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THE SYNTHESIS OF 4,6-Di-O-(α-D-GLUCOPYRANOSYL)-D-GLUCOPYRANOSE, THE BRANCH POINT OF GLYCOGEN AND AMYLOPECTIN

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Although there seems little reason to doubt that the principal interchain linkage in glycogen and amylopectin is of the α-D-(1→6)-glucosidic type, the isolation and characterization of a branched oligosaccharide[1] from a controlled acid fragmentation of these polysaccharides has not been recorded. However, oligosaccharides containing the α-D-(1→6)-linkage have been isolated from glycogen and amylopectin by partial acid hydrolysis. Thus isomaltose (1, 2, 3), panose (3, 4) and isomaltotriose (5) all have been obtained as pure preparations.

It became of interest to attempt isolation of the branched trisaccharide Ia from a limited acid hydrolysate of starch or glycogen.

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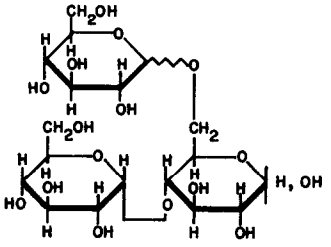
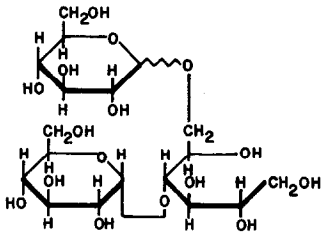
[1] The term branched oligosaccharide will be used to refer to saccharides in which at least two sugar residues are linked glycosidically to a third sugar residue.



Ia α-1,6- LINKAGE

Ib β-1,6- LINKAGE



IIa α-1,6- LINKAGE

IIb β-1,6- LINKAGE

In order to ascertain the feasibility of such a study it was decided to synthesize the trisaccharide so as to determine its stability in acidic media. Previous attempts to synthesize such a substance resulted in the formation of the β-isomer (O-α-D-glucopyranosyl-(1→4)-O-[β-D-glucopyranosyl-(1→6)]-D-glucopyranose) (6, 7, 8), Ib, recognized by its relatively low specific optical rotation, $[\alpha]_D^{20}$ + 84°, degradation by emulsin, and identification of gentiobiose (6-O-β-D-glucopyranosyl-D-glucose) as a product of partial hydrolysis.

The synthesis of Ia has now been achieved by employing a Koenigs-Knorr condensation of 1, 2, 3, 2', 3', 4', 6'-hepta-O-acetyl-β-maltose (6, 8) and 3, 4, 6-tri-O-acetyl-2-O-nitro-β-D-glucopyranosyl chloride (9). The 2-O-nitro derivative prevents the undesirable neighboring group participation in the condensation reaction and results in the formation of an α-D-glucosidic linkage. Removal of the nitro group by catalytic hydrogenation, deacetylation with sodium methoxide and isolation by preparative chromatography on thick filter paper afforded the sirupy trisaccharide, Ia, in 12% yield.

The following evidence is presented in support of structure Ia for the trisaccharide:

1. On paper chromatograms the trisaccharide travelled as a single component in three different solvent systems. Its mobility was similar to, but different from the isomeric trisaccharides, panose and isopanose. The mobility of Ia is also distinct from its β-isomer, Ib.

2. The trisaccharide, Ia, in common with saccharides carrying glycosyl substituents at C-4 of the reducing residue gave a purple color with the diphenylamine-aniline spray reagent (10). Panose and maltose reacted in similar fashion whereas isomaltose and isopanose afforded the characteristic green (sometimes yellow) color for saccharides with a free hydroxyl group at C-4 of the reducing unit. Trisaccharide Ia stained red with triphenyltetrazolium chloride reagent indicating the

trisaccharide to be unsubstituted at C-2 of the reducing moiety (11, 12).

3. Analysis for total carbohydrate (phenol-sulfuric acid procedure) (13) before and after borohydride reduction of Ia revealed the recovery of 2/3 of its carbohydrate content indicating the substance to be a trisaccharide.

4. Treatment with sweet almond emulsin for 24 hours gave unchanged trisaccharide whereas controls (cellobiose, methyl β-D-glucopyranoside and Ib) were cleaved to give the expected products.

5. The high specific optical rotation ($[\alpha]_D^{22}$ + 125° water, c, 0.9) suggests the presence of two α-D-glucosidic linkages in Ia. As mentioned above the β-isomer (Ib) has $[\alpha]_D^{20}$ + 84° (7).

6. Paper chromatographic analysis of a partial hydrolysate of Ia yielded glucose, maltose, isomaltose and unchanged Ia. Isomaltose was isolated by paper chromatography and found to have $[\alpha]_D^{30}$ + 123° water, c, 0.1; reported value $[\alpha]_D^{20}$ + 127° (14); gentiobiose has $[\alpha]_D$ + 11.8° (15). The isolated isomaltose was unattacked by emulsin whereas a gentiobiose control was cleaved to glucose. Complete hydrolysis of Ia gave only glucose.

7. Reduction of Ia with sodium borohydride gave the alditol IIa having a relatively high specific optical rotation ($[\alpha]_D^{27}$ + 110°) compared with the corresponding alditol (IIb) from the β-isomer, Ib, ($[\alpha]_D^{20}$ + 53°).

8. Partial hydrolysis of IIa gave glucose as the only reducing saccharide (p-anisidine spray reagent).

9. Periodate oxidation of IIa resulted in the consumption of 5.91 moles of periodate and the liberation of 2.92 moles of formic acid and 1.0 molecular proportion of formaldehyde. Theory requires 6:3:1, respectively.

10. Reduction of periodate-oxidized IIa followed by hydrolysis gave glycerol and erythritol as the only products.

These data together with the mode of synthesis confirm the structure of the trisaccharide as Ia and of its alditol as IIa.

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