635. *The Mannans of Ivory Nut* (Phytelephas macrocarpa). *Part I. The Methylation of Mannan A and Mannan B.*

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Mannans A and B from ivory nut (*Phytelephas macrocarpa*) gave on hydrolysis D-mannose (97·6%), D-galactose (1·8%), and D-glucose (0·8%), and D-mannose (98·3%), D-galactose (1·1%), and D-glucose (0·8%), respectively. Hydrolysis of methylated mannan A gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose (7·3%), 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (1·7%), 2 : 3 : 6-tri-*O*-methyl-D-mannose (83·0%), 2 : 3 : 4-tri-*O*-methyl-D-mannose (6·8%), and a di-*O*-methyl-D-mannose (1·2%). Hydrolysis of methylated mannan B gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose (1·3%), 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (1·3%), 2 : 3 : 6-tri-*O*-methyl-D-mannose (81·8%), 2 : 3 : 4-tri-*O*-methyl-D-mannose (14·3%), and a di-*O*-methyl-D-mannose (1·3%). 2 : 3 : 4-Tri-*O*-methyl-D-mannose was isolated from both hydrolysates as the non-reducing disaccharide 2 : 3 : 4-tri-*O*-methyl-D-mannopyranosyl 2 : 3 : 4-tri-*O*-methyl-D-mannopyranoside. It is concluded that mannan A and mannan B both contain at least two types of molecule, one terminated by a D-mannopyranose residue and the other terminated by a D-galactopyranose residue. The majority of the mannopyranose residues are linked through positions 1 and 4 but 1 : 6-linkages are also present. Mannans A and B differ only in molecular size having average chain lengths of 10—13 and 39—40, respectively.

The chemistry of the endosperm of ivory nut (*Phytelephas macrocarpa*) was first studied by Reiss (*Ber.*, 1889, **22**, 609) who obtained on hydrolysis a sugar that was subsequently identified as mannose by Fischer and Hirschberger (*Ber.*, 1889, **22**, 1155). Several investigators (Johnson, *J. Amer. Chem. Soc.*, 1896, **18**, 214; Baker and Pope, *J.*, 1900, **77**, 676; Ivanov, *Journ. f. Landw.*, 1908, **56**, 217; Pringsheim and Seifert, *Z. physiol. Chem.*, 1922, **123**, 205) studied the polysaccharide extracted from ivory nuts with alkali and showed that it yielded mainly mannose on hydrolysis. Patterson (*J.*, 1923, 1139) methylated the polysaccharide and from the hydrolysate isolated a syrupy trimethylmannose which could be quantitatively converted into crystalline methyl tetra-*O*-methyl-α-D-mannoside. Patterson concluded, therefore, that all the mannose residues were of the ordinary stable type.

Further investigations by Lüdtke (*Annalen*, 1927, **456**, 201) showed that two different mannans could be obtained from delignified ivory nuts by dissolution in cuprammonium solution followed by fractional precipitation with sodium hydroxide. The mannan mixture was separated into two fractions, mannan A (soluble in aqueous sodium hydroxide) and mannan B (insoluble in aqueous sodium hydroxide).

Methylation studies of mannans A and B were carried out by Klages (*Annalen*, 1934, **509**, 159; **512**, 185) who concluded that mannan A consisted of a chain of 80 1 : 4-linked β-D-mannopyranose residues and postulated that mannan B was similarly constituted. More recent investigations by Ward (M.Sc. Thesis, Manchester, 1947) indicated that mannan A had a repeating unit of 15 and that the 1 : 4-linkage was not the only linkage present. The present investigation was undertaken to determine whether the two mannans were similarly constituted and with the aid of modern chromatographic techniques to determine the fine structure of each.

Mannan A was extracted from delignified ivory nut shavings with cold aqueous potassium hydroxide and purified by two precipitations, as the copper complex, with Fehling's solution. Hydrolysis of the mannan, $[\alpha]_D^{15}$ −46° (*c*, 0·7 in N-NaOH), gave D-mannose (97·6%), D-galactose (1·8%), and D-glucose (0·8%), estimated by quantitative paper chromatography (Hirst and Jones, *J.*, 1949, 1659; Duff and Eastwood, *Nature*, 1950, **156**, 848).

The mannan was methylated under nitrogen with sodium hydroxide and methyl sulphate, and subsequently with methyl iodide and silver oxide, to give a product (OMe,

43·5%), which was fractionated by dissolution in chloroform–light petroleum. The methylated mannan A {$[\alpha]_D^{17}$ −22·5° (*c*, 1·0 in $CHCl_3$), OMe, 44·3%)} was hydrolysed successively with anhydrous formic acid and dilute sulphuric acid (Jones, *J.*, 1950, 3292), and the mixture of sugars separated on cellulose. The following fractions were obtained: (1) tetra-*O*-methylmannose (7·3%); (2) tetra-*O*-methylgalactose (1·7%); (3) 2 : 3 : 6-tri-*O*-methylmannose (83·0%); and (4) a mixture of a trimethylmannose and a dimethylmannose. 2 : 3 : 4 : 6-Tetra-*O*-methyl-D-mannose was identified as its aniline derivative, and as the phenylhydrazide of the corresponding 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannonic acid. 2 : 3 : 4 : 6-Tetra-*O*-methyl-D-galactose was identified as its aniline derivative. 2 : 3 : 6-Tri-*O*-methyl-D-mannose was identified as its aniline derivative and as the γ-lactone and phenylhydrazide of the corresponding 2 : 3 : 6-tri-*O*-methyl-D-mannonic acid.

Fraction 4 was further separated into a trimethylmannose (6·8%) and a dimethylmannose (1·2%). The trimethylmannose, which was chromatographically pure, crystallised to give a non-reducing hexamethyl-disaccharide *X*, m. p. 148—150°, $[\alpha]_D^{18}$ +55° (*c*, 1·4 in H_2O). The substance *X* was methylated and gave on hydrolysis only 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose, indicating that both mannose residues were in the pyranose form. The trimethylmannose was regenerated on hydrolysis of substance *X* and travelled on the chromatogram at a speed different from 2 : 3 : 6- and 3 : 4 : 6-tri-*O*-methylmannoses. Oxidation with lead tetra-acetate showed that the sugar could not be 2 : 4 : 6-tri-*O*-methylmannose, and the detection of formadehyde on oxidation with sodium metaperiodate (Chanda, Hirst, Percival, and Ross, *J.*, 1952, 1833) indicated that the sugar was 2 : 3 : 4-tri-*O*-methyl-D-mannose. The non-reducing substance *X* was therefore 2 : 3 : 4-tri-*O*-methyl-D-mannopyranosyl 2 : 3 : 4-tri-*O*-methyl-D-mannopyranoside. The dimethyl sugar travelled on the chromatogram at the same rate as 2 : 3-di-*O*-methyl-D-mannose, but the small quantity isolated was of little structural significance and probably arose from undermethylation of the polysaccharide and/or demethylation during hydrolysis. No methylated glucoses were isolated.

Although it was not found possible to obtain a complete paper chromatographic separation of all the methylated sugars, hypoiodite oxidation (cf. Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289) indicated a ratio of tetramethyl sugar : trimethyl sugar of 1 : 9, a figure in close agreement with the quantities of methylated sugars isolated from the column.

The isolation of tetramethylgalactose (1·7%) accounted for all the galactose present in the original polysaccharide. The isolation of both tetramethylmannose and tetramethylgalactose together with the absence of significant quantities of any dimethyl sugar indicates that at least two molecular types are present in mannan A, both comprising an average of 10—13 mannose residues, one terminated at the non-reducing end by a mannopyranose residue and the other by a galactopyranose residue. From the present evidence, it is not possible to indicate whether 1 : 6-linked mannose residues are randomly distributed in both molecular types or are present only in one. The stability of mannan A to acid hydrolysis together with its optical rotation indicates that the majority of mannose residues are joined by β-1 : 4-linkages.

Mannan B was isolated by dissolution of the residue after extraction of mannan A in cuprammonium solution and precipitation by the addition of sodium hydroxide solution, and purified by dissolution in anhydrous formic acid. The mannan gave on hydrolysis D-mannose (98·3%), D-galactose (1·1%), and D-glucose (0·8%).

Methylation of the polysaccharide gave methylated mannan B, which was hydrolysed as before to give a mixture of methylated sugars, separation of which gave the following fractions: (1*a*) 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose (1·3%); (1*b*) 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (1·3%); (2) 2 : 3 : 6-tri-*O*-methyl-D-mannose (81·8%); (3) and (4*a*) 2 : 3 : 4-tri-*O*-methyl-D-mannose (14·3%); and (4*b*) 2 : 3-di-*O*-methyl-D-mannose (1·3%). Paper chromatographic estimation indicated a ratio of tetramethyl sugar : trimethyl sugar of 1 : 40, a value in close agreement with the quantities of methylated sugars isolated from the column.

As in the case of mannan A, all the galactose residues of mannan B were accounted for as tetramethylgalactose. The isolation of both tetramethylmannose and tetramethylgalactose may be explained in two ways: (*a*) mannan B consists of equal amounts of two

molecular species of average chain length 38—40, terminated at the non-reducing end by mannopyranose and galactopyranose residues, respectively; (*b*) mannan B consists of a singly branched molecule of 75—80 D-mannopyranose residues, one of the two non-reducing end groups being a galactopyranose residue. In the absence of a physical determination of molecular size it is impossible to distinguish finally between these two alternatives, but as at least part of the dimethylmannose isolated probably arose from undermethylation of the polysaccharide and/or demethylation during hydrolysis, the first explanation seems more probable.

The results of the present investigation indicate that mannans A and B are chemically similar and differ only in molecular size, having average chain lengths of 10—13 and 38—40, respectively. It is probable that both mannans contain at least two molecular types differing in the terminal non-reducing sugar residue. Further investigations will be necessary in the case of both mannans to decide whether the 1 : 6-linked mannose residues are regularly or randomly distributed.

Experimental

Mannan A

Preparation of Mannan A.—Ivory nut shavings were extracted successively with benzene and methanol to remove waxy and colouring materials, and were delignified by Wise's method (*Ind. Eng. Chem. Anal.*, 1945, **17**, 63) as modified by Chanda, Hirst, Jones, and Percival (*loc. cit.*). The holocellulose was extracted with potassium hydroxide solution (7%), the extract acidified with glacial acetic acid, and the crude mannan precipitated by the addition of an equal volume of ethanol. The polysaccharide, obtained in 42% yield after 3 extractions, had $[\alpha]_D^{13}$ −39° (*c*, 1·0 in N-NaOH). Purification was effected by two successive precipitations of the copper complex formed on addition of Fehling's solution to a solution of the polysaccharide in aqueous potassium hydroxide (7%), followed by decomposition of the copper complex with dilute hydrochloric acid and precipitation of the regenerated polysaccharide with ethanol. The purified mannan A had $[\alpha]_D^{15}$ −46° (*c*, 0·7 in N-NaOH), and $[\alpha]_D^{15}$ −28° (*c*, 0·8 in anhydrous formic acid; unchanged after 70 hr.). Chromatographic examination of the hydrolysate (Hirst and Jones, *loc. cit.*; Duff and Eastwood, *loc. cit.*) showed the presence of mannose (97·6%), galactose (1·8%), and glucose (0·8%).

Methylation of Mannan A.—Mannan A (20 g.) was methylated eleven times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature and once with methyl iodide and silver oxide, giving a product (9 g.; Found: OMe, 43·5%), isolated by dissolution in hot chloroform.

Fractionation was effected by refluxing chloroform–light petroleum (b. p. 60—65°) mixtures of differing composition. Two main fractions were obtained and these were combined for subsequent work.

Fraction	% $CHCl_3$ in solvent	$[\alpha]_D^{17}$ (*c*, 1·0 in $CHCl_3$)	OMe, %	Wt. (g.)
1	10	−22°	44·4	2·66
2	15	−23	44·2	4·80

Hydrolysis of Methylated Mannan A.—Methylated mannan A (4 g.) was heated with anhydrous formic acid (25 c.c.) at 100° for 8 hr. The formic acid was removed under reduced pressure and the resultant syrup heated with N-sulphuric acid (10 c.c.) on the water-bath for 6 hr. to hydrolyse formyl esters and neutralised with Amberlite resin IR-4B, and the solution concentrated to a syrup. Paper chromatographic examination, butanol–ethanol–water (4 : 1 : 5; top layer) being used as solvent, showed the presence of tetramethylmannose and trimethylmannose, together with smaller quantities of tetramethylgalactose and a dimethylmannose.

Separation of Methylated Sugars and Examination of Fractions.—The syrupy hydrolysate (3·43 g.) was fractionated on cellulose (90 × 3 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–*n*-butanol (6 : 4), saturated with water, as eluant to give four fractions.

Fraction 1. The syrup (242 mg.) was partly crystalline and after recrystallisation from ether had m. p. 109—111°. The mixture of syrup and crystals had $[\alpha]_D^{20}$ +23° ⟶ +10° (*c*, 0·7 in H_2O) (Found: OMe, 50·4. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). Chromatographic examination showed only 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose, and the aniline derivative

had m. p. 144—145° (not depressed on admixture with an authentic sample) and $[\alpha]_D^{18}$ −94° ⟶ −42° (*c*, 0·8 in $COMe_2$) (Found: C, 61·8; H, 8·0. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·0%). A portion of the syrup was converted into the δ-lactone {$[\alpha]_D^{15}$ +148° (*c*, 1·0 in H_2O)} which was characterised by conversion into 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannonic acid phenylhydrazide, m. p. and mixed m. p. 185—186° (Found: N, 7·8. Calc. for $C_{16}H_{26}O_6N_2$: N, 8·2%).

Fraction 2. The syrup (58 mg.) was chromatographically pure and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 195—196°, $[\alpha]_D^{16}$ −136° (*c*, 0·4 in C_5H_5N), $[\alpha]_D^{14}$ −76° (*c*, 0·3 in $COMe_2$).

Fraction 3. The syrup (2·623 g.) had $[\alpha]_D^{13}$ −7° (*c*, 4·6 in H_2O) (Found: OMe, 38·6. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Chromatographic examination showed only 2 : 3 : 6-tri-*O*-methylmannose, and hypoiodite oxidation indicated 92% purity. A sample was converted into 2 : 3 : 6-tri-*O*-methyl-*N*-phenyl-D-mannosylamine, m. p. and mixed m. p. 127—128°. A second sample was converted into the γ-lactone, m. p. and mixed m. p. 81—82°, $[\alpha]_D^{16}$ +64·5° (*c*, 3·2 in H_2O), part of which was converted into 2 : 3 : 6-tri-*O*-methyl-D-mannonic acid phenylhydrazide, m. p. and mixed m. p. 132—133°.

Fraction 4. Paper chromatography showed the presence of two sugars, and the syrup (240 mg.) was separated on Whatman 3MM paper with butanol–ethanol–water (4 : 1 : 5; top layer) as eluant to give fractions (4*a*) (206 mg.) and (4*b*) (34 mg.). Fraction 4*a*, obtained as a syrup, travelled on the chromatogram as a trimethylhexose but at a speed different from those of 2 : 3 : 6- and 3 : 4 : 6-tri-*O*-methylmannoses. After several weeks the syrup crystallised almost completely to a non-reducing *substance X*, m. p. 148—150°, $[\alpha]_D^{18}$ +55° (*c*, 1·4 in H_2O, unchanged after 48 hr.), $[\alpha]_D^{18}$ +65° (*c*, 0·7 in 1% methanolic hydrogen chloride, unchanged after 100 hr.) (Found: C, 50·5; H, 7·9; OMe, 42·9. $C_{18}H_{34}O_{11}$ requires C, 50·7; H, 8·0; OMe, 43·7%). Demethylation (Hough, Jones, and Wadman, *J.*, 1950, 1702) showed *X* to be a mannose derivative, and the molecular weight, obtained by Barger's isopiestic method (cf. Caesar, Gruenhut, and Cushing, *J. Amer. Chem. Soc.*, 1947, **69**, 617), corresponded to that of a hexamethyldisaccharide.

Fraction 4*b*, which remained as a syrup, was shown by demethylation to be a mannose derivative and travelled on the chromatogram at the same rate as 2 : 3-di-*O*-methyl-D-mannose.

Substance *X* (60 mg.) was methylated with methyl iodide and silver oxide, and the product (69 mg.) was hydrolysed on the water-bath with sulphuric acid (1 c.c.; N). The hydrolysate was neutralised with Amberlite resin IR-4B and concentrated to a syrup (60 mg.). Chromatographic examination showed only tetramethylmannose, and the syrup was converted into 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine (70 mg.), m. p. and mixed m. p. 144—145°.

Hydrolysis of *X* gave a chromatographically pure trimethylmannose which was oxidised by lead tetra-acetate (cf. Buchanan, Dekker, and Long, *J.*, 1950, 3162). Oxidation with sodium metaperiodate solution liberated formaldehyde, which was detected with phenylhydrazine hydrochloride, potassium ferricyanide, and concentrated hydrochloric acid (cf. Chanda, Hirst, Percival, and Ross, *loc. cit.*). The trimethylmannose was therefore 2 : 3 : 4-tri-*O*-methyl-D-mannose, and X was 2 : 3 : 4-*tri*-O-*methyl*-D-*mannopyranosyl* 2 : 3 : 4-*tri*-O-*methyl*-D-*mannopyranoside*.

Quantitative Examination of the Methylated Sugars.—A portion of the hydrolysate of methylated mannan A was separated chromatographically (Hirst, Hough, and Jones, *J.*, 1949, 298), benzene–ethanol–water (169 : 47 : 15; top layer) being used as solvent, and the tetramethyl and trimethyl sugars estimated by alkaline hypoiodite (cf. Chanda, Hirst, Jones, and Percival, *loc. cit.*) [Found (results expressed as c.c. of 0·01N-iodine consumed): "tetra," 0·16, 0·41; "tri," 1·43, 3·68]. These figures correspond to a molecular ratio of tetramethyl sugars : trimethyl sugars of 1 : 9.

Mannan B

Preparation of Mannan B.—The residue remaining after extraction of mannan A was extracted with potassium hydroxide solution (14%) to remove further mannan A, washed with water to remove alkali, and shaken in the dark with cuprammonium solution to which sucrose had been added to minimise oxidation of the polysaccharide. After removal of undissolved materials at the centrifuge, sodium hydroxide solution was added until the mixture was 0·2N with respect to sodium hydroxide. The bulky complex which separated was stirred with water and decomposed with glacial acetic acid, the mannan being precipitated by the addition of an equal volume of ethanol. The material thus obtained was subjected to a second precipit-

ation by the same procedure and crude mannan B isolated. Chromatographic examination of the hydrolysate showed the presence of mannose, glucose, and galactose. Further purification was effected by re-extraction with potassium hydroxide solution (7%) followed by dissolution in anhydrous formic acid (Dr. J. K. N. Jones, personal communication), mannan B {$[\alpha]_D^{15}$ −26° (*c*, 0·8 in anhydrous formic acid, unchanged after 70 hr.)} being obtained by precipitation with ethanol. Chromatographic examination of the hydrolysate showed the presence of mannose (98·3%), galactose (1·1%), and glucose (0·8%).

Methylation of Mannan B.—Mannan B (11 g.) was methylated 12 times with methyl sulphate and sodium hydroxide and twice with methyl iodide and silver oxide to give a product (3·5 g.; Found: OMe, 44·9%) isolated by dissolution in hot chloroform. Fractionation by dissolution in chloroform–light petroleum mixtures gave a main fraction {2·8 g.; $[\alpha]_D^{15}$ −20° (*c*, 1·0 in $CHCl_3$); Found: OMe, 45·0%}.

Hydrolysis of Methylated Mannan B and Separation of Methylated Sugars.—Methylated mannan B (2·5 g.) was hydrolysed successively with anhydrous formic acid (15 c.c.) and sulphuric acid (7 c.c.; N) as described for methylated mannan A. The hydrolysate (2·0 g.) was fractionated on cellulose as described previously and four fractions collected.

Fraction 1. Chromatographic examination of the syrup (55 mg.) showed the presence of two sugars, which were separated on Whatman 3MM paper with benzene–ethanol–water as eluant to give fractions 1*a* (27 mg.) and 1*b* (28 mg.). Fraction 1*a* was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose by conversion into its aniline derivative, m. p. and mixed m. p. 144—145°, $[\alpha]_D^{17}$ −96° ⟶ −41° (*c*, 0·1 in $COMe_2$). Fraction 1*b* was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into its aniline derivative, m. p. and mixed m. p. 195—196°, $[\alpha]_D^{16}$ −137° (*c*, 0·5 in C_5H_5N).

Fraction 2. The syrup (1·640 g.) had $[\alpha]_D^{17}$ −10° (*c*, 6·0 in H_2O). Chromatographic examination showed only 2 : 3 : 6-tri-*O*-methyl-D-mannose, and hypoiodite oxidation indicated 95% purity (Found: OMe, 39·8. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The identity of the sugar was confirmed by conversion into 2 : 3 : 6-tri-*O*-methyl-*N*-phenyl-D-mannosylamine, m. p. and mixed m. p. 127—128°, $[\alpha]_D^{16}$ −155° ⟶ −40° (*c*, 0·1 in MeOH) (Found: C, 60·9; H, 7·9; N, 4·1. Calc. for $C_{15}H_{23}O_5N$: C, 60·6; H, 7·8; N, 4·7%), 2 : 3 : 6-tri-*O*-methyl-D-mannono-γ-lactone, m. p. and mixed m. p. 80—81°, $[\alpha]_D^{20}$ +69·5° (*c*, 0·6 in H_2O), and 2 : 3 : 6-tri-*O*-methyl-D-mannonic acid phenylhydrazide, m. p. and mixed m. p. 130—131°, $[\alpha]_D^{16}$ −17° (*c*, 0·4 in H_2O).

Fraction 3. The syrup (66 mg.) crystallised slowly and after recrystallisation from ether had m. p. 149—151° (undepressed on admixture with fraction 4*a* of the methylated mannan A hydrolysate).

Fraction 4. The syrup (232 mg.) which partly crystallised on standing was hydrolysed with N-sulphuric acid, and chromatographic examination showed the presence of two sugars. Separation was effected on Whatman 3MM paper, butanol–ethanol–water being used as eluant to give fractions 4*a* (203 mg.) and 4*b* (29 mg.). Fraction 4*a* had m. p. 148—150° (undepressed on admixture with fraction 3). Fraction 4*b* was demethylated to give mannose and travelled on the chromatogram at the same rate as 2 : 3-di-*O*-methyl-D-mannose.

Quantitative Examination of the Methylated Sugars.—A portion of the hydrolysate of methylated mannan B was separated chromatographically and estimated by alkaline hypoiodite as described previously [Found (results expressed as c.c. of 0·01N-iodine consumed): "tetra," 0·085, 0·075; "tri," 3·50, 2·96]. These figures correspond to a molecular ratio of tetramethyl sugar : trimethyl sugar of 1 : 40.

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