4-O-methyl-D-glucosazone, mp 154–155°, after recrystallization from 30% aqueous acetone (lit.[39] mp 158°).

A portion of the insoluble methylated dextran (0.157 g) which resisted methanolysis (fraction ii) was solubilized in 72% sulfuric acid (2 ml) at 5° for 3 days. The solution was diluted with water to give 1 *N* sulfuric acid and refluxed for 11 hr. The hydrolysate was neutralized with barium carbonate, filtered, and concentrated. The methylated sugars were separated by chromatography (Whatman 3 MM paper, solvent C) giving 2,3,4,6-tetra- (0.006 g), 2,3,4-tri- (0.077 g), 2,4-di- (0.013 g), 3,4-di- (0.008 g), and 4-O-methyl-D-glucose (0.004 g). Each sugar was identified by paper electrophoresis and paper chromatography as described previously.

Methyl 2,3,4,6-tetra-O-methyl-α-D-glucoside was subjected to the two procedures that were used for cleavage of the methylated dextran, namely, (A) methanolysis followed by 1 *N* sulfuric acid hydrolysis (for fraction i) and (B) 72% sulfuric acid followed by 1 *N* sulfuric acid hydrolysis (for fraction ii). The extent of demethylation and degradation of the methylated sugar was ascertained by quantitative analysis (phenol–sulfuric acid method[40]) of each component of the hydrolysates after separation by paper chromatography (solvent C). Methanolysis (procedure A) resulted in a loss of 10% of the tetra-O-methyl-D-glucose, whereas 7% was lost in the 72% sulfuric acid method (procedure B); in both procedures tri-O-methyl-D-glucose was the major methylated sugar formed by demethylation.

(40) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

Registry No.—2,3,4-Tri-O-methyl-D-glucose α-1,6-di-*p*-nitrobenzoate, 16780-52-2.

Acknowledgments.—This work was supported by funds from the U. S. Public Health Service, Department of Health, Education and Welfare, Grant No. GM 11737-01. M. J. St. Cyr was the recipient of a fellowship under Pathology Training Grant No. 5T1, GM 628-04, U. S. Public Health Service. The authors thank Dr. E. J. Hehre for providing the crude polysaccharide. The elemental analyses were performed by the Scandinavian Microanalytical Laboratory, Herlev, Denmark.

The Structure of *Leuconostoc mesenteroides* Strain C Dextran. II. Fragmentation Analysis[1a]

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Received June 2, 1967

Partial acid hydrolysis of the dextran produced by *Leuconostoc mesenteroides* strain C (NRRL B-1298) has afforded the homologous series of isomaltodextrins, whereas acetolysis gave kojibiose, nigerose, isomaltose, and, in addition, trisaccharides derived from the branch points. Further evidence for the detailed structure of the dextran was obtained from periodate oxidation studies.

Methylation studies (part I of this series[2]) have shown that the dextran synthesized by *Leuconostoc mesenteroides* strain C (NRRL B-1298) is a highly branched D-glucan (CL 4-5) linked predominantly by 1 → 6 glycosidic bonds. Twelve per cent of the D-glucose units have branches at C-2 and 7.7% at C-3, while a few units appear to be doubly branched at these positions. To obtain further information on the sequential arrangement of the linkages and to confirm the α-anomeric configuration suggested by the high positive rotation of the dextran and its derivatives, the dextran was fragmented by partial acid hydrolysis, by partial acetolysis, and by Smith degradation.

Since the order of stability of glycosidic linkages involving primary and secondary hydroxyls is reversed in acid hydrolysis compared with acetolysis, these two reactions are complimentary methods for fragmentation of glycans containing mixed linkages. Thus (1 → 6)-linked oligosaccharides are obtained from such glycans by partial acid hydrolysis, whereas glycosidic linkages involving secondary hydroxyls tend to be preserved in the oligosaccharides resulting from partial acetolysis.[3,4]

When the dextran was heated in 0.1 *N* oxalic acid, the homologous series of isomaltodextrins from isomaltose to isomaltooctaose was obtained in addition to D-glucose. Each oligosaccharide was identified by paper chromatography and by partial acid hydrolysis to give the lower homologs of the series. Isolation of this homologous series of oligosaccharides establishes the presence in this dextran of sequences of consecutive (1 → 6)-linked D-glucopyranose units.

Acetolysis of the dextran afforded D-glucose pentaacetate and a mixture of oligosaccharide acetates. After deacetylation the oligosaccharides were separated by gradient elution from a charcoal column and purified further by paper chromatography. The disaccharides were obtained in good yield and were characterized by conversion into crystalline derivatives. In two separate experiments kojibiose and nigerose were isolated in yields of 7–13 and 2%, respectively, whereas isomaltose was obtained in only 2–3% yield. The yield of kojibiose in particular (35% of the maximum yield theoretically possible on the basis of the methylation data) confirms previous observations[5,6] on the value of this procedure for obtaining non-(1 → 6)-linked oligosaccharides. These results are in accordance with those of Suzuki and Hehre[5] who isolated kojibiose and nigerose from this dextran in yields of 11.7 and 2.5%, respectively, under similar acetolysis conditions.

The trisaccharide fraction obtained from the ace-

(1) (a) This paper, was presented in part at the National Meeting of the American Chemical Society, Phoenix, Ariz., 1966, and forms part of the thesis submitted by M. J. S. to the Graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D., 1966. (b) Deceased.

(2) B. A. Lewis, M. J. St. Cyr, and F. Smith, *J. Org. Chem.*, **33**, 3136 (1968).

(3) K. Matsuda, H. Watanabe, K. Fujimoto, and K. Aso, *Nature*, **191**, 278 (1961).

(4) J. K. N. Jones and W. H. Nicholson, *J. Chem. Soc.*, 27 (1958).

(5) H. Suzuki and E. J. Hehre, *Arch. Biochem. Biophys.*, **104**, 305 (1964).

(6) I. J. Goldstein and W. J. Whelan, *J. Chem. Soc.*, 170 (1962).

tolysis reaction contained six components of which four were obtained sufficiently pure for structural studies. Altering the acetolysis conditions increased the yield of the trisaccharide fraction somewhat while lowering the yield of kojibiose. The repeated paper chromatographic separations required to give pure components, however, led to poor recoveries of the individual trisaccharides. Three trisaccharides were identified as isomaltotriose, *O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl-(1 → 6)-D-glucopyranose (**1**), and 2,6-di-*O*-(α-D-glucopyranosyl)-D-glucopyranose (**2**). A fourth trisaccharide (**3**) corresponded to either *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucopyranose or 3,6-di-*O*-(α-D-glucopyranosyl)-D-glucopyranose. These structural assignments are based on the following observations.

Trisaccharides **1** and **2** had identical mobilities on paper chromatography in three different solvent systems but could be distinguished by paper electrophoresis in 0.1 *M* borate buffer and by paper chromatography of the borohydride-reduced trisaccharides. The structural studies were carried out on a mixture of the two trisaccharides. Partial acid hydrolysis afforded glucose, kojibiose, and isomaltose as revealed by paper chromatography, whereas the borohydride-reduced trisaccharides gave rise to glucose, sorbitol, kojibiose, and isomaltitol indicating that the reducing glucose unit in each trisaccharide was substituted at C-6.

Trisaccharides **1** and **2** were methylated by the Kuhn and Purdie techniques until no hydroxyl absorbance was detected in the infrared spectrum. Acid hydrolysis of the methylated trisaccharides afforded 2,3,4,6-tetra, 2,3,4-tri-, 3,4,6-tri-, and 3,4-di-*O*-methyl-D-glucoses which were isolated and identified by paper chromatography and paper electrophoresis. The identification of 3,4-di-*O*-methyl-D-glucose established the branched structure of trisaccharide **2** while the two tri-*O*-methyl-D-glucoses must originate from a trisaccharide having 1 → 6 and 1 → 2 linkages. From the partial acid hydrolysis data then, trisaccharide **1** must have the structure assigned with isomaltose at the reducing terminal. The ratio of di- to tri- to tetra-*O*-methyl-D-glucose of 1.00:1.74:3.11 suggests that the two trisaccharides were present in approximately equal amounts.

Partial acid hydrolysis of trisaccharide **3** gave isomaltose, nigerose, and glucose as revealed by paper chromatography. It was established that the 1 → 3 linkage was at the reducing end of the trisaccharide since periodate oxidation, reduction, and hydrolysis afforded glycerol and arabinitol but no glucose. However, these data do not distinguish between the linear and the branched trisaccharide.

Another trisaccharide fraction isolated in small quantity gave glucose, nigerose, and kojibiose on partial acid hydrolysis. When this fraction was treated successively with periodate, sodium borohydride, and refluxing methanolic hydrogen chloride, arabinitol, methyl α-D-glucopyranoside, and glycerol were obtained. This evidence suggests that this fraction was a mixture of the two linear trisaccharides containing both 1 → 2 and 1 → 3 linkages [D-G_p-(1 → 3)-D-G_p-(1 → 2)-D-G_p and D-G_p-(1 → 2)-D-G_p-(1 → 3)-D-G_p]. It does not rule out the alternate possibility that there was present a mixture of nigerotriose and kojitriose. However, there is no evidence from periodate oxidation studies for the presence of consecutive 1 → 3 linkages which would give rise to nigerotriose. In view of the small proportion of this trisaccharide fraction containing both (1 → 2)- and (1 → 3)-linked glucopyranose units and the relative stability to acetolysis of these linkages compared with the 1 → 6 linkages, it would appear that sequences of consecutive 1 → 2 and 1 → 3 linkages are not a common structural feature in this dextran.

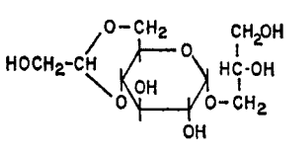
Additional evidence for the structure of the dextran was provided by periodate oxidation studies. The dextran was oxidized by periodate with a reduction of 1.58 mol of periodate per glucose unit while 0.66 mol of formic acid was liberated per glucose unit. These values are in good agreement with the values predicted by the methylation data (1.59 and 0.73, respectively). The dextran polyaldehyde showed little tendency to overoxidize; the periodate consumption reached a maximum in 40 hr and remained constant for an additional 55 hr.

For further studies the dextran polyaldehyde was reduced to the polyalcohol with sodium borohydride. On complete hydrolysis of the polyalcohol glucose and glycerol were obtained in the mole ratio 1.00:7.60 as determined by colorimetric analysis after separation of the two components by paper chromatography. This ratio is somewhat different from that indicated by the methylation results (1.00:5.98) but it is shifted in the direction expected if part of the 2,4,6-tri-, 2,4-di-, or 4-*O*-methyl-D-glucose had arisen by either incomplete methylation of the dextran or by partial demethylation of the methylated sugars during cleavage of the methylated dextran.

Partial fragmentation of the dextran polyalcohol was accomplished most satisfactorily with methanolic hydrogen chloride at room temperature. The methanolysate was examined by gas–liquid partition chromatography revealing glycerol, glycolaldehyde dimethyl acetal, and glyceraldehyde dimethyl acetal, the latter arising from 1 → 2 linkages. Several less volatile components were detected by paper chromatography. The components of the methanolysate were isolated by paper chromatography and identified as follows.

Glycerol, the major component, was identified by chromatography and as the tri-*p*-nitrobenzoate. It is derived from the linear segments of the dextran chain, the nonreducing terminal units, and the units branched at C-2.

1-*O*-α-D-Glucopyranosyl-L-glycerol was isolated along with two of its acetals containing glycolaldehyde (**4** and **5**). These three glucosides arise from glucose



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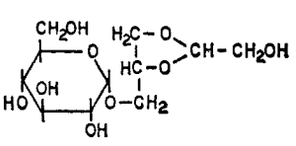

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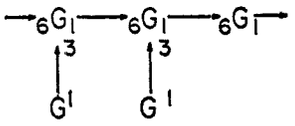
units substituted at C-3 and at C-1 by periodate-labile units. Since the methylation study revealed that most, if not all, of the 3-substituted glucose units represent branch points, then C-6 of this glucose unit is also substituted by an oxidizable residue. These periodate-

sensitive units can be nonreducing terminal units, linear 1 → 6 units, or the C-2 branched units.

A fifth component in the methanolysate was identified as 1-*O*-α-isomaltosyl-L-glycerol (**6**) by chromatographic comparison with the authentic compound. In addition partial acid hydrolysis of **6** afforded isomaltose, 1-*O*-α-glucopyranosylglycerol, glucose, and glycerol while complete hydrolysis liberated glucose and glycerol in the mole ratio of 2:1. If **6** is structurally significant and is not an artifact created by incomplete oxidation, then it must originate from two adjacent glucose units in the (1 → 6)-linked chain each branched at C-3 as in the linkage sequence depicted by **7**. This structural feature has been observed previously in a dextran.[7] A trace of 1-*O*-α-isomaltotriosyl-L-glycerol was obtained also.



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The isolation of **6** suggests some randomness in the distribution of C-3 branches. However the preponderance of the glucosylglycerol and its acetals **4** and **5** indicates that most of the 3-linked branches occur in isolated rather than in adjacent positions along the (1 → 6)-linked chain. No glycosides of nigerose were isolated from the polyalcohol suggesting that consecutive 1 → 3 linkages are not present in the dextran.

Cleavage of the dextran polyalcohol with aqueous acid, the method used previously for fragmenting these polyalcohols, gave the same components and, in addition, several other compounds which were condensation products with glyceraldehyde or glycol aldehyde. This complicated both the isolation and identification procedures. It seems advisable therefore to subject polyalcohols derived from (1 → 2)-linked polysaccharides to methanolysis thereby converting glyceraldehyde into the less reactive dimethyl acetal.

The structures of the two glycolaldehyde acetals of 1-*O*-α-D-glucopyranosyl-L-glycerol were established in the following manner. The rapid mobility of **4** and **5** on paper chromatograms and their acid lability suggested their acetal nature. Acetal **4** on partial acid hydrolysis gave 1-*O*-α-glucopyranosylglycerol, glucose, and glycerol. Methylation by the Kuhn procedure gave the methyl ether of **4** which on hydrolysis with dilute sulfuric acid afforded 2,3-di-*O*-methylglucose. The methyl ether of **4** was methanolyzed giving rise to methoxyacetaldehyde dimethyl acetal and 1,2-di-*O*-methylglycerol which were identified by glpc. The identification of these components establishes that **4** is 1-*O*-[4,6-*O*-(2-hydroxyethylidene)-α-D-glucopyranosyl]-L-glycerol.

Acetal **5** on partial acid hydrolysis gave 1-*O*-α-glucopyranosylglycerol which was identified by paper chromatography. Glycolaldehyde dimethyl acetal was detected in the methanolysate of **5** by glpc. Methylation of **5** and subsequent hydrolysis gave 2,3,4,6-tetra-*O*-methylglucose and glycerol, while methoxyacetaldehyde dimethyl acetal was obtained by methanolysis. The structure *O*-α-D-glucopyranosyl-(1 → 1)-2,3-*O*-(2-hydroxyethylidene)-L-glycerol was assigned to **5** from these results. Although the stereochemistry of the glycerol moiety in acetals **4** and **5** and in isomaltosylglycerol (**6**) has not been established for the isolated compounds, the configuration was assigned on the basis of its derivation from a 6-substituted D-glucopyranose unit.

Acetals **4** and **5** apparently arise through incomplete methanolysis of the dextran polyalcohol. The possibility that they were synthesized from glucosylglycerol and glycolaldehyde during methanolysis seems unlikely under the dilute conditions employed for methanolysis. Indeed when a mixture of 1-*O*-α-D-glucopyranosyl-L-glycerol and glycolaldehyde was treated with methanolic hydrogen chloride under conditions similar to those used for the polyalcohol, acetals **4** and **5** were not detected. The structural significance of **4** and **5** then depends on whether acid-catalyzed structural rearrangements took place during methanolysis. If acid-catalyzed rearrangements of the glycolaldehyde moiety of **4** and **5** did not occur, then these two compounds would have additional structural significance since the glycolaldehyde moiety of **4** and **5** can only be derived from a linear (1 → 6)-linked glucose unit and not from a C-2 branched unit.

Authentic 1-*O*-α-D-glucopyranosyl-L-glycerol was prepared from isomaltitol by oxidation with 1 mol of lead tetraacetate, followed by reduction with sodium borohydride. The oxidant attacks the glucitol moiety preferentially[8] and a mixture of glycosides is produced; 1-*O*-α-D-glucopyranosyl-L-glycerol was the major product isolated after reduction, and it was characterized as the crystalline hexa-*p*-nitrobenzoate.

Definitive evidence for the fine structure of dextrans has been lacking particularly with reference to the nature of the branches. Physical studies have suggested the presence of single unit branches as well as long branches.[9] The pattern of oligosaccharides formed by the action of dextranases[7,10] and recent chemical studies[7] indicate that the majority of branches in certain dextrans consist of a single α-D-glucopyranosyl unit.

The evidence obtained from these studies on the strain C dextran does not distinguish between single-unit branches and long branches or a ramified structure. The oligosaccharides obtained by acetolysis and periodate fragmentation could arise from C-2 and C-3 branch points terminated by either single α-D-glucopyranosyl units or chains of (1 → 6)-linked α-D-glucopyranose units.

Experimental Section

The general conditions are described in part I of this series.[2] The following chromatography solvent systems were used in addition to these described previously: (E) 1-propanol–ethyl acetate–water (65:10:25), (F) isoamyl alcohol–pyridine–water (1:1:1, upper phase), (G) pyridine–ethyl acetate–water (4:10:3), (H) 1-butanol–pyridine–water (6:4:3). Mobilities are expressed relative to glucose (R_G), isomaltose (R_{IM}), isomaltotriose (R_{IMT}), and glycerol (R_{GEY}).

Unless stated otherwise partial acid hydrolyses were performed with Amberlite IR-120 (H^+) cation-exchange resin as catalyst,

(7) D. Abbott, E. J. Bourne, and H. Weigel, *J. Chem. Soc., Sect. C*, 827 (1966).

(8) A. J. Charlson and A. S. Perlin, *Can. J. Chem.*, **34**, 1200 (1956).

(9) W. B. Neely, *Advan. Carbohydrate Chem.*, **15**, 341 (1960).

(10) R. W. Bailey, D. H. Hutson, and H. Weigel, *Biochem. J.*, **80**, 514 (1961); D. H. Hutson and H. Weigel, *ibid.*, **88**, 588 (1963).

and the reaction mixtures were heated in a boiling-water bath. Sulfuric acid hydrolyses were neutralized with barium carbonate or ion-exchange resins.

An F & M 500 gas chromatograph equipped with a thermal conductivity detector and a column (1.8 m × 6 mm) of 20% diethylene glycol succinate on acid-washed Chromosorb W, 60–80 mesh, was used for gas–liquid partition chromatography (glpc). Helium was used as carrier gas at a flow rate of 1 ml/sec and inlet pressure of 30 psi. All runs were linearly programmed at a rate of 7.9°/min from 40 to 200°. The components are defined by their retention time (R_t) in minutes and the temperature (T) at which they emerged from the column.

Fragmentation of the *L. mesenteroides* Strain C Dextran by Acid Hydrolysis.—Dextran (0.10 g) was heated in 0.1 N oxalic acid (2 ml) in a sealed tube in a boiling water bath for 5 hr. The acid was neutralized with calcium carbonate, and the solution was filtered and concentrated. The components were separated by chromatography on Whatman 3 MM paper using solvent G. Paper chromatography on solvents A, E, and F indicated that the components represented the homologous series of isomaltodextrins from isomaltose to isomaltooctaose as well as free glucose. Partial hydrolysis of each oligosaccharide at 95° for 2 hr with Amberlite IR-120 (H^+) resin as catalyst gave rise to the lower molecular weight homologs of the series as revealed by paper chromatography.

Fragmentation by Acetolysis.—Dextran acetate (12.4 g) was dissolved in acetic anhydride (170 ml) at 5° and treated with a cold 5:1 mixture of acetic anhydride–sulfuric acid (78 ml) for 15 min. The reaction mixture was allowed to warm to room temperature (2 hr) and then held at 35° for 45 hr. The acetates were recovered by centrifugation after pouring the reaction mixture into water, and the supernatant was extracted with chloroform to remove the remaining acetates. The combined acetates were dissolved in chloroform, washed with sodium bicarbonate solution and water, and concentrated to a syrup (yield 14.0 g). The syrup was extracted with methanol and the methanol-soluble material was deacetylated catalytically with sodium methoxide (yield 4.0 g). The methanol-insoluble acetate fraction was deacetylated in chloroform with methanolic potassium hydroxide and shown by chromatography to be primarily large oligosaccharides (yield 1.5 g). This latter fraction was not investigated further.

The deacetylated acetolysate (4.0 g) was resolved by cocoanut charcoal column chromatography,[11] giving the disaccharides in a yield of about 17% (1.16 g) and the trisaccharides in 5% yield (0.30 g).[12] The yields are calculated as the percentage by weight of the dextran (12.4 g of dextran acetate correspond to 7.1 g of dextran). Fractions eluted from the carbon column were purified further by chromatography on Whatman 3 MM paper with solvents E and G giving the following oligosaccharides.

A. **3-*O*-α-D-Glucopyranosyl-D-glucopyranose (Nigerose).**—Fraction a (yield 0.14 g, 2%) was chromatographically (paper) identical with nigerose: R_G 0.51, 0.74, and 0.85 in solvents A, E, and F, respectively; $[\alpha]^{26}D$ +128.2° (c 1.2, water) (lit.[6] $[\alpha]^{18}D$ +131° in water). Acetylation with acetic anhydride and sodium acetate afforded the β-octaacetate: mp 145–147°; $[\alpha]^{26}D$ +89° (c 0.6, chloroform) after recrystallization from ethanol (lit.[6,13] mp 149, 152–154; $[\alpha]D$ +83° in chloroform).

B. **2-*O*-α-D-Glucopyranosyl-α-D-glucopyranose (Kojibiose).**—Fraction b (yield 0.51 g, 7%) was recrystallized from aqueous ethanol: mp 186–187°; $[\alpha]^{25}D$ +161° (10 min) → +136.5° (80 min) (c 0.9, water) (lit.[14] α-kojibiose: anhydrous, mp 193–194°; hydrate, mp 195–196°, $[\alpha]D$ +145.8° → +135.2° in water). This fraction was identical with kojibiose by paper chromatography in solvents A, E, and F (R_G 0.37, 0.68, and 0.76, respectively). Acetylation with acetic anhydride and sodium acetate afforded the β-octaacetate which was recrystallized from ethanol: mp and mmp 117–119°; $[\alpha]^{26}D$ +130.5° (c 0.2, chloroform) (lit.[15] mp 118°; $[\alpha]D$ +113° in chloroform).

Kojibiose was reduced with sodium borohydride to the syrupy kojibiitol, $[\alpha]^{23}D$ +83.5° (c 1.5 in water) and R_G 0.70 in solvent E. On oxidation with 0.015 M periodic acid at 5°, kojibiose reduced 2.9 mol of periodate in 23 hr, unchanged after 42 hr, and thereafter was overoxidized reducing 5.2 mol in 76 hr and 6.5 mol in 165 hr.

Methylation of kojibiose (0.072 g) using dimethylformamide (6 ml), methyl iodide (1.8 ml), and silver oxide (3.5 g)[16] gave the octamethyl ether (0.074 g): $[\alpha]^{27}D$ +113° (c 1.9, chloroform). The octa-*O*-methylkojibiose was refluxed for 8 hr and the methylated sugars were separated by paper chromatography (solvent C) giving equimolar quantities of 2,3,4,6-tetra-*O*-methyl-D-glucose, mp 88–90°, and 3,4,6-tri-*O*-methyl-D-glucose, $[\alpha]^{29}D$ +55° (c 0.7, water) (lit.[17] $[\alpha]D$ +78° in water). The mobility of the 3,4,6-tri-*O*-methyl-D-glucose on paper electrophoresis in 0.1 M borate buffer established that C-2 was not methylated since the other tri-*O*-methyl-D-glucoses do not form borate complexes under these conditions.[18]

C. **6-*O*-α-D-Glucopyranosyl-D-glucopyranose (Isomaltose).**—Fraction c (yield 0.23 g, 3%) was identical with isomaltose by paper chromatography in solvents A, E, and F (R_G 0.26, 0.58, and 0.64, respectively), and G (R_{IMT} 1.90). Acylation with *p*-nitrobenzoyl chloride in pyridine at 85° for 3.5 hr gave the β-octa-*p*-nitrobenzoate which was recrystallized from 2-butanone: mp 226–228°; $[\alpha]^{22}D$ +83.8° (c 0.3 in acetonylacetone) {lit.[19] mp 188°; $[\alpha]D$ +22.0° (c 1.3 in acetonylacetone) after recrystallization from acetone}. Authentic isomaltose β-octa-*p*-nitrobenzoate prepared in this laboratory with *p*-nitrobenzoyl chloride and pyridine and recrystallized from 2-butanone had mp 223–225° and $[\alpha]^{23}D$ +78.8° (c 0.6 in acetonylacetone).

Anal. Calcd for $C_{68}H_{46}N_8O_{35}$: C, 53.2; H, 3.0; N, 7.3. Found: C, 53.0; H, 3.3; N, 7.2.

Recrystallization of the β-octa-*p*-nitrobenzoate from acetone gave crystals which melted at 189–190°, solidified at 200°, and remelted at 236–237°.

Fraction c was transformed into isomaltitol by reduction with sodium borohydride. Recrystallization of the isomaltitol from methanol gave crystals with mp 137–138° and $[\alpha]^{29}D$ +90.3° (c 0.7, water) (lit.[20] mp 165.5–167° and $[\alpha]^{28}D$ +89° in water).

D. **Isomaltotriose.**—Fraction d (0.05 g) was chromatographically identical with isomaltotriose in solvents E, F, and G and had $[\alpha]^{27}D$ +142° (c 0.8, water) (lit.[21] $[\alpha]D$ +142° in water). Partial hydrolysis liberated glucose and isomaltose only.

E. **Trisaccharides 1 and 2.**—Fraction e (0.060 g), $[\alpha]D$ +83° (c 0.8, water), contained two components which had similar mobilities on paper chromatograms in solvents E, F, and G (R_{IMT} 1.12 in solvent G) but were distinguished by paper electrophoresis. When this fraction was partially hydrolyzed, glucose, kojibiose, and isomaltose were detected by paper chromatography. Treatment of fraction e with sodium borohydride afforded the two reduced trisaccharides with R_{IM} 0.68 and 0.79 in solvent E and R_{IMT} 1.08 and 1.38 in solvent G. Partial hydrolysis of the mixture of reduced trisaccharides gave glucose, sorbitol, kojibiose, and isomaltitol as revealed by paper chromatography.

The trisaccharide mixture (36 mg) was subjected to three Kuhn and five Purdie methylations until no hydroxyl absorption was observed in the infrared. The methylated trisaccharides were hydrolyzed in refluxing 1 N sulfuric acid, and the methylated sugars were isolated by paper chromatograph in solvent C. Assay of each fraction by the phenol–sulfuric acid method[22] gave the ratio 1.00:1.74:3.11 for the the di-, tri-, and tetra-*O*-methyl-D-glucoses. The following components of the hydrolysate were identified by comparison with the authentic compounds by paper chromatography in solvents B and C and by paper electrophoresis in 0.1 M borate buffer: (i) 3,4-di-*O*-methyl-D-glucose, (ii) 3,4,6-tri-*O*-methyl-D-glucose, (iii) 2,3,4-tri-*O*-methyl-D-glucose, and (iv) 2,3,4,6-tetra-*O*-methyl-D-glucose. Reduction of

(11) F. W. Barth and T. E. Timell, *Can. J. Chem.*, **36**, 1321 (1958).

(12) When the dextran was subjected to acetolysis using a longer reaction time and a higher concentration of acid (similar to the conditions described by Suzuki and Hehre[6]), the oligosaccharides were isolated in the following yields: isomaltose (2%), kojibiose (13%), nigerose (2%), and trisaccharides (1%).

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iii with sodium borohydride and subsequent oxidation with 0.037 *M* periodic acid at 5° for 40 hr afforded 2,3,4-tri-*O*-methylxylose as revealed by paper chromatography. This establishes conclusively that iii is 2,3,4-tri-*O*-methyl-D-glucose.

No glucose was detected by paper chromatography when the trisaccharide mixture was oxidized with periodic acid for 24 hr, reduced with borohydride, and hydrolyzed.

F. Trisaccharide 3.—Fraction f (0.043 g) had R_{IM} 0.63 and R_{IMT} 1.31 on paper chromatography in solvent G. Partial hydrolysis released glucose, isomaltose, and nigerose. The trisaccharide was oxidized with 0.1 *M* periodic acid at 5° for 24 hr, and the product was reduced with sodium borohydride for 3 days. The reduced product was hydrolyzed with 0.1 *N* hydrochloric acid at room temperature for 22 hr. Paper chromatography of the deionized hydrolysate showed considerable streaking of the components and the hydrolysate was re-treated with sodium borohydride and again deionized. Glycerol and arabinitol were the only components detected by paper chromatography.

G. Trisaccharides 8 and 9.—Fraction g (0.013 g) showed two components by paper chromatography in solvent G (R_{IMT} 1.52 and 1.74). Partial hydrolysis of fraction g yielded glucose, nigerose, and kojibiose. Fraction g was oxidized with 0.1 *M* periodic acid at 5° for 15 hr, and the product was reduced with sodium borohydride and refluxed with 1 *N* methanolic hydrogen chloride for 2 hr. The deionized methanolysate was shown by paper chromatography (solvents A, B, and H) to contain arabinitol, methyl α-D-glucopyranoside, and glycerol.

Periodate Oxidation of Dextran.—Dextran (0.2044 g), purified *via* the acetate, was oxidized with 0.075 *M* periodic acid (100 ml) at 5°. Aliquots (2 ml) were titrated for periodate by the Fleury–Lange method,[23] and formic acid was titrated on 5-ml aliquots to the phenolphthalein end point with 0.009 *N* sodium hydroxide after reduction of the excess periodate with ethylene glycol (Table I).

TABLE I

	Time, hr					
	22	26	39	63	65	113
Periodate reduced, mol/glucose unit	1.44	1.45	1.58	1.56	...	...
Formic acid released, mol/glucose unit	...	...	...	...	0.65	0.66

For further studies dextran (5.11 g) was oxidized with 0.1 *M* periodic acid (1 l.) at 5° for 95 hr (1.58 mol of periodate reduced/glucose unit). The acid was neutralized with barium carbonate, and the solution was filtered and treated with sodium borohydride (5 g) for 22 hr. The excess borohydride was destroyed with acetic acid, and the solution was concentrated to 200 ml.

Methanolysis of the Dextran Polyalcohol.—A portion of the solution (100 ml) was passed through a column of Amberlite IR-120 (H^+), and the eluate was concentrated nearly to dryness and treated with methanol (75 ml). The solution was concentrated to a syrup and again treated with methanol; this process was repeated four times. The residue was dissolved in methanol (80 ml) and treated with 1 *N* methanolic hydrogen chloride (30 ml) for 16 hr at 23°. The acid was neutralized with silver carbonate, the mixture was filtered, and the solution was concentrated to 10 ml. Examination of this methanolysate by glpc revealed glycolaldehyde dimethyl acetal (R_t 8.6 min, *T* 120°), glyceraldehyde dimethyl acetal (R_t 16.6 min, *T* 180°), and glycerol, which were confirmed by cochromatography[24] with the authentic compounds. The remainder of the methanolysate was concentrated to dryness (yield 0.93 g).

A. Glucose–Glycerol Ratio.—A portion of the methanolysate (19 mg) was hydrolyzed in refluxing 1 *N* sulfuric acid for 21 hr. The solution was deionized [Duolite A4 (OH^-)] and concentrated, and the components were separated by paper chromatography in solvent A. Glucose and glycerol were each eluted with water and analyzed respectively by the phenol–sulfuric acid[22] and the periodate–chromotropic acid[25] methods giving a glucose–glycerol molar ratio of 1.00:7.60.

B. Identification of the Components of the Methanolysate. The methanolyzate (0.745 g) was separated on Whatman 3 MM paper using solvent A. The components were eluted with water and identified as follows.

i. Glycerol (0.197 g).—Fraction i was chromatographically identical with glycerol in solvents A, B, and H and gave the tri-*p*-nitrobenzoate, mp 192–193°.

ii. 1-*O*-[4,6-*O*-(2-Hydroxyethylidene)-α-D-glucopyranosyl]-L-glycerol (4) (0.054 g).—Compound **4** had $[\alpha]^{28}D$ +79° (*c* 0.3, methanol) and R_{GEY} 0.81 in solvent A. Partial hydrolysis of **4** for 30 min gave 1-*O*-α-D-glucopyranosylglycerol, glycerol, and glucose as revealed by paper chromatography. The original compound could not be detected after hydrolyzing for 60 min.

Compound **4** (6 mg) was methylated twice by the Kuhn procedure, and a portion was hydrolyzed with 1 *N* sulfuric acid at 100° for 12 hr. Examination of the hydrolysate by paper chromatography in solvents C and H and by electrophoresis revealed one visible component corresponding to 2,3-di-*O*-methyl-D-glucopyranose. The remainder of the methyl ether of **4** was heated in 1 *N* methanolic hydrogen chloride in a sealed tube in a boiling-water bath for 14 hr. Examination of the methanolysate by glpc showed peaks corresponding to methoxyacetaldehyde dimethyl acetal (*T* 91°, R_t 7.4 min) and 1,2-di-*O*-methylglycerol (*T* 76°, R_t 2.5 min) which were confirmed by cochromatography.

iii. 1-*O*-α-D-Glucopyranosyl-2,3-*O*-(2-hydroxyethylidene)-L-glycerol (5) (0.042 g).—Compound **5** had $[\alpha]^{18}D$ +44° (*c* 1.6, methanol) and R_{GLY} 0.45 in solvent A. Partial hydrolysis of **5** for 15 min gave 1-*O*-α-D-glucopyranosylglycerol and a trace of glucose as revealed by paper chromatography; the original compound could not be detected. Methanolysis of **5** in a sealed tube at 95° for 6 hr afforded glycolaldehyde dimethyl acetal (*T* 122°) detected by glpc and confirmed by cochromatography.

Compound **5** was methylated two times by the Kuhn procedure and subjected to methanolysis. Glpc of the methanolysate showed a peak corresponding to methoxyacetaldehyde dimethyl-acetal (*T* 98°, R_t 7.1 min). Mono- and di-*O*-methylglycerol were not detected. The remainder of the methanolysate was refluxed with 1 *N* sulfuric acid for 9 hr. Paper chromatography of the concentrated hydrolysate using solvents A and C revealed 2,3,4,6-tetra-*O*-methyl-D-glucose and glycerol.

iv. 1-*O*-α-D-Glucopyranosyl-L-glycerol (0.084 g).—Fraction iv had $[\alpha]^{29}D$ +122.8° (*c* 1.4, water); it was chromatographically identical with the authentic compound on solvents A, B, and H (R_G 0.71, 1.04, and 0.95, respectively) and on partial hydrolysis afforded glucose and glycerol in addition to iv. Acylation with *p*-nitrobenzoyl chloride and pyridine gave the hexa-*p*-nitrobenzoate which was recrystallized from acetone and from 2-butanone: mp and mmp 219–220° (melted at 134–136° and resolidified); $[\alpha]^{25}D$ +53.9° (*c* 0.6, acetone).

v. *O*-α-D-Glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 1)-L-glycerol (1-*O*-α-Isomaltosyl-L-glycerol) (6).—Fraction v was purified further by paper chromatography giving 15 mg of **6**. Compound **6** was chromatographically identical with authentic 1-*O*-α-isomaltosyl-L-glycerol[26] in solvents A, E, and F (R_G 0.24, 0.57, and 0.60, respectively) and had $[\alpha]^{28}D$ +109° (*c* 0.2, water); authentic, $[\alpha]D$ +165° (*c* 0.7, water). Partial acid hydrolysis of **6** for 30 min afforded isomaltose, 1-*O*-glucopyranosylglycerol, glucose, and glycerol which were revealed by paper chromatography. The original compound was still detected after 2 hr but not after 3 hr. Complete hydrolysis of **6** with refluxing 1 *N* sulfuric acid for 12 hr afforded glucose and glycerol in the mole ratio 1.00:0.55; glucose was analyzed by the phenol–sulfuric acid method[22] and glycerol by the periodate–chromotropic acid technique.[25]

vi. 1-*O*-α-Isomaltotriosyl-L-glycerol.—Fraction vi was chromatographically identical with 1-*O*-α-isomaltotriosyl-L-glycerol[26] on solvents A, E, and F (R_{IM} 0.28, 0.42, and 0.55, respectively). Partial hydrolysis for 30 min gave 1-*O*-isomaltosylglycerol, isomaltose, 1-*O*-glucopyranosylglycerol, glucose, and glycerol which were detected by paper chromatography.

(23) R. D. Guthrie in "Methods in Carbohydrate Chemistry," Vol. I, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press Inc., New York, N. Y., 1962, p 437.

(24) Cochromatography refers to chromatography of an admixture of the authentic specimen and the compound being examined.

(25) B. A. Lewis, F. Smith, and A. M. Stephen in "Methods in Carbohydrate Chemistry," Vol. I, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press Inc., New York, N. Y., 1962, p 472.

(26) The 1-*O*-α-isomaltosyl-L-glycerol and 1-*O*-α-isomaltotriosyl-L-glycerol required as reference compounds were prepared by partial periodate oxidation of NRRL B-512 dextran.

C. Attempted Condensation of Glycolaldehyde with 1-*O*-α-D-Glucopyranosyl-L-glycerol.—A mixture of 1-*O*-α-D-glucopyranosyl-L-glycerol (21 mg, 0.082 mmol) and glycolaldehyde (32 mg, 0.53 mmol) in methanol (1 ml) was treated with 1 *N* methanolic hydrogen chloride (0.38 ml) for 18 hr at 20°. The reaction mixture was neutralized with silver carbonate and filtered, and the solution was concentrated. The unreacted glucosylglycerol was the only component visible by paper chromatography in solvents A and E. Acetals **4** and **5** could not be detected.

Hydrolysis of the Dextran Polyalcohol.—The dextran polyalcohol was hydrolyzed with 0.1 *N* hydrochloric acid at 25° for 18 hr, and the reaction mixture was neutralized and treated with sodium borohydride. The components of the deionized hydrolysate were separated by paper chromatography. The same components (i–vi) were identified, but in addition there were small amounts of several components which appeared to be condensation products of these components with glycolaldehyde or glyceraldehyde. These were not investigated further.

Synthesis of 1-*O*-α-D-Glucopyranosyl-L-glycerol.—A solution of isomaltitol (0.245 g) in water (1 ml) was diluted with acetic acid (75 ml) and treated with lead tetraacetate (0.350 g, 1.1 molecular proportions). The reaction mixture was shaken vigorously until the lead tetraacetate had dissolved and, after 2.5 hr at room temperature, oxalic acid (0.368 g) in acetic acid (10 ml) was added. The mixture was filtered and the solution was concentrated. The residue was dissolved in water and deionized with Amberlite IR-120 (H^+) and Duolite A-4 (OH^-) resins. Sodium borohydride (0.20 g) was added, and after 15 hr the solution was neutralized with acetic acid and the sodium ions were removed with Amberlite IR-120 (H^+). The solution was concentrated, and the residue was treated with methanol to remove boric acid.

Preparative paper chromatography (solvent G) of the syrupy product (0.204 g) afforded pure 1-*O*-α-D-glucopyranosyl-L-glycerol (0.070 g) in addition to isomaltitol (0.054 g), 1-*O*-α-D-glucopyranosyl-L-erythritol (0.011 g), and hydroxyethyl α-D-glucopyranoside (0.030 g). The 1-*O*-α-D-glucopyranosyl-L-glycerol had $[\alpha]^{29}D$ +123.9° (*c* 1.2, water) and on heating with pyridine and *p*-nitrobenzoyl chloride at 95° for 3.5 hr it gave a hexa-*p*-nitrobenzoate which was recrystallized from acetone–ethanol (1:1): mp 121–125°, solidifying and remelting at 222–223°; $[\alpha]^{24}D$ +57.4° (*c* 1.0, acetone).

Anal. Calcd for $C_{51}H_{36}N_6O_{26}$: C, 53.3; H, 3.2; N, 7.3. Found: C, 53.2; H, 3.2; N, 7.1.

Registry No.—Isomaltose β-acta-*p*-nitrobenzoate, 16780-53-3; 1-*O*-α-D-glucopyranosyl-L-glycerol hexa-*p*-nitrobenzoate, 16808-40-5.

Acknowledgments.—This work was supported by funds from the U. S. Public Health Service, Department of Health, Education and Welfare, Grant No. GM 11737-01. M. J. St. Cyr was the recipient of a fellowship under Pathology Training Grant No. 5T1, GM 628-04, U. S. Public Health Service. The authors thank Dr. E. J. Hehre for the polysaccharide.

Deoxophylloerythroetioporphyrin[1a]

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Received September 14, 1967

A method of preparation of deoxophylloerythroetioporphyrin using the chlorophyll derivative, pheophytin, as the starting material has been worked out. In the procedure pheophytin is first converted into pyropheophorbide which is further degraded to deoxophylloerythrin in a single composite reaction based on the Wolff–Kishner reduction. Deoxophylloerythrin is then decarboxylated to yield deoxophylloerythroetioporphyrin by a seven-step reaction which includes Curtius rearrangement, Hofmann degradation, and catalytic hydrogenation.

The natural occurrence of deoxophylloerythroetioporphyrin (DPEP) was suspected by A. Treibs when he extracted from a Swiss marl a porphyrin with a visible spectrum identical with that of deoxophylloerythrin (for structures see Table I) and chemical properties indicating the absence of carboxyl groups.[2] The central position of DPEP in the geochemistry of the fossil porphyrins[3] required that the suggested structure be confirmed by synthesis. Using the usual synthetic approach of the Fischer school based on the appropriate pyrromethenes, an authentic sample of DPEP was prepared. However, the yields were dishearteningly low; for example, 12 mg of DPEP was obtained from 30 g of pyrromethenes.[4] A later attempt by other workers to repeat the synthesis produced only fractional milligrams of the desired porphyrin and they reported that the major product was etioporphyrin.[5] The comparative ease with which quantities of the chlorophyll derivative, pheophytin, can be obtained from natural sources suggested that a different approach might be more fruitful. Since it already contains the required carbon skeleton, the choice of pheophytin as the starting point would avoid much of the tedium of the pyrromethene synthesis. Furthermore, now that the structure of chlorophyll has been confirmed,[6] it is sound to use it as a starting point for the synthesis of compounds of related structures.

Thus, a logical starting point for the synthesis of DPEP was pheophytin a + b (see Table II). Pheophytin is produced by extraction from chlorophyll-rich plants and may be obtained commercially. It is known to be readily converted into pyropheophorbide a + b by refluxing in concentrated HCl, and so could be made available in quantity without undue labor. A glance

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