160. *Polysaccharides. Part XXIV. Yeast Mannan.*

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The polysaccharide constituents of yeast include glycogen and the so-called " yeast gum," which occurs mainly in the cell wall and was first isolated by Salkowski (*Ber.*, 1894, **27**, 497, 925). It was later recognised as a derivative of mannose and is of special interest on this account, since few of the naturally occurring mannans have so far been the subject of investigation. The mannan of ivory-nut was found by Klages (*Annalen*, 1934, **509**, 159) to consist of a chain of β-mannopyranose units linked through the 1 : 4-positions. It has therefore a structure similar to that of cellulose. In contrast with this the mannan described by Haworth, Raistrick, and Stacey (*Biochem. J.*, 1935, **29**, 612) had a chain-structure in which α-mannopyranose units are linked together through positions 1 and 6. Yeast

mannan, as will appear from the present paper, differs fundamentally in structure from both these and contains a chain of mannose units linked through positions 1 and 6, but with a mannopyranose unit linked glycosidically to the second carbon atom of each alternate mannose unit in the chain. The full implications of this will be discussed when the evidence on which this conclusion is based has been presented.

The yeast mannan was extracted from the cell wall by the action of boiling dilute alkali on baker's yeast. It was separated from other extracted materials in the form of its copper hydroxide complex and after removal of the copper was purified by repeated precipitation from aqueous solution by alcohol. The white powder so obtained was essentially homogeneous and could not be separated into fractions having different properties. It gave only mannose on hydrolysis.

It could be readily acetylated by treatment with pyridine and acetic anhydride and the *acetate* resisted all attempts to separate from it fractions showing different properties. Either from this acetate, by simultaneous de-acetylation and methylation, or by direct methylation of the polysaccharide, the same methylated derivative was obtained. Rigorous fractionation of this material failed to give portions differing materially from one another and it appeared to be essentially homogeneous. This methylated mannan gave solutions of low viscosity compared with similar solutions of methylated starch or methylated cellulose, but in view of the chemical constitution no attempt has been made to apply Staudinger's equation for molecular weight determinations. But the particle weight determined by osmotic pressure measurements (Carter and Record, *J. Soc. Chem. Ind.*, 1936, **55**, No. 11, " Chemistry & Industry," p. 218) is very large (*ca.* 100,000, *i.e.*, 500 mannose units).

Like the original polysaccharide, methylated yeast mannan showed resistance to weak or highly dilute hydrolytic agents, but it was hydrolysed almost quantitatively and without appreciable decomposition by treatment with methyl-alcoholic hydrogen chloride at 150° under pressure. There were three main products, obtained in approximately equimolecular proportions, namely 2 : 3 : 4 : 6-tetramethyl methylmannopyranoside, 2 : 3 : 4-trimethyl methylmannopyranoside, and 3 : 4-dimethyl methylmannopyranoside. Proof of the identity of these substances was obtained in the following ways :

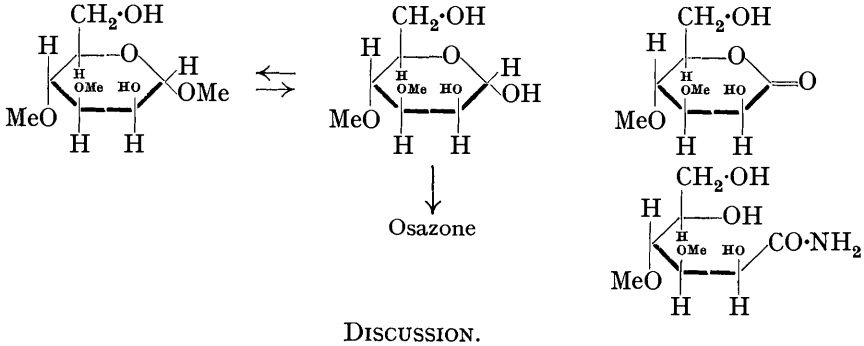
(a) The tetramethyl methylmannoside gave on hydrolysis 2 : 3 : 4 : 6-tetramethyl mannose, recognised both as the sugar and its crystalline anilide and by its transformation by oxidation into the crystalline 2 : 3 : 4 : 6-tetramethyl mannonolactone (Drew, Goodyear, and Haworth, J., 1927, 1243). There can be no doubt, therefore, of the identity of this constituent.

(b) Equally convincing proof of the identity of the trimethyl methylmannoside was obtained by its conversion into the crystalline sugar, from which on oxidation by bromine water the crystalline lactone of 2 : 3 : 4-trimethyl mannonic acid was obtained. Finally the sugar gave on oxidation by nitric acid 2 : 3 : 4-trimethyl mannosaccharic acid. The last substance was formed without loss of a methoxyl group and position 6 must therefore have been a primary alcoholic group. The lactone obtained by oxidation with bromine water was definitely a δ-lactone and it follows immediately that the three methyl groups were severally situated at C_2, C_3, and C_4.

(c) The dimethyl methylmannoside gave on hydrolysis a crystalline dimethyl mannose which, on oxidation by bromine water, gave a crystalline dimethyl mannonolactone and from its rate of hydrolysis in aqueous solution the lactone was identified as belonging to the δ-series. This indicated that a methoxyl group was attached to C_4, since under the conditions adopted for lactonisation of the dimethylmannonic acid it is expected that a γ-lactone would be formed if this mode of lactonisation were possible. Further decisive evidence on this point was provided by a study of the reaction between the free dimethylmannose and cold methyl-alcoholic hydrogen chloride. It is known that in the mannose series formation of a methyl mannofuranoside readily and fairly rapidly takes place under these conditions. In the present instance no evidence of furanoside formation could be observed and no reaction took place until the solution was heated and conditions for pyranoside formation were employed.

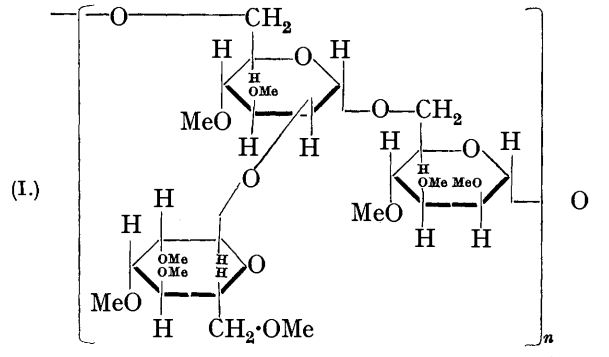
One of the methoxyl groups is therefore attached to C_4. The other cannot be at C_6,

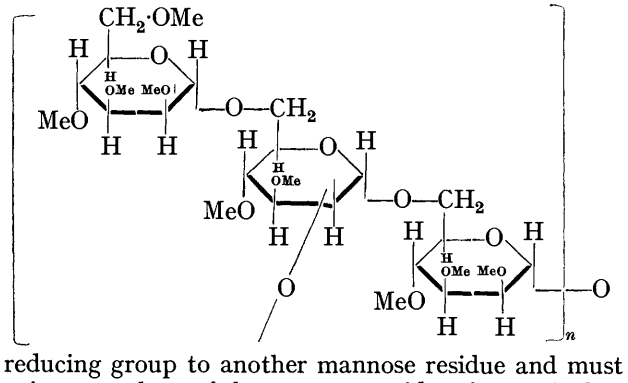
because 4 : 6-dimethyl mannose is known (Ault, Haworth, and Hirst, J., 1935, 517) and is obviously not identical with the sugar now under discussion. There remains only the choice between C_2 and C_3 and this is easily made in view of the observations that the dimethyl mannose gives an osazone without loss of a methoxyl group and that the amide prepared from the dimethyl mannonolactone gives a strong positive Weerman reaction with sodium hypochlorite. There is, therefore, no methoxyl group at C_2 and the dimethyl sugar must be identified as 3 : 4-*dimethyl mannose.*



Discussion.

These results show that methylated mannan contains in its structure three types of residues which are united in the polysaccharide molecule. The size of this molecule is unknown but it is probably large. In any possible structural formulæ the following conditions must be satisfied : (a) one in every three of the mannose residues must be





attached by its reducing group to another mannose residue and must form a terminated side chain, (b) one in every three of the mannose residues is attached at positions C_1 and C_6 to other residues, and (c) the third mannose residue is attached to other mannose residues at positions C_1, C_2, and C_6.

Two types of structure will satisfy these conditions and these are represented in their simplest form in (I) and (II).

The formula (I) comprises a chain of α-mannopyranose units linked through the 1 : 6-positions with a mannose unit joined at C_2 of every alternate residue. The formula (II) postulates a chain of mannopyranose units joined alternately by 1 : 6 and 1 : 2 links, but at the 6-position of the latter unit a further mannopyranose residue is attached through its reducing group. There are at present no polysaccharides of this type of structure known, but on the present evidence such a constitution cannot be excluded. For each bracketed structure where $n = 1$ in formulation (II) a trisaccharide anhydride is represented, but the minimum molecular weight determined by the Rast method rules out the possibility at once and the properties of the methylated mannan indicate that n is probably a large number. Since n is greater than unity, various possibilities present themselves. (a) The molecule may exist as a large ring. (b) The molecule may be a terminated chain and in this case it is to be remarked that a terminated chain would reveal itself on hydrolysis, not necessarily by the presence of additional tetramethyl mannose but by the presence of a molecule of 3 : 4 : 6-trimethyl mannose (I) or 2 : 3 : 4-trimethyl mannose (II). Beyond the fact that the observed amount of trimethyl mannose is slightly greater than that of the tetramethyl derivative, nothing can be said on this point at the moment. The search for a small amount of 3 : 4 : 6-trimethyl mannose in an excess of 2 : 3 : 4-trimethyl mannose is not an easy problem to solve. (c) The molecule may be essentially as described under (b), but aggregation to larger units, comprising some 500 mannose residues, may occur by one or other of the mechanisms suggested by Haworth, Hirst, and Oliver (J., 1934, 1917) as applicable to xylan, starch, and other polysaccharides.

Although the formula (I) shows the 1 : 6-union of adjoining mannopyranose groups as α, this stereochemical arrangement is based only on the high dextrorotation of the mannan and on the fall in rotation on complete hydrolysis. This does not exclude the possibility of the presence of some β-linkages.

Experimental.

Preparation of Yeast Mannan.—Baker's yeast (7 kg.) was boiled for 8 hours with 6% sodium hydroxide solution (15 l.). The solution was centrifuged, and the clear liquid concentrated under diminished pressure to 3·5 l. After acidification by addition of acetic acid sodium nucleate was deposited as a slime. The solution was then centrifuged and separated into three layers: (a) a sticky solid in the upper portion of the tubes, (b) a clear liquid, (c) a granular precipitate collected on the bottom of the tubes. (a) and (c) were combined, stirred with dilute acetic acid, and centrifuged again. The clear liquid from this operation was combined with (b), filtered through glass wool, neutralised with sodium hydroxide, and concentrated to 1 l. Addition of alcohol (to make the final concentration of alcohol 65%) precipitated crude mannan, which was separated and washed with 60% alcohol. It was dissolved in water (800 c.c.), the solution adjusted until it was only slightly alkaline, and Fehling's solution was added until the colour of the liquid above the grey-blue precipitate was deep blue. The precipitate was thoroughly washed with warm water, then suspended in water (1 l.), and concentrated hydrochloric acid was slowly added with stirring, the solution being kept greenish-grey in colour. When all the mannan–copper complex had dissolved, a little more acid was added and the solution was poured into alcohol (3 vols.). The precipitate, after being washed with alcohol, was dissolved in water and reprecipitated with alcohol (this precipitation proceeds best if the aqueous solution is slightly acid). The precipitate was then dissolved in water (1 l.) and the solution, rendered just alkaline by potassium hydroxide, was poured into alcohol (3 l.). The precipitate of crude mannan was washed with alcohol and dried at 50° under diminished pressure. Yield, 150 g. $[\alpha]_D^{20°}$ + 73° in water (*c*, 1·0).

The crude mannan (400 g., collected by repetitions of the above procedure) was dissolved in warm water (2·5 l.). Glacial acetic acid (750 c.c.) was then added and the solution was treated with charcoal, filtered, and poured slowly into methylated spirit (10 l.). The precipitate was collected, and the procedure repeated twice. The *mannan* (260 g.) obtained after the third precipitation had the following properties: white hygroscopic powder, readily soluble in cold water; no colour with iodine in aqueous solution; iodine number negligible; non-reducing to Fehling's solution. On titration with sodium hydroxide the amount of alkali used was negligible; when the mannan was boiled with 12% hydrochloric acid, the amount of furfural liberated was negligible (absence of pentose sugars and uronic acids); $[\alpha]_D^{24°}$ + 89° in water (*c*, 1·4) [Found: C, 44·5; H, 6·1; N, nil. $(C_6H_{10}O_5)_n$ requires C, 44·4; H, 6·2%].

Fractional precipitation of yeast mannan from aqueous solution by gradual addition of alcohol gave a series of fractions with properties identical with those just described. Hydrolysis of yeast mannan with fuming hydrochloric acid in the cold was unsatisfactory. Better results were obtained by hydrolysis with 2*N*-sulphuric acid at 95°. In the course of 5 hours the rotation fell regularly from $[\alpha]_D$ + 80° to the constant value + 12° (for mannose the value of $[\alpha]_D$ is + 15°). The final solution was dark and some decomposition had taken place. After neutralisation with barium carbonate and treatment with charcoal, the solution was evaporated to a syrup (diminished pressure). This syrup slowly crystallised. After some days the syrupy portion was washed away by 50% acetic acid, leaving crystalline mannose (m. p. 131°; $[\alpha]_D^{20°}$ + 15°, equilibrium value in water, *c*, 3·0; yield, 25% of the theoretical). On addition of phenylhydrazine to the acetic acid solution of the syrup a copious precipitate of mannose phenylhydrazone was obtained (yield, 50% of the theoretical). Control experiments in which mannose was subjected to exactly the above procedure showed that the recovery of mannose amounted to 82% of the theoretical. Using this correction, we find that in the hydrolysis of mannan a minimum yield of 92% of the theoretical quantity of mannose was obtained. It may be concluded, therefore, that in all probability yeast mannan is composed entirely of mannose residues.

Acetylation of Yeast Mannan.—(a) Mannan (15 g.) was dissolved in warm water (100 c.c.) and alcohol (900 c.c.) was added slowly to the solution. The precipitated mannan was separated and immediately acetylated by acetic anhydride (150 c.c.) and pyridine (200 c.c.). The mixture was kept at 80° for 30 hours with stirring. Nearly all the mannan dissolved and the small residue was filtered off. The solution was poured into water, and the *acetate* isolated in the usual way. It was a white powder soluble in acetone, chloroform and hot methyl alcohol, insoluble in water and in cold alcohol. $[\alpha]_D^{24°}$ + 62° in chloroform (*c*, 1·7). $\eta_{sp.}^{20°}$ 0·25 in *m*-cresol (0·05 g. in 5 c.c.). Yield, 85% of the theoretical (Found: CH_3·CO, 45·1. $C_{12}H_{16}O_8$ requires CH_3·CO, 44·8%).

(b) Mannan (10 g.) was treated in exactly the same way and acetylated by a mixture of acetic acid (65 c.c.) containing a little chlorine and acetic anhydride (100 c.c.) containing sulphur dioxide. Acetylation was complete in 2 hours at 65°. The acetate (yield, 70% of the theoretical) had properties the same as those described under (a) except that it had a greater solubility in chloroform and showed a smaller viscosity in *m*-cresol ($\eta_{sp.}^{20°}$ 0·12; 0·05 g. in 5 c.c.).

Mannan acetate [200 g., prepared by the method (a) involving the use of pyridine and acetic anhydride] was carefully fractionated by the gradual addition of light petroleum to a chloroform solution. The data recorded in the accompanying table show that no serious differences are observable in the properties of the fractions, except that the small fraction I contained most of the mineral impurities and is not comparable with the others.

Fraction.	Wt., g.	Ash, %.	$[\alpha]_D^{20°}$ (in $CHCl_3$).	$\eta_{sp.}^{20°}$.*	CH_3·CO, %.
1	12	0·6	+ 52°	0·56	44·4
2	115	0·15	+ 61	0·28	44·6
3	54	0·10	+ 60	0·23	44·0
4	17	0·04	+ 61	0·17	44·6

* 0·05 G. in 5 c.c. of *m*-cresol. The meaning of the viscosity figures is not clear: they appear to be connected closely with the ash content and their possible interpretation in terms of chain length is deferred until further data are available.

Methylation of Yeast Mannan.—(a) Fractions 2 and 3 of the acetate were combined and used for methylation. The acetate (10 g.) in acetone (200 c.c.) was simultaneously de-acetylated and methylated at 55° by the gradual addition during 2 hours of sodium hydroxide (320 c.c., 30% solution) and methyl sulphate (120 c.c.). When all the reagents had been added, the mixture was heated at 100° for 30 minutes. Usually the methylated mannan separated from the hot solution and was filtered off. Occasionally it separated as an emulsion. In such cases the solution was then brought almost to neutrality by addition of sulphuric acid and concentrated, alcohol added to precipitate most of the sodium sulphate, the aqueous alcoholic solution evaporated to dryness, and the residue treated again with methyl sulphate and sodium hydroxide in the presence of acetone. After 8 successive methylations the crude methylated mannan was dissolved in chloroform, the solution was filtered to remove mineral impurities, and after evaporation of the solvent the methylated mannan was dissolved in ether containing a little acetone. Addition of excess of light petroleum threw down methylated yeast mannan as a pale yellow, amorphous powder (yield, 70% of the theoretical), soluble in acetone, chloroform, and

methyl alcohol, insoluble in light petroleum. $[\alpha]_D^{24°}$ + 85° in chloroform (*c*, 1·96); + 84° in methyl alcohol (*c*, 1·5) (Found : OMe, 43·6%).

(b) Methylated mannan is obtainable also by direct methylation in aqueous solution. Mannan (16 g.) in water (100 c.c.) was treated in the usual way at 50° with methyl sulphate (200 c.c.) and sodium hydroxide (400 c.c., 30% solution). When the solution was heated at 100° at the end of the reaction, methylated mannan separated. After 4 methylations (of which 2 were conducted in the presence of acetone) a product identical with that described above was obtained. $[\alpha]_D^{20°}$ + 85° in chloroform (*c*, 1·2); $\eta_{sp.}^{20°}$ 0·08 in *m*-cresol (0·02 g. in 5 c.c.) (yield, 92% of the theoretical) (Found : OMe, 43·5%).

Fractionation of Methylated Yeast Mannan.—Methylated mannan [46 g., prepared by method (a)] was dissolved in a mixture of ether (350 c.c.) and acetone (110 c.c.) and to the solution light petroleum was slowly added, the following fractions (which differed inappreciably from one another) being obtained :

Fraction.	Wt., g.	$[\alpha]_D^{25°}$ (in $CHCl_3$).	$\eta_{sp.}^{20°}$.*	OMe, %.
1	27	+ 89°	0·26	44·1
2	12	+ 86	0·25	43·8
3	7	+ 86	0·19	43·8

* In *m*-cresol (0·05 g. in 5 c.c.).

Molecular weight estimations (fraction 2) by Rast's method in camphor indicated a minimum molecular weight of 3000. The real particle weight may however be very much greater than this.

Hydrolysis of Methylated Yeast Mannan.—After many trial experiments had been carried out it was found that the method of hydrolysis most suitable for the present investigation consisted in heating methylated mannan (21·1 g.) in 1% methyl-alcoholic hydrogen chloride (800 c.c.) at 150° under pressure for 20 hours. Hydrolysis was completed in this way without appreciable decomposition. The hydrochloric acid was removed as silver chloride in the usual way and the product was a pale yellow syrup (22·7 g.; $n_D^{20°}$ 1·4595). This syrup was submitted to systematic fractional distillation through a specially designed, vacuum-jacketed column. The following fractions were ultimately separated :

Fraction.	Bath temp. and pressure.	Wt., g.	$n_D^{21°}$.	OMe, %.
1	120—125°/0·07 mm.	4·9	1·4475	59·8
2	135—145 /0·07 mm.	2·8	1·4500	58·0
3	145—155 /0·10 mm.	4·3	1·4574	52·4
4	155—165 /0·10 mm.	2·7	1·4620	50·1
5	165—180 /0·05 mm.	1·2	1·4670	44·1
6	138—145 /0·04 mm.*	4·4	1·4705	41·1
7	Residue	1·4		

* Distilled without fractionating column : hence lower bath temperature.

Fractions 1, 3, and 6 consisted of tetramethyl methylmannopyranoside, trimethyl methylmannoside, and dimethyl methylmannoside respectively. By calculation from the refractive indices and methoxyl contents of fractions 2, 4, and 5 the compositions of these fractions were estimated, giving the following results : Fraction 2, 2·1 g. of tetramethyl methylmannoside and 0·7 g. of trimethyl methylmannoside; fraction 4, 1·9 g. of trimethyl methylmannoside and 0·8 g. of dimethyl methylmannoside; fraction 5, 0·3 g. of trimethyl methylmannoside and 0·9 g. of dimethyl methylmannoside. The still residue probably retained some dimethyl methylmannoside which could not be separated from the high-boiling residue owing to the decomposition of the latter when heated strongly enough to distil away the last traces of dimethyl methylmannoside (dimethyl methylmannoside was being collected in fraction 6 when the distillation had to be stopped owing to onset of decomposition in the flask). The composition of the mixture of hydrolysis products was therefore : Tetramethyl methylmannoside 7·0 g., trimethyl methylmannoside, 7·2 g., and dimethyl methylmannoside 6·1 g. In view of the difficulty experienced in collecting the last-named substance these results may be held to show that the hydrolysis product contained the three sugars in equimolecular proportions.

Proof of Identity of Tetramethyl Methylmannopyranoside.—Fraction 1 (4·8 g.) was heated with 2*N*-sulphuric acid (200 c.c.) at 95° for 4 hours. After neutralisation with barium carbonate the solution was evaporated under diminished pressure. The residue was extracted with boiling chloroform and after removal of the solvent tetramethyl mannose was obtained as a syrup (yield, 95% of the theoretical), $[\alpha]_D^{20°}$ + 4° in water (*c*, 4·2), $n_D^{19°}$ 1·4610.

When boiled with aniline, this syrup gave tetramethyl mannose anilide, which was recrystallised from ether (yield, 82% of the theoretical), m. p. alone or when mixed with an authentic specimen, 142—143° (Found : C, 61·3; H, 8·2; N, 4·8; OMe, 38·8. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·0; N, 4·5; OMe, 39·9%).

Another sample of the syrupy tetramethyl mannose was oxidised by bromine water at 40° for 80 hours. After aeration to remove bromine, neutralisation with silver carbonate to remove hydrobromic acid, and treatment with hydrogen sulphide to precipitate silver, the solution was evaporated, and the product extracted with chloroform. After removal of the chloroform a syrup was obtained (yield, 85% of the theoretical), which was distilled and the distillate was allowed to react with phenylhydrazine at 15° for 12 hours. This gave the phenylhydrazide of 2 : 3 : 4 : 6-tetramethyl mannonolactone, m. p. (after recrystallisation from benzene) 184—185°. Yield, 70% of the theoretical, calculated on the weight of tetramethyl mannose oxidised (Found : C, 56·0; H, 7·6; N, 8·4; OMe, 35·0. Calc. for $C_{16}H_{26}O_6N_2$: C, 56·1; H, 7·6; N, 8·2; OMe, 36·3%).

The phenylhydrazide was hydrolysed by heating it with *N*/3-sulphuric acid at 80° for 3 hours. After neutralisation (barium carbonate) the solution was extracted repeatedly with ether, the organic acid liberated by addition of the exact quantity of sulphuric acid, the solution evaporated to dryness (diminished pressure), and the product dissolved in chloroform. After removal of the chloroform a syrup remained which distilled at 100°/0·05 mm. The distillate (2 : 3 : 4 : 6-tetramethyl mannonolactone) crystallised immediately, m. p. 25°, $[\alpha]_D^{20°}$ + 150° in water (*c*, 2·0) (initial value; + 67°, final equilibrium value, attained after 100 hours at 15°) (Found : C, 51·2; H, 7·8; OMe, 50·5. Calc. for $C_{10}H_{18}O_6$: C, 51·3; H, 7·7; OMe, 53·0%).

Proof of Identity of 2 : 3 : 4-*Trimethyl Methylmannoside.*—(a) 2 : 3 : 4-*Trimethyl mannose.* A portion of fraction 3 (1·13 g.) was heated with 2*N*-sulphuric acid at 95°, the reaction being followed polarimetrically : $[\alpha]_{5461}^{19°}$ + 54° (initial value); 45° (15 mins.); 38° (30 mins.); 33° (45 mins.); 29° (60 mins.); 24° (90 mins.); 19° (120 mins.); 16° (150 mins.); 14° (180 mins.); 12° (240 mins., constant value). This rate of hydrolysis is characteristic of a mannopyranoside. The solution was neutralised (barium carbonate) and evaporated to dryness (diminished pressure). The product was extracted with chloroform and on removal of the solvent 2 : 3 : 4-trimethyl mannose was obtained as a syrup (0·93 g.) which slowly crystallised; m. p. 102—103° (after recrystallisation from ether–light petroleum), $[\alpha]_D^{20°}$ + 7°, equilibrium value in water (*c*, 5·0) (Found : C, 49·0; H, 7·9; OMe, 41·8. Calc. for $C_9H_{18}O_6$: C, 48·7; H, 8·1; OMe, 41·9%).

(b) 2 : 3 : 4-*Trimethyl mannonolactone.* 2 : 3 : 4-Trimethyl mannose obtained by hydrolysis of fraction 3 was oxidised by bromine water in the usual way. The product was distilled, giving 2 : 3 : 4-trimethyl mannonolactone as a viscid non-reducing liquid, b. p. 135°/0·02 mm., $n_D^{12°}$ 1·4776, $[\alpha]_D^{20°}$ + 137° in water (*c*, 0·9) (yield, 50% of the theoretical). When kept, this syrup crystallised slowly but almost completely. The lactone was then recrystallised from ether, m. p. 91—92°, $[\alpha]_D^{20°}$ + 138° in water (*c*, 1·0) (initial value); 116° (10 hours); 99° (20 hours); 72° (40 hours); 55° (60 hours); 47° (80 hours); 45° (90 hours); 44° (110 hours, constant value). The rate of hydrolysis is characteristic of a δ-mannonolactone. The rotation of the corresponding acid, 2 : 3 : 4-trimethyl mannonic acid, $[\alpha]_D$ + 18° (*c*, 0·2), was determined by preparing a solution of the lactone in sodium hydroxide, acidifying it, and taking the rotation immediately. The lactone (0·10 g.) required 4·50 c.c. of *N*/10-sodium hydroxide for neutralisation (calc., 4·54 c.c.). The physical constants given here should replace those recorded by Haworth, Raistrick, and Stacey in the course of work on the syrupy lactone encountered during investigations on mannocarolose (Found : C, 49·0; H, 7·3; OMe, 42·0. $C_9H_{16}O_6$ requires C, 49·1; H, 7·3; OMe, 42·3%).

(c) 2 : 3 : 4-*Trimethyl mannosaccharic acid.* Further confirmation of the identity of the 2 : 3 : 4-trimethyl mannose was obtained by oxidising it with nitric acid (*d* 1·42) at 100° for 2 hours. The nitric acid was removed, and the product isolated as the methyl ester (for details of the method, consult previous papers from this laboratory), b. p. 110°/0·05 mm., $n_D^{14°}$ 1·4540 (yield, nearly quantitative). On treatment with methyl-alcoholic ammonia this ester gave 2 : 3 : 4-*trimethyl mannosaccharodiamide*, m. p. 191° (yield, almost quantitative) (Found : C, 43·2; H, 6·9; N, 11·3; OMe, 37·2. $C_9H_{18}O_6N_2$ requires C, 43·2; H, 7·2; N, 11·2; OMe, 37·2%).

Proof of Identity of 3 : 4-*Dimethyl Methylmannoside.*—A portion of fraction 6 was hydrolysed by heating it with 2*N*-sulphuric acid at 95°, the reaction being followed polarimetrically : $[\alpha]_D^{18°}$ + 33° (initial value); 25° (15 mins.); 19° (30 mins.); 14° (45 mins.); 10° (60 mins.); 5° (90 mins.); 2·5° (120 mins.); 1° (180 mins.); ± 0° (240 mins., constant value). The rate of hydrolysis indicates the presence of a mannopyranoside structure. The acid was neutralised

with barium carbonate, the solution evaporated under diminished pressure, and the sugar dissolved in chloroform. Evaporation of the solvent left a syrup, which crystallised completely when left in contact with wet ether (yield, 80%). Recrystallisation from ether–ethyl acetate gave the *monohydrate* of 3 : 4-*dimethyl mannose*, m. p. 107—109°, $[\alpha]_D$ + 3°, equilibrium value in water (*c*, 1·8) (Found : C, 42·6; H, 7·45; OMe, 26·8. $C_8H_{16}O_6,H_2O$ requires C, 42·5; H, 7·2; OMe, 27·2%).

3 : 4-Dimethyl mannose monohydrate (1·2 g.) was oxidised by bromine water at 40° for 16 hours. The solution was then neutralised with silver carbonate and filtered (charcoal). The filtrate was rendered slightly acid with hydrochloric acid and, after filtration, was concentrated to a syrup under diminished pressure. The lactone was extracted by boiling chloroform and on removal of the solvent crystallised completely (yield, 80% of the theoretical). M. p. 157—158° (after recrystallisation from alcohol), $[\alpha]_D^{20°}$ + 174° in water, *c*, 1·0 (initial value); 160° (5 hours); 150° (10 hours); 145° (15 hours); 142° (20 hours); 135° (30 hours); 132° (40 hours); 129° (50 hours, constant value). The rate of hydrolysis indicates a δ-lactone. The rotation of 3 : 4-dimethyl mannonic acid in water was $[\alpha]_D^{20°}$ + 32° (*c*, 0·2). The *lactone* (0·1 g.) required 4·78 c.c. of *N*/10-sodium hydroxide for neutralisation (calc., 4·80 c.c.) (Found : C, 46·6; H, 7·2; OMe, 30·2. $C_8H_{14}O_6$ requires C, 46·6; H, 6·8; OMe, 30·1%).

When the lactone was allowed to react with methyl-alcoholic ammonia at 0° for 3 days, it was transformed almost quantitatively into 3 : 4-*dimethyl mannonamide*, m. p. 140° (after recrystallisation from a mixture of acetone, methyl alcohol and light petroleum). $[\alpha]_D^{20°}$ + 22° in water (*c*, 2·7). (The amide, therefore, does not follow the amide rotation rule; compare Harris, Hirst, and Wood, J., 1935, 1658.) When treated with sodium hypochlorite under standard and carefully controlled conditions (Weerman, *Rec. trav. chim.*, 1917, **37**, 16), the amide gave a copious yield of sodium cyanate, recognised in the usual way as hydrazodicarbonamide, m. p. 254° (yield, 40% of the theoretical). The amide therefore possessed a free hydroxyl group at C_2 [Found (for amide) : C, 43·3; H, 8·0; N, 6·1; OMe, 28·1. $C_8H_{17}O_6N$ requires C, 43·1; H, 7·6; N, 6·3; OMe, 27·7%].

Further evidence of the presence of a free hydroxyl gro C_2 was obtained from the preparation of an osazone without loss of methoxyl. Dimeth nose (0·2 g.), phenylhydrazine (0·4 c.c.), and glacial acetic acid (0·4 c.c.) were heated in water (10 c.c.) at 80° for 4 hours. A red oil separated which crystallised when kept at room temperature. Recrystallisation could not be carried out, the *osazone* separating always as an oil which subsequently crystallised. On this account the osazone could not be obtained analytically pure, but the figures given show clearly that the substance was an osazone containing two methoxyl groups (Found : C, 61·6; H, 6·6; OMe, 15·6. $C_{20}H_{36}O_4N_4$ requires C, 62·2; H, 6·7; OMe, 16·1%).

The presence of a methoxyl group at C_4 was indicated in the following way. Methylated derivatives of mannose in which the hydroxyl at C_4 is unsubstituted react readily with methyl-alcoholic hydrogen chloride in the cold, giving methyl mannofuranosides, the rotation first of all diminishing to a minimum negative value owing to the more rapid formation of the β-derivative at the beginning of the reaction. Thereafter the rotation slowly increases in the positive direction. When this reaction was tried with 3 : 4-dimethyl mannose monohydrate (0·4 g.) in 0·6% methyl-alcoholic hydrogen chloride (10 c.c.), the initial rotation ($[\alpha]_D^{20°}$ + 27°) remained unaltered during 60 hours and no formation of methylmannoside took place. The temperature was raised to 70° and the usual slow formation of a methylmannopyranoside was then observed : $[\alpha]_D^{20°}$ + 27° (initial value); 44° (4 hours); 57° (6 hours); 61° (8 hours); 64° (10 hours); 65° (12 hours); 67° (16 hours, constant value; solution non-reducing).

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