Carbohydrate Research, 27 (1973) 39–46

THE POLYSACCHARIDES OF MANGO SEEDS (*Mangifera indica*, VAR. BULLOCK'S HEART)

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(Received August 8th, 1972; accepted August 23rd, 1972)

ABSTRACT

Mangoes (*Mangifera indica*) are grown in many warm regions of the world; for example, in Australia, Brazil, India, South America, and the United States (California and Florida), and along the shores of the Mediterranean sea (Egypt, Italy, and Spain). In Egypt, many varieties yielding delicious fruits are grown in large quantities (50,000 tons annually). Mango kernels are used[1] for feeding mammals and chickens. Starch has been isolated[2] from mango and its physical properties studied.

The aim of the present work was to study the polysaccharides present in mango kernels.

INTRODUCTION AND DISCUSSION

1. Polysaccharides of the endocarp. — The endocarp was extracted with hot water for 24 h, and material was precipitated from the extract with acetone. The precipitate was purified, and dried, to give ~3% of a yellowish white, amorphous substance (**1**). After extraction with water, the residue was extracted with hot, 25% sodium hydroxide solution for 3 h. The protein in the extract was precipitated with acetic acid in the cold, and removed by centrifugation. The polysaccharide was then precipitated with acetone, purified, and dried, to give ~9% of a yellowish white, amorphous substance (**2**).

Polysaccharide **1** had $[\alpha]_D^{23}$ −80° (*c* 0.4, water). Hydrolysis with acid, followed by paper chromatography of the hydrolyzate, indicated the presence of about 93% of xylose.

Polysaccharide **2** had $[\alpha]_D^{23}$ −83° (*c* 0.4, water). Hydrolysis with acid, followed by paper chromatography of the hydrolyzate, indicated the presence of about 93% of xylose.

Complete methylation of these xylans (**1** and **2**) with methyl sulfate and sodium hydroxide, followed by methyl iodide and silver oxide in *N*,*N*-dimethylformamide, gave viscous products having $[\alpha]_D^{23}$ −145° and −150° (*c* 0.1, chloroform), which, on hydrolysis and paper chromatography, showed two major components, namely, a 2,3-di-*O*-methylxylose, indicating (1→4) linkage; a 2-*O*-methylxylose, indicating that

C-3 is the point of branching; and a faint spot of a 2,3,4-tri-*O*-methylxylose, indicating a pyranose structure.

The results of assay of end-groups by periodate oxidation showed that the polysaccharide molecule is composed of ~71 sugar residues for xylan **1**, and ~79 for xylan **2**.

2. Polysaccharides from the kernels. — The kernels were successively extracted with acetone, ether, and methanol to remove lipids and related compounds. The dry kernels were then extracted with cold water containing 0.2% of sulfur dioxide. The aqueous extract, on treatment of a sample with iodine solution, gave a dark-blue color, indicating the presence of starch. The isolated starch was purified several times to give 64.5% of a yellowish white, amorphous substance, in comparison to 64% of starch in potatoes. Acid hydrolysis and paper chromatography indicated the presence of only one component corresponding to glucose. The starch was fractionated into its components, giving about 65% of amylose and 35% of amylopectin, compared to 25 and 75%, respectively, for potato starch. Mango-kernel amylose is a pale-brown, amorphous substance. It dissolves with difficulty in sodium hydroxide solution, giving a pale-brown, opaque solution. It has $[\alpha]_D^{23}$ +118° (*c* 0.1, 4% sodium hydroxide).

Acid hydrolysis followed by paper chromatography indicated the presence of glucose only; quantitative estimation indicated 93% of glucose. Complete methylation with methyl sulfate and sodium hydroxide, and then with methyl iodide and silver oxide in *N*,*N*-dimethylformamide, gave a product having $[\alpha]_D^{23}$ +171°, which, on hydrolysis and paper chromatography, showed mainly 2,3,6-tri-*O*-methylglucose, besides a little 2,3,4,6-tetra-*O*-methylglucose, indicating (1→4)-linkages. Quantitative estimation of the two components indicated a chain length of about 400 glucose residues.

The amylopectin of mango kernel is a pale-grey, amorphous substance which dissolves in sodium hydroxide to give a clear solution. It has $[\alpha]_D^{23}$ +122° (*c* 0.1, 4% sodium hydroxide). Acid hydrolysis followed by paper chromatography indicated the presence of glucose only; on quantitative estimation, it gave 94% of glucose. Complete methylation with methyl sulfate and sodium hydroxide, and then with methyl iodide and silver oxide in *N*,*N*-dimethylformamide, gave a product having $[\alpha]_D^{23}$ +176°, which, on hydrolysis and paper chromatography, showed a large spot for a 2,3,6-tri-*O*-methylglucose and two other (faint) spots for 2,3-di-*O*-methylglucose and 2,3,4,6-tetra-*O*-methylglucose, indicating (1→4)-linkages in the chain, with a point of branching on C-6. Quantitative estimation of the three components indicated repeating units of ~19 sugar residues.

End-group analysis by periodate oxidation indicated the presence of about 414 glucose units per molecule of amylose, and the repeating units of the amylopectin molecule contained 21 glucose residues, compared to 420 and 27 glucose units for potato amylose and amylopectin, respectively.

EXPERIMENTAL

Isolation of the xylans. — Mango endocarps were cut into thin slices. After defatting, 100 g was minced in hot water (one liter) for 24 h. The aqueous extract was filtered and centrifuged, and the clear solution was poured into acetone (3 ml/ml) to precipitate polysaccharide **1**.

The residue was then minced in 25% sodium hydroxide solution, and heated on a boiling-water bath for 3 h. The aqueous extract was centrifuged, the clear solution made neutral with M acetic acid, and the precipitated protein removed by centrifugation. The clear, supernatant liquor was poured into acetone (3 ml/ml) to precipitate polysaccharide **2**.

Purification[3] was effected by dissolving each polysaccharide in M sodium hydroxide (200 ml), acidifying the solution with acetic acid, and centrifuging; the clear solution was poured into acetone (3 ml/ml), giving an amorphous product.

The amorphous products were dialyzed against water for 24 h, precipitated with acetone, and dried. Polysaccharide **1** (3 g) was a yellowish white, amorphous substance which readily dissolved in water; it had $[\alpha]_D^{23}$ −80° (*c* 0.4, water). (Found: ash, 0.04; N, 0.03%.).

Polysaccharide **2** (9 g) was a yellowish white, amorphous substance which dissolved in M sodium hydroxide solution; it had $[\alpha]_D^{23}$ −83° (*c* 0.4, M sodium hydroxide). (Found: ash, 0.05; N, 0.06%).

Acid hydrolysis of the xylans (**1** *and* **2**). — A portion (400 mg) of each polysaccharide was heated with 0.5M sulfuric acid (30 ml) in a sealed tube for 10 h at 100°. The resulting mixture was filtered, and the filtrates rendered neutral with 0.05M barium hydroxide. Barium sulfate was removed, and the solution was evaporated to dryness. The residue was extracted several times with boiling ethanol, and the ethanol extracts were combined, de-ionized with a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) ion-exchange resins, and evaporated to a yellow syrup (320 mg and 340 mg, respectively).

Chromatography and estimation of sugars. — Portions of the acid hydrolyzates were examined on paper chromatograms with the upper layer of 5:1:4 butanol–ethanol–water as the mobile phase, and *p*-anisidine hydrochloride as the spray reagent; this showed only one spot, corresponding to xylose (R_G 0.15). After separation of the sugar on the paper chromatograms, quantitative estimation[4] of the sugar indicated about 93% of xylose for each extract from **1** and **2**. Other portions of the syrups were separated on paper chromatograms and the sugar (D-xylose) eluted: $[\alpha]_D^{23}$ +19° (equil. water). It was converted into D-xylose benzoylhydrazone (m.p., and mixed m.p., 167°).

Anal. Calc. for $C_{12}H_{16}N_2O_5$: C, 53.7; H, 6.0; N, 10.44. Found: C, 53.5; H, 6.2; N, 10.21.

Acetylation of the xylans[5] (**1** *and* **2**). — A portion (1.0 g) of each xylan was heated with pyridine (40 ml) for 2 h at 70°, and the mixture was cooled and kept overnight at room temperature.

Acetic anhydride (20 ml) was then added dropwise during 30 min, and the mixture was kept for 3 days at 52°. The resulting, clear solution was diluted with acetic acid (40 ml) and poured, with stirring, into ethanol. The precipitates were dried, and the products re-acetylated, giving 0.5 g and 0.48 g, respectively, of white, amorphous materials having $[\alpha]_D^{23}$ −120° and −124° (*c* 0.1, chloroform), respectively.

Anal. Calc. for $C_9H_{12}O_6$: *O*-acetyl, 39.82. Found: 37.03 for acetylated xylan **1**, and 40.00 for acetylated xylan **2**.

Methylation of the xylans (**1** *and* **2**). — A portion (3 g) of each xylan was suspended in water (30 ml) and allowed to swell for 5 h at 5°. It was then methylated[6] ten times with methyl sulfate and sodium hydroxide solution under an atmosphere of nitrogen, the mixture was dialyzed, and the product re-methylated[7] with methyl iodide and silver oxide in *N*,*N*-dimethylformamide. The products were extracted with chloroform, giving pale-yellow syrups (yield 0.8 and 0.6 g) having $[\alpha]_D^{23}$ −145° and −150° (*c* 0.1, chloroform), respectively.

Anal. Calc. for $C_7H_{12}O_4$: OMe, 38.8 Found: 38.0 for methylated xylan **1** and 36.3 for methylated xylan **2**.

Methanolysis and hydrolysis of the methylated xylans. — The methylated product (0.5 g each) was heated for 12 h at 100° with methanolic hydrogen chloride (3%), and the mixture was made neutral with cold, ethereal diazomethane[8]. The solution was evaporated at room temperature, and a solution of the product in 4% hydrochloric acid was heated for 7 h at 100° in a sealed tube. The acid was neutralized with silver carbonate, the filtrate treated with hydrogen sulfide, and the suspension filtered. The filtrate was de-ionized with a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) ion-exchange resins, and evaporated to a syrup (0.5 mg).

Chromatography of the hydrolysis products of the methylated xylans. — Examination of the hydrolysis products on paper chromatograms, with the upper layer of 5:1:4 butanol–ethanol–water as the mobile phase and *p*-anisidine hydrochloride as the spray reagent, showed three components, corresponding to a 2-*O*-methylxylose (R_G 0.38), 2,3-di-*O*-methylxylose (R_G 0.74), and 2,3,4-tri-*O*-methylxylose (R_G 0.94). Quantitative estimation of the three components indicated average repeating-units of ~64 and ~70 sugar units, respectively.

Periodate oxidation of the xylans (**1** *and* **2**)[8,9]. — A series of samples (20 mg) of each polysaccharide (**1** and **2**) was dissolved in water (20 ml) and treated with potassium chloride (0.5 g) followed by 0.05 mol. of sodium metaperiodate in water (10 ml). The mixtures were shaken in the dark. After 5 h, the solutions were treated with ethylene glycol (distilled from potassium hydroxide, and neutral to methyl red) (1 ml). The mixtures were allowed to stand for 1 h. The formic acid produced was then titrated against 0.01M sodium hydroxide, using Methyl Red as the indicator. The results obtained indicated chain-lengths of ~71 and ~79 sugar residues, respectively.

Isolation of mango starch. — Fresh mango kernels (2 kg) containing 48% of water were cut into thin slices and minced in acetone (6 kg) for 2 weeks; they were then filtered off, and the filtrate was evaporated to dryness to give a dark-brown

residue; the kernels were then successively treated with ether, and methanol, for 2 weeks.

The dry, defatted kernels (880 g) were treated with water (6 liters) containing 0.2% of sulfur dioxide and kept overnight, and the mixture was filtered on coarse cloth, and then centrifuged to give crude starch (800 g; 77%).

Examined under the microscope the starch granules were seen to be small (1–5 μm), with an elliptic shape compared with potato starch granules (20 μm).

Purification of the starch. — A solution of the crude starch (800 g) in M sodium hydroxide solution in an ice bath was rendered neutral with acetic acid. The precipitated proteins were removed by centrifugation, and the clear solution treated with acetone; the precipitate was dried, giving 690 g of starch (66.3%). Purification was effected by dialyzing an aqueous solution of the isolated starch in a cellophane bag for 24 h, reprecipitating with acetone, washing and drying, to give 680 g of product. The dried starch was extracted with ether in a Soxhlet apparatus for 24 h. The yield of pure starch was 670 g (64.5%). It was a creamy white, amorphous substance, soluble in alkali solution. Found: ash, 0.1; N, 0.06.

Determination of amylose content of the starch. — *A. Colorimetrically*[6]. A sample of the starch (100 mg) was dissolved in 100 ml of 0.1M sodium hydroxide. A portion (5 ml, equivalent to 5 mg of the alkaline starch solution) was introduced into a 500-ml volumetric flask, water (~100 ml) was added, and the mixture was slightly acidified with 3 drops of M hydrochloric acid; 5 ml of 0.1M iodine solution was added, and the volume was made to the mark with water. The experiment was repeated for pure amylose, and the intensity of the blue color formed was estimated, by use of a spectrophotometer, at 680 nm, against a "blank" iodine solution. The starch solution gave a blue value of 0.81 (based on a blue value of 1.2 for amylose); this corresponds to an amylose content of 67.5%, after correction for the non-starch components of the mixture.

B. Potentiometrically[10]. — A solution of dry starch (40 mg) in 5 ml of M potassium hydroxide solution was kept for 1 h, and made neutral to methyl orange with 0.5M hydrochloric acid; 0.5M potassium iodide (10 ml) was added, and the solution was diluted to 100 ml with water. The experiment was repeated with a sample of pure amylose. Each solution was titrated potentiometrically with 0.05M iodine solution. The results indicated that 100 mg of starch combined with 13.12 g of iodine, which corresponds to an amylose content of 66.6%.

Fractionation of the starch[11] *by selective precipitation.* — The defatted starch (100 mg) was gelatinized with distilled water (4 liters) on a boiling-water bath, and then autoclaved for 3 h at 18–20 lb.in.$^{-2}$, the pH before and after autoclaving being ~5.9–6.3. The solution was then centrifuged to remove cellular materials (5 g), the hot solution was added to butanol (400 ml), and the mixture allowed to cool slowly to room temperature, with continuous stirring, and kept overnight in a refrigerator. The precipitate of amylose–butyl alcohol complex was separated by centrifugation, washed several times with ethyl alcohol (to remove butyl alcohol) and then with acetone, and dried. The supernatant liquor (which contained the amylopectin fraction)

was treated with an excess of ethyl alcohol, and the precipitate was centrifuged off, washed with acetone, and dried. The properties of the two fractions were as follows.

	Amylose	*Amylopectin*
Yield from 100 g of starch	61.75	33.25
%	65	35
Color	pale brown	pale grey
$[\alpha]_D^{23}$ (*c* 0.1, 4.5M NaOH)	+118°	+122°
Blue value	1.2	0.1
Amylose value	19.8	0.4
η_{sp}	0.96	0.93

Acid hydrolysis of the amylose and amylopectin. — A portion (0.5 g) of each polysaccharide was hydrolyzed with 0.5M sulfuric acid (35 ml) for 10 h at 100° in a sealed tube. The resulting mixture was filtered, and the filtrate was made neutral by addition of 0.05M barium hydroxide. The barium sulfate was removed by filtration, and the clear filtrate was evaporated to dryness. The residue was extracted several times with hot methanol, and the extracts were combined, de-ionized with a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) ion-exchange resins, and evaporated to a yellow syrup (500 mg).

Paper chromatography of the sugar hydrolyzates. — The acid hydrolyzates were examined on paper chromatograms by use of the upper layer of 5:4:1 butanol–water–ethanol, and *p*-anisidine hydrochloride as the spray agent; this showed only one component (glucose; R_G 0.09). Quantitative[4] estimation of the sugar indicated 93% of glucose for the amylose fraction, and 94% of glucose for the amylopectin. Another portion of the syrup was isolated on a paper chromatogram, and the glucose eluted from the paper had $[\alpha]_D^{23}$ +53° (equilibrium; water); it was converted into its phenylosazone, m.p. and mixed m.p. with authentic D-glucose phenylosazone, 205°.

Anal. Calc. for $C_{18}H_{22}N_4O_4$: C, 60.30; H, 6.2; N, 15.6. Found: C, 60.0; H, 6.1; N, 15.5.

Acetylation of the isolated amylose and amylopectin[5]. — Each of the dry, powdered polysaccharides (2 g) was heated with pyridine (40 ml) for 2 h at 70°, and the mixture was cooled and kept overnight at room temperature. Acetic anhydride (20 ml) was then added dropwise during 30 min, and the mixture was kept for 3 days at 52°. The resulting, clear solution was diluted with acetic acid (40 ml) and poured, with stirring, into ethanol. The precipitates were dried, and the products were re-acetylated, giving 0.7 g of acetylated amylose and 0.72 g of acetylated amylopectin. The former had $[\alpha]_D^{23}$ +196°, and the latter +200° (*c* 0.1, chloroform).

Anal. Calc. for $C_{12}H_{16}O_8$: *O*-acetyl, 44.8 Found: For acetylated amylose, *O*-acetyl, 44.9; and for acetylated amylopectin, *O*-acetyl, 44.7.

Methylation of mango amylose and amylopectin. — Each of the powdered polysaccharides (2 g) was suspended in water (30 ml) and allowed to swell for 2 h at 5°. Each was then methylated[6] ten times with methyl sulfate and sodium hydroxide

solution under an atmosphere of nitrogen. The mixture was dialyzed, and the product was methylated[7] with methyl iodide and silver oxide in *N,N*-dimethylformamide. The product was extracted with chloroform, and the extract evaporated to dryness. The products, which were pale-yellow, viscous substances, were fractionated by successive extractions with light petroleum ether (b.p. 60–80°)–chloroform in the proportions 9:1, 17:3, and 4:1. The main products were obtained from the 17:3 mixture, respectively affording 0.6 g of methylated amylose and 0.7 g of methylated amylopectin. Methylated amylose, $[\alpha]_D^{23}$ +171° (*c* 0.1, chloroform), and methylated amylopectin, $[\alpha]_D^{23}$ +176° (*c* 0.1, chloroform).

Anal. Calc. for $C_9H_{16}O_5$: OMe, 45.6. Found: For methylated amylose, OMe, 44.5, and, for methylated amylopectin, OMe, 41.3.

Methanolysis and hydrolysis of the methylated products. — Each of the methylated products (0.5 g) was heated with methanolic hydrogen chloride (3%), for 12 h at 100°, and the mixture made neutral with cold, ethereal diazomethane[8]. The solution was evaporated at room temperature, and a solution of the product in 4% hydrochloric acid was heated for 7 h in a sealed tube at 100°. The acid was neutralized with silver carbonate, and the filtrate treated with hydrogen sulfide gas; the filtrate was de-ionized with a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) ion-exchange resins, and evaporated to a yellow syrup (350 mg from amylose, and 390 mg from amylopectin).

Chromatography of the hydrolyzates. — Examination of these hydrolyzates on paper chromatograms, with the upper layer of 5:1:4 butanol–ethanol–water as the mobile phase, and *p*-anisidine hydrochloride as the spray reagent, showed, for methylated amylose, a main component corresponding to 2,3,6-tri-*O*-methylglucose (R_G 0.83) and another faint spot corresponding to 2,3,4,6-tetra-*O*-methylglucose (R_G 1.0). Quantitative estimation of these two components indicated an average chain-length of 400 sugar units.

For methylated amylopectin, paper chromatography showed three spots, corresponding to a 2,3-di-*O*-methylglucose (R_G 0.57), 2,3,6-tri-*O*-methylglucose (R_G 0.83), and 2,3,4,6-tetra-*O*-methylglucose (R_G 1.0). Quantitative estimation of the three components indicated an average repeating unit of 19 sugar residues.

Periodate oxidation of mango amylose and amylopectin[8,9]. — A series of samples (20 mg) of the polysaccharides were dissolved in water (20 ml), and treated with potassium chloride (0.5 g) followed by 0.05 molar proportion of sodium metaperiodate in water (10 ml). A blank experiment was performed with potassium chloride (0.5 g) in water (20 ml) followed by 0.05 molar proportion of sodium metaperiodate in water (10 ml). The mixtures were shaken in the dark, and, after 5 h, the solutions were treated with ethylene glycol (distilled from potassium hydroxide, and neutral to Methyl Red) (1 ml), and the mixtures were kept at room temperature for 1 h. The formic acid present was then titrated against 0.01M sodium hydroxide, with Methyl Red as the indicator. The results obtained indicated a chain length of about 414 sugar units for amylose, and repeating units of about 21 sugar units for amylopectin.

REFERENCES

1 E. G. WILKINS, *Indian Farming*, 3 (1942) 636.
2 H. C. SRIVASTAVA, S. N. HARSHA, AND F. KIRITKUMER, *Ahmadabad Textile Ind. Res. Assoc.*, 111 (1970) 321.
3 EL S. AMIN, *J. Chem. Soc.*, (1956) 828.
4 K. H. MEYER, G. NOELTING, AND P. BERNFELD, *Helv. Chim. Acta*, 31 (1948) 103.
5 E. L. HIRST, J. K. N. JONES, AND A. J. ROUDIER, *J. Chem. Soc.*, (1948) 1779.
6 C. E. BALLOU AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1952) 1054.
7 J. K. N. JONES AND L. E. WISE, *J. Chem. Soc.*, (1952) 3389.
8 S. K. CHANDA, E. L. HIRST, J. K. N. JONES, AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1950) 1289.
9 T. G. HALSALL, E. L. HIRST, AND J. K. N. JONES, *J. Chem. Soc.*, (1947) 1399.
10 F. L. BATES, D. FRENCH, AND R. E. RUNDLE, *J. Amer. Chem. Soc.*, 65 (1963) 142.
11 T. J. SCHOCH, *J. Amer. Chem. Soc.*, 64 (1942) 2957.