34. *Mannose-containing Polysaccharides. Part IV.* The Glucomannans of Lily Bulbs.*

By P. ANDREWS, L. HOUGH, and J. K. N. JONES.

Glucomannans isolated from bulbs of *Lilium candidum*, *L. henryii*, and *L. umbellatum* showed similar physical properties and were each constituted of D-glucose and D-mannose (*ca.* 1 : 2 parts) but apparently in slightly different proportions.

Structural studies of *L. umbellatum* and *L. henryii* glucomannan polysaccharides suggested that the majority of the hexose units were linked together by 1 : 4-β-glycosidic bonds to form long chains which were terminated at their non-reducing ends by D-glucopyranosyl units. These two polysaccharides differed in their average chain lengths. *L. umbellatum* glucomannan had a small number of glucopyranosyl units as branching points linked through $C_{(1)}$, $C_{(3)}$, and $C_{(6)}$.

FEW glucomannans have been examined in detail although several have been isolated from plants as, for example, by extraction of the tubers of *Amorphophallus konjak* [1] and *A. oncophyllus*,[2] the leaves of *Aloe vera*,[3] the roots of *Cremastra variabilis* and *Bletilla striata*,[4] and the bulbs of *Narcissus tazetta*.[5] None of these glucomannans appeared to contain more glucose than mannose but, since physical homogeneity was not established, they could have been mixtures. "Iles Mannan," extracted from tubers of *Amorphophallus* plants, has been separated into an amylose-type polysaccharide (1 part) and a glucomannan (6 parts), the latter containing mainly D-glucopyranosyl and D-mannopyranosyl units (in approximately 1 : 2 ratio) joined by 1 : 4-β-glycosidic linkages.[6]

Lily bulbs contain in addition to starch a reserve polysaccharide, which exists as a water-soluble mucilage in the parenchymatous cells of the bulb scales.[7] Takahashi [8] found twice as much mannose as glucose in the polysaccharide which formed an insoluble copper complex.

Extraction of the bulbs of *L. umbellatum*, *L. henryii*, and *L. candidum* with cold water gave in each case a glucomannan (the yield varying with the species), but none of these three products contained starch components. After purification *via* their copper complexes, these polysaccharides became insoluble in water, perhaps because of a change in the conformation of the hexopyranoside rings during complex formation, as has been observed in the case of amylose in alkaline solution.[9] The three glucomannans showed similar optical rotations ($[\alpha]_D$ *ca.* −26°, −21°, −25° respectively) and resemble the glucomannan ($[\alpha]_D$ −21°) from *Amorphophallus*,[6] in that they all contained D-glucose and D-mannose in the approximate ratio of 1 : 2 respectively; however, in the case of the three *Lilium* glucomannans, the proportions differed slightly (1 : 1·83; 1 : 1·93; 1 : 2·00 respectively). When the *Lilium* glucomannans were heated in 0·01N-sulphuric acid for 7 hr. at 100°, the solutions showed but little increase in reducing power and did not liberate any monosaccharide; thus it was assumed that little, if any, furanose forms of the sugars were present.

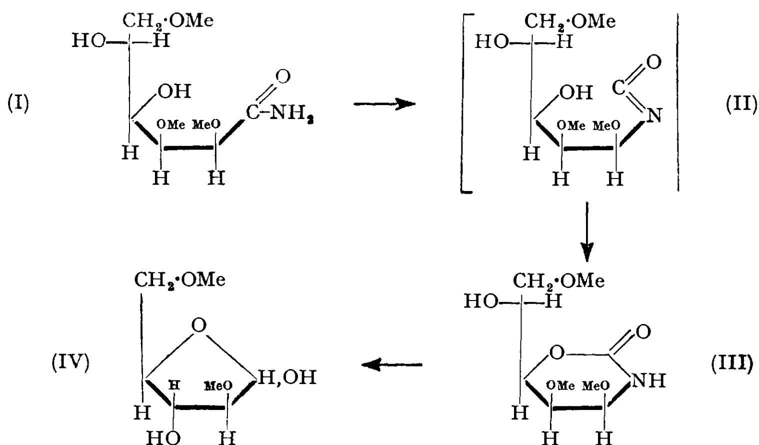
The glucomannans from *L. umbellatum* and *L. henryii* were methylated exhaustively to give methylated products ($[\alpha]_D$ −20°, −23°, in $CHCl_3$, respectively), which on hydrolysis

* Part III, *J.*, 1953, 1186. Some aspects of this work were presented at the 12th International Congress of Pure and Applied Chemistry, New York, 1951.

[1] Otsuki, *Acta Phytochim., Japan*, 1928, **4**, 1; Nishida and Hoshima, *J. Dept. Agric. Kyushu Imp. Univ.*, 1930, **2**, 277.
[2] Wise, *Arch. Biochem.*, 1949, **23**, 127.
[3] Roboz and Haagen-Smit, *J. Amer. Chem. Soc.*, 1948, **70**, 3248.
[4] Otsuki, *Acta Phytochim., Japan*, 1937, **10**, 1, 29.
[5] Kihara, *J. Agric. Chem. Soc. Japan*, 1931, **7**, 1061.
[6] Rebers and Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 6097.
[7] Parkin, *Proc. Cambridge Phil. Soc.*, 1901, **11**, 139.
[8] Takahashi, *J. Agric. Chem. Soc. Japan*, 1930, **6**, 791, 861; 1931, **7**, 219.
[9] Reeves, *J. Amer. Chem. Soc.*, 1954, **76**, 4595.

yielded similar mixtures of *O*-methyl sugars, consisting mainly of tri-*O*-methylhexoses with small amounts of tetra- and di-*O*-methylhexoses, which were separated by chromatography on cellulose. In the tetra-*O*-methyl fractions, only 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose could be detected and a quantitative estimate suggested that it formed 3·7% and 1·3% of the respective *O*-methyl sugar mixtures. Although the tri-*O*-methylhexose fractions showed on paper chromatograms only one spot, which corresponded with that given by both 2 : 3 : 6-tri-*O*-methyl-D-mannose ($[\alpha]_D$ −7°) and 2 : 3 : 6-tri-*O*-methyl-D-glucose ($[\alpha]_D$ +70·5°), the optical rotation (+ 19°) of each fraction was suggestive of mixtures of these two monosaccharides in the approximate ratio of 2 : 1 respectively. The difficulty in separating, and thus identifying, these two *O*-methyl sugars was circumvented in each case by oxidation to their corresponding mixed lactones, which were separated by chromatography on cellulose and then converted into characteristic crystalline derivatives. Rebers and Smith,[6] who worked with a similar mixture, overcame this difficulty by treating the mixture with cold methanolic hydrogen chloride, when only the tri-*O*-methylglucose derivative reacted, thus giving a mixture of methyl 2 : 3 : 6-tri-*O*-methyl-D-glucofuranoside and 2 : 3 : 6-tri-*O*-methyl-D-mannose which were separable by chromatography on cellulose.

2 : 3 : 6-Tri-*O*-methyl-D-mannose was characterised as crystalline 2 : 3 : 6-tri-*O*-methyl-D-mannonamide (I). When this amide (I) was degraded with hypochlorite by the Weerman procedure, a crystalline cyclic urethane derivative (III) was produced, presumably *via* the acyclic *iso*cyanate (II) (cf. Ault, Haworth, and Hirst [10]). Degradation of the cyclic urethane derivative (III) with aqueous base gave 2 : 5-di-*O*-methyl-D-arabinose (IV). Haworth, Peat, and Whetstone [11] carried out a similar degradation using 2 : 3 : 6-tri-*O*-methyl-D-gluconamide.



Demethylation of the di-*O*-methyl fraction from the methylated *L. henryii* glucomannan revealed the presence of both glucose and mannose derivatives. At least three di-*O*-methyl derivatives were detected on paper chromatograms, but these were not identified because of the small quantities available, and hence their significance cannot be assessed. They could have arisen from incomplete methylation of the polysaccharide, from demethylation during hydrolysis, and from branching points in the polysaccharide. In contrast, the *L. umbellatum* methylated glucomannan yielded a di-*O*-methylhexose fraction which contained mainly 2 : 4-di-*O*-methyl-D-glucose. On paper chromatograms, it gave only one spot which was indistinguishable from that from an authentic specimen, and on demethylation only glucose was detected; its derived lactone showed a rapid change in optical rotation in aqueous solution (indicating a 4-methoxyl group), and the derived amide gave only a very slight Weerman test for an α-hydroxy-amide (indicating a

[10] Ault, Haworth, and Hirst, *J.*, 1934, 1722.
[11] Haworth, Peat, and Whetstone, *J.*, 1938, 1975.

2-methoxyl group). 2 : 4-Di-*O*-methyl-D-glucose could not have arisen by either undermethylation or demethylation from a 1 : 4-linked polymer (cf. Andrews, Hough, and Jones [12]) and must therefore represent a branching point.

Thus methylation studies of *L. umbellatum* glucomannan indicated that it contained chains of D-glucopyranosyl and D-mannopyranosyl units linked through $C_{(1)}$ and $C_{(4)}$ with an average of about 27 hexose units per non-reducing end group. Only D-glucopyranosyl units were detected as non-reducing end groups, and the chains were joined by a small number of D-glucopyranosyl units linked through $C_{(1)}$, $C_{(3)}$, and $C_{(6)}$ (branching points). The molecular ratio of tetra-*O*-methylglucose to di-*O*-methylglucose was found to be 1·0 to 0·65, suggesting an average of about two branching points per molecule. Methylation evidence suggested that *L. henryii* glucomannan contained mainly 1→4 linked hexopyranosyl units, but with an average chain length of about 75 hexose units per D-glucopyranosyl end group. Attempts to obtain structural information by periodate oxidation of the polysaccharides were unsuccessful, since only a steady liberation of formic acid was observed. The optical rotations of the glucomannans and of their methylated derivatives suggested that the majority of the inter-glycosidic linkages were of the β-type.

The *Lilium* glucomannans thus show a marked structural resemblance to the *Amorphophallus* glucomannan,[6] although in this case neither end groups nor branching points were detected. It is of interest to compare the *Lilium* glucomannans with the polysaccharides of Iris seeds,[13] since both are constituted of chains of D-glucopyranosyl and D-mannopyranosyl units linked through $C_{(1)}$ and $C_{(4)}$. However, in *L. umbellatum* and *L. henryii* glucomannans, the non-reducing end groups were mainly, if not all, D-glucopyranosyl, whereas only D-galactopyranosyl was detected as non-reducing end group in the *Iris* polysaccharides. In mannans A and B from ivory nut the majority of the D-mannopyranosyl residues are linked through $C_{(1)}$ and $C_{(4)}$, and both D-mannopyranosyl and D-galactopyranosyl units were found as end groups.[14]

Experimental

Paper chromatography was by the descending method,[15] on Whatman No. 1 filter paper and with the following solvent systems: (*a*) ethyl acetate–acetic acid–water (9 : 2 : 2 v/v); (*b*) butan-1-ol–pyridine–water (10 : 3 : 3 v/v); (*c*) butan-1-ol–ethanol–water (40 : 11 : 19 v/v); (*d*) benzene–ethanol–water (34 : 10 : 3 v/v; top layer, clarified with a little ethanol) (for methylated sugars only). Sugars were detected on the chromatograms with ammonical silver nitrate,[15] aniline phthalate or *p*-anisidine hydrochloride.[16] The rate of movement of methyl sugars is quoted relative to that of tetra-*O*-methylglucopyranose ($R_G = 1{\cdot}00$).

Unless otherwise stated, solutions were concentrated under reduced pressure and optical rotations were determined in water at $20° \pm 2°$.

Preliminary Treatment of the Bulbs.—The bulbs of each species were shredded, washed with methanol, and then macerated with this solvent. After 7 days, the insoluble material was collected on a cheese-cloth, and the extraction process repeated. The insoluble material was then dried under reduced pressure first at room temperature and then at 50°. The crisp solids so obtained were milled and gave cream-coloured powders. The methanolic filtrates when evaporated yielded thick, brown syrups in which the presence of sucrose, glucose, fructose, and amino-acids was indicated by paper chromatography. Treatment of the macerated bulbs with methanol for two weeks appeared to inactivate the polysaccharidases as no degradation of the glucomannans was noted during aqueous extraction.[17]

Preparation of the Glucomannans.—The glucomannans were isolated by extraction of the bulb powder with cold water (3 l. per 100 g. of powder) for 18 hr. Starch, the other main polysaccharide component, was not extracted by this process, as indicated by the absence of blue colour when iodine solution was added to the aqueous extract. The starch could be isolated by extraction with hot water or dilute alkali. After clarification of the cold-water extracts on the

[12] Andrews, Hough, and Jones, *J.*, 1954, 806.
[13] *Idem*, *J.*, 1953, 1186.
[14] Aspinall, Hirst, Percival, and Williamson, *J.*, 1953, 3184.
[15] Partridge, *Biochem. J.*, 1948, **42**, 238.
[16] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[17] Husemann, *J. prakt. Chem.*, 1940, **155**, 241.

centrifuge, the supernatant liquors were poured into ethanol to precipitate the glucomannans. After several re-precipitations by slow addition of ethanol to their aqueous solutions, the glucomannans were isolated on the centrifuge, washed with ethanol, and ether, and dried over phosphoric oxide at 20° under reduced pressure. The resultant pale, buff-coloured powders dissolved slowly in water to give opaque solutions, probably owing to the presence of a little protein. They gave no colour with iodine solution and had the following properties :

Glucomannan from	Yield (%) from dried bulb	$[\alpha]_D$	N (%)	Sulphated ash (%)
L. umbellatum	5	−26° ± 5° (*c*, 0·4)	1·5	2·9
L. henryii	20	−21° ± 8° (*c*, 0·5)	1·0	2·2
L. candidum	14	−25° ± 5° (*c*, 0·7)	1·0	0·6

The glucomannans were precipitated as blue copper complexes from aqueous solution by Fehling's solution (equal parts of A and B). The copper complexes were soluble in ice-cold 0·1N-hydrochloric acid, from which solutions the glucomannans were precipitated with ethanol. However, even before drying, the glucomannans so treated had become insoluble in water, but remained soluble in sodium hydroxide solution and in formic acid. The mother-liquors from which the insoluble copper complexes had been removed were neutralised with dilute mineral acid, and after dialysis the solutions were evaporated. Hydrolysis of the residues with N-sulphuric acid at 100° for 16 hr., followed by chromatography of the neutralised (barium carbonate) hydrolysates, indicated the presence in each of amino-acids and very small amounts of arabinose; mannose and glucose were absent.

Hydrolysis of the Glucomannans.—The water-soluble glucomannans were hydrolysed in N-sulphuric acid at 100° for 15 hr., and the hydrolysates were neutralised (barium carbonate), filtered, and concentrated. Paper chromatography of each hydrolysate revealed the presence of glucose and mannose, in the approximate ratio of 1 : 2, with traces of arabinose and xylose; uronic acids were not detected.

The two hexoses present in each glucomannan were isolated by hydrolysing each polysaccharide (1—2 g.) as above, and separating the sugars on a column of cellulose (30 × 1·5 cm.) using butan-1-ol, half-saturated with water, as the mobile phase. In each case crystalline D-glucose and D-mannose were isolated and characterised as tabulated, after recrystallisation from methanol.

	D-Glucose			D-Mannose		
Glucomannan from	m. p.	mixed m. p.	$[\alpha]_D$ (equil.)	m. p.	mixed m.p.	$[\alpha]_D$ (equil.)
L. umbellatum	144°	145°	+52·5°	132°	132°	+14°
L. henryii	145	145	+52	132	131	+15
L. candidum	146	145	+52	132	132	+14

For quantitative analyses, the glucomannans (*ca.* 50 mg.) were hydrolysed in sealed tubes with N-sulphuric acid (2 c.c.) as above. To each hydrolysate was added D-ribose (*ca.* 20 mg.) and, after removal of anions with the minimum amount of Amberlite resin IR-4B (carbonate form), the solutions were evaporated. Portions of each of the residual sugar syrups were separated on large sheet-paper chromatograms with solvent (*c*). After recovery from the appropriate paper strips by Soxhlet-extraction with water (5 c.c.), the sugars were estimated in duplicate by the periodate oxidation method of Hirst and Jones.[18] It being assumed that there was complete recovery of the ribose, and that none of the sugars was lost preferentially, the annexed results were obtained.

Glucomannan from	Wt. hydrolysed (mg.)	Hexose found (mg.) (calc. as $C_6H_{10}O_5$) Glucose	Mannose	Recovery * (%)	Mean sugar ratios Glucose : Mannose
L. umbellatum	57·2	17·8, 17·1	32·2, 31·5	87, 85	1·00 : 1·83
L. henryii	49·1	14·2, 14·5	27·0, 28·2	84, 87	1·00 : 1·93
L. candidum	47·2	14·8, 14·6	29·2, 29·3	93, 93	1·00 : 2·00

* Not corrected for ash and protein content.

An estimate of the glucose : mannose ratio in the *L. umbellatum* glucomannan by Jones and Pridham's benzidine colorimetric method [19] gave a value of 1·00 : 1·88.

[18] Hirst and Jones, *J.*, 1949, 1659.
[19] Jones and Pridham, *Biochem. J.*, 1954, **58**, 288.

Periodate Oxidations.—As in the method described by Brown, Halsall, Hirst and Jones,[20] *L. henryii* glucomannan (1·654 g.) was dissolved in water (150 c.c.), and potassium chloride (10 g.) and 0·23M-sodium metaperiodate solution (80 c.c.) were added. The mixture was kept in the dark and frequently shaken. At intervals, portions (10 c.c.) of the clear solution were withdrawn, ethylene glycol (3 c.c.) was added, and the formic acid titrated with 0·009N-sodium hydroxide, methyl-red screened with methylene-blue being used as indicator.

Period of oxidation (hr.)	168	216	240	288	314	360	407	461
Titre (c.c.)	0·77	1·34	1·50	1·52	1·76	1·91	2·22	2·43
Average no. of hexose units yielding 1 mole of formic acid	63	36	33	32	28	26	22	20

The uptake of periodate by the *L. henryii* glucomannan was determined as follows: in each of several bottles was placed glucomannan (43·7 mg.) in water (25 c.c.), together with potassium chloride (0·3 g.) and 0·452N-sodium metaperiodate (3·00 c.c.). After various periods, the mixture in one bottle was made just alkaline with dilute sodium hydroxide, saturated sodium hydrogen carbonate (10 c.c.), excess of potassium iodide, and 0·097N-sodium arsenite (15 c.c.) were added, and the excess of arsenite was titrated with 0·167N-iodine [titres (c.c.): 2·86 (144 hr.), 2·91 (186 hr.), 3·15 (307 hr.), 3·29 (432 hr.)]. These titres correspond to periodate uptakes (mol. per $C_6H_{10}O_5$ unit) of: 0·89, 0·90, 0·98, and 1·02 respectively.

L. candidum glucomannan (1·2064 g.) was suspended in 0·15M-sodium metaperiodate (100 c.c.) at 18° shaken in a brown bottle. At intervals, portions (10 c.c.) were withdrawn, ethylene glycol (2 c.c.) was added, and after 10 min. the solution titrated with 0·01N-sodium hydroxide to pH 6·25 (cf. Anderson, Greenwood, and Hirst [21]) with the following results:

Period of oxidation (hr.)	15	19	21	41	46	65	89	137
Titre (c.c.)	2·1	2·1	2·15	3·7	4·3	5·65	7·2	9·8

In a similar experiment, *L. umbellatum* (1·0787 g.) gave the following results:

Period of oxidation (hr.)	15	19	22	41	46	65	89	137
Titre (c.c.)	3·0	3·2	3·35	4·35	5·3	6·4	7·4	9·1

Methylation.—The *L. umbellatum* and the *L. henryii* glucomannan were treated with methyl sulphate and sodium hydroxide in the usual way.[13] The *L. umbellatum* glucomannan (6 g.) after six methylations gave a product (5 g.) [Found: OMe, 44·6. Calc. for $C_6H_7O_2(OMe)_3$: OMe, 45·6%] which was extracted from the dialysed mixture with chloroform. It was fractionated under reflux for 1 hr. with mixtures (100 c.c.) of chloroform and light petroleum (b. p. 40—60°). In the first extraction pure light petroleum was used. Thereafter the solid was extracted with light petroleum containing 10%, then 20%, and then 30% of chloroform. Each extraction was carried out twice. The main fraction of methylated polysaccharide was a crisp yellow solid (4·2 g.) and was soluble in the mixture containing 80% of light petroleum, but insoluble in the mixture containing 90% of light petroleum.

After 9 methylations, the *L. henryii* glucomannan (3 g.) similarly gave a chloroform-soluble product (1·6 g.) (Found: OMe, 41·9%), which when fractionated with chloroform–light petroleum gave, as the main fraction, a crisp buff solid (1·28 g.) with the same solubility properties as the main *L. umbellatum* fraction. The methylated polysaccharides had the annexed properties.

	Main fraction of methyl derivative			
Glucomannan from	Yield (%) *	OMe (%)	$[\alpha]_D$ (in $CHCl_3$)	Sulphated ash (%)
L. umbellatum	56	44·7	−20° (*c*, 1·6)	0·3
L. henryii	34	44·0	−23° (*c*, 1·0)	1·2

* From the original glucomannan.

Fission of the Methylated Glucomannans.—Methanolysis was accomplished by boiling the methylated polysaccharide in methanolic hydrogen chloride (2% w/w) under reflux for 16 hr., and then the resultant methylglycosides were hydrolysed in aqueous N-hydrochloric acid at 95—100° for 16 hr. (for further details, see ref. 13). The yields of syrupy methyl-sugar mixtures were 90—95%.

[20] Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27.
[21] Anderson, Greenwood, and Hirst, *J.*, 1955, 225.

Examination of the Methyl-sugar Mixtures.—Paper chromatograms [solvent (*c*)] indicated that each of the two mixtures consisted largely of tri-*O*-methylhexose (R_G 0·89), which moved at the same rate as the 2 : 3 : 6-tri-*O*-methyl derivatives of glucose and mannose. Small amounts of tetra-*O*-methyl- (R_G 1·00) and di-*O*-methyl-hexose (R_G *ca.* 0·6) were also present.

The sugar mixtures were fractionated by partition chromatography on hydrocellulose columns (28 × 4 cm.), with benzene–ethanol (4 : 1 v/v), nearly saturated with water, as the mobile phase. Butan-1-ol was added to this mixture to elute the di-*O*-methylhexoses.

The mixture of methyl-sugars (2·06 g.) from *L. umbellatum* gave the following fractions :

Fraction (1) (120) mg. was a syrup, containing compounds which gave two spots on the chromatogram, with R_G 1·00 and 0·89, in the approximate proportions of 3 : 1 respectively. After hydrolysis in N-hydrochloric acid at 100° for 5 hr., the proportions of the two substances had changed to *ca.* 2 : 1 respectively, indicating the presence of methyl tri-*O*-methylhexoside, but further hydrolysis effected no further change. Anions were removed from the hydrolysate with Amberlite resin IR-4B (carbonate form), the solution was evaporated, and the resultant sugar mixture (110 mg.) separated into the following two fractions on a large sheet-paper chromatogram, with solvent (*d*).

Fraction (1a) (65 mg.) [Found : OMe, 49·8. Calc. for $C_6H_8O_2(OMe)_4$: OMe, 52·5%] had R_G 1·00 and $[\alpha]_D$ +74° (*c*, 1·1). Demethylation of a portion (5 mg.) with hydrobromic acid (48% w/w; 1 c.c.) at 100° for 10 min. indicated that glucose was the parent sugar; mannose may have been present in much smaller amount, but could not be detected with certainty. The fraction was dissolved in ether–light petroleum (b. p. 60—80°) and, when seeded, gave a small crop of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. and mixed m. p. 85—87° [Found : OMe, 52·8. Calc. for $C_6H_8O_2(OMe)_4$: OMe, 52·5%]. The sugar (47 mg.) remaining in the mother-liquors was boiled in alcoholic solution with aniline (18 mg.) and yielded *N*-phenyl-D-glucopyranosylamine tetramethyl ether (25 mg.), which after recrystallisation from ethanol had m. p. and mixed m. p. 137—138° and $[\alpha]_D$ +235° (init. value; *c*, 0·2 in $COMe_2$).

Fraction (1b) (30 mg.) had R_G 0·89; it was combined with Fraction (2).

Fraction (2) (1·75 g.) [Found : OMe, 39·0. Calc. for $C_6H_9O_3(OMe)_3$: OMe, 41·8%] was a syrup, with $[\alpha]_D$ +19° (*c*, 2·5), which on the paper chromatogram with solvent (*c*) gave only one reddish-brown spot (R_G 0·89) with the *p*-anisidine hydrochloride reagent. A portion of this fraction (250 mg.) was boiled under reflux with a solution of aniline (100 mg.) in ethanol, and yielded *N*-phenyl-D-mannopyranosylamine 2 : 3 : 6-trimethyl ether (135 mg.), which after recrystallisation from ether–light petroleum (b. p. 40—60°) had m. p. 123°, raised to 125° on further recrystallisation, and $[\alpha]_D$ −152° (init. value; *c*, 0·6 in MeOH) ⟶ −39° (equil. value) (Found : N, 4·6. Calc. for $C_{15}H_{23}O_5N$: N, 4·7%). The mixture from which this derivative had crystallised was heated in N-sulphuric acid at 95° for 10 min., and the solution extracted continuously with chloroform. This gave a syrup (150 mg.) whcih was dissolved in ether and, when seeded, gave a very small crop (*ca.* 3 mg.) of 2 : 3 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 115—118°.

A portion (1·10 g.) of the fraction was oxidised with excess of bromine water in the usual way, and the resultant mixture of lactones examined on the paper chromatogram [solvent (*c*)] (cf. Abdel-Akher and Smith[22]). Two spots (R_G 0·92 and 1·00) were detected with ammoniacal silver nitrate, the former being the larger. The mixture of lactones was dissolved in ether, and when seeded the solution yielded 2 : 3 : 6-tri-*O*-methyl-D-mannono-γ-lactone (400 mg.), which after recrystallisation from ether had m. p. and mixed m. p. 79—80° and $[\alpha]_D$ +73° (init. value; *c*, 0·9) ⟶ +70° (90 hr.) [Found : OMe, 42·2. Calc. for $C_6H_7O_3(OMe)_3$: OMe, 42·3%]. The remaining lactone mixture (*ca.* 600 mg.) was chromatographed on large sheets of filter paper [solvent (*c*)], giving 2 fractions :

Fraction (2a) (210 mg.) consisted mainly (*ca.* 90%) of the compound with R_G 1·00, the remainder having R_G 0·92. It had $[\alpha]_D$ +54° (init. value; *c*, 1·1) ⟶ +44° (96 hr.). The syrup (190 mg.) could not be induced to crystallise, but when boiled in ethanol with phenylhydrazine (105 mg.) it gave crystalline 2 : 3 : 6-tri-*O*-methyl-D-gluconophenylhydrazide (105 mg.) which after recrystallisation from ethanol had m. p. 145—146° and $[\alpha]_D$ +18° (*c*, 1·0) (Found : N, 8·2. Calc. for $C_{15}H_{24}O_6N_2$: N, 8·5%).

Fraction (2b) (290 mg.) consisted mainly of the lactone (R_G 0·92) with a little of the glucono-lactone (R_G 1·00). The former corresponded to 2 : 3 : 6-tri-*O*-methyl-D-mannono-γ-lactone, and when the fraction (80 mg.) was boiled in alcohol with phenylhydrazine (40 mg.) it yielded 2 : 3 : 6-tri-*O*-methyl-D-mannonophenylhydrazide, which crystallised from ethanol as the

[22] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.

monohydrate, m. p. 134—135° (m. p. 133—134° on admixture with an authentic specimen of m. p. 132°) and $[\alpha]_D$ −21° (*c*, 0·5) (Found: C, 52·2; H, 7·2; N, 7·9; loss of wt. on drying *in vacuo* at 110°, 4·6. Calc. for $C_{15}H_{24}O_6N_2,H_2O$: C, 52·0; H, 7·5; N, 8·1; H_2O, 5·2%). No other crystalline material was isolated from the mixture of phenylhydrazides.

The remainder of this fraction (2b) (210 mg.) was converted into the corresponding mixture of amides by dissolution in methanolic ammonia; after evaporation of the solution, the residual crystalline material (260 mg.) was dissolved in water (3 c.c.), and 0·6N-sodium hypochlorite (5 c.c.) added at 0°. Excess of sodium thiosulphate solution was added after 1 hr. at 0°, and the mixture then extracted continuously with chloroform. Evaporation of the chloroform gave crystalline material (80 mg.), which after 2 recrystallisations from acetone yielded colourless needles (25 mg.) of the cyclic *urethane* (III) derived from 2 : 3 : 6-tri-*O*-methyl-D-mannonamide, with m. p. 153° and $[\alpha]_D$ +37° (*c*, 1·0) [Found: C, 46·1; H, 7·4; N, 5·8; OMe, 38·7. $C_6H_8O_3N(OMe)_3$ requires C, 45·95; H, 7·2; N, 5·95; OMe, 39·6%]. The material from the chloroform extract was recombined (60 mg.) and dissolved in 0·3N-sodium hydroxide (10 c.c.), and the solution kept at room temperature for 48 hr. It was then neutralised with dilute sulphuric acid and evaporated to dryness, and the residue extracted with boiling ether. Evaporation of the ether gave a di-*O*-methylpentose (31 mg.) [Found: OMe, 33·3. Calc. for $C_5H_8O_3(OMe)_2$: OMe, 34·8%], $[\alpha]_D$ +30° (*c*, 1·2), which gave on the paper chromatogram one spot [R_G 0·71 in solvent (*c*)] which was purplish-brown with *p*-anisidine hydrochloride and indistinguishable from that of 2 : 5-di-*O*-methylarabinose. The sugar was oxidised with bromine water, but the derived lactone (18 mg.) did not recrystallise satisfactorily and accordingly was boiled in ethanol with phenylhydrazine (12 mg.). Evaporation of the ethanol gave a syrup which crystallised slowly; recrystallisation from ethanol afforded 2 : 5-di-*O*-methyl-D-arabonophenylhydrazide as white needles, m. p. 162°.

Fraction (3) (40 mg.) [Found: OMe, 28·4. Calc. for $C_6H_{10}O_4(OMe)_2$: OMe, 29·8%] had $[\alpha]_D$ +56° (*c*, 0·9). A portion (5 mg.) on demethylation with hydrobromic acid (48% w/w; 1 c.c.) at 100° for 3 min. showed only glucose on paper chromatograms. On the paper chromatogram [solvent (*c*)] it gave a purple-brown spot (R_G 0·61) with *p*-anisidine hydrochloride, thus resembling 2 : 4-di-*O*-methylglucose, and differing from the 2 : 3-, 3 : 4-, and 4 : 6-isomers, which had R_G 0·67, 0·63, 0·54 and gave dark brown, light brown, and light brown spots respectively. When oxidised with bromine water in the usual way, the fraction (20 mg.) gave a syrupy lactone (16 mg.), $[\alpha]_D$ +77° (init. value; *c*, 0·8) ⟶ +57° (18 hr.) ⟶ +54° (48 hr.; equil. value). The lactone was converted, by treatment with methanolic ammonia, into a syrupy amide (20 mg.). The amide was dissolved in 0·6N-sodium hypochlorite (1 c.c.) and after 0·5 hr. at 0° excess of sodium thiosulphate solution was added, followed by sodium acetate (20 mg.) and semicarbazide hydrochloride (30 mg.). A slight precipitate (*ca.* 0·2 mg.), m. p. *ca.* 250°, had formed after 18 hr. at 0°. Under similar conditions, gluconamide (8 mg.) gave hydrazodicarbonamide (3·5 mg.), m. p. 255°.

The *L. henryii* mixture of methyl sugars (1·222 g.) gave the following fractions:

Fraction (1′) (20 mg.) was a syrup which on the paper chromatogram gave only one spot [R_G 1·00 in (*c*)], but hydrolysis [see under Fraction (1)] revealed that it contained *ca.* 20% of methyl tri-*O*-methylhexoside. The resultant mixture of tetra- and tri-*O*-methylhexoses was fractionated on a large sheet-paper chromatogram [solvent (*d*)] and the tetra-*O*-methylhexose (12 mg.) isolated. It had n_D^{20} 1·4560 and $[\alpha]_D$ +70° (*c*, 0·6). Demethylation of a portion (3 mg.) [see Fraction (1a)] showed glucose on the paper chromatogram, but mannose was not detected. Treatment of the tetra-*O*-methyl sugar (9 mg.) with aniline (5 mg.) in ethanol yielded *N*-phenyl-D-glucopyranosylamine 2 : 3 : 4 : 6-tetramethyl ether (5 mg.), m. p. and mixed m. p. 135—136°, $[\alpha]_D$ +240° (*c*, 0·6 in Me_2CO).

Fraction (2′) (1·140 g.) [Found: OMe, 40·7. Calc. for $C_6H_9O_3(OMe)_3$: OMe, 41·8%] was a syrup with $[\alpha]_D$ +19° (*c*, 1·6) which gave only one spot (R_G 0·89) on the paper chromatogram [solvent (*c*)]. Oxidation of a portion (500 mg.) with bromine water in the usual way yielded a mixture of two lactones [R_G 1·00 and 0·92 in solvent (*c*)]. This mixture was separated into two fractions by chromatography on large paper sheets with solvent (*c*):

Fraction (2′a) (120 mg.) consisted mainly of 2 : 3 : 6-tri-*O*-methyl-D-glucono-γ-lactone (R_G 1·00), $[\alpha]_D$ +52° (init. value; *c*, 2·0) ⟶ +42° (120 hr.). The lactone (70 mg.), when treated with phenylhydrazine (35 mg.) in boiling ethanol, gave 2 : 3 : 6-tri-*O*-methyl-D-gluconophenylhydrazide (35 mg.) which after recrystallisation from ethanol had $[\alpha]_D$ +17° (*c*, 0·5) and m. p. 145°, undepressed on admixture with the specimen prepared from the *L. umbellatum* methyl-sugars.

Fraction (2′b) (295 mg.) consisted mainly of 2 : 3 : 6-tri-*O*-methyl-D-mannono-γ-lactone

(R_G 0·92) which crystallised when seeded. After recrystallisation from acetone–ether–light petroleum (b. p. 60—80°) it had m. p. and mixed m. p. 79—80° and $[\alpha]_D$ +70° (init. value; *c*, 1·2) ⟶ +68° (92 hr.). The material in the mother-liquors of the recrystallisation yielded 2 : 3 : 6-tri-*O*-methyl-D-mannonophenylhydrazide, which crystallised from ethanol as the monohydrate, m. p. and mixed m. p. 131—132°, $[\alpha]_D$ −18° (*c*, 0·5) [Found (on an anhydrous sample): N, 8·4; OMe, 28·4. Calc. for $C_{15}H_{24}O_6N_2$: N, 8·5; OMe, 28·4%].

Fraction (3′) (35 mg.) was a syrupy mixture of at least three di-*O*-methylhexoses [R_G 0·58—0·62 in solvent (*c*)] which were incompletely separated on the paper chromatogram. Demethylation of a portion with hydrobromic acid (48% w/w) for 3 min. at 100° showed both glucose and mannose on the paper chromatogram. It was not further examined.

Quantitative Analyses of the Methyl-sugar Mixtures.—The relative amounts of tetra-, tri-, and di-*O*-methylhexose in each methylated polysaccharide (*ca.* 20 mg. *L. umbellatum*; *ca.* 30 mg. *L. henryii*) were estimated, after methanolysis and hydrolysis as described above, by chromatographic separation on filter paper with solvent (*d*), and the separated methyl-sugars were then determined by alkaline hypoiodite.[23] 0·1N-Iodine (2 c.c.) in water (5 c.c.) with sodium hydroxide–sodium phosphate buffer (4 c.c.; pH 11·4) were used to oxidise the tetra- and the di-*O*-methyl sugars, and five times these amounts for the oxidation of the tri-*O*-methylhexoses. The oxidations were allowed to proceed for 18 hr. in the dark, and blanks were run concurrently. After acidification, the excess of iodine was titrated with 0·01N-sodium thiosulphate except in the case of tri-*O*-methyl sugars for which 0·1N-sodium thiosulphate was used. The results are summarised in the Table.

	Consumption of 0·01N-I_2 (c.c.)			Molecular ratios			
Glucomannan from	"Tetra"	"Tri"	"Di"	"Tetra"	"Tri"	"Di"	Average chain length
L. umbellatum ...	0·55, 0·60	13·1, 15·5	0·32, 0·43	1·0, 1·0	24, 26	0·6, 0·7	26, 28
L. henryii	0·49, 0·34	34·3, 24·8	1·24, 0·97	1·0, 1·0	70, 73	2·5, 2·8	74, 77

THE UNIVERSITY, BRISTOL.
[Present address (J. K. N. J.): QUEEN'S UNIVERSITY, KINGSTON, ONTARIO, CANADA.
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[23] Hirst, Hough, and Jones, *J.*, 1949, 928; Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.