McLean, J. R., Cohn, G. L., Brandt, I. K., and Simpson, M. V. (1958), *J. Biol. Chem. 233*, 657.
Margrath, A., and Novello, F. (1964), *Exptl. Cell Res. 35*, 38.
Maxwell, E. S. (1962), *Proc. Natl. Acad. Sci. U.S. 48*, 1639.
Muscatello, V., Andersson-Cedergren, E., Azzone, G. F., and Von der Decken, A. (1961), *J. Biophys. Biochem. Cytol. 10* (Supplement), 201.
Perry, S. V., and Zydowo, M. (1959), *Biochem. J. 72*, 682.
Porter, K. R., and Palade, G. E. (1957), *J. Biophys. Biochem. Cytol. 3*, 269.
Roth, J. S., and Milstein, S. W. (1952), *J. Biol. Chem. 196*, 489.
Schmidt, G. (1964), *Ann. Rev. Biochem. 33*, 667.
Shemin, D., and Rittenberg, D. (1944), *J. Biol. Chem. 153*, 401.
Siekevitz, P. (1952), *J. Biol. Chem. 195*, 549.
Walls, E. W. (1960), *Struct. Funct. Muscle 1*, 21.
Weinstein, I. B., and Schechter, A. N. (1962), *Proc. Natl. Acad. Sci. U.S. 48*, 1686.

Preparation of Mannobiose, Mannotriose, and a New Mannotetraose from *Saccharomyces cerevisiae* Mannan

Yuan-Chuan Lee and Clinton E. Ballou

ABSTRACT: The deacetylated products of an acetolysate of yeast mannan have been separated on a Sephadex column to yield pure di-, tri-, and tetrasaccharides in high yield. The structure of each was established by methylation and periodate oxidation, as well as by comparison with authentic compounds. The substances isolated were the disaccharide *O*-α-D-mannopyranosyl-(1→2)-D-mannopyranose, the trisaccharide *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannopyranose, and a tetrasaccharide *O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannopyranose. The tetrasaccharide cannot be fitted as a unit into the most recent structure proposed for yeast mannan, and this suggests that alternative structures must be considered for this polysaccharide.

Yeast mannan, one of the main soluble polysaccharides of yeast cell wall, can be obtained easily by autoclaving whole yeast cells with dilute alkali or neutral buffer. Because bakers' yeast, *Saccharomyces cerevisiae*, is so readily available, most structural studies have been carried out on mannan extracted from this microorganism.

Several investigators have studied the mannan structure by methylation; and, in general, the results are consistent despite the different methods of preparation (Table I). It is clear from these studies that yeast mannan is highly branched and contains 1,2′-, 1,3′-, and 1,6′- linkages. On the basis of methylation data, Haworth *et al.* (1941) proposed several possible structures, the fundamental feature being an α-1,2′-linked backbone with branch points involving α-1,6′- linkages.

Recently, however, Peat *et al.* (1961b) have isolated a series of α-1,6′-linked oligomannosides by acid hydrolysis of yeast mannan, and, in conjunction with methylation work (Peat *et al.*, 1961a), they proposed a structure in which an α-1,6′-linked chain forms the backbone of the polysaccharide, the 1,2′- linkages being at branch points.

TABLE I: Methylation Analysis of Yeast Mannan.

O-Methyl-mannose	Molar Ratios Reference		
	Haworth *et al.* (1941)	Cifonelli and Smith (1955)	Peat *et al.* (1961a)
2,3,4,6-Tetra-	2	2	15
3,4,6-Tri-	1	1	7
2,4,6-Tri-	1	1	7
2,3,4-Tri-	Trace	Trace	1
3,4-Di-	2	2	14

In what began as a routine study, we undertook the acetolysis of yeast mannan to prepare authentic mannose oligosaccharides to be used as proton magnetic resonance standards in the determination of the chemi-

* From the Department of Biochemistry, University of California, Berkeley. *Received October 14, 1964.*

TABLE II: Gas Chromatography of Methyl and Trimethylsilyl Derivatives of Mannose.

Derivatives		Retention Times (min)		
		Column and Temperature		
		1.3% QF-1	2.3% Neopentylglycol Succinate	
		130°	128°	140°
CH_3O-	$(CH_3)_3SiO$-			
1,2,3,4,6-		4.1	10.8	6.6
1,3,4,6-		5.8	23	13.5
1,2,3,4-		6.0	23	13.5
2,3,4-	1,6-	5.8	7.8	4.6
3,4,6-	1,2-	4.7	4.9	3.2
1,2,3,4-	6-		7.8	
1,3,4,6-	2-		5.6	

cal shifts of anomeric protons. In agreement with previous work, we were able to isolate the α-1,2′-linked disaccharide, *O*-α-D-mannopyranosyl-(1→2)-D-mannopyranose (I), and the trisaccharide, *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannopyranose (II). However, we obtained in addition, and in good yield, a tetrasaccharide, *O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannopyranose (III). The presence of this tetrasaccharide in the mannan acetolysate invalidates the structure proposed by Peat *et al.* (1961a), since their structure does not contain such a building unit. Therefore we are reporting at this time on the isolation, proof of structure, and significance of the mannotetraose in the elucidation of the yeast mannan structure.

Experimental Procedure

General Procedures. Carbohydrate was analyzed by the phenol–sulfuric acid method (Dubois *et al.*, 1956). The following solvent mixtures (in volume ratios) were used for descending paper chromatography on Whatman No. 1 paper: (A) 1-butanol-ethanol-water, 3:1:1; (B) the same, 2:1:1; (C) ethyl acetate–pyridine–water, 10:4:3. Periodate-benzidine dip reagents (Gordon *et al.*, 1956) were used for location of oligosaccharides on a paper chromatogram.

Procedures for methylation and gas chromatography of methylated mannoses have been described (Lee and Ballou, 1964). Optical rotations of the oligosaccharides were determined in 1% aqueous solution with a Rudolph photoelectric polarimeter.

Gas Chromatography of Trimethylsilyl Derivatives of Methyl O-Methyl-D-mannosides. Under the conditions for gas chromatography described in the previous work (Lee and Ballou, 1964), methyl 2,3,4-tri-*O*-methyl-D-mannoside could not be separated from methyl 3,4,6-tri-*O*-methyl-D-mannoside. An improved procedure has been developed for qualitative analysis of these sugars. The methanolysate, after removal of methanol and hydrochloric acid, was trimethylsilylated according to Sweeley *et al.* (1963). The reaction mixture was centrifuged and a small portion of the supernatant was injected onto a column of 2% neopentylglycol succinate operated at about 140°. The retention times are shown in Table II.

To reduce the solvent peak and noise caused by the reagents, the supernatant was evaporated to dryness at room temperature, and the residue was dissolved in a few drops of carbon disulfide, which solution was injected for chromatography (Y. C. Lee and C. E. Ballou, unpublished data). The effect of this treatment is demonstrated by comparing parts B and C of Figure 2.

Isolation of Mannan. Bakers' yeast, 2.5 kg, was crumbled into 1 liter of 6% sodium hydroxide and the mixture was autoclaved at 110° for 2 hours. The mannan was precipitated and purified according to the method described by Cifonelli and Smith (1955). The final product, 12.5 g, gave only mannose on acid hydrolysis.

Acetolysis of Mannan. Mannan, 12 g, was dissolved in a mixture of 70 ml of glacial acetic acid and 70 ml of acetic anhydride, and 7 ml of 98% sulfuric acid was added to the solution. During the process the temperature of the reaction mixture was kept under 25° by cooling the reaction vessel in ice. After 5 days at room temperature, the dark-brown reaction mixture was centrifuged. The supernatant was poured into 2 liters of an ice-water mixture, and the *p*H of the diluted solution was adjusted to 5.5 by addition of sodium hydroxide. The chilled mixture was filtered and the precipitate was dissolved in chloroform. The chloroform solution was decolorized with charcoal, washed with 1 N sodium bicarbonate until neutral, dried over anhydrous sodium sulfate, and evaporated to dryness to yield 22.2 g of a mixture of sugar acetates.

Deacetylation. The acetates, 22 g, were dissolved in 150 ml of dry methanol and deacetylated by addition of 4 meq of barium methoxide. The precipitate formed upon deacetylation was washed with methanol and

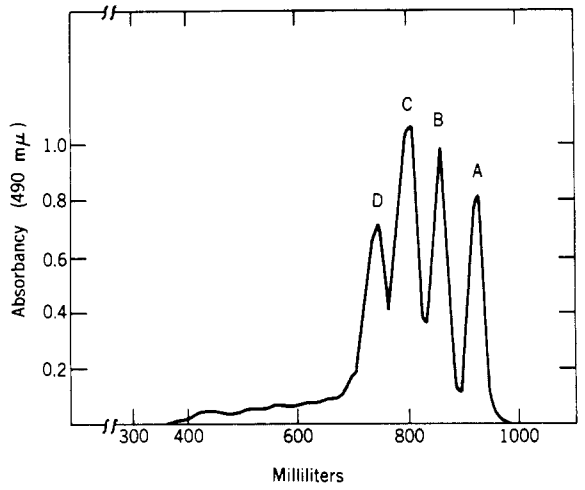


FIGURE 1: Separation of oligomannosides on Sephadex G-25 by elution with water. Peak A is mannose, B is mannobiose, C is mannotriose, and D is mannotetraose.

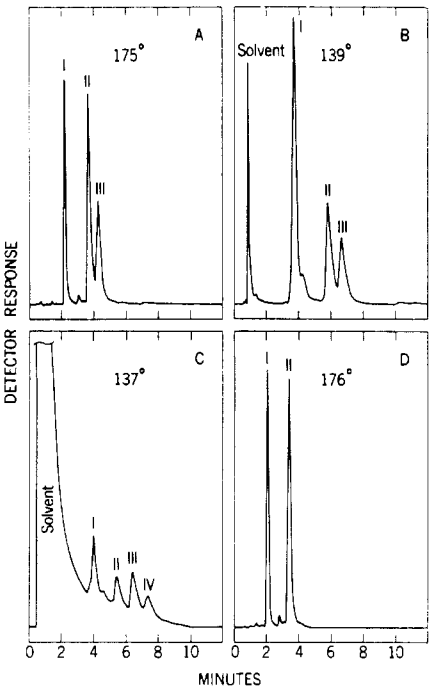


FIGURE 2: Gas-chromatographic tracings of the separations of partially methylated methyl mannosides. (A) Methanolysate of permethylated mannotetraose: methyl 2,3,4,6-tetra-*O*-methylmannoside, I; methyl 3,4,6-tri-*O*-methylmannoside, II; and methyl 2,4,6-tri-*O*-methylmannoside, III. (B) Trimethylsilyl derivatives of (A): methyl 3,4,6-tri-*O*-methyl-2-*O*-trimethylsilylmannoside, I; methyl 2,4,6-tri-*O*-methyl-3-*O*-trimethylsilylmannoside, II; methyl 2,3,4,6-tetra-*O*-methylmannoside, III. (C) Trimethylsilyl derivatives of methanolysate of permethylated reduced mannotetraose: methyl 3,4,6-tri-*O*-methyl-2-*O*-trimethylsilylmannoside, I; 1,3,4,5,6-penta-*O*-methyl-2-*O*-trimethylsilylmannitol, II; methyl 2,4,6 tri-*O*-methyl-3-*O*-trimethylsilylmannoside, III; and methyl 2,3,4,6-tetra-*O*-methylmannoside, IV. (D) Methanolysate of permethylated mannotriose obtained by emulsin digestion of the mannotetraose: methyl 2,3,4,6-tetra-*O*-methylmannoside, I; and methyl 3,4,6-tri-*O*-methylmannoside, II.

dissolved in water. The solution was treated with Dowex 50 (H^+ form), and freeze-dried to yield 2.2 g of product. The methanol supernatant, together with the washing, was poured into a suspension of 4 g of Dowex 50 in 200 ml of water to remove barium ion, and the solution was freeze-dried to obtain a second fraction of 6.5 g. When the methanol-soluble and the methanol-insoluble oligosaccharide mixtures were examined by paper chromatography (solvent A), mannose, mannobiose ($R_{mannose}$ = 0.74), mannotriose ($R_{mannose}$ = 0.55), and mannotetraose ($R_{mannose}$ = 0.39) were observed as the main spots revealed by the periodate-benzidine dip reagents.

Gel Filtration of the Oligosaccharides. Gel filtration of the oligosaccharides was carried out through a 150 × 3-cm column of Sephadex G-25 (fine mesh), with an elution rate of 40 ml of water per hour. A typical pattern of fractionation, in which 2 g of the methanol-soluble fraction of oligosaccharides was applied to the column, is shown in Figure 1.

The methanol-insoluble fraction gave essentially the same pattern. Peak A was found to be mannose and peaks B, C, and D corresponded with mannobiose, mannotriose, and mannotetraose. By taking the non-overlapping portion of each peak, and rechromatographing the remainder, mannobiose, mannotriose, and mannotetraose were obtained in good yield (Table III). Oligosaccharides larger than the tetraose were found only in small amount in both the methanol-soluble and methanol-insoluble fractions.

Characterization of Mannobiose and Mannotriose. The mannobiose and the mannotriose could not be distinguished from the authentic samples of compounds I and II (Gorin and Perlin, 1956, 1957) by paper chromatography in solvents A and C. The specific rotations of these oligosaccharides (Table III) are also in agreement with the values reported previously (Peat *et al.*, 1961a; Gorin and Perlin, 1956, 1957). On methylation of the mannobiose, only 2,3,4,6-tetra-*O*-methylmannose and 3,4,6-tri-*O*-methylmannose were found, and in equivalent amounts. Similarly, the mannotriose produced the same tetramethylmannose and trimethylmannose in a molar ratio of 1:2.

Characterization of Mannotetraose. When the molecular rotations for mannose, the mannobiose, and the mannotriose were plotted against the degree of polymerization (French, 1954), the value for the tetraose did not fall on the line formed by the values for the lower oligosaccharides. This indicated that the tetraose was not in the same homologous series, that is, it did not contain only α-1,2′- linkages. On methylation analysis (Figure 2A), 1 mole of methyl 2,3,4,6-tetra-*O*-methyl-

TABLE III: Mannose Oligosaccharides from Yeast Mannan.

Oligosaccharide	$R_{mannose}$[a]	Yield[b] (%)	[α]D (water) Found (deg)	[α]D (water) Reported (deg)
Mannose	1.00			+14.2
Mannobiose	0.74	19	+46.8	+48.0[c]
				+40.0[d]
Mannotriose	0.55	22	+59.3	+55.0[d]
Mannotetraose	0.39	17	+76.2	

[a] Solvent mixture A. [b] On weight basis. [c] Peat *et al.* (1961a). [d] Gorin and Perlin (1957).

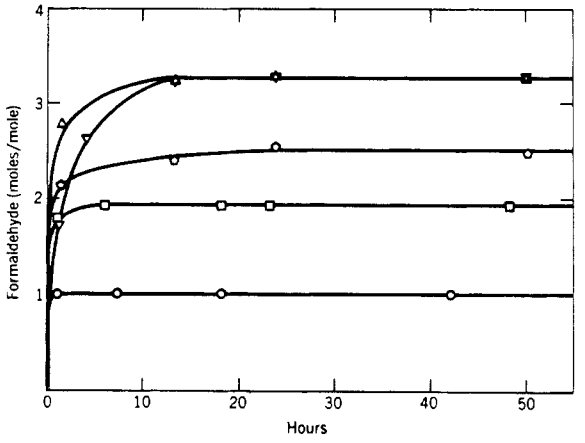


FIGURE 3: Alkaline periodate oxidation of oligosaccharides. ○, mannose; □, mannobiose; ⬡, mannotriose; △, mannotetraose; ▽, maltotetraose.

mannoside, 2 moles of methyl 3,4,6-tri-*O*-methylmannoside, and 1 mole of methyl 2,4,6-tri-*O*-methylmannoside were found. Identification of the 3,4,6-tri-*O*-methyl derivative was made by gas chromatography after trimethylsilylation of the mannoside mixture (Figure 2B). The 2,4,6-tri-*O*-methyl derivative was identified initially by a process of elimination, since the retention time of this component did not correspond to any of the other possible methyl tri-*O*-methylmannosides.

A portion of the permethylated mannotetraose was hydrolyzed with 1 N sulfuric acid and the hydrolysate was oxidized with an excess of sodium periodate. The oxidation product was chromatographed on Whatman 3MM paper with solvent A to remove oxidized fragments, and the fraction corresponding to unoxidized trimethylmannose was isolated. After conversion to its methyl glycoside, the substance was found by gas chromatography to correspond to peak III (Figure 2A). Since 2,4,6-trimethylmannose is the only trimethylmannopyranose which could survive periodate oxidation, the identity assigned to peak III is confirmed.

The tetraose, 100 mg, was reduced with 150 mg of sodium borohydride in 10 ml of water in the cold for 2 days, and the excess of sodium borohydride was removed. The reduced tetraose was methylated and the product was analyzed by gas chromatography. The chromatogram of the methanolyzate appeared to be the same as that obtained from the tetraose before reduction. However, after trimethylsilylation, a new peak appeared (retention time 0.74 relative to methyl 2,3,4,6-tetra-*O*-methylmannoside), while the peak area of methyl 3,4,6-tri-*O*-methyl-2-*O*-trimethylsilylmannoside decreased (Figure 2C).

A portion of the tetraose (100 mg) was digested with 75 mg of sweet almond emulsin (Worthington Biochemical Corp. "Beta Glucosidase" which contains also an α-D-mannosidase) at *p*H 4.1 and 37°, for 14 hours, and the products formed by enzyme action were isolated by chromatography on Whatman 3MM paper in solvent B for 6 days. Half of the tetrasaccharide was recovered, the yield of trisaccharide was 10 mg, and 35 mg of a mixture of mono- and disaccharides was obtained. The mannotriose had the same R_F value as compound II and, on methylation analysis, no methyl 2,4,6-tri-*O*-methylmannoside could be detected (Figure 2D).

Alkaline Periodate Oxidation. Periodate oxidation was carried out in an alkaline medium according to Hough and Perry (1956). Sugar equivalent to 4 μmoles of hexose unit was oxidized at room temperature with 60 μmoles of sodium periodate in 2 ml of 0.1 M potassium bicarbonate containing 0.5 mg of *p*-hydroxybenzaldehyde. At intervals, aliquots of the reaction mixture were withdrawn for determination of formaldehyde by the chromotropic acid method (Hanahan and Olley, 1958). The results are given in Figure 3.

Proton Magnetic Resonance. Proton magnetic resonance spectra of the saccharides were measured with a Varian A-60 spectrometer in deuterium oxide solution. Exchangeable hydrogens were replaced by the evaporation of the sugar solutions in deuterium oxide. Acetic acid (2.10 ppm) was used as an internal standard, and the chemical shifts are expressed in ppm, downfield from tetramethylsilane.

Portions of mannobiose, mannotriose, and mannotetraose were reduced with sodium borohydride as

TABLE IV: Chemical Shifts of Anomeric Hydrogens of Some Mannose Oligosaccharides.

Compounds	Groups[a]			
	Man-A	Man-B	Man-C	Man-D
Mannose	5.2			
methyl α-glycoside	4.9			
Mannobiose (I)	5.4	5.1		
methyl α-glycoside	5.1[b]	5.1[b]		
"mannobiitol"		5.1		
Mannotriose (II)	5.4	5.3	5.1	
"mannotriitol"		5.3	5.1	
Mannotetraose (III)	5.4	5.3	5.2	5.1
"mannotetritol"		5.3	5.2	5.1

[a] See Figure 5. [b] A broad signal.

described. The methyl glycoside of mannobiose was prepared by treating the sugar with 3.5% methanolic hydrochloride at room temperature for 4 days, followed by purification of the product by paper chromatography.

The results of proton magnetic resonance measurement of the reducing mannose oligosaccharides are shown in Figure 4 and Table IV. The coupling constants of the signals due to anomeric hydrogens were less than 3 cps in all cases.

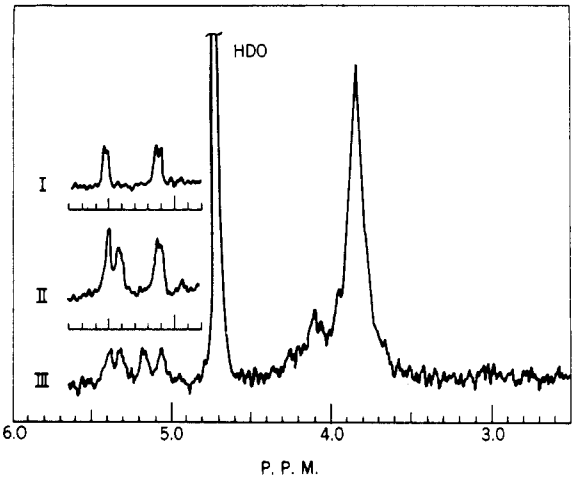


FIGURE 4: Proton magnetic resonance spectra of anomeric protons of mannobiose (I), mannotriose (II), and mannotetraose (III).

Discussion

Isolation of the Oligosaccharides. Classical procedures for isolation of oligosaccharides from partially degraded polysaccharides involve fractional precipitation, fractional crystallization, or chromatography on charcoal, cellulose, or other adsorbents. In general, these methods are tedious and recoveries are low. The value of Sephadex G-25 for fractionation of lower homologous oligosaccharides has been demonstrated (Flodin and Aspberg, 1961) and, in the present work, the gel filtration technique was used with great effectiveness. The advantages of Sephadex columns in such fractionations have been discussed (Lee and Montgomery, 1965).

Although the peaks obtained in gel filtration are not completely resolved, their sharpness makes it possible to isolate over 75% of each component without contamination. Thus the oligosaccharides isolated by taking only the sharp portions of the peaks appeared to be pure by paper chromatography in solvents A, B, and C. The overlapping portions of the peaks were combined and subjected to a second gel filtration, thus increasing the yield. The yields of mannobiose, mannotriose, and mannotetraose from the mannan were 19, 22, and 17%, respectively, totaling nearly 60% of the starting mannan. This contrasts with the yields of 0.5–1.0% of α-1,6′-linked mannobiose obtained by acid hydrolysis (Peat *et al.*, 1961a).

Structure of Mannose Oligosaccharides. Identity of the di- and trisaccharides with authentic α-1,2′-linked oligomannosides was established by cochromatography with the known compounds, by their optical rotations, and by methylation analysis.

Methylation of the new mannotetraose gave one part of methyl 2,4,6-tri-*O*-methylmannoside which demonstrated the existence of 1 mole of 1,3′- linkage. The two parts of methyl 3,4,6-tri-*O*-methylmannoside come from the two 1,2′- linkages. A trisaccharide was obtained from the emulsin digest of the tetraose. This compound was identical by paper chromatography with compound II. Methylation analysis of compound II and of this sample gave the same results, each producing 1 mole of methyl 2,3,4,6-tetra-*O*-methylmannoside and 2 moles of methyl 3,4,6-tri-*O*-methylmannoside. Absence of the 2,4,6-tri-*O*-methyl derivative in the trisaccharide produced by emulsin action indicates that the 1,3′- linkage was at the nonreducing end

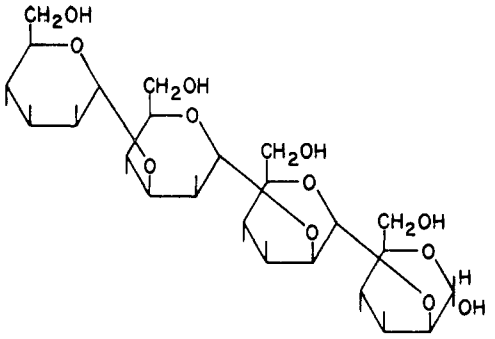


FIGURE 5: Structure of mannotetraose (III).

of the chain, since the enzyme hydrolyzes from that end of an oligosaccharide.

In confirmation of this conclusion, reduction of the mannotetraose eliminated one of the 1,2′- linkages and proved that the mannose unit at the reducing end was substituted at the C-2 position. From the combined evidence, it is clear that the tetraose has the structure in Figure 5 and the definitive name assigned to compound III.

Alkaline Periodate Oxidation. Alkaline periodate oxidation of oligosaccharides has been used for the detection of 1,6′- linkages (Hough and Perry, 1956; Hough *et al.*, 1957; Bines and Whelan, 1960). Under these conditions, 1,2′-, 1,3′- and 1,4′-linked oligosaccharides are completely oxidized through the formation of a malondialdehyde derivative, thus producing formaldehyde from each unsubstituted C-6 carbon. If the "overoxidation" proceeds according to the accepted scheme (Cantley *et al.*, 1963), 2, 3, and 4 moles of formaldehyde, respectively, should be produced by 1 mole each of di-, tri-, and tetrasaccharide containing such linkages. In reality, the yield of formaldehyde is always lower than the theoretical value, even in the presence of *p*-hydroxybenzaldehyde (Hough and Perry 1956; Hough *et al.*, 1957; Bines and Whelan, 1960).

Under the conditions shown in Figure 3, each mole of mannose produced 1.03 moles of formaldehyde, while each mole of mannobiose, mannotriose, and mannotetraose produced only 1.82, 2.55, and 3.28 moles of formaldehyde, respectively. Chromatographically pure maltotetraose also produced only 3.28 moles of formaldehyde per mole under the same condition, indicating that the low yield is not characteristic of the mannose series alone. The low yield of formaldehyde cannot be attributed to contamination by 1,6′-linked oligosaccharide, since the compounds were shown by gas chromatographic analysis of methylation products to be free of 1,6′- linkage.

However, if the "overoxidation" via the foregoing scheme proceeds incompletely (for example, only 90% for each of the steps) the calculated yield of formaldehyde from the di-, tri-, and tetrasaccharide would be 1.90, 2.71, and 3.44 moles of formaldehyde per mole, or $[1 + (0.90)^{n-1}]$ moles of formaldehyde per mole, where n is degree of polymerization. Thus, the longer the molecule, the lower the yield of formaldehyde. Our findings are in keeping with this assumption. Whether the speculation is correct or not, the foregoing observations point the need for caution in the interpretation of the results of alkaline periodate oxidation, particularly of the higher oligosaccharides.

Proton Magnetic Resonance of Anomeric Hydrogens. The positions of the anomeric hydrogen signals from the oligosaccharides (Figure 4) indicate that these sugars contain only α- linkages (Van der Veen, 1963). The small coupling constants (less than 3 cps) suggest that the mannosyl residues are in the C-1 chair conformation, using the same arguments described previously (Lee and Ballou, 1964). All of the signals of anomeric hydrogens of the oligosaccharides studied here were cleanly separated (Table IV), and assignments were made with reasonable certainty.

The anomeric hydrogen in α-D-mannopyranose gives a signal at 5.2 ppm (Table V). Conversion to the methyl α-D-glycoside results in an upfield shift of 0.3 ppm (δ = 4.9 ppm) owing to the shielding effect of the methyl group. The α-1,2′-linked mannobiose (I) has two anomeric signals, one at 5.1 and one at 5.4 ppm. In the methyl glycoside of compound I, the signal at 5.4 ppm is shifted upfield to 5.1 ppm as would be expected if it were due to the anomeric hydrogen of Man-A. Confirmation of this assignment comes by reduction of the mannobiose to mannobiitol (Man-H_2), in which case the signal at 5.4 ppm is eliminated.

The effect on the anomeric hydrogen signal of one mannose caused by substitution at the 2- position by another mannose can be evaluated by comparing the methyl glycosides. Thus methyl α-D-mannopyranoside gives a signal at 4.9 ppm, while the hydrogen of the mannobiose which has been converted to the methyl glycoside appears at 5.1 ppm. The net effect on Man-B is a downfield shift of 0.2 ppm caused by deshielding by the Man-B residue.

The trisaccharide (II) has signals at 5.1, 5.3, and 5.4 ppm. The last is lost on reduction and is assigned to the anomeric hydrogen of the reducing carbon atom of Man-A. The signal at 5.3 ppm is assigned to the middle mannose (Man-B) whose anomeric hydrogen has been deshielded (0.2 ppm) by substitution at the 2- position by Man-C, and the signal at 5.1 is from the mannose at the terminal nonreducing end (Man-C). These assignments are completely consistent with those for the mannobiose.

The tetrasaccharide (III) has signals at 5.1, 5.2, 5.3, and 5.4 ppm. The last is again lost on reduction, and can be assigned to the reducing Man-A residue. The signal at 5.3 ppm is due to the second mannose from the reducing end of the tetrasaccharide (Man-B). The signal at 5.2 ppm would appear to be due to the Man-C, which is slightly less deshielded than Man-B, since the former is substituted in the 3- position by the terminal mannose unit (Man-D). Finally, the signal at 5.1 ppm is assigned to the terminal α-1,3′-linked mannose (Man-D).

These last two assignments are not certain, since we would have expected a slight difference between

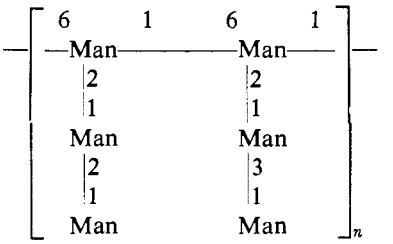
TABLE V: Assignments of Anomeric Proton Magnetic Resonance Signals.

Mannose Series	Mannobiose Series	Mannotriose Series	Manno-tetraose Series
			Man-D (5.1) 1 ↓ 3
		Man-C (5.1) 1 ↓ 2	Man-C (5.2) 1 ↓ 2
	Man-B (5.1) 1 ↓ 2	Man-B (5.3) 1 ↓ 2	Man-B (5.3) 1 ↓ 2
Man-A (5.2)	Man-A (5.4)	Man-A (5.4)	Man-A (5.4)
			Man-D (5.1) 1 ↓ 3
		Man-C (5.1) 1 ↓ 2	Man-C (5.2) 1 ↓ 2
	Man-B (5.1) 1 ↓ 2	Man-B (5.3) 1 ↓ 2	Man-B (5.3) 1 ↓ 2
	Man-H_2	Man-H_2	Man-H_2
	Man-B (5.1) 1 ↓ 2		
Man-(OCH_3) (4.9)	Man-(OCH_3) (5.1)		

terminal α-1,2′- and α-1,3′-linked mannoses, the former being attached to an axial hydroxyl group and the latter to an equatorial group. Until an α-1,3′-linked mannobiose is obtained as a reference, this point must be left open.

The sensitivity of the chemical shift to 1,2′- and 1,3′-linkages is emphasized by comparison with a homologous series of α-1,6′-linked glucosaccharides (Table VI). These all give a single anomeric hydrogen signal at about 5.1 ppm.

Structure of the Mannan. The structure for yeast mannan proposed by Peat *et al.* (1961a) is shown by IV. Despite the high yield of oligosaccharides we obtained, none containing the 1,6′- linkage could be detected in the acetolyzed product of yeast mannan, since these are apparently selectively cleaved in the acetolysis reaction. Under similar conditions, Gorin and Perlin (1956) also failed to isolate any 1,6′-linked oligosaccharide from the *S. rouxii* mannan, although such a linkage had been demonstrated to be present.



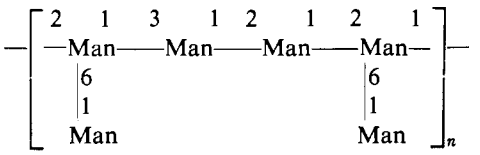
IV

The most significant finding is the isolation of a tetrasaccharide containing α-1,2′- and α-1,3′- linkages. The presence of this tetrasaccharide in yeast mannan demands revision of the structure proposed by Peat *et al.* (1961a). One of the structures depicted by Haworth *et al.* (1941) (V) could yield the mannotetraose

TABLE VI: Anomeric PMR[a] Signals for Homologous α-1,6′-linked Oligoglucosides.

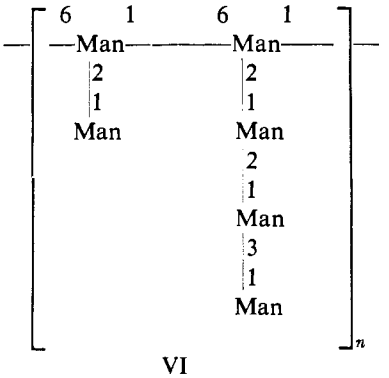
Compound	Chemical Shifts[b] Non-reducing	Reducing α	Reducing β
Isomaltose	5.07	5.35	4.75
Isomaltotriose	5.08	5.40	
Isomaltotetraose	5.10	5.45	
Isomaltopentaose	5.10	5.45	

[a] PMR = proton magnetic resonance. [b] Measured in deuterium oxide solution relative to tetramethylsilane.



V

we have isolated, but not the series of α-1,6′-linked oligosaccharides reported by Peat *et al.* (1961a). A minor revision of structure IV, represented by structure VI, would also fit the results.



VI

With the available data, several alternative structures still are possible for yeast mannan. Studies are underway to gain more insight on this problem.

Acknowledgment

We wish to thank Dr. A. J. Perlin for samples of α-1,2′-mannobiose and mannotriose; Dr. J. K. N. Jones for α-1,6′-mannobiose; Dr. J. D. Commerford for maltotetraose, -pentaose, and -hexaose; and Dr. W. J. Whelan for an isomaltose homologous series.

References

Bines, B. J., and Whelan, W. J. (1960), *Biochem. J. 76*, 253.

Cantley, M., Hough, L., and Pittet, A. O. (1963), *J. Chem. Soc.*, 2527.

Cifonelli, J. A., and Smith, F. (1955), *J. Am. Chem. Soc. 77*, 5682.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956), *Anal. Chem. 28*, 350.

Flodin, P., and Aspberg, K. (1961), *Biol. Struct. Function IUB/IUBS Intern. Symp. 1st, Stockholm, 1960, 1*, 345.

French, D. (1954), *Advan. Carbohydrate Chem. 9*, 149.

Gordon, H. T., Thornburg, W., and Werum, L. N. (1956), *Anal. Chem. 28*, 848.

Gorin, P. A. J., and Perlin, A. S. (1956), *Can. J. Chem. 34*, 1796.

Gorin, P. A. J., and Perlin, A. S. (1957), *Can. J. Chem. 35*, 262.

Hanahan, D. J., and Olley, J. N. (1958), *J. Biol. Chem. 231*, 813.

Haworth, W. N., Heath, R. L., and Peat, S. J. (1941), *J. Chem. Soc.*, 833.

Hough, L., and Perry, M. B. (1956), *Chem. Ind. (London)*, 768.

Hough, L., Woods, B. M., and Perry, M. B. (1957), *Chem. Ind. (London)*, 1100.

Lee, Y. C., and Ballou, C. E. (1964), *J. Biol. Chem. 239*, 1316.

Lee, Y. C., and Montgomery, R. (1965), *Methods Carbohydrate Chem.* (in press).

Peat, S., Turvey, J. R., and Doyle, D. (1961a), *J. Chem. Soc.*, 3918.

Peat, S., Whelan, W. J., and Edwards, T. E. (1961b), *J. Chem. Soc.*, 29.

Sweeley, C. C., Bentley, R., Makita, M., and Wells, W. W. (1963), *J. Am. Chem. Soc. 85*, 2497.

Van der Veen, J. M. (1963), *J. Org. Chem. 28*, 564.