Note

The hemicellulose of pollen grains of *Phoenix dactylifera*

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The Egyptian date palm (*Phoenix dactylifera*), a plant distributed in North Africa and South Western Asia, is also grown in Mexico and the United States of America. It is widely distributed in Egypt, and its dates are extensively used as human food.

The aim of the present work was to study the hemicellulose extracted from the pollen grains of this palm with 4% sodium hydroxide and precipitated from the extract with ethanol. The hemicellulose is a pale-yellow, amorphous material having $[\alpha]_D^{23}$ −173° (*c* 0.01, sodium hydroxide) that slowly dissolves in hot water to form a viscous solution, but is readily soluble in alkali. Paper chromatography of the hydrolyzate obtained by hydrolysis with acid indicated the presence of 45.8% of an arabinose, 25.4% of a galactose, 17.9% of a xylose, 8.9% of a rhamnose, and 2.16% of uronic acids. Exhaustive methylation of the hemicellulose with dimethyl sulfate and sodium hydroxide, followed by methyl iodide and silver oxide in *N*,*N*-dimethylformamide, gave a product having $[\alpha]_D^{24}$ −140° (*c* 0.1, chloroform). This was hydrolyzed with acid, and paper chromatography of the hydrolyzate showed three major components, namely, 2,3-di-*O*-methylarabinose [indicating a (1→5)-linkage], 2,3,5 tri-*O*-methylarabinose (indicating that arabinose residues are present in the hemicellulose as terminal groups), and 2,3,6-tri-*O*-methylgalactose [indicating (1→4)-linkages]; a minor component was found to be 3,4-di-*O*-methylrhamnose [indicating a (1→2)-linkage].

Results of end-group analysis by periodate oxidation showed that the hemicellulose molecule is composed of ~36 sugar residues.

EXPERIMENTAL

Isolation of the hemicellulose. — Pollen grains of the Egyptian date palm (*Phoenix dactylifera*, var. El-Hayani), collected at the end of April, 1968, in El-Saadate, Egypt, were dried (830 g), and defatted by being kept under acetone for one week. After filtration, the pollen was extracted for 4 h with boiling water (10 liters), and then with 1.5% sodium carbonate for 2 days at room temperature. The residue was dried, extracted for 3 h at 100° with 4% sodium hydroxide solution (4.5 liters), the

suspension filtered through muslin, and the filtrate centrifuged. The extract was cooled in ice, made acid with glacial acetic acid, and centrifuged; the clear centrifugate was poured into ethanol (6 parts), with stirring. The resulting, amorphous precipitate was washed with ethanol, and dried (yield 5.2 g).

Purification[1] was effected by dialysis for 48 h, and reprecipitation with ethanol. Further purification was achieved by dissolving the precipitate in M sodium hydroxide, acidifying the solution with acetic acid, and precipitating the hemicellulose with ethanol.

Attempted fractionation by use of 25% aqueous cupric chloride gave no precipitate. The hemicellulose was reprecipitated from ethanol as a pale-yellow, amorphous material readily soluble in cold water (Found: ash, 0.0952%; N, 0.077%; reducing power after hydrolysis, 98.63%).

Acid hydrolysis of the hemicellulose. — A mixture of the purified hemicellulose (360 mg) with 25 ml of 0.5M sulfuric acid was heated in a sealed tube for 12 h at 100°. The solution, in which was suspended a small amount of flocculent material (8 mg), was filtered, and the filtrate was made neutral with 5mM barium hydroxide. The barium sulfate was removed by filtration, and the filtrate was evaporated to dryness under diminished pressure at 80°. The sugars were extracted from the residue with fresh portions of boiling methanol, until the last extract was nonreducing, leaving a brown residue. The extract was de-ionized with a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) ion-exchange resins, and evaporated to a syrup (300 mg).

The brown residue was tested for uronic acids with the benzidine reagent and by the Lefèvre–Tollens test[2]; it was found to contain a uronic acid.

Chromatography, and estimation of sugars in the hemicellulose. — A portion of the acid hydrolyzate was examined on a paper chromatogram by use of 5:4:1 butyl alcohol–water–ethanol, with *p*-anisidine hydrochloride as the spray reagent. The major components were arabinose and galactose; xylose and rhamnose were minor components. After separation of the sugars by chromatography, quantitative estimation by the method of Meyer *et al.*[3] was performed; the results indicated the presence of 45.8% of an arabinose, 25.4% of a galactose, 17.9% of a xylose, and 8.9% of a rhamnose. The uronic acids were determined by the Lefèvre–Tollens method, which showed the presence of 2.16% thereof.

Another portion of the syrup was separated on a paper chromatogram, and the individual components were extracted with methanol. The sugar in the first spot was converted into galactose benzoylhydrazone (m.p. and mixed m.p., 184°), and that in the second spot was converted into arabinose benzoylhydrazone (m.p. and mixed m.p., 200°).

Acetylation of the hemicellulose[4]. — The powdered, dry, purified hemicellulose (1 g) was warmed with pyridine (50 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 clear solution was decanted, diluted with acetic acid (50 ml), and poured in ethyl alcohol (1 liter), with stirring; the resulting, white, amorphous precipitate was collected by centrifuga-

tion, successively washed with alcohol and ether, and dried (yield 0.8 g). Reacetylation was conducted in the same way, giving 0.4 g of product, $[\alpha]_D^{23}$ −121° (*c* 0.1, chloroform).

Methylation of the hemicellulose. — The powdered hemicellulose (1.5 g) was suspended in water (20 ml) and allowed to swell for 5 h at 5°; it was then methylated[5] eight times with dimethyl sulfate and sodium hydroxide solution under an atmosphere of nitrogen, the mixture was dialyzed, and the dry product was methylated twice with methyl iodide and silver oxide in *N*,*N*-dimethylformamide. The product (isolated by extraction with chloroform) was a yellow, amorphous material (yield 0.32 g) that had $[\alpha]_D^{24}$ −140° (*c* 0.1, chloroform).

Methanolysis and hydrolysis of the methylated hemicellulose. — The methylated product (0.1 g) was heated for 12 h at 100° with methanolic hydrogen chloride (3%), and the mixture was made neutral with cold, ethereal diazomethane[6]. The solution was evaporated at room temperature, and a solution of the product in 4% hydrochloric acid was boiled for 7 h under reflux. The acid was neutralized with silver carbonate, and 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.

Examination of the hydrolyzate on a paper chromatogram, with the upper layer of 5:1:4 butanol–ethanol–water as the mobile phase and *p*-anisidine hydrochloride as the spray reagent, showed spots corresponding to 2,3-di-*O*-methylarabinose (R_G 0.64), 2,3,6 tri-*O*-methylgalactose (R_G 0.71), 2,3,5 tri-*O*-methylarabinose (R_G 0.95), and 3,4-di-*O*-methylrhamnose (R_G 0.84).

Periodate oxidation of the hemicellulose. — A series of samples (50 mg) of the hemicellulose were dissolved in M ammonia, and each solution was rendered neutral with dilute hydrochloric acid, made up to 5 ml with 3% aqueous sodium chloride, and oxidized with 0.37M sodium metaperiodate (2 ml) at room temperature. The excess of periodate was decomposed with ethylene glycol, and the formic acid liberated was titrated with 0.05M barium hydroxide. The results obtained (5-h intervals) indicated the presence of chains having ~36 sugar residues.

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