ON THE STRUCTURE OF CELL WALLS AND CELL WALL MANNANS FROM IVORY NUTS AND FROM DATES

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INTRODUCTION

As early as 1889 REISS[1] found that the cell walls of vegetable ivory nut (*Phytelephas macrocarpa* Ruiz et Pav.) yielded mostly mannose (Seminose) after hydrolysis. LÜDTKE[2] has shown that there are two different mannans in the cell walls of ivory nuts, *viz.* mannans A and B, which differ from each other in their solubility in dilute alkali. He pointed out that there is also about 7% cellulose in the endosperm. The chemical structure of the two mannans has been studied by KLAGES[3] and more recently by ASPINALL *et al.*[4]. According to the latter these mannans both contain two types of molecules, one with a mannopyranose residue as non-reducing end-group, the other with a galactopyranose residue. As well as the 1,4-linkages between the mannopyranose residues, there were also 1,6-linkages present (6.8% in mannan A and 14.3% in mannan B). According to ASPINALL *et al.*[4], the two mannans differ mainly in molecular size, mannan A having a degree of polymerisation ($\overline{DP}_n$) of 10–13 and mannan B of 40.

Chemical composition

All the date and ivory-nut samples which were studied microscopically, by X-rays, and osmometrically for $\overline{DP}_n$ determination, were analysed by quantitative paper chromatography. Tables I and II show the results. There is no essential difference in chemical composition between the date and ivory-nut endosperms.

The approximate percentages of mannan A, mannan B and cellulose in ivory nut endosperm are given in Table III, which gives figures that were calculated from the yields of the residues after extraction.

TABLE I

CARBOHYDRATE ANALYSES OF DATE-ENDOSPERM MATERIAL

Sample		*Galactose* %	*Glucose* %	*Mannose* %	*Arabinose* %
D_1	Native	3.5	6.4	87.4	2.7
D_2	D_1 extracted with acetone–water, acetone and ether		(7.3)	90.7	2.0
DA	Mannan A extracted with 7% KOH from D_2		(0.5)	99.5	trace
DB	Residue after several extractions of D_2 with 7% and 14% KOH		(12.8)	83.8	3.4

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TABLE II

CARBOHYDRATE ANALYSES OF IVORY-NUT ENDOSPERM MATERIAL

Sample		*Galactose/Glucose* %		*Mannose* %	*Arabinose* %
I_1	Native	(10.7)		88.0	1.3
I_2	I_1 extracted with acetone and ether (yield 84.3 % from I_1)	2.5	6.1	90.3	1.1
I_3	I_2 treated with sodium chlorite (yield 79 % from I_1)	2.2	6.9	89.7	1.2
IA_1	Crude mannan A extracted with 7 % KOH from I_2	(0.5)		98.1	1.4
IA_2	IA_1 reprecipitated twice with Fehling's solution	(trace)		100	—
IA_3	Crude mannan A extracted with 7% KOH from I_3	(2.5)		97.5	trace
IA_4	IA_3 reprecipitated twice with Fehling's solution	(0.4)		99.6	—
IB_1	Residue after 3 extractions of I_2 with 7 % KOH	(19.6)		80.4	trace
IB_2	Residue after 4 extractions of IB_1 with 14 % KOH (yield 30.8 % from I_1)	2.6	17.6	79.8	trace
IB_3	Residue after 2 extractions of I_3 with 7 % KOH	(18.5)		80.6	0.9
IB_4	Residue after extraction of I_3 with 7, 14, 18% KOH and twice with 24% KOH	(22.1)		77.9	trace
IB_p	Mannan B purified	1.8	2.4	93.4	2.4

TABLE III

APPROXIMATE COMPOSITION OF IVORY-NUT ENDOSPERM

Material extracted with acetone and ether	cell contents, mainly oils	16%
Material removed afterwards by sodium chlorite treatment	other cell contents	5 %
Material removed by dilute alkali	mannan A	48 %
Material not removed by dilute alkali	mannan B	24 %
	cellulose	6 %

Microscopic and submicroscopic morphology of the endosperm cell walls

The endosperm cell wall of the ivory nut is generally described as consisting of three layers (*cf.* REISS[1], GILSON[5]): a thin middle lamella, a thick intermediate lamella (Zwischenlamelle), and a thin inner lamella bordering the cell lumen. From the strongly positive reaction of the inner lamella with iodine and zinc chloride it has been assumed to consist of cellulose. However, LÜDTKE[2] has shown that mannan B reacts with iodine and zinc chloride in the same way as cellulose. Investigation by the present author confirmed this for mannan B in the native as well as in the regenerated state. It is therefore very difficult to localise the small percentage of cellulose present in the cell walls.

Microscopically, the cell walls of date (*Phoenix dactylifera* L.) and ivory-nut endosperm differ mainly in their thickness and in the orientation of their birefringent constituents.

Fig. 1(a) shows a transverse section through date endosperm in polarised light.

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Only the thin middle lamella appears isotropic; the other layers are strongly birefringent. A longitudinal section (Fig. 2(a)) shows that only the outer part of the walls (primary wall, probably together with a transition lamella), which also forms the pit membranes, is birefringent. No isotropic middle lamella is recognisable. In those parts of the longitudinal section where the cell walls are seen from the surface and not in section (arrows in Fig. 2(a)) a colour plate (Red I) shows that the double refraction is oriented negatively to the longitudinal axis of the cells. Figs. 1 and 2 show that a cell wall separated from the neighbouring wall by a thin isotropic middle lamella consists of a primary wall (probably together with a transition lamella) and of a thick secondary wall in which the birefringent elements are oriented more or less transversely to the cell axis and have a circular texture round the pits. Polarisation optical studies alone do not show whether a tertiary wall exists.

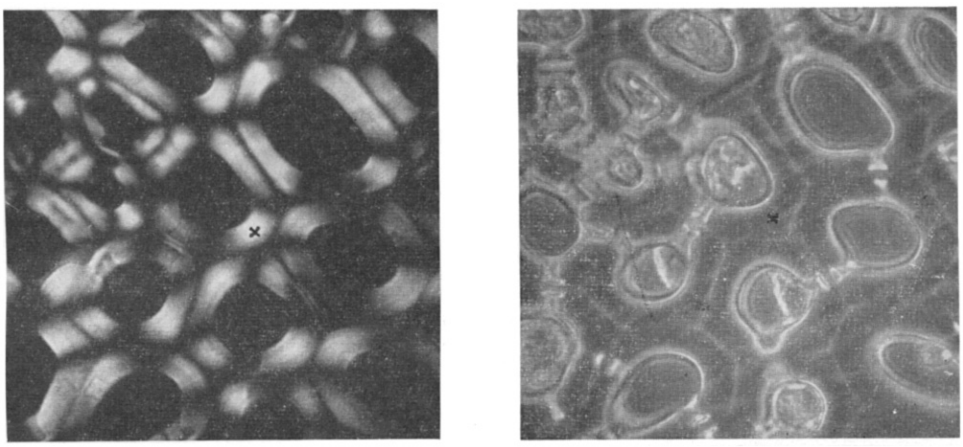

Fig. 1(a) and 1(b). Transverse section through native date endosperm with polarised light (1(a)) and phase contrast (1(b)). (x = identical cells). × 540.

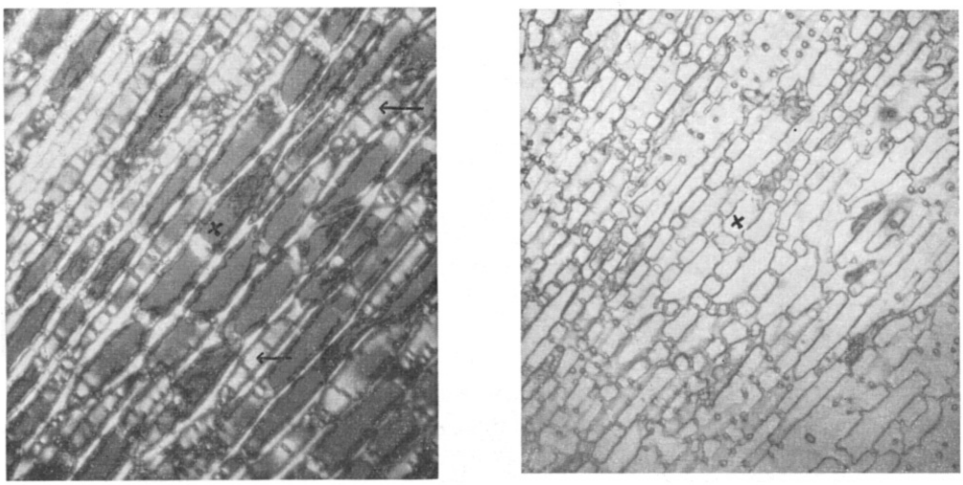

Fig. 2(a) and 2(b). Longitudinal section through native date endosperm with polarised light (2(a)) and phase contrast (2(b)). (x = identical cells). × 140.

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A transverse section through ivory-nut endosperm shows in polarised light the same features as a date transverse section. A longitudinal section, however, reveals some differences (Fig. 3). Not only the primary wall (possibly with the transition lamella) but also the secondary wall is birefringent in section. In a surface view, however, the secondary wall is—except for the regions round the pits (arrows in Fig. 3)—almost isotropic. This may be caused by a dispersed micellar texture or by a crossed lamellar texture. But since there are no dispersed micellar textures known in secondary walls, a crossed lamellar structure with the birefringent elements of each lamella more or less perpendicular to each other is more probable. The strong double refraction of the secondary wall in transverse as well as in longitudinal sections can probably be interpreted as indicating that the angle of the birefringent elements in the secondary wall must be at about 45° to the cell axis.

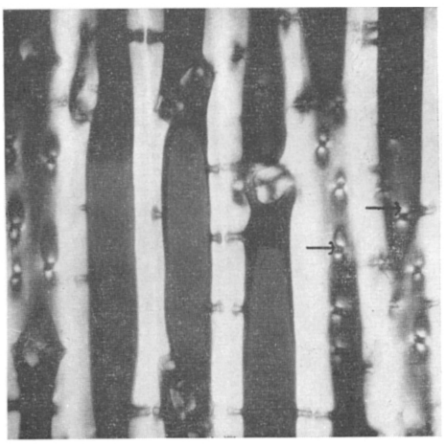

Fig. 3. Longitudinal section through native ivory-nut endosperm with polarised light. ×140.

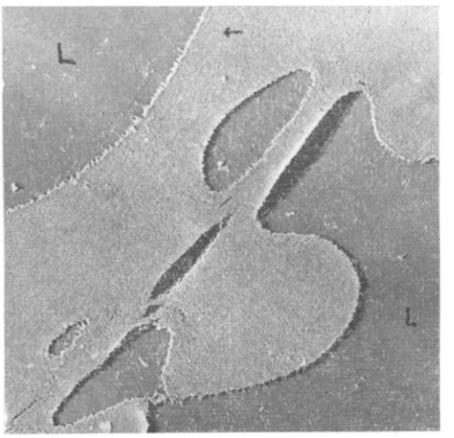

Fig. 4. Oblique transverse section through two neighbouring cell walls of date endosperm. (L = cell lumen) Electron micrograph. × 3,800.

The question might be raised whether the double refraction of the endosperm cell walls is caused by the mannan A, by the mannan B, or by both. Specimens from which the mannan A had been extracted with dilute alkali were therefore studied, using the same methods as were used for the unextracted ones. However, no differences were observed either in the transverse or in the longitudinal section.

For further investigation of the submicroscopic structure of the cell walls, ultra-thin sections were studied with the electron microscope. Fig. 4 shows an oblique transverse section through two neighbouring date-cell walls with a pit. The pit membrane is rather thick and probably includes not only the primary wall but also the transition lamella. In the secondary wall a fibrillation concentric to the cell lumen can be recognised (arrow in Fig. 4).

A longitudinal section through a swollen date-cell wall (Fig. 5) shows clearly that the wall consists at least partly of fibrillar elements. A similar picture is visible in a section through an ivory nut cell wall. Fig. 6 shows a crossed fibrillar texture that can probably be attributed to the secondary wall, as was assumed from studies in polarised light.

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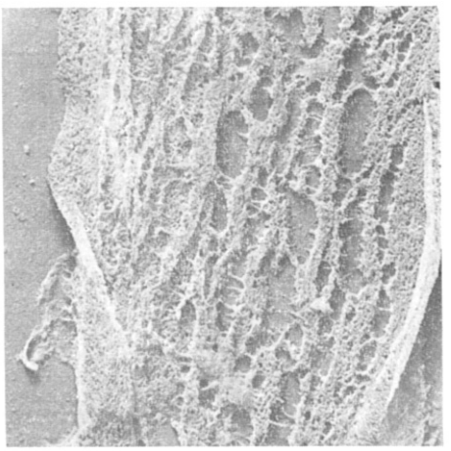

Fig. 5. Longitudinal section through a swollen cell wall of date endosperm. Note the microfibrils between the swollen layers. Electron micrograph. × 15,000.

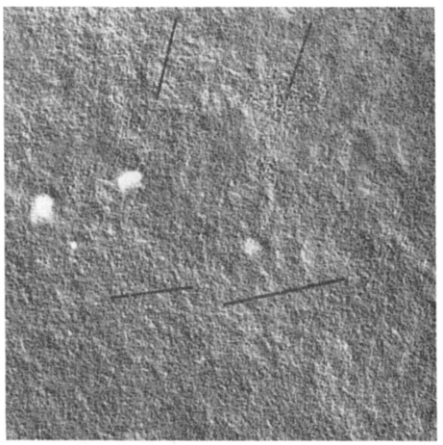

Fig. 6. Section through the secondary wall of ivory-nut endosperm. Note the two different orientations of the microfibrils. Electron micrograph. × 15,000.

Submicroscopic structure of mannan A and mannan B

As has been shown in an earlier paper (MEIER[6]), mannans A and B differ considerably in their submicroscopic structure. A sample (I_3, Table II) containing both mannan A and mannan B in the native state was disintegrated in a Turmix blender and with ultrasonic waves and studied in the electron microscope. It showed two different structural elements (Fig. 7): small grains often aggregated to greater particles and microfibrillar elements. When a sample (IB_3, Table II) from which the mannan A has been extracted with dilute potassium hydroxide, is prepared in the same way as I_3, the electron microscope picture shows that after extraction of mannan A most

Fig. 7. Ivory-nut endosperm (sample I_3) after Turmix treatment but before extraction of mannan A. Electron micrograph. × 22,500.

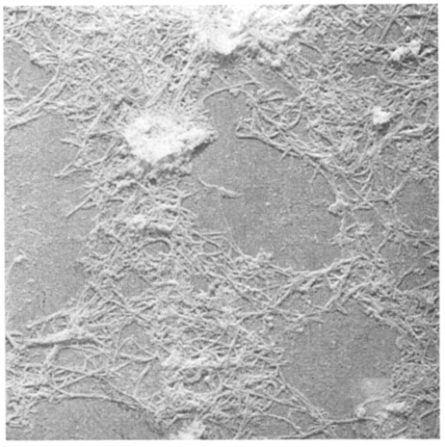

Fig. 8. Ivory-nut endosperm (sample IB_3) after extraction of mannan A and after Turmix and ultrasonic treatment. Electron micrograph. × 22,500.

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of the granular material has disappeared (Fig. 8). Practically only the microfibrillar material is left. It may therefore be concluded that mannan A is built into the cell walls in the form of small and (as will be shown below) crystalline grains with a diameter of 100 to 200 Å, while mannan B lies in the walls in the form of microfibrils. It is difficult to decide whether these are pure mannan or mixed mannan/cellulose microfibrils. However, the latter seems less probable because the mannan B, after prehydrolysis of the material with 1% hydrochloric acid, can be extracted completely with 24% potassium hydroxide, whereas the cellulose is resistant to this treatment. This would hardly be possible if there were mixed microfibrils of about 80% mannan and 20% cellulose. It might be noted here that without prehydrolysis mannan B cannot be extracted with potassium hydroxide concentrations up to 24% (*cf.* Table II, sample IB_4).

The submicroscopic structure of the isolated and purified mannans A and B was, as had been expected, somewhat different from the structure in the native state. The isolated mannan A was composed of small crystallites which were birefringent in the polarising microscope. In the electron microscope they were more or less cubic and about 1 to 2 μ in diameter (Fig. 9). Ultra-thin sections of these particles reveal a granular structure in the interior that is somewhat similar to that of the granular mannan A in its native state (Fig. 10).

Regenerated and purified mannan B (sample IB_p, Table II) precipitated without the influence of orienting forces showed, as might be expected, no fibrillar structure.

HERZOG AND GONELL[7] obtained X-ray diagrams of vegetable ivory nut and found that the material was crystalline. Since the heterogeneous character of the ivory-nut mannan was unknown at that time, their paper does not show whether mannans A *and* B or only one of them is crystalline. HESS AND LÜDTKE[8] have studied mannan A and mannan B with X-rays. According to them, both were crystalline and showed similar diffraction diagrams, the differences being mainly in the different intensities of the diffraction rings. Mannan B lacked some diffractions given by mannan A. HESS AND LÜDTKE[8] believed that the similarity of both diagrams was fortuitous.

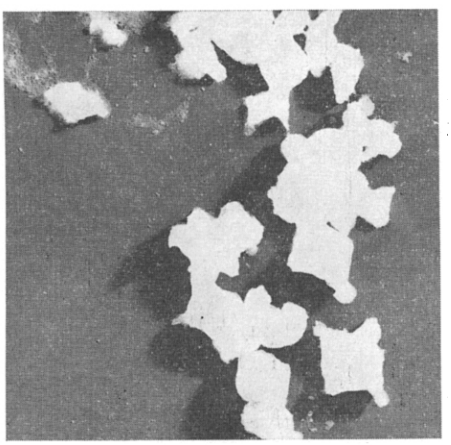

Fig. 9. Isolated date mannan A after Turmix and ultrasonic treatment. Electron micrograph. × 15,000.

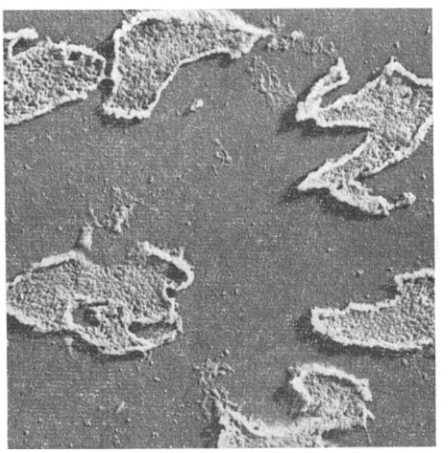

Fig. 10. The same as in Fig. 9 but sectioned. Electron micrograph. × 15,000.

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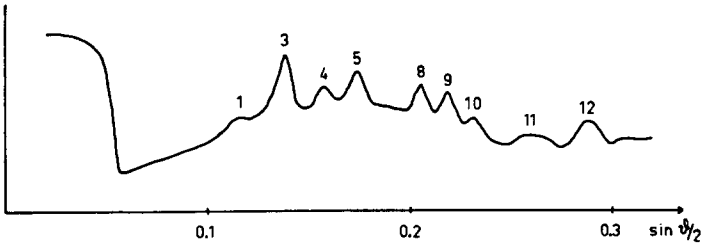

Fig. 11. Photometer curve of the X-ray diagram of native ivory-nut endosperm (sample I_1)

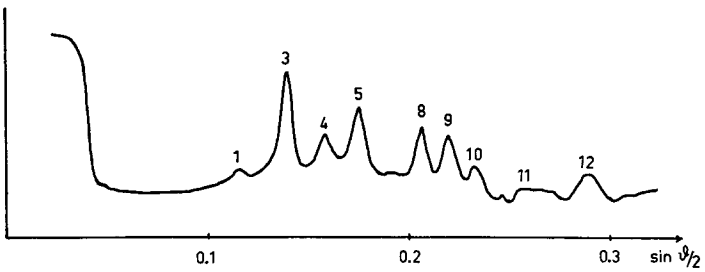

Fig. 12. Photometer curve of the X-ray diagram of isolated and purified mannan A (sample IA_2).

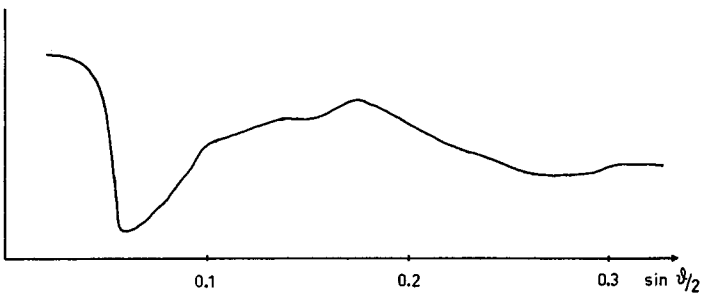

Fig. 13. Photometer curve of the X-ray diagram of ivory-nut endosperm after extraction of mannan A (sample IB_4).

However, it is rather likely that the mannan B specimen of these authors contained mannan A as impurity. HESS AND LÜDTKE[8] investigated only regenerated, that is, dissolved and reprecipitated samples of mannan A and mannan B. The question might therefore be asked whether the two mannans are also crystalline in the native state and whether the crystal structure was the same then as in the regenerated state.

Fig. 11 shows the photometer curve of the X-ray diagram of native ivory-nut endosperm, and Fig. 12 shows the curve obtained from isolated mannan A. Comparison of the two curves shows that the interferences in Fig. 11 must be caused by the same crystal lattice as those in Fig. 12, that is, by the mannan A lattice. (The lattice-plane distances of mannan A are given in Table IV.) Mannan A is therefore crystalline both in its native state and after dissolution and reprecipitation. Fig. 13 shows the curve of the X-ray diagram of ivory-nut endosperm after complete removal of mannan A by extraction. From this curve it can be concluded that mannan B in its native state is amorphous or paracrystalline. The X-ray diagram of the isolated and regenerated mannan B (sample IB_9) likewise gives no indication of crystalline structure.

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TABLE IV

LATTICE-PLANE DISTANCES AS DEDUCED FROM TWO X-RAY DIFFRACTION DIAGRAMS OF ISOLATED MANNAN A

The diagram "Graz" shows three faint interference rings more than the diagram "Uppsala" The photometer curve of the latter is shown in Fig. 12.

Ring	*Lattice plane distances in Å*	
	"Graz"	"Uppsala"
1	6.84	6.73
2	6.19	
3	5.60	5.56
4	4.94	4.89
5	4.47	4.43
6	4.22	
7	3.95	
8	3.80	3.79
9	3.56	3.54
10	3.38	3.34
11	3.01	2.96
12	2.72	2.72

By analogy with cellulose a microfibrillar structure might be assumed to imply X-ray crystallinity. The X-ray amorphous structure of the microfibrillar mannan B is therefore somewhat surprising. It might, however, be explained by the presence of over 14% 1,6-linkages in addition to the 1,4-linkages in mannan B (*cf.* ASPINALL *et al.*[4]).

The cell walls of date and ivory-nut endosperm are still highly birefringent after extraction of the X-ray crystalline mannan A. Measurement by the immersion method (cinnamon oil–amyl alcohol) of the refractive indices in a longitudinal section through a cell wall of sample IB_4 gave $n'_\gamma = 1.540$ ($n'_\gamma < n_\gamma$) and $n_\alpha = 1.518$ (cellulose: $n_\gamma = 1.599$, $n_\alpha = 1.531$). Since in this mannan A-free cell wall there is about 20% cellulose as well as the 78% mannan B, and since it is not known whether the cellulose is localised in the primary wall and the transition lamella or is also present in the secondary wall where the n_γ and n_α were actually measured, these values might be influenced by the cellulose. A considerable part of the double refraction must, however, come from the mannan B. Mannan B in its native state is therefore a typical paracrystalline substance with optical anisotropy resulting from a parallel arrangement of long-chain macromolecules without the definite crystal lattice which would give a crystalline X-ray diagram.

It is often uncritically assumed that amorphous polysaccharides are easily extractable from cell walls, while those extracted only with difficulty are supposed to be crystalline. The behaviour of mannan A and mannan B demonstrates that such a generalisation is not universally true.

$\overline{DP}_n$ measurements on mannan A and mannan B

As already pointed out in the introduction, ASPINALL *et al.*[4] have determined $\overline{DP}_n$ (= numerical average degree of polymerisation) of mannan A and mannan B by end-group determination. Recently, TIMELL[9] has published viscosimetric $\overline{DP}_w$ (= weight-average degree of polymerisation) determinations of the ivory nut components. In the course of the present investigation, osmometric $\overline{DP}_n$ determinations

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TABLE V

OSMOMETRIC DP_n DETERMINATIONS

Sample	*Solvent*	*Membrane*	$\overline{DP}_n$	*Nitrogen content %*
IA_1	Acetone	"U. allerf."	21.2	12.52
	Acetone	D-60	20.3	12.52
IA_2	Acetone	"U. allerf."	18.8	14.07
	Butyl acetate	"U. allerf."	17.4	14.07
	(sodium borohydride method)		16.0	
IA_3	Acetone	"U. allerf."	19.1	12.13
IA_4	Acetone	"U. allerf."	22.2	11.88
IB_p	Acetone	"U. allerf."	86.7	12.06
	Butyl acetate	"U. allerf."	80.0	12.06

were made with purified mannan A and mannan B samples (see Table V). TIMELL[9] emphasised that pre-treatment of the material with sodium chlorite might have a great influence on the degree of polymerisation of the mannans. Since the present author also considered it possible that the sodium chlorite treatment of ASPINALL *et al.*[4] might have influenced the results of their DP determinations, mannan A was extracted from sodium chlorite-treated ivory-nut meal and also from untreated material. Both the mannan A samples were nitrated, one part directly and one part after two reprecipitations with Fehling's solution. $\overline{DP}_n$ for all four samples (IA_1 — IA_4) was then determined and, as can be seen from Table IV, there was almost no difference in $\overline{DP}_n$. This shows that at least the mannan A is not sensitive to sodium chlorite treatment or to reprecipitation from Fehling's solution.

According to TIMELL[9] the $\overline{DP}_w$ of mannan A lies between 5 and 7, and according to ASPINALL *et al.*[4] $\overline{DP}_n$ is 10–13. The values of TIMELL[9] were calculated from viscosity determinations. Since the theoretical as well as the experimental background for a calculation of the $\overline{DP}_w$ from the viscosity is rather deficient for such low-polymer components, TIMELL's figures are questionable. The $\overline{DP}_n$ values of ASPINALL *et al.* are certainly more reliable. They are somewhat lower then the osmometrically determined $\overline{DP}_n$ values in Table V, and also lower than the values given by an end-group determination using the sodium borohydride method (LINDBERG AND THEANDER[10]), which gave $\overline{DP}_n$ 16 for sample IA_2. Both the osmometric and the sodium borohydride method have a tendency to give values that are too high. The figures of ASPINALL *et al.*[4], on the other hand, might be somewhat too low, since a slight degradation of the samples during the lengthy methylation procedure can hardly be prevented. It may therefore be assumed with fairly high certainty that mannan A must have a degree of polymerisation of about 15.

For mannan B, ASPINALL *et al.*[4] give a $\overline{DP}_n$ of about 40, making the assumption that the molecules are not branched. TIMELL[9] determined the $\overline{DP}_w$ of the higher-polymer ivory-nut carbohydrates, including mannan B and cellulose, viscosimetrically and found values between 300 and 1200. Since he had no pure mannan B fractions (they all contained considerable amounts of cellulose), it is difficult to draw any conclusions from his results as far as the DP of mannan B is concerned. The long-chain

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cellulose molecules certainly had a large effect on TIMELL's viscosimetric determinations. By calculation it can be shown that a mixture of 13.6% glucan and 86.4 mannan B as in TIMELL's fractions 26–27 (Table V in TIMELL's publication), would give a $\overline{DP}_w$ of 477 if mannan B has a $\overline{DP}_n$ of 80 (as the present author has found) and the glucan a $\overline{DP}_n$ of 3000 (as an average cellulose would have). This explains the high $\overline{DP}_w$ values reported by TIMELL.

Since small amounts of long-chain cellulose have almost no influence on osmometric $\overline{DP}_n$ determinations, the latter will give more reliable figures for the DP of mannan B. A cellulose content of 2.4% as in sample IB_p should have almost no influence. That ASPINALL's DP values for mannan B are lower than those of the present author might be due to the same reasons as were discussed above for mannan A. However, as ASPINALL *et al.*[4] point out, the possibility must be kept in mind that mannan B might have a slightly branched structure.

EXPERIMENTAL

The experimental figures will be given for the ivory-nut material only. The date-mannan investigations that were made were done in a similar way.

Quantitative paper chromatography

The quantitative paper chromatography was carried out by the method of SAEMAN *et al.*[11], using the solvent system, ethyl acetate–acetic acid–water (3:1:3). As this mixture does not provide a good separation for galactose and glucose, the system ethyl acetate–pyridine–water (2:1:2) was used parallel with the first for some of the samples.

Preparation of the starting material

The brown seed coat and the germ of the ivory nuts were removed from the endosperm. The latter was ground in a Wiley mill and extracted with acetone and ether in a Soxhlet apparatus. The extracted material was screened and the fraction between 13–40 mesh was used for experimental work.

Chlorite treatment

Part of the acetone–ether-extracted material (I_2) was treated with sodium chlorite according to the method of CHANDA *et al.*[12] and then washed thoroughly with tap water and finally with large quantities of distilled water to give I_3. The yield was rather high (93.7% from I_2). Microscopic investigation of I_2 and I_3 suggests that the sodium chlorite treatment removes mainly the cell contents and probably only very minor amounts of material from the cell walls, which contain no lignin and, as shown by TIMELL[9], hardly any pectic substances.

Isolation of mannan A

Samples I_2 and I_3 were extracted three times with 7% potassium hydroxide overnight on a shaker in sealed glass flasks. The extracts were neutralised with acetic acid and the mannan A was precipitated with ethanol. Part of the mannan A extracted from I_2 and I_3 (samples IA_1 and IA_3, respectively) was reprecipitated twice with Fehling's solution by the method of ASPINALL *et al.*[4] to give IA_2 (from IA_1) and IA_4 (from IA_3). After reprecipitation through the copper complex and drying, the mannan A dissolved very badly in 7% potassium hydroxide. The solubility could be somewhat improved by not drying the mannan A precipitated with ethanol.

Isolation of mannan B

The isolation of mannan B was based on the method used by LÜDTKE[2]. Sample IB_3 (residue after the extraction of mannan A) was extracted once more with 7% and then with 14% potassium hydroxide to remove mannan A as completely as possible. The residue was washed with dilute acetic acid and then with large quantities of distilled water. It was dissolved in "cuoxam" and the copper complex of mannan B was precipitated by adding 1 *N* sodium hydroxide until the sodium hydroxide concentration of the solution reached 0.2 *N*. The precipitate was centrifuged, washed with ammonia and 1 *N* sodium hydroxide, and dispersed in water. The copper complex was

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destroyed by addition of acetic acid (5%) and the mannan B was precipitated with ethanol. The precipitate was centrifuged, washed with dilute acetic acid, ethanol, and ether, and dried to give IB_p.

Preparation for electron microscope studies

The samples to be sectioned were embedded according to the method of NEWMAN *et al.*[13] in a mixture of butyl and methyl methacrylate (5:1) with the addition of 0.5% dichlorobenzoyl-peroxide. The samples were polymerised in a thermostat at 55 to 60°. The ultra-thin sections were made on a Sjöstrand microtome with glass knives. After dissolving away the embedding material with amyl acetate the sections were shadowed with chromium at an angle of about 30° and photographed with an RCA-microscope.

Nitration of the mannans for osmometric $\overline{DP}_n$ determinations

The nitration mixture consisting of nitric acid, phosphoric acid and phosphorus pentoxide (48:50:2) was cooled down to —10°. About two grams of the samples to be nitrated were then added to about 50 ml of the mixture in a suction flask, which was put in a refrigerator for four hours, evacuated several times and shaken by hand. The nitration mixture was then removed through a glass filtre and the nitrated samples were washed with dilute acetic acid cooled down to —10° and with large quantities of distilled water. They were then left overnight in two litres of distilled water and the following day they were boiled twice in water for three hours under a reflux condenser and then dried over phosphorus pentoxide.

Osmometric $\overline{DP}_n$ determinations

Osmometric $\overline{DP}_n$ determinations were made as described in an earlier paper (LINDBERG AND MEIER[14]). Since, however, ivory-nut mannan A has a $\overline{DP}_n$ as low as 15, the difficulty arises that "Ultracellafilter allerfeinst" (Membranfiltergesellschaft Göttingen) membranes leak. The osmotic pressure time curves were therefore not horizontal and had to be extrapolated to time zero. According to a theory of STAVERMANN[15], osmotic pressures measured with non-selective membranes are always too low and the values for $\overline{DP}_n$ therefore too high. To check the possibility of the effect postulated by STAVERMANN's theory, an especially tight membrane, D-60 from Membranfiltergesellschaft Göttingen, was used parallel with the "Ultracellafilter allerfeinst". The membrane D-60 was tight for nitromannan A, but it needed an extremely long time to reach equilibrium pressure. The approximate equilibrium rise of a sample was therefore first determined with an "Ultracellafilter allerfeinst", so that when making a measurement with a D-60 membrane the meniscus of the solution could be brought as close as possible to the equilibrium height at the start. Fig. 14 shows for comparison the curves for the height differences as a function of time for one concentration of the nitrated sample IA_2 with "Ultracellafilter allerfeinst" and a D-60 membrane (solvent: acetone). As can be seen from these curves the equilibrium height with "Ultracellafilter allerfeinst" when extrapolated to zero time is not very different from the equilib-

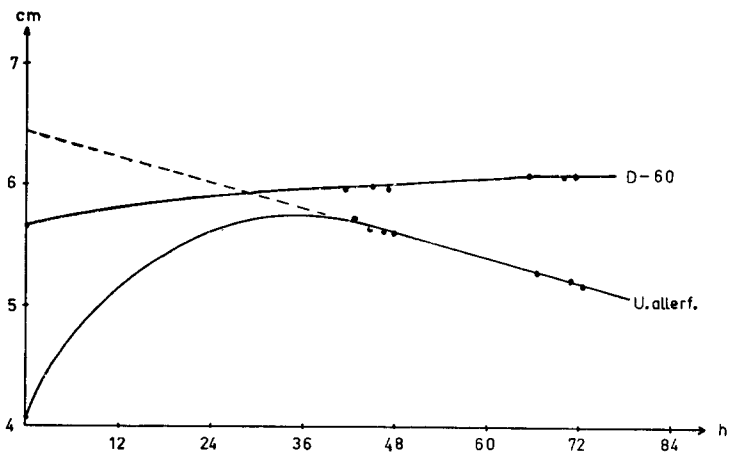

Fig. 14. Height difference as a function of time for the nitrated sample IA_1 (11.6 g/l acetone) with the two membranes D-60 and "Ultracellafilter allerfeinst." The curve "U. allerf." is extrapolated to zero time.

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rium height for the D-60 membrane. The $\overline{DP}_n$ values gained with "Ultracellafilter allerfeinst" were not more than about 5% too high.

Table V shows that with the two samples IA_2 and IB_4 where the osmotic pressure was determined with acetone as solvent as well as with butyl acetate, the $\overline{DP}_n$ values were lower with butyl acetate. Butyl acetate is therefore a better solvent than acetone and is to be preferred. Since the nitrogen content of most of the samples was rather low, it is possible that the solutions were not completely molecularly dispersed and the $\overline{DP}_n$ values have therefore a tendency to be too high.

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SUMMARY

The submicroscopic structure of date and ivory-nut-endosperm cell walls has been studied with polarisation and electron microscopes. The structure of the two main components of these cell walls, mannan A and mannan B, has been investigated. Mannan A was found to be a granular material that was X-ray crystalline both in the native and in the isolated state. Native mannan B was found to be microfibrillar and to show birefringence; it was X-ray amorphous both before and after isolation and purification.

The degree of polymerisation ($\overline{DP}_n$) of mannans A and B was determined osmometrically. This gave values between 17 and 21 for mannan A and about 80 for mannan B. The actual values, however, might be somewhat lower.

Carbohydrate analyses of the isolated and purified mannan A showed only traces of galactose and glucose; mannan B after purification contained 6.6% of sugars other than mannose.

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