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STUDIES OF THE POLYSACCHARIDES FROM SUNFLOWER HEADS

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ABSTRACT

Extraction of sunflower heads with ammonium oxalate afforded water-soluble pectin material and water-insoluble glycoprotein material, the carbohydrate portion of which consisted of galacturonic acid and xylose residues; the pectin material defied fractionation with cetylpyridinium chloride. Extraction with hydrochloric acid (pH 1.5) afforded water-soluble and water-insoluble polysaccharide materials. The former, when fractionated with cetylpyridinium chloride, gave a glycoprotein, the carbohydrate moiety of which was composed of galacturonic acid, galactose (major), glucose, arabinose, and xylose, and also a rhamnan. The latter was a glycoprotein, the carbohydrate portion of which consisted of galactose (major), glucose, xylose, and rhamnose residues. Extraction of the sunflower heads with water also gave glycoprotein material, which was fractionated by paper electrophoresis into a glycoprotein, the carbohydrate moiety of which was composed of galacturonic acid (minor), galactose, glucose, xylose, arabinose, and rhamnose (major) residues, and a heteropolysaccharide composed of galactose (major), glucose, xylose, and arabinose residues.

INTRODUCTION

Most of the studies on the carbohydrates of sunflower heads have been mainly concerned with the pectic substances[1,2]. The fractionation of pectins from sunflowers and from other sources, using sodium acetate, has been reported[2]. Each of the pectic acids contained a galacturonan free of neutral sugars and a galacturonan to which neutral sugars were attached, probably as side chains[2].

We have reported[3] that local sunflower heads contained residues of galacturonic acid, galactose, glucose, arabinose, xylose, and rhamnose (major). The presence of galactose and rhamnose in such high proportions indicates that they are not entirely components of the sunflower galacturonan.

We now report on the isolation of the polysaccharide materials from local sunflower heads.

EXPERIMENTAL

General. — Chromatography on Whatman No. 1 paper was performed with *A* 1-butanol–pyridine–water[4] (6:4:3), *B* 1-butanol–acetic acid–water[5] (4:1:5, upper layer), and *C* phenol–water[6] (80:20), and detection was effected with aniline hydrogen phthalate and ninhydrin reagents[7]. Paper electrophoresis was effected (5 h, room temperature) with an Elphor apparatus and acetic acid–acetate buffer (0.02M, pH 3.42), at a potential of 300 V, giving a current of 0.2 mamp/strip. Staining was effected with Amido Black dye and with the Toluidine Blue reagent[8].

Ash contents were determined by heating polysaccharide samples to constant weight at 800°. Protein was determined for water-soluble and water-insoluble samples by the method of Lowry *et al.*[9] and by the micro-Kjeldahl method, respectively. Complete, acid hydrolysis of polysaccharide samples was performed with sulphuric acid[10]. Sugars in the hydrolysates were determined, after ascending p.c. (solvent *A*) and elution from the chromatograms; galacturonic acid was determined by reaction with carbazole[11], arabinose and xylose with orcinol[12], and galactose, glucose, and rhamnose with L-cysteine–sulphuric acid[13,14]. Methoxyl content was determined according to the procedure of Myers and Baker[15]. All solutions were concentrated *in vacuo* at 40°.

Sunflower (*Helianthus annuus*) heads, obtained from the Imbaba locality at Giza, Cairo, in August 1973, were freed from foreign substances, air-dried, and milled.

Extractions with ammonium oxalate and hydrochloric acid. — (*a*) Sunflower-head material was twice extracted with 0.3% ammonium oxalate at 90° for 1.5 h. After filtration, the combined extracts were dialysed against distilled water for 72 h; a precipitate formed in the dialysis bag. The water-insoluble residue (*A*) was isolated by centrifugation of the dialysed solution. The supernatant solution was treated with 1 vol. of ethanol acidified with HCl (pH 2), and the precipitate (*B*) was repeatedly washed with ethanol until free from chloride ions. *A* and *B* were dried under reduced pressure at room temperature.

(*b*) The plant material was twice extracted at 80° for 1.5 h with water adjusted to pH 1.5 with HCl. The clarified, combined extracts were neutralised with saturated, aqueous sodium carbonate, dialysed against distilled water for 72 h, and centrifuged. The supernatant solution was lyophilized and the residue was dried under reduced pressure at room temperature to give the water-soluble (*C*) and water-insoluble (*D*) polysaccharide materials, respectively.

Extraction with water. — Plant material (5 g) was twice ground with distilled water (50 ml) for 20 min at room temperature. The filtered extract was dialysed against distilled water at $\sim -5°$ and then centrifuged, and the supernatant solution was lyophilized to give the water-extractable polysaccharide material (*E*).

Fractionation of the polysaccharide materials. — (*a*) *With cetylpyridinium chloride.* A solution of *B* or *C* (1 g) in water (100 ml) containing sodium sulphate

(0.4 g) was treated with aqueous 2.5% cetylpyridinium chloride until no further precipitation occurred. Each precipitate was isolated by centrifugation in the presence of Celite. The supernatant was dialysed against distilled water and freeze-dried. Polysaccharide material was recovered from each precipitate by several extractions with 4M potassium chloride. Excess cetylpyridinium chloride was then removed by precipitation with M potassium thiocyanate, each supernatant was dialysed against distilled water, concentrated to 25 ml, and treated with 4 vol. of ethanol, and the precipitate was isolated and dried; *B* defied fractionation, whereas *C* afforded acidic (*C1*) and neutral (*C2*) fractions.

(*b*) *By paper electrophoresis.* By this process, *E* was fractionated into a component which moved towards the cathode and stained with Amido Black and Toluidine Blue reagents, and one which migrated towards the anode and stained purple with Toluidine Blue. The two components were eluted from unstained pherograms with water, and the solutions were concentrated to dryness to afford *E1* and *E2*, respectively.

RESULTS AND DISCUSSION

The ammonium oxalate-extractable, water-soluble polysaccharide material (*B*, 12%) from sunflower heads contained protein (1.9%) and methoxyl groups (9.0%), and was rich in carbohydrate (78%) of which galacturonic acid comprised 73%. *B* had $[\alpha]_D$ +250° and can thus be considered as pectin material. These results are in agreement with those of Zitko and Bishop[2]. *B* also contained minor amounts of glucose, arabinose, and rhamnose, but not galactose or xylose, which were found in the original plant-material[3]. Glucuronic acid (or its lactone) was not found in the acid hydrolysates of *B* or the original plant-material.

Two-dimensional p.c. (solvents *B* and *C*) of a hydrolysate of *B* indicated the presence of aspartic acid, glutamic acid, serine, glycine, threonine, alanine, lysine, histidine, arginine, proline, valine, methionine, tyrosine, leucine, and isoleucine. *B* defied fractionation with cetylpyridinium chloride, but this result does not mean that it is a single heteropolysaccharide.

Unlike *B*, the ammonium oxalate-extractable, water-insoluble material *A* (1.5%) was rich in protein (34.5%) and contained a minor carbohydrate component (1%). Acid hydrolysis of *A* gave (p.c., solvent *A*) minor amounts of galacturonic acid (0.7%) and xylose (0.4%); xylose was not a component of *B*. *A* contained no methoxyl groups but all the amino acids found in *B*, and also cystine and phenylalanine. Thus, *A* is an acid glycoprotein containing galacturonic acid (major) and xylose (minor).

The hydrochloric acid-extractable, water-soluble polysaccharide material (*C*, 4.5%) differed from *B* in that it contained no methoxyl groups, more protein (12.5%), and less carbohydrate (54%). *C* contained the amino acids aspartic acid, glutamic acid, cystine, serine, glycine, threonine, alanine, lysine, histidine, arginine, proline, methionine, tyrosine, and phenylalanine.

Acid hydrolysis of *C* gave (p.c., solvent *A*) galacturonic acid (13%), galactose (13%), glucose (trace), arabinose (trace), xylose (trace), and rhamnose (26%). The characteristic feature of *C* is its high content of rhamnose (48% of the total carbohydrate). Hydrochloric acid was more efficient than ammonium oxalate in extracting polysaccharide material rich in rhamnose, whereas ammonium oxalate extracted polysaccharide material rich in galacturonic acid. No glucuronic acid was found in acid hydrolysates of *A*, *B*, or *C*.

Fractionation of *C* with cetylpyridinium chloride gave a major, acidic fraction (*C1*, 57%) and a minor, neutral fraction (*C2*, 16%); *C1*, but not *C2*, gave a positive reaction for protein.

Hydrolysis of *C1* gave (p.c., solvent *A*) galacturonic acid (15%), galactose (34%), and minor amounts of glucose, arabinose, and xylose; galactose comprised 63% of the total carbohydrate. On the other hand, acid hydrolysis of *C2* gave only rhamnose and a trace of arabinose; *C2* is therefore a rhamnan.

Thus, *C* comprises an acidic glycoprotein (*C1*) containing galactose as the major component, and a rhamnan (*C2*).

The hydrochloric acid-extractable, water-insoluble material (*D*, 4%) was similar to *A* in being rich in ash and protein, but contained a higher proportion of carbohydrate (9.7%). The amino acid composition of *D* was similar to that of *A*, except for the lack of glutamic acid.

Acid hydrolysis of *D* gave (p.c., solvent *A*) galactose (7%) and trace amounts of glucose, xylose, and rhamnose; galactose comprised 72% of the total carbohydrate. However, the most characteristic feature of *D* was its lack of galacturonic acid. Thus, *D* is a neutral glycoprotein.

Extraction of sunflower heads with water afforded a glycoprotein *E* (15.0%) which contained ~64% of carbohydrate, 29% of protein, and all the sugars found in the sunflower heads[3]. Unlike *B* and *C*, *E* contained only a little galacturonic acid, and, on acid hydrolysis, gave galacturonic acid (minor), glucose, galactose (major), xylose, arabinose, and rhamnose.

E was fractionated by paper electrophoresis (0.02M acetate buffer, pH 3.42) into two components, one of which (*E1*) moved towards the cathode and the second (*E2*) migrated towards the anode. Variation of the pH and the concentration of the buffer did not give further fractionation. *E1* stained with both Amido Black and Toluidine Blue, indicating the presence of protein and carbohydrate moieties, whereas *E2* stained (purple) only with Toluidine Blue.

E1 contained 48% of protein and, on acid hydrolysis, afforded galacturonic acid (4%), glucose (3%), galactose (5%), xylose (9%), arabinose (11%), and rhamnose (20%), and is therefore an acid glycoprotein.

E2 gave a negative reaction for protein and, on acid hydrolysis, afforded glucose (27%), galactose (42%), xylose (12%), and arabinose (19%). The most characteristic feature of *E2* was its lack of galacturonic acid. Although *E2* contained only neutral sugars, it migrated towards the anode on paper electrophoresis and hence bears a negative charge.

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