with capillary columns. Formate columns were not suitable for some separations involving such pairs of epimers as *altro*- and *ido*-octulose phosphates or Alt 6-P and All 6-P. This problem was solved by a modification of the procedure of Lefebvre *et al.*[25], using DEAE-Sephadex columns and borate buffers. In agreement with these workers, we obtained sharper peaks with triethylamine tetraborate than with ammonium tetraborate as solvent. Nevertheless, the latter was chosen for routine work as it proved easier to remove by vacuum distillation with methanol. DEAE-Sephadex A-25 used for these separations consistently gave better resolution than borate ion-exchange resins. A major disadvantage involved its notably lower capacity than that of the Dowex resins. Hence, in some separations (the synthesis of All 6-P), it was necessary to resort to rather large columns (2.6 × 100 cm) to prevent overleading. In contrast to formate columns, very little improvement in resolution was observed by increasing the column size. Consequently, the final

choice of column was determined by the amount of material to be chromatographed.

A p.c. and t.l.c. method was also developed using the GW3 solvent[17] modified by the inclusion of 2% of phenylboronic acid which enhanced the mobility of certain sugar phosphates. Such compounds as D-ribose and D-*g*-D-*ido*-oct, or their phosphates, formed stable complexes with boronate which had much higher mobilities (Table I). The data of Table I were obtained after two ascending developments. For the isolation of Rib 5-P from other pentose phosphates, chromatograms run for 16 h in the descending mode gave the best resolution.

Phenylboronic acid was easily removed from the eluted sugar phosphates by extraction with ether. The phenylboronic acid-containing solvent was most useful for the resolution, analysis, and purification of octulose diphosphates by t.l.c. when only small amounts of material were available. Fig. 2 shows the excellent separation of Rib 5-P, D-*g*-D-*alt*-oct 1,8-P_2, and D-*g*-D-*ido*-oct 1,8-P_2 by two-dimensional chromatography using the GW3 solvent and then the GW3–phenylboronic acid solvent.

The application of phenylboronic acid in the chromatography of free sugars was first reported by Bourne *et al.*[26]; we believe that the results reported here represent its first application to phosphorylated sugars.

EXPERIMENTAL

Materials. — Yeast transketolase was obtained from Sigma; all other enzymes were from Boehringer Mannheim. P.c. was performed on Whatman 3MM paper and t.l.c. on cellulose-coated plates (Merck).

Enzymic analysis of octulose phosphates. — A Gilford-2600 single-beam spectrophotometer was used.

(*a*) Octulose diphosphates were assayed by measuring the DHAP liberated in an aldolase-catalysed reaction. The mixture (total volume of 1 mL) contained 100mM TEA-HCl buffer (pH 7.6), 0.16mM NADH, α-glycerol 3-phosphate dehydrogenase (EC 1.1.1.8, 1.7 U), rabbit muscle aldolase (1.8 U), oct 1,8-P_2 (0.04–0.1 μmol); 9 units of aldolase were used for the assays of the *ido* epimer, to ensure rapid completion of the reaction.

(*b*) Octulose 8-phosphates were determined by measuring the amount of Fru 6-P and/or Glc 6-P formed in a transketolase-catalysed reaction with Ery 4-P as the aldehyde acceptor. The reaction mixture (total volume of 1.0 mL) contained 80mM TEA-HCl buffer (pH 7.6), 0.3mM $NADP^+$, 0.3mM Ery 4-P, 10mM $MgCl_2$, 0.1mM TPP, yeast transketolase (0.5 U), glucosephosphate isomerase (1.7 U), and D-glucose 6-phosphate dehydrogenase (EC 1.1.1.49, 1.4 U).

(*c*) Fructose 1,6-diphosphate was assayed as described by Lang and Michal[16], except that the reaction mixture contained rabbit-muscle fructose 1,6-diphosphatase (0.5 U).

Colorimetric reactions of sugar phosphates. — Cysteine–sulphuric acid tests were performed by the method of Paoletti *et al.*[13], and the spectra of the products

were recorded by using the Gilford 2600 single-beam spectrophotometer coupled to a Hewlett–Packard 7225A plotter. The orcinol reaction for pentose phosphates[27] and the anthrone reactions for hexose phosphates[28] were carried out as described. Inorganic phosphate was measured by the method of Tashima and Yoshimura[29]. Phosphate-containing compounds after p.c. or t.l.c. were visualised using the phosphate staining reagent[30].

^{13}C-N.m.r. spectroscopy. — The spectrum of D-*g*-D-*alt*-oct 8-P (85 μmol) was recorded with a Jeol FX 200 spectrometer (50.3 MHz) and a solution in D_2O (0.5 mL; internal MeOH, δ 49.7 relative to the signal for Me_4Si) adjusted to pH 7.

Synthesis of sugar phosphates. — D-*g*-D-*alt*-Oct 1,8-P_2 was prepared by reacting Rib 5-P (600 mg, 2 mmol), Fru 1,6-P_2 (75 μmol), aldolase (45 U), and triosephosphate isomerase (500 U) in 50mM Tris-HCl buffer (20 mL, pH 7.6) for 3 h at 25°. After deproteinisation with 0.6M perchloric acid and KOH, the mixture at pH 6.8 was eluted from a column (1.5 × 90 cm) of ($HCOO^-$)-resin, using a linear gradient generated by the addition to H_2O (1.6 L) of 4M HCOOH–M $HCOONH_4$ at 0.65 mL.min^{-1}. Ammonium formate was removed from the combined fractions containing oct 1,8-P_2 by using an (H^+)-resin and continuous extraction with ether. The pH of the extracted solution was adjusted to 6.8 with sodium hydroxide and the water was removed by lyophilisation. The yield of the oct 1,8-P_2 was 80–90% based on Fru 1,6-P_2. D-Allose 6-phosphate was synthesised[31] from Rib 5-P. Condensation of the Rib 5-P with cyanide afforded a mixture of epimeric hexonitrile phosphates which were catalytically hydrogenated without prior purification. The resulting mixture of allose and altrose 6-phosphates was fractionated on a column (2.6 × 100 cm) of DEAE-Sephadex A-25, using a 0.05→0.4M linear gradient (3.5 L) of ammonium tetraborate (pH 8.4). The individual sugar phosphates were then passed through a column (2.6 × 100 cm) of Sephadex G-10 in order to remove residual salts. Colorimetric analysis (anthrone) indicated a 20% yield of allose 6-P. The details of the synthesis as well as the ^{13}C-n.m.r. structural analysis of this sugar will be reported elsewhere.

D-*g*-D-*alt*-Oct 8-P was prepared by reacting allose 6-P (100 μmol) and β-hydroxypyruvate (300 μmol) with transketolase (20 U), as described for the synthesis of the *ido* epimer except that the incubation time was extended to 3 h. The deproteinised mixture was eluted from a column of ($HCOO^-$)-resin, using a linear gradient of formic acid–ammonium formate as described for the syntheses of octulose diphosphates, to give 80% of D-*g*-D-*alt*-oct 8-P. ^{13}C-N.m.r. analysis indicated no contamination by D-allose 6-P.

D-*g*-D-*ido*-Oct 1,8-P_2 was synthesised as follows. A mixture (total volume, 10 mL) at pH 7.6 containing Tris-HCl (500 μmol), Ara 5-P (800 μmol), Fru 1,6-P_2 (25 μmol) (equivalent to 50 μmol of triose-P), aldolase (45 U), and triosephosphate isomerase (500 U) was incubated at 25° for 16–18 h. The reaction was terminated by ultrafiltration to remove protein, using an Amicon PM-10 membrane (molecular weight cut-off, 10,000). The filtrate was applied to a column (1.5 × 90 cm) of ($HCOO^-$)-resin and eluted as described for D-*g*-D-*alt*-oct 1,8-P_2. Arabinose 5-P was

located in the fractions by using the orcinol reagent[27], and the sugar diphosphate fractions were identified by using the cysteine–sulphuric acid reagent[13] and by specific enzymic assay[14] (Fru 1,6-P_2 only). The combined fractions containing D-*g*-D-*ido*-oct 1,8-P_2 (60–65% yield) were processed as described below.

D-*g*-D-*ido*-Oct 8-P was prepared by reacting (total volume, 15 mL), at pH 7.6, Glc 6-P (80 μmol), β-hydroxypyruvate (240 μmol), transketolase (20 U), TEA-HCl (1200 μmol, pH 7.4), $MgCl_2$ (150 μmol), and TPP (1.5 μmol) for 24 h at 30°. The reaction was terminated by removal of enzyme protein using the ultrafiltration procedure described above. The filtrate was pumped onto a column (1.5 × 90 cm) of ($HCOO^-$)-resin and eluted as described for the synthesis of D-*g*-D-*alt*-oct 1,8-P_2. Following removal of the solvent (for details see below), D-*g*-D-*ido*-oct 8-P was recovered (60% yield). Specific enzymic analysis showed there was no contamination of the product with Glc 6-P.

Formate ion-exchange chromatography. — The separation of sugar phosphates was carried out on columns (1.5 × 90 cm) of AG-1 X8 ($HCOO^-$)-resin (200–400 mesh). Sugar phosphates were pumped onto the column which was then washed with deionised water (150 mL). A linear formate gradient (0.65 mL.min^{-1}, using an LKB Microperspex peristaltic pump) from 1.6 L of H_2O and 1.6 L of 4M HCOOH–M $HCOONH_4$ was applied. For quantitative analysis, 4 L of eluent were used which notably improved the resolution of the monophosphate region of the chromatogram. Fractions (6.5 mL) were collected and each second fraction was tested with the appropriate colour reagents for sugar phosphates. Fractions containing sugar phosphates were combined and passed through a column (80 mL) of Dowex 50W (H^+) resin (100–200 mesh) together with 1.5 bed-volumes of deionised H_2O. The eluate was concentrated to 50 mL at 40°, then continuously extracted with ether for 12–14 h to remove formic acid, neutralised with NaOH to pH 6.8, and lyophilised. The recoveries of sugar phosphates were 70–95%, depending upon the sugar being processed.

Ion-exchange chromatography on DEAE-Sephadex A-25. — DEAE-Sephadex A-25 was pre-swollen and converted into the borate form by repeated washings with 0.1M ammonium tetraborate (pH 8.2–8.4). Excess of tetraborate was removed by washing with deionised water (2 L). The gel was degassed under vacuum and packed into columns (1.5 × 40 cm) which were adequate for the resolution of a total of 35 mequiv. of sugar phosphate. For up to 265 mequiv. of sugar phosphate, 2.6 × 100-cm columns were used. Ideally, only ~15% of these quantities should be applied to the columns. The pH of the sample was adjusted to 8.2–8.4 with NH_4OH. Sugar phosphates were eluted (0.65 mL.min^{-1}) by using a linear gradient of 0.05 and 0.4M ammonium tetraborate. A total of 1.5 L of gradient was used for the smaller columns and 3.5 L for the larger column. Sugar phosphate-containing fractions were located with the appropriate colour reagents, combined, and concentrated to near dryness. Boric acid was then removed[32] by repeated evaporation of methanol (100-mL portions) from the residue. In some instances, a significant amount of salt was left and such samples were passed through a column

(2.6 × 100 cm) of Sephadex G-10 using deionised water. Sugar phosphates obtained in this way (85–95% recoveries) were converted into the sodium salt by passing through an (H^+)-resin and adjusting the pH of the eluate to 6.8 with NaOH.

P.c. and t.l.c. — The GW3 solvent[17] or its modified version containing 2% of phenylboronic acid was used. The latter solvent permitted clear separations of epimeric sugar phosphates, such as Rib 5-P, Ara 5-P, or D-*g*-D-*ido*-oct 1,8-P_2 and D-*g*-D-*alt*-oct 1,8-P_2. Chromatograms were run twice in the ascending mode and marker compounds were visualised with the molybdate reagent[30]. Appropriate sections of the paper chromatograms were excised and eluted with deionised water for 6–8 h. Residual trichloracetic acid and phenylboronic were then removed by continuous extraction with ether. Although t.l.c. was used only for analytical purposes, sugar phosphates could be recovered by removing the cellulose coating from the plate, extracting it with water, and then processing as described for p.c.

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