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Composition and Structure of Glucofructans from Durum Wheat Flour¹

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ABSTRACT

Flour from *Triticum durum* (variety Leeds) was extracted with aqueous ethanol and the glucofructan fraction precipitated as the barium hydroxide complex. After acetylation, the glucofructan acetates were fractionally precipitated from acetone-ethanol with petroleum ether. Nine acetate fractions were obtained varying in specific optical rotation in chloroform from -3° to $+48^{\circ}$. The major fraction (20% of the total acetate yield) was structurally characterized by partial acid and enzymatic hydrolysis, periodate oxidation, and methylation. These data indicated that this fraction was composed of D-fructofuranose units linked beta-2,6 with a sucrose end unit. Single unit, beta-1,2-linked D-fructofuranosyl branches were also found. The remaining acetate fractions were methylated and the methylated sugars identified after hydrolysis by GLC. Analysis of these data indicated that the nine fractions obtained in this work were composed of two structurally different series of glucofructan molecules. In one (62% of the total including the major fraction), the primary D-fructose linkage was beta-2,6. In the other fractions (38% of the total), the primary linkage between D-fructose units was beta-1,2.

Fructose polymers found in plants generally are one of two types. Either they are composed of beta-1,2-linked fructose units as in inulin (1) or beta-2,6-linked fructose units as has been found in several plant species (2,3).

In wheat flour, a series of oligosaccharides composed of D-glucose and D-fructose have been found (4,5,6,7). They are a group of extremely water-soluble, nonreducing carbohydrates which extend in molecular weight to around 2,000. Their content in wheat flour is reported to be about 1% (4).

Montgomery and Smith (8) studied the structure of a glucofructan isolated from U.S. HRS wheat flour. It was found to be a branched polymer with the main chain composed of D-fructofuranosyl units linked beta-2,6. Schulbach and Müller (9) used a very similar procedure to isolate a glucofructan fraction from a European wheat flour. This polymer was found to have a main chain composed of beta-1,2-linked D-fructose units.

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Such a difference between two wheat glucofructans, isolated in essentially the same manner, has not been explained previously. It is conceivable that different structures are produced by different wheat varieties, or by the different climatic conditions prevailing in Europe and America. Another explanation was suggested by the work of White and Secor (10), who obtained paper chromatographic evidence for two homologous series of glucofructans in wheat.

Triticum durum is a different species from common bread wheat. Relatively little is known about the minor carbohydrate constituents in this species. The study reported here was undertaken to obtain structural information concerning the glucofructans from durum wheat.

By use of newer analytical techniques, an attempt was made to clearly establish whether two structurally different glucofructan series were present. The work on durum wheat may help explain the difference between the bread wheat samples noted above (8,9).

MATERIALS AND METHODS

Paper Chromatography

Paper chromatography was performed on Whatman No. 1 paper with the following solvents: A, ethyl acetate:acetic acid:water (3:3:1 v./v.); B, methyl ethyl ketone:water azeotrope. Spots were visualized with either silver nitrate spray reagent (11) or *p*-anisidine hydrochloride reagent (12).

Extraction of Glucofructans

The procedure was essentially as described by Montgomery and Smith (8). Durum wheat flour (*Triticum durum*, var. Leeds) (600 g.) and calcium carbonate (5 g.) were stirred under reflux with 82% ethanol (2 liters) for 1 hr. The alcohol was diluted to 70% and stirring continued for 15 hr. at room temperature. The alcohol was removed by centrifugation and the residue again extracted with 70% ethanol (2 liters) for 8 hr. After centrifugation, the alcohol extracts were combined and evaporated to 50 ml. under vacuum at 40°C. The precipitated protein was periodically removed by centrifugation as the evaporation proceeded. This solution was diluted with 95% ethanol until slightly turbid. A hot, clear solution of barium hydroxide octa-hydrate (20 g. in 75 ml. water) was added immediately. A precipitate formed at once and the resulting mixture was diluted to 2 liters with 95% ethanol. After standing overnight, a second addition of barium hydroxide solution was made as before. The insoluble barium "complex" of the glucofructan fraction was isolated by centrifugation.

The glucofructan-barium hydroxide complex was suspended in 100 ml. of ice-cold water and neutralized with dilute sulfuric acid. After removal of the barium sulfate precipitate by centrifugation, the clear supernatant solution was deionized by passing through a column of Dowex-50(H⁺) cation exchange resin and then through a column of Duolite A-4(OH⁻) anion cation exchange resin. The deionized solution was concentrated to dryness under reduced pressure.

The extraction of 3,600 g. of flour yielded 35 g. of a pale yellow syrup of crude glucofructans.

Acid and Enzymatic Hydrolysis

Acid hydrolysis was performed as described by Adams (13,14). Component

TABLE I. FRACTIONATION OF ACETYLATED GLUCOFRUCTANS

Fraction	Yield g.	$[\alpha]_D^{25}$ (CHCl ₃)
1	0.13	-3.0 ^o
2	0.80	+16.4 ^o
3	1.60	+20.4 ^o
4	4.80	+25.8 ^o
5	3.60	+30.1 ^o
6	2.40	+35.0 ^o
7	3.70	+41.0 ^o
8	3.00	+43.0 ^o
9	3.80	+48.0 ^o

sugars were identified by paper chromatography with solvent A and the silver nitrate spray.

Enzymatic hydrolysis with invertase² was performed on paper as described by Williams and Bevenue (15). Sucrose was used as a standard and the papers were developed in solvent A and sprayed with silver nitrate reagent.

Acetylation and Fractionation

The crude glucofructan syrup was acetylated with acetic anhydride and pyridine as described previously (8). A total of 30 g. of syrupy glucofructan acetate was isolated.

Glucofructan acetate (30 g.) was dissolved in 100 ml. of chloroform-acetone (1:1 v./v.). Petroleum ether was added slowly from a buret with stirring until a slight turbidity was produced. The mixture was then set aside for several hours in order for the precipitate to form and settle. The clear solution was decanted and subjected to further addition of petroleum ether.

Each glucofructan acetate fraction was dried under vacuum over anhydrous phosphorus pentoxide and its specific optical rotation in chloroform determined. Nine fractions were obtained in the initial precipitation. Fractions with similar rotations were combined and all fractions were redissolved in chloroform-acetone and fractionated again with petroleum ether. Twelve subfractions were obtained and those with similar specific rotations were combined to give a total of nine separate fractions (Table I).

Characterization of the Major Glucofructan Acetate Fraction

Deacetylation. A portion (1 g.) of fraction 4 (Table I) was dissolved in absolute ethanol (50 ml.) and treated with clean sodium metal (8). The precipitated glucofructan was isolated as a hygroscopic, colorless powder (0.5 g.); $[\alpha]_D^{25} +3^0$ (c.0.1, water).

Preliminary Analysis. A portion of the free glucofructan fraction was hydrolyzed with dilute sulfuric acid and its component sugars identified by paper chromatography in solvent A. Quantitative analysis of the component sugars was achieved by the phenol-sulfuric acid method (16). Enzymatic hydrolysis was performed as described above and autohydrolysis was done as described by Aspinall and Telfer (17).

²"Melibiase free," obtained from Nutritional Biochemical Corp., Cleveland, Ohio.

Periodate Oxidation. Periodate oxidation of the major glucofructan fraction was performed on the deacetylated product by the procedure described by Hay et al. (18). The periodate consumption was determined by the iodometric method and formic acid production was determined by titration with 0.005N NaOH solution. Periodate uptake was constant after 60 hr. and the formic acid production was constant after 100 hr.

Methylation. Methylation was done by the dimethylsulfinyl carbanion procedure of Hakamori (19). The anion was produced as described by Corey and Chaykovsky (20). The concentration of the anion in dimethyl sulfoxide was determined by titration of a 1-ml. aliquot with 0.1N hydrochloric acid. The procedure followed was that described by Sandford and Conrad (21). A light yellow syrup (0.6 g.) was obtained from the methylation of 1.5 g. of glucofructan acetate. Infrared analysis of the methylated product showed a complete absence of the strong OH absorption band at 3,600 to 3,200 cm^{-1} .

Hydrolysis of the Methylated Product and Identification of Methylated Sugars. Methylated glucofructan (0.3 g.) was dissolved in methanol and hydrolyzed with oxalic acid as described by Montgomery and Smith (8). After methylation, the hydrolyzed syrup was examined by paper chromatography in solvent B. The methylated sugars were visualized by the *p*-anisidine hydrochloride spray reagent.

Methanolysis and Quantitative Estimation of Methylated Sugars. Methylated samples were methanolized with 3% hydrogen chloride in absolute methanol (22). The methanolysis solution was not neutralized and was injected directly into the gas chromatograph.

The procedure used for the separation and quantitative estimation of the methyl glycosides of the methylated sugars was as described by Aspinall (22).

Gas chromatography was performed with a Pye Series 104 Chromatograph (Model 64) equipped with dual columns and heated dual-flame ionization detectors. Nitrogen was used as the carrier gas. Two column packings were used in 0.4-cm. stainless-steel columns, 150 cm. in length. Column A was 15% butane-1,4-diol succinate (BDS) on 80- to 100-mesh acid-washed Chromosorb W (Johns-Manville Products Corp.). Column B was 10% polyphenyl ether [*m*-bis-(*m*-phenoxy-phenoxy)-benzene] on 80- to 100-mesh acid-washed Chromosorb W. Column conditions were adjusted so that the values for standard methyl 2,3,4,6-tetra-O-methyl-beta-D-glucopyranoside and methyl 2,3,4,6-tetra-O-methyl-alpha-D-glycopyranoside corresponded to those reported by Aspinall (22). Column A was operated at 175°C. with a carrier gas flow of 60 ml. per min. Column B was operated at 200°C. with a carrier gas flow of 80 ml. per min. The detector temperature was maintained at 200°C. in all cases. Molar ratios for the various sugars were determined by carefully excising the corresponding peaks on the recorder chart paper and weighing them accurately on an analytical balance.

Characterization of Other Glucofructan Acetate Fractions

All other glucofructan acetate fractions were characterized only by methylation followed by hydrolysis and methanolysis of the methylated products. Hydrolysis products were identified by paper chromatography in solvent B and methanolysis products were identified by GLC. All procedures were as described above for the major glucofructan fraction.

RESULTS AND DISCUSSION

The crude glucofructan syrup yielded only D-glucose and D-fructose after acid hydrolysis as shown by paper chromatography. Results from the enzymatic hydrolysis with invertase gave evidence for D-glucose, D-fructose, and traces of sucrose. The fact that these glucofructans were readily hydrolyzed by invertase indicated that the D-fructose units were in the furanose form and that the glycosidic linkages were principally of the beta-type.

The fractionation data for the acetylated glucofructans are shown in Table I. Fraction 4, the major fraction, represented 20% of the total material isolated after fractionation. Structural studies were performed on this fraction after deacetylation.

The ratio of component sugars was determined after both acid and enzymatic hydrolysis using the phenol-sulfuric acid method. In both cases, a glucose:fructose ratio of 1:4 was determined. The complete hydrolysis by invertase suggested that all fructose linkages involved a beta-fructofuranosidyl bond.

After autohydrolysis with hot water (17), sucrose was one of the first products formed. This suggested that D-glucose was involved as a sucrose residue in the glucofructan molecule.

Periodate uptake on this fraction was 1.2 moles of periodate per anhydrohexose unit. In addition, 1 mole of formic acid was liberated for every 5 moles of anhydrohexose. If all D-fructose units were in the furanose form as indicated by acid and enzymatic hydrolysis, only D-glucose would produce formic acid provided it was unbranched at carbons 2, 3, and 4. The production of 1 mole of formic acid per 5 sugar units was in good agreement with the hydrolysis data which suggested a glucose:fructose ratio of 1:4. The fact that 1.2 moles of periodate were consumed per hexose unit indicated that each D-fructose unit was susceptible to periodate cleavage and was, therefore, unbranched at carbons 3 and 4.

The methylated glucofructan was subjected to mild acid hydrolysis with oxalic acid. Methylated sugars were identified by paper chromatography in solvent B. Four components were detected that were identical in rate of movement with authentic samples of 2,3,4,6-tetra-O-methyl-D-fructofuranose, 1,3,4,6-tetra-O-methyl-D-fructofuranose, 1,3,4-tri-O-methyl-D-fructofuranose, and 3,4-di-O-methyl-D-fructofuranose.

The methanolized methylated glucofructan was injected directly into the gas chromatograph for quantitative estimation of the methylated sugars. It was necessary to use two column types to obtain quantitative data on all four of the methylated sugars indicated by paper chromatography. This procedure was developed by Bishop and Cooper (23). Column conditions were adjusted with suitable standards so that the relative retention times were identical to those reported by Aspinall (22). The GLC data confirmed the paper chromatographic results with regard to component methylated sugars. Quantitative analysis of the molar ratio of the various methylated sugars is shown in Table II.

No trace of methyl 3,4,6-tri-O-methyl-D-fructofuranoside was detected by either column. This indicated the absence of any 1,2-linkages in the main chain of this fraction. A structure that will account for all the observed facts is shown in Fig. 1. Comparison of this structure with that found for the major glucofructan fraction of a HRS wheat flour (8) suggests that the two glucofructans were similar with regard to component units, glycosidic linkages, and branching pattern. The primary

TABLE II. MOLAR RATIO OF METHYLATED SUGARS (FRACTION 4)

Methyl Glycoside of	Mole Proportion
2,3,4,6-Tetra-O-methyl-D-glucose	1
1,3,4,6-Tetra-O-methyl-D-fructose	2
1,3,4-Tri-O-methyl-D-fructose	1
3,4-Di-O-methyl-D-fructose	1

difference observed was that the glucofructan from durum wheat contained one less branched repeating unit.

In previous structural studies on wheat glucofructans (8,9), only the major fraction was investigated. The one-step methylation procedure used in this work, combined with the relative ease of GLC analysis of the methylated product, facilitated some structural analysis on all the fractions isolated. Fraction 1 was obtained in low yield and has not been included in the methylation analysis. The other seven fractions were methylated, hydrolyzed, methanolized, and examined by paper chromatography (solvent B) and GLC.

Fractions 2, 6, 8, and 9 were similar to fraction 4, the major glucofructan fraction (Table III). The four methylated sugars were identified by comparison with authentic samples on both paper and gas chromatographic analysis. Fraction 2 gave an identical ratio of methylated sugars to that reported by Montgomery and Smith (8) for the major glucofructan from HRS wheat flour.

Fractions 3, 5, and 7, which together represented 38% of the total glucofructan acetate after fractionation, were similar to each other but quite different from fractions 2, 4, 6, 8, and 9. Paper chromatography in solvent B showed spots identical in rate of movement and color reaction with the *p*-anisidine spray reagent to authentic samples of 2,3,4,6-tetra-O-methyl-D-glucose, 1,3,4,6-tetra-O-methyl-D-fructose, 1,3,4-tri-O-methyl-D-fructose, 3,4,6-tri-O-methyl-D-fructose, and 3,4-di-O-methyl-D-fructose. The 3,4,6-tri-O-methyl-D-fructofuranose appeared to be the major fraction in all cases. These same sugars were identified by gas chromatography after methanolysis. Quantitative data were not obtained on the molar ratios of the various sugars due to incomplete resolution of all the peaks. However, an approximation of the relative peak areas indicated that 3,4,6-tri-O-methyl-D-fructofuranose made up more than 50% of the methylated sugars in these fractions.

The identification of 3,4,6-tri-O-methyl-D-fructose as the major methylated sugar indicated that the main glycosidic linkages in fractions 3, 5, and 7 were of the beta-1,2-type rather than the beta-2,6-type found in fractions 2, 4, 6, 8, and 9. This

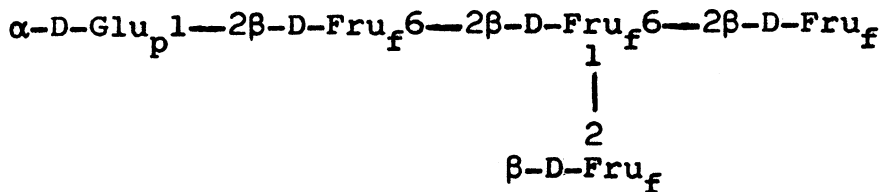


Fig. 1. Proposed structure for the major glucofructan fraction from durum wheat flour.

TABLE III. MOLAR RATIO OF METHYLATED SUGARS IN FRACTIONS 2, 4, 6, 8, and 9

Fraction	Relative Mole Proportion of the Methyl Glycoside of			
	2,3,4,6-Tetra-O-methyl-D-glucose	1,3,4,6-Tetra-O-methyl-D-fructose	1,3,4-Tri-O-methyl-D-fructose	3,4-Di-O-methyl-D-fructose
2	1	3	2	2
4	1	2	1	1
6	1	3	1	2
8	1	3	1	2
9	1	2	1	1

linkage is similar to that reported by Schulbach and Müller (9) for European bread flour glucofructan.

There are, therefore, two series of glucofructan molecules in the wheat sample studied in this work, one with primarily 1,2-linkages and one with primarily 2,6-linkages. It is probable that this was true also for the glucofructans investigated by Montgomery and Smith (8) and Schulbach and Müller (9) but the fact was missed because only the major fraction was studied.

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