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Note

A study of the polysaccharides of the kernel and endocarp of the fruit of the Doum palm (*Hyphaene thebaica*)

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Research on the fruit of the Doum palm (*Hyphaene thebaica*) has been performed by many investigators[1–13] The mannan component of the kernel has been studied by Maymone *et al*[7], as well as by El Khadem and Sallam[11], however, a complete investigation has not been conducted on the remaining polysaccharides of the kernel On the other hand, the polysaccharides of the woody endocarp have not been studied at all up to the present

In the present work, a higher yield of mannan (30%) was obtained from the kernel than in previous studies[7 11] by refluxing it with 25% sodium hydroxide after defatting Moreover, the residue obtained (37 2%) was identified, and found to consist of cellulose

The polysaccharide component of the woody endocarp was investigated, and found to contain about 23% of xylan and 52 3% of cellulose The negative optical rotation of the xylan indicated that the linkages are in the β form After methylation and hydrolysis of the xylan, chromatography revealed two major components, corresponding to 2,3-di-*O*-methyl-D-xylose [indicating (1→4)-linkages] and 2-*O*-methyl-D-xylose (showing that C-3 is a point of branching), as well as a faint spot corresponding to 2,3,4-tri-*O*-methyl-D-xylose (indicating the pyranose structure) The degree of polymerization was found to be about 80

EXPERIMENTAL

Analysis of the kernel

Chemical analysis of the white kernel showed it to have the following percentage composition oils, 11 3, proteins, 7 0, ash, 1 72, moisture, 11 76, cellulose, 37 2, and mannans, 30%

The finely ground, white kernel (5 g) was hydrolyzed with 25 ml of 70% sulfuric acid for 7 h at 25°, water (325 ml) was added, and the solution was heated for 7 h in a boiling-water bath It was then cooled, and made neutral with barium hydroxide and barium carbonate After filtration of the suspension, the filtrate was concentrated to a small volume An aliquot of the resulting solution was subjected to paper chromatography by using 5.1 4 butanol–ethanol–water and subsequently spraying with aniline–diphenylamine–phosphoric acid reagent This revealed a major

zone corresponding to glucose, and another, slightly fainter, one corresponding to mannose

Isolation of the cellulose — A defatted sample of the finely ground, white kernel was boiled under reflux with 25% sodium hydroxide for 3 h The mixture obtained was highly gelatinous It was acidified with 50% acetic acid, with cooling, and then diluted with boiling water After centrifugation, the sticky residue was copiously washed with tap water, and dried, yielding 37 2% of a white powder which was found to be soluble only in the Schweitzer reagent

Acid hydrolysis of the isolated cellulose — A sample of this product was hydrolyzed for 6 h with 70% sulfuric acid as already described Paper chromatography under the same conditions revealed only one spot, corresponding to glucose, the identity of which was confirmed by preparing the osazone in pale-yellow needles having the same shape as those of glucosazone and having m p and mixed m p of 205°

Anal Calc for $C_{18}H_{22}N_4O_4$ C, 60 3, H, 6 2, N, 15 6 Found C, 60 0, H, 6 1, N, 16 0.

Methylation of the cellulose — The residue (6 g) was suspended in water (40 ml), and allowed to swell for 2 h at ~10° The swollen product was then methylated eight times by the gradual addition of methyl sulfate and 30% aqueous sodium hydroxide solution under an atmosphere of nitrogen The mixture was dialyzed, and the product was remethylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide By extraction with chloroform, about 2 5 g was obtained, $[\alpha]_D^{25}$ −15 2° (*c* 1 8, chloroform)

Anal Calc for $C_9H_{16}O_5$ OMe, 45 6, C, 54 1, H, 8 1 Found OMe, 42 0, C, 53 3; H, 6 8

Hydrolysis and chromatography of the methylated cellulose — The methylated product (~1 g) was heated with 3% methanolic hydrogen chloride in a sealed tube for 7 h at 100° The solution was then made neutral with cold, ethereal diazomethane, concentrated at room temperature, and the residue refluxed with 4% hydrochloric acid for 7 h, and the solution made neutral with silver carbonate, the suspension filtered, and the filtrate de-ionized and evaporated to a syrup Paper chromatography of the hydrolyzate with 5 1 4 butanol–ethanol–water, with *p*-anisidine hydrochloride as the spray reagent, revealed the presence of 2,3,6-tri-*O*-methyl-D-glucose (R_G 0 83), the identity of which was confirmed by a m p and mixed m p of 120°, $[\alpha]_D^{22}$ +69° (*c* 1 5, water)

Anal Calc for $C_9H_{18}O_6$ OMe, 41 9, C, 48 7, H, 8 20 Found OMe, 39 5, C, 48 5, H, 7 7

There was another faint spot, for 2,3-di-*O*-methylglucose (R_G 0 57) Rough quantitative estimation of these components indicated that the average chain-length is ~170 glucose units (*cf*, 200 units for cotton cellulose)

Identification of the mannan. — The acidified, 25% sodium hydroxide extract was treated with an excess of acetene, giving a yield of 30% of mannan; its identity was proved by acid hydrolysis as already described, followed by paper chromatography, and formation of the phenylhydrazone (m p and mixed m p , 205°)

Anal Calc for $C_{12}H_{18}N_2O_5$ C, 53 3, H, 6 7, N, 10 3 Found C, 53 0, H, 6 2; N, 10 0

Analysis of the endocarp

Isolation of the xylan — The woody endocarp (15 g) was dried to constant weight at ~100°, and then defatted by boiling under reflux for 3 h with dry benzene After air-drying, the residue was treated overnight with 25% sodium hydroxide, and the suspension was filtered. The proteins were precipitated from the filtrate with acetic acid, and then the polysaccharide was precipitated with ethanol, yielding 4 4 g (23%) of an amorphous, yellow material

Acid hydrolysis of the isolated xylan — A sample of this polysaccharide was hydrolyzed with 0 5M sulfuric acid in a sealed tube for 15 h at 100° and, after neutralization with dilute barium hydroxide, the hydrolyzate was paper-chromatographed, using 5 1 4 butanol–ethanol–water, and *p*-anisidine hydrochloride as the spray reagent This revealed one zone corresponding to xylose, the identity of which was confirmed by preparing the corresponding osazone (m p and mixed m p 165°)

Anal Calc for $C_{17}H_{20}N_4O_3$ C, 62 0, H, 6 1, N, 17 1 Found C, 61 5, H, 6 2, N, 17 2

Methylation of the isolated xylan — The xylan (2 g) was suspended in water (25 ml) and allowed to swell for 5 h at 50° The swollen product was then methylated eight times by the gradual addition of methyl sulfate and 30% aqueous sodium hydroxide solution under an atmosphere of nitrogen The mixture was dialyzed, and the product remethylated with methyl iodide and silver oxide in *N,N*-dimethylformamide By extraction with chloroform, about 0 5 g of a yellowish material was obtained, $[\alpha]_D^{25}$ −150° (*c* 0 1, chloroform)

Anal Calc for $C_7H_{12}O_4$ OMe, 38 7, C, 52 5, H, 7 9 Found OMe, 38 2, C, 52 5, H, 7 3

Hydrolysis of the methylated xylan — The methylation product was heated for 12 h at 100° with 3% methanolic hydrogen chloride The mixture was made neutral with cold, ethereal diazomethane, and concentrated at room temperature The product was then heated with 4% hydrochloric acid in a sealed tube for 7 h at 100° The acid was neutralized with silver carbonate, and the filtrate was treated with hydrogen sulfide and filtered The filtrate was de-ionized, and evaporated to a syrup

Chromatography of the hydrolyzate — Examination of the hydrolyzate on a paper chromatogram with 5 1 4 butanol–ethanol–water, and *p*-anisidine hydrochloride as the spray reagent, showed three spots, corresponding to (*a*) 2-*O*-methylxylose (R_G 0 38)

Anal Calc for $C_6H_{12}O_5$ OMe, 18 9, C, 43 9, H, 7 2 Found OMe, 18 5, C, 43 6, H, 7 0

(*b*) 2,3-di-*O*-methylxylose (R_G 0 74)

Anal Calc for $C_7H_{14}O_5$ OMe, 34 8, C, 47 2, H, 7 9 Found OMe, 34 5, C, 47 0, H, 7 5

and (*c*) a faint spot of 2,3,4-tri-*O*-methylxylose (R_G 0 94)

Quantitative estimation of the three components indicated that the average chain length is about 80 xylose units

Identification of the residue — The fibrous residue obtained after treatment with 25% sodium hydroxide and filtration was washed with glacial acetic and then with water to neutrality, it was dried, giving a yield of 7 85 g (52 3%) of whitish, fibrous residue, which was found to be soluble only in the Schweitzer reagent

This material (5 g) was hydrolyzed with 25 ml of 70% sulfuric acid for 7 h as already described Paper chromatography under the conditions already given revealed only one spot, corresponding to glucose This conclusion was confirmed by preparing the osazone, pale-yellow needles having the same shape as those of glucosazone, and having m p and mixed m p 205°

Anal Calc for $C_{18}H_{22}N_4O_4$ C, 60 3, H, 6 2; N, 15 6 Found C, 60 0, H, 6 1; N, 16 0.

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