THE SYNTHESIS OF SUCROSE 6′-PHOSPHATE

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ABSTRACT

Sucrose 6′-phosphate, the monophosphate of sucrose which is an intermediate in the biosynthesis of sucrose, has been synthesised by an unambiguous route. The known sucrose 2,3,6,3′,4′-penta-acetate (3) was converted into 6′-*O*-tritylsucrose (5) which, on per-*O*-acetylation and detritylation, afforded crystalline sucrose 2,3,4,6,1′,3′,4′-hepta-acetate (8). Phosphorylation of **8** by means of cyanoethyl phosphate and dicyclohexylcarbodiimide, followed by removal of protecting groups, yielded sucrose 6′-phosphate (1), isolated as the barium and potassium salts. The synthetic compound was enzymically active towards wheat-germ UDP-D-glucose:D-fructose 6-phosphate 2-D-glucosyltransferase (sucrose 6′-phosphate synthetase).

INTRODUCTION

The existence of a sucrose monophosphate in sugar-beet leaves was demonstrated by Calvin, Benson and their co-workers[1,2] in 1952 and its role in sucrose biosynthesis postulated[1-4]. It was shown that the phosphate group was attached to the D-fructose residue, but the precise location was not proved. The tentative conclusion[2] that it was sucrose 1′-phosphate[†] is rendered unlikely because many workers have now shown that the sucrose phosphate from several sources[6-11], including sugar beet[7], is the 6′-phosphate. The enzymic synthesis of sucrose 6′-phosphate from "uridine diphosphate glucose" and D-fructose 6-phosphate is now well-established[6-11].

The work described in this paper was directed towards a synthesis of sucrose 6′-phosphate (1). A synthetic sucrose monophosphate had previously been prepared by Neuberg and Pollak in 1910 by treatment of an aqueous solution of sucrose with phosphorus oxychloride in chloroform, in the presence of calcium oxide. The product was examined by Hatano[13] and by Courtois and Ramet[14-17]; acid hydrolysis yielded D-fructose and a mixture of D-glucose phosphates (Hatano's ester), later shown to contain D-glucose 2-phosphate[16]. More recently, modifications of the Neuberg synthesis have been reported in patents issued to the Colonial Sugar Refining Co.

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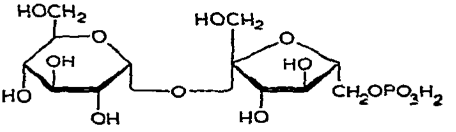
[*] To whom enquiries should be addressed, at the Department of Chemistry, Heriot-Watt University, Edinburgh EH1 1HX, Great Britain. A brief account of this work is given in J. Yudkin, J. Edelman, and L. Hough (Eds.), *Sugar*, Butterworths, London, 1971, Chapter 8.

[†] The system of nomenclature for the sucrose molecule, in which primed numbers refer to the D-fructose component, is that of Hockett and Zief[5].

Ltd., of Australia[18], and the resulting calcium sucrose phosphate has been used as an inhibitor of dental caries[19,20]. There is no doubt that all of these preparations contain mixtures of sucrose phosphates[17,21]. No unambiguous synthesis of a single isomer of sucrose monophosphate has previously been reported; MacDonald and Wong have described a synthesis of trehalose 6-phosphate, an intermediate in the biosynthesis of trehalose[22].



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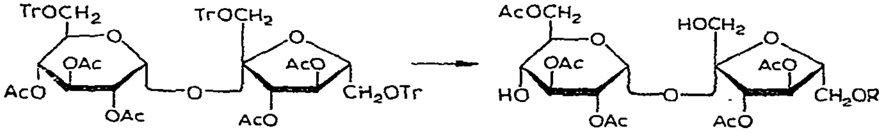
RESULTS AND DISCUSSION

The essential problem to be solved in a rational synthesis of sucrose 6′-phosphate was that of discriminating between the three primary hydroxyl groups in the sucrose molecule. A suitable intermediate appeared to be the sucrose penta-acetate **3** described by McKeown *et al.*[23]. When the triphenylmethyl ether **2** is heated in aqueous acetic acid, acetyl migration occurs as well as detritylation[24–26]. The resulting penta-acetate **3** now has only two primary hydroxyl groups, and there was a possibility that the C-6′ hydroxyl group might be the less-hindered of the two. The route to sucrose 6′-phosphate which was evisaged was **2**→**3**→**4**→**7**→**8**→**10**→**1**.



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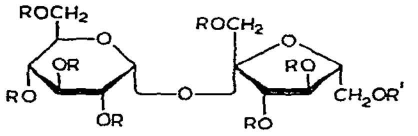
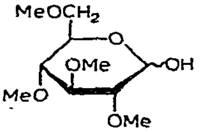
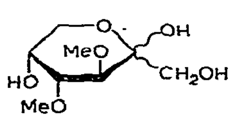
3 R = H

4 R = Tr







5 R = H, R′ = Tr

6 R = Me, R′ = Tr

7 R = Ac, R′ = Tr

8 R = Ac, R′ = H

9 R = Ac, R′ = p-$SO_2C_6H_4NO_2$

10 R = Ac, R′ = $P(O_2H)OCH_2CH_2CN$

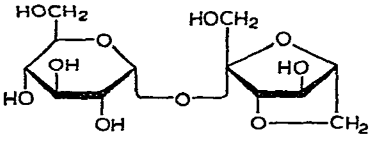
11

12

The trityl ether **2** was prepared in 66% yield from sucrose by a modification of the original procedure[23]. It was converted into the penta-acetate **3**, by treatment with 98% acetic acid, in 43% yield. Many trials were made to establish the optimal conditions for the monotritylation of **3** by chlorotriphenylmethane in pyridine. The effect of variation in temperature and in the concentration of reactants was studied by t.l.c. The best method for isolation of a monotrityl ether was found to be to deacetylate the crude product, containing mainly **4**; crystalline 6'-O-tritylsucrose (**5**) was isolated in 53% yield. The structure of **5** was proved by methylation. Treatment of a solution of **5** in *N,N*-dimethylformamide with methyl iodide and silver oxide[27] afforded the permethyl ether **6**, acid hydrolysis of which yielded the methyl ethers **11** and **12**, both in crystalline form.

Acetylation of the trityl ether **5** gave the hepta-acetate **7** in high yield. A careful study was made to determine the optimal conditions for effecting the conversion of **7** into **8**. When **7** was heated with 98% aqueous acetic acid at 100° for 1 h, detritylation was complete, but although the product was mainly the sucrose hepta-acetate **8** it also contained the octa-, hexa-, and penta-acetates. Hydrolysis with 80% acetic acid at 40° for 15 h gave a chromatographically homogeneous preparation of hepta-acetate. A more rapid, and equally clean, detritylation was obtained by using[25] a solution of hydrogen bromide in acetic acid at 0°. However, if this reagent is kept in contact with the product for longer than 2 min, conversion into other sucrose acetates takes place.

Although it was very unlikely that any acetyl migration had taken place during the conversion of **7** into **8**, a confirmation of the structure of **8** was carried out. Treatment of **8** with *p*-nitrobenzenesulphonyl chloride in pyridine yielded a syrupy product, presumably **9**, which was converted by means of sodium ethoxide into the anhydrosucrose **13**. An acid hydrolysate of **13**, when examined by paper chromatography, showed the presence of glucose and an unknown sugar, but no fructose. The unknown sugar was probably the expected 3,6-anhydro-D-fructose (**14**), because when the acid hydrolysate was reduced with sodium borohydride the products were D-glucitol, 1,4-anhydro-D-mannitol (**15**), and 3,6-anhydro-D-glucitol (**16**). The anhydrohexitols were identified by paper chromatography and by g.l.c.



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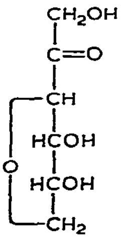

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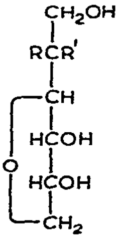

15 R= OH, R'= H
16 R= H, R'= OH

The hepta-acetate **8** was treated with cyanoethyl phosphate and dicyclohexylcarbodiimide in pyridine[28]. The crude product, containing the phosphodiester **10**, was treated with an excess of aqueous barium hydroxide in order to remove the protecting groups and to hydrolyse the excess of reagent. Sucrose 6'-phosphate (**1**) was isolated as the barium salt, which was an amorphous solid. Attempts to form a crystalline cyclohexylamine salt were unsuccessful, but a microcrystalline potassium salt was prepared. Enzymic dephosphorylation yielded sucrose and inorganic phosphate, and acid hydrolysis gave glucose and a fructose phosphate having the chromatographic properties of fructose 6-phosphate.

Dr. A. J. Keys, of Imperial College, very kindly examined the synthetic sucrose 6′-phosphate in the wheat-germ UDP-D-glucose:D-fructose 6-phosphate 2-D-glucosyltransferase system ("sucrose 6′-phosphate synthetase")[6,10], which mediates the reaction D-fructose 6-phosphate + "uridine diphosphate glucose" $\rightleftharpoons$ sucrose 6′-phosphate + uridine diphosphate.

When the synthetic compound **1**, uridine diphosphate, and ^{14}C-labelled D-fructose 6-phosphate were added to the enzyme, t.l.c. clearly showed incorporation of radioactivity into the sucrose phosphate, due to the reversibility of the reaction. No incorporation was observed in the absence of any of the components. When the ^{14}C-sucrose phosphate was isolated, it was found to yield ^{14}C-sucrose on enzymic dephosphorylation. Subsequent hydrolysis of the ^{14}C-sucrose by invertase showed that only the D-fructose moiety contained ^{14}C.

Since the completion of the work described in this paper[29], Otake[30] has published the preparation of some of the intermediates we have used. Some discrepancies in physical properties call for comment. Otake subjected sucrose to monotritylation and, by countercurrent distribution, crystallisation, and chromatography on silica gel, was able to isolate 6′-*O*-tritylsucrose (**5**) as a solid, "softening point" 149°, $[\alpha]_D^{20}$ +31.9° (*c* 4.0, *N*,*N*-dimethylformamide). In our hands, **5** crystallised as a hydrate, m.p. 221–223° (with shrinkage at 110°), $[\alpha]_D^{20}$ +40.1° (chloroform). We are in agreement that the heptamethyl ether **6** is a glass (there appears to be a transposition of the compound numbers in the Experimental section of Otake's paper). We found that the hepta-acetate **7** crystallised; from aqueous methanol, it had m.p. 68–70°, possibly a hydrate; from methanol, it had m.p. 118°. The 100-MHz n.m.r. spectrum of **7** is identical (in the region τ 4–7) with that published by Otake[30]. The sucrose hepta-acetate **8**, crystallised from ethyl acetate–light petroleum, had m.p. 160°. Otake's preparation, crystallised from aqueous ethanol, had m.p. 93–94°, but again the n.m.r. spectrum of **8** is identical to the published spectrum[30]. These discrepancies must be due to differences in crystalline form, or in solvation[31].

EXPERIMENTAL

Evaporations were carried out by using a rotary evaporator at bath temperatures below 40°. Melting points are uncorrected. Infrared spectra were measured for potassium bromide discs. Light petroleum refers to the fraction of b.p. 60–80°.

Chromatographic methods. — Adsorption chromatography was carried out on silica gel (Hopkin and Williams); when trityl ethers were chromatographed, the silica gel was pretreated with ammonia[32]. T.l.c. used Kieselgel G (Merck) as adsorbant, detection being with anisaldehyde–sulphuric acid[33].

Paper chromatography was carried out on Whatman No. 1 paper, using the following solvent systems: (*A*) butyl alcohol–pyridine–water (6:4:3, v/v); (*B*) propyl alcohol–conc. ammonia–water (6:3:1, v/v); (*C*) butanone saturated with water;

(*D*) methanol–conc. ammonia–water (6:3:1, v/v); *tert*-butyl alcohol–water–picric acid (80 ml:20 ml:2 g); (*F*) butyl alcohol–pyridine–water (6:3:1, v/v). The following spray reagents were used: (*1*) periodate and Schiff's reagent for α-glycols[34]; (*2*) *p*-aminophenol–phosphoric acid for reducing sugars and readily hydrolysable glycosides[35]; (*3*) alkaline silver nitrate for sugars and alditols[36]; (*4*) molybdic acid for phosphoric esters[37]; (*5*) α-naphthol–phosphoric acid for fructose derivatives[38]; (*6*) aniline hydrogen phthalate for reducing sugars[39].

*2,3,4,3',4'-Penta-*O*-acetyl-6,1',6'-tri-*O*-tritylsucrose* (**2**). — Dry, finely ground sucrose (20 g) was dissolved in dry pyridine (400 ml) by agitation at room temperature for 24–36 h. Chlorotriphenylmethane (57.2 g, 3.5 molar equiv.) was added, the mixture was shaken until the solid had dissolved, and the resulting solution was heated at 80° for 2 h. After cooling, the mixture was treated with acetic anhydride (100 ml) for 24 h at room temperature, and then poured into water (4 litres). The resulting solid was filtered off and washed with water. After recrystallisation from acetone–methanol (charcoal), the trityl ether **2** (49.4 g, 66%) had m.p. 231–233° and was indistinguishable from a sample prepared by the published method[23].

*2,3,6,3',4'-Penta-*O*-acetylsucrose* (**3**). — The trityl ether **2** (44 g) was dissolved in hot glacial acetic acid (1.1 litres), water (22 ml) was added, and the solution was heated under reflux for 30 min. After cooling, the mixture was evaporated to dryness, ether (300 ml) was added to the residue, and the mixture was left overnight. The crystals were filtered off, washed with ice-cold ether, and recrystallised from chloroform–ether. The penta-acetate **3** (8.2 g, 43%), m.p. 154–156°, was chromatographically homogeneous and indistinguishable from an authentic sample[23].

*6'-*O*-Tritylsucrose* (**5**). — The penta-acetate **3** (6.92 g) was dissolved in pyridine (140 ml) and treated with chlorotriphenylmethane (3.52 g, 1.01 molar equiv.) at 50° for 20 h. More chlorotriphenylmethane (1.80 g, 0.51 molar equiv.) was added, and heating was continued for a further 12 h. By means of chloroform, the product was isolated as a syrup, which was heated under reflux for 2 h with methanol containing a catalytic amount of sodium methoxide. Evaporation of the solvent left a colourless solid which was dissolved in ethyl acetate (250 ml), and the solution was washed twice with water and dried (Na_2SO_4). The filtered solution was evaporated and the residue crystallised from ethyl acetate containing a little methanol. Recrystallisation afforded chromatographically homogeneous 6'-*O*-tritylsucrose (**5**) (3.9 g, 52%), m.p. 221–223° (shrinks at *ca.* 110°), $[\alpha]_D^{20}$ +40.1° (*c* 0.86, chloroform) (Found: C, 61.2; H, 6.1. $C_{31}H_{36}O_{11}\cdot H_2O$ calc.: C, 61.8; H, 6.3%).

*Methylation and hydrolysis of 6'-*O*-tritylsucrose* (**5**). — The trityl ether **5** (250 mg) was dissolved in *N*,*N*-dimethylformamide (6 ml), and methyl iodide (2 ml) was added. The solution was stirred vigorously at room temperature (magnetic stirrer) while freshly prepared silver oxide (2 g) was added in portions over 45 min. Further quantities of methyl iodide (0.5 ml) and silver oxide (0.5 g) were added after 8 and 24 h. After 48 h, the reaction mixture was filtered and the precipitate washed with *N*,*N*-dimethylformamide (10 ml) and chloroform (10 ml). The combined filtrate was mixed with water (50 ml), and potassium cyanide (0.5 g) was added. The mixture was extracted with chloroform (5 × 10 ml), and the combined chloroform extracts were washed with aqueous potassium cyanide (25 ml of 2%) and water (4 × 10 ml). The dried chloroform solution was evaporated to dryness, and final traces of *N*,*N*-dimethylformamide were removed by evaporation with butyl alcohol. The resulting yellow oil was shown by t.l.c. to consist mainly of a single trityl ether contaminated with triphenylmethanol and some partially methylated derivatives. The product was dissolved in light petroleum–benzene (3:7) and chromatographed on neutral silica gel[32]. Benzene–ether (17:3) eluted the permethyl ether **6** (200 mg, 69%) as a chromatographically homogeneous, pale-yellow syrup, $[\alpha]_D^{20}$ +55.3° (*c* 7.2, chloroform). The trityl ether **6** was dissolved in acetone (8.3 ml), 2M hydrochloric acid (1.7 ml) was added, and the solution was heated in a sealed tube at 55° for 10 h. T.l.c. (ether) showed the presence of triphenylmethanol, 2,3,4,6-tetra-*O*-methyl-D-glucose (**11**) (double spot), and 1,3,4-tri-*O*-methyl-D-fructose (**12**), indistinguishable from authentic samples.

The hydrolysate was neutralised with Dowex-2(HCO_3^-) resin and filtered, and the filtrate was evaporated to dryness. The residue was dissolved in benzene and chromatographed on silica gel. Benzene–chloroform (19:1) eluted triphenylmethanol. Benzene–chloroform (4:6) eluted 2,3,4,6-tetra-*O*-methyl-D-glucose (**11**, 63 mg) which, after distillation (80°/0.001 mmHg), crystallised in the α-form (37 mg), m.p. 86–88°, $[\alpha]_D^{20}$ +92.5° (10 min)→+82° (24 h) (*c* 0.38, water), identical (t.l.c., mixed m.p., i.r. spectrum) with an authentic sample[40,41]. Benzene–chloroform (1:4) eluted 1,3,4-tri-*O*-methyl-D-fructose (**12**) which, after distillation (85°/0.001 mmHg), was a syrup, crystallising from ether as prisms (30 mg), m.p. 75–76°, $[\alpha]_D^{20}$ −59.9° (19 h) (*c* 0.7, water), identical (t.l.c., mixed m.p., i.r. spectrum) with an authentic sample[42] given to us by Dr. D. J. Bell.

*2,3,4,6,1′,3′,4′-Hepta-*O*-acetyl-6′-*O*-tritylsucrose* (**7**). — The trityl ether **5** (2 g) was treated with pyridine (26 ml) and acetic anhydride (20 ml) overnight at room temperature. The solution was poured, with stirring, into iced water (200 ml), and the solid was filtered off and washed with water. Recrystallisation from aqueous methanol gave the hepta-acetate **7** as needles (3.0 g, 99%), m.p. 68–70°. When crystallised from methanol, it had m.p. 118°, $[\alpha]_D^{20}$ +58.5° (*c* 0.96, chloroform) (Found: C, 61.7; H, 5.2. $C_{45}H_{50}O_{18}$ calc.: C, 61.5; H, 5.7%). The n.m.r. spectrum at 100 MHz in $CDCl_3$ solution showed a complex multiplet at τ 2.50–2.80 (15 protons, trityl) and a group of partially superimposed singlets at τ 7.92–8.10 (21 protons, 7 C-Me); the remainder of the spectrum was identical to that published by Otake[30].

*2,3,4,6,1′,3′,4′-Hepta-*O*-acetylsucrose* (**8**). — (*a*) The trityl ether **7** (2.8 g) was dissolved in glacial acetic acid (5 ml) and cooled in ice. A solution of hydrogen bromide in acetic acid [45%(w/v), 0.65 ml] was added; a precipitate of trityl bromide formed immediately. After 1–2 min at 0°, the mixture was filtered into a Buchner flask containing ice and solid sodium hydrogen carbonate. The product was isolated by using chloroform and crystallised from ethyl acetate–light petroleum, giving the heptaacetate **8** (2.0 g, 98%), m.p. 160°, $[\alpha]_D^{20}$ +49.5° (*c* 0.4, chloroform) (Found: C, 49.1; H, 5.8. $C_{26}H_{36}O_{18}$ calc.: C, 49.1; H, 5.7%). The n.m.r. spectrum at 100 MHz in $CDCl_3$ solution showed a group of partially superimposed singlets at τ 7.82–8.00 (21 protons, 7C-Me); the remainder of the spectrum was identical to that published by Otake[30].

(*b*) The trityl ether **7** (2.0 g) was dissolved in aqueous acetic acid [80%(v/v), 50 ml] and heated at 40° for 15 h. The solvent was evaporated at 50°, leaving a syrupy residue which was crystallised from ethyl acetate–light petroleum to give the hepta-acetate **8** (1.2 g, 83%) identical to the compound prepared in (*a*).

3′,6′-Anhydrosucrose (**13**). — The hepta-acetate **8** (204 mg) was dissolved in pyridine (4 ml) and nitrobenzene-*p*-sulphonyl chloride (148 mg) was added at 0°. After 3 days at room temperature, the product was isolated, using chloroform, to give an orange syrup, which was heated under reflux with ethanol (25 ml) containing sodium ethoxide (from 0.52 g of sodium) for 2 h. After addition of solid carbon dioxide, the mixture was evaporated to dryness, and the residue was dissolved in water. The solution was passed through columns of Dowex-50(NH_4^+) and Dowex-2(HCO_3^-) resins, and the final eluate, with aqueous washings, was evaporated to dryness. Paper chromatography (solvents *A* and *B*) showed one spot with reagents *1*, *2*, and *3*. Crystallisation from methanol afforded 3′,6′-anhydrosucrose (**13**) (78 mg, 71%), m.p. 146°, $[\alpha]_D^{20}$ +104° (*c* 1.2, methanol) (Found: C, 41.9; H, 6.7. $C_{12}H_{20}O_{10}\cdot H_2O$ calc.: C, 42.1; H, 6.4%). The same anhydrosucrose was prepared from the corresponding bromobenzene-*p*-sulphonate.

Acid hydrolysis of 3′,6′-anhydrosucrose (**13**). — The sugar **13** (40 mg) was heated with 0.1M hydrochloric acid (8 ml) at 100° for 5 min. After evaporation to dryness, the residue was examined by paper chromatography in solvent *A*. Reagent *1* showed the presence of glucose (R_{SUC} 1.24) and a compound giving a yellow colour[34] (R_{SUC} 3.04), presumably 3,6-anhydro-D-fructose (**14**).

The residue was dissolved in water (8 ml), sodium borohydride (40 mg) in water (2 ml) was added, and the solution was left overnight at room temperature. The solution was neutralised (pH 7) with acetic acid, passed through a column of Dowex-50 (NH_4^+) resin, and then concentrated to 2 ml. Methanol (10 ml) was added and the solution evaporated to dryness at 70°, a process that was repeated four times. Paper chromatography in solvent *C*, using reagent *1*, showed the presence of D-glucitol (intense purple spot appearing rapidly), 3,6-anhydro-D-glucitol, and 1,4-anhydro-D-mannitol ($R_{Glucitol}$ 4.61 and 5.49, respectively). The spots due to the anhydrohexitols were yellow, changing to dark green[34], and were identified by means of authentic samples.

The syrupy residue was dissolved in water and fractionated on a small column of Dowex-1(HO^-) resin[43]. Fractions were examined by paper chromatography in solvent *C*. The fractions containing D-glucitol were combined and evaporated, and the syrupy residue was acetylated by using acetic anhydride (0.5 ml) and pyridine (1 ml). The hexa-acetate, isolated by means of chloroform, crystallised from aqueous ethanol and had m.p. 98–100°; it was indistinguishable from an authentic sample (i.r., mixed m.p.). Later column fractions contained the mixture of anhydrohexitols. The fractions were combined and examined by g.l.c. as trimethylsilyl ethers[44], using a Pye 104 gas chromatograph with flame-ionisation detector. The stationary phase was poly(ethylene glycol succinate), the carrier gas nitrogen, and the operating temperature 182°. The two peaks corresponded exactly to 3,6-anhydro-D-glucitol and 1,4-anhydro-D-mannitol, the latter in larger amount. The authentic compounds were used for co-chromatography.

Sucrose 6'-phosphate (**1**). — (*a*) *Barium salt.* The hepta-acetate **8** (318 mg, 0.5 mmole) was dissolved in pyridine (5 ml) and added to the pyridinium salt of 2-cyanoethyl phosphate prepared from the barium salt (322 mg, 1 mmole) by the use of Dowex-50 (H^+) resin, neutralisation with pyridine, and evaporation. The solution was evaporated to dryness at 30°, and the process was repeated twice with 5-ml portions of dry pyridine. The residue was dissolved in pyridine (5 ml), dicyclohexylcarbodiimide (619 mg, 3 mmoles) was added, and the solution was kept at room temperature for 2 days. Water (3 ml) was added, the *N,N'*-dicyclohexylurea was removed by filtration and washed with water (5 ml), and the combined filtrate was evaporated to dryness at 30°. A hot, saturated, aqueous solution of barium hydroxide (25 ml) was added to the residue, and the solution was kept at 90° for 1 h; during this time a white precipitate separated. The mixture was neutralised (CO_2) and then heated at 100° for 10 min. The precipitate was filtered off and washed with water. The combined filtrates were concentrated to 10 ml, and the slight precipitate was removed by centrifugation. Ethanol (30 ml) was added to the supernatant, and the mixture was kept at 4° overnight. The precipitate was collected by centrifugation and washed with ethanol, acetone, and finally ether. The barium salt (178 mg) was examined by paper chromatography in solvent *B*. The only phosphate-containing compound detected by reagent *4* was sucrose phosphate **1**, but barium acetate was present as an impurity.

The crude barium salt was dissolved in water (10 ml), the solution was clarified by centrifugation, and the barium salt was precipitated with ethanol (30 ml). After centrifugation of the barium salt, the purification was repeated three times before the pure barium salt was isolated and dried by washing with ethanol, acetone, and finally ether. The barium salt of sucrose 6'-phosphate (**1**) (134 mg, 44% from **8**), $[\alpha]_D^{20}$ +35.4° (*c* 1.1, water), was homogeneous when examined by paper chromatography in solvents *B*, *D*, and *E*, using reagents *1*, *3*, *4*, and *5* (Found: C, 23.5; H, 4.4; P, 4.7. $C_{12}H_{21}BaO_{14}P \cdot 3H_2O$ calc.: C, 23.6; H, 4.4; P, 5.1%).

(*b*) *Potassium salt.* The barium salt was converted into the dipotassium salt by passing an aqueous solution through a column of Dowex-50 (K^+) resin. It was obtained as a microcrystalline powder from aqueous ethanol (2:5, v/v) and collected by centrifugation. The solid, dried by means of ethanol, acetone, and finally ether, had $[\alpha]_D^{20}$ +34° (*c* 1.0, water) (Found: C, 26.4; H, 4.9; P, 5.5. $C_{12}H_{21}K_2O_{14}P \cdot 3H_2O$ calc.: C, 26.1; H, 4.9; P, 5.6%).

Enzymic dephosphorylation of sucrose 6'-phosphate. — The above potassium salt (1 mg) was dissolved in water (0.5 ml). A solution of ammonium carbonate (5 mg) in water (0.5 ml) was added until the pH of the resultant solution was 9. Calf-intestinal phosphatase (Sigma Chemical Company, 0.5 mg) was added, and the solution was incubated at 37° for 18 h. Paper chromatography in solvent *B* (reagents *1*, *4*, and *5*) showed that only sucrose and inorganic phosphate were present in the final solution.

Acid hydrolysis of sucrose 6'-phosphate. — The barium salt (1 mg) was dissolved in 0.1M hydrochloric acid (0.4 ml) and heated in a sealed tube at 100° for 5 min. The evaporated solution was examined by paper chromatography in solvent *B*, in comparison with D-glucose and D-fructose 6-phosphate. Sprays *1*, *3*, and *6* showed the presence of glucose, and *1*, *3*, *4*, and *5* the presence of fructose 6-phosphate, in the hydrolysate. Paper chromatography in solvent *F*, using reagent *6*, showed that glucose, not fructose, was present in the hydrolysate.

ACKNOWLEDGMENTS

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