6. *Polysaccharides of Baker's Yeast. Part IV.*[1] *Mannan.*

By Stanley Peat, W. J. Whelan, and T. E. Edwards.

The structure of the mannan from baker's yeast has been examined by partial acid-hydrolysis and fractionation of the products. A homologous series of oligosaccharides containing 1,6-linkages has been isolated, and the structure of the disaccharide member confirmed by chemical synthesis. The repeating linkages in these oligasaccharides are believed to have the α-configuration.

Yeast mannan is one of the three main polysaccharides of baker's yeast, the other two being glycogen [2] and the cell-wall glucan.[1,3] The mannan constitutes about 14% of the dry weight of the yeast.[4] Haworth, Hirst, and Isherwood [5] examined the structure of the mannan by methylation analysis and found the components of methylated and hydrolysed mannan to be 2,3,4,6-tetra-, 2,3,4-tri-, and 3,4-di-*O*-methylmannose in approximately equimolar ratios. Haworth, Heath, and Peat [6] later re-examined the methylated sugars and found that the tri-*O*-methyl fraction contained only 10% of the 2,3,4-isomer, the remaining 90% consisting of equimolar proportions of 2,4,6- and 3,4,6-tri-*O*-methylmannose. It was therefore concluded that the mannan contained 1,6-, 1,2- and 1,3-linkages in the proportions 2 : 3 : 1. Periodate oxidation gave results in agreement with this conclusion.[7] We have re-investigated the structure of this polysaccharide by the method we had used for the other two polysaccharide constituents, namely, partial acid-hydrolysis, fractionation, and examination of the fragments (linkage analysis).[1,3]

Previous investigators [5,6] extracted the mannan by prolonged boiling of the yeast with 6% aqueous sodium hydroxide. The mannan is, however, freely soluble in water and it seemed necessary only to disrupt the yeast cells in order to extract the polysaccharide. This was achieved by heating the yeast in an autoclave at pH 7, and the possibility of alkaline degradation was thus avoided. The extract contained glycogen and mannan, which were separated by forming the insoluble copper complex of the latter. Despite repeated purification by this method the acid-hydrolysate of the mannan always contained a trace of glucose. The polysaccharide was not stained with iodine nor did α- or β-amylase liberate maltose. The glucose did not therefore arise from glycogen and since the partial acid-hydrolysate of the mannan appeared not to contain an oligosaccharide in which glucose and mannose were combined together, it was concluded that the glucose had no structural significance in relation to the mannan but perhaps represented an impurity of cell-wall glucan.[1,3]

The mannan (33·7 g.) was hydrolysed with dilute sulphuric acid until the apparent conversion into mannose was 67%. Fractionation of the hydrolysate on charcoal–Celite yielded mannose (20·4 g.), containing a trace of glucose, and various oligosaccharides which were further fractionated where necessary in order to obtain chromatographically pure material. Also present was a non-reducing lævorotatory substance which emerged from the charcoal column just before the disaccharide fraction. In view of our finding of lævoglucosan as a product of the acid-reversion of glucose [8] it seemed likely that this material was lævomannosan. It had the same R_F value as and a similar value of $[\alpha]_D$ to authentic material. Immediately following the mannose anhydride was a reducing disaccharide (380 mg.) having a high M_G value,[9] suggestive of a 1,3- or 1,6-linkage, which

[1] Part III, *J.*, 1958, 3868.
[2] Part I, *J.*, 1955, 355.
[3] Part II, *J.*, 1958, 3862.
[4] Trevelyan and Harrison, *Biochem. J.*, 1952, **50**, 298.
[5] Haworth, Hirst, and Isherwood, *J.*, 1937, 784.
[6] Haworth, Heath, and Peat, *J.*, 1941, 833.
[7] Lindstedt, *Arkiv Kemi, Min., Geol.*, 1954, A20, No. 13.
[8] Peat, Whelan, Edwards, and Owen, *J.*, 1958, 586.
[9] Foster, *Adv. Carbohydrate Chem.*, 1957, **12**, 81.

on periodate oxidation gave 4·8 mol. of formic acid (calc. for the 1,6-linked disaccharide, 5 mol.). The sugar had $[\alpha]_D$ +58° and formed a crystalline octa-acetate having the properties reported by Talley, Reynolds, and Evans [10] for the acetate of 6-*O*-β-D-mannopyranosyl-D-mannose, prepared by reaction between 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide and 1,2,3,4-tetra-*O*-acetyl-D-mannose. We repeated this synthesis and obtained the same product. Talley *et al.*[10] assigned the β-configuration to the linkage on the grounds that such condensation of an α-halogeno-sugar is usually accompanied by Walden inversion. However, the high $[\alpha]_D$ of the disaccharide in relation to that of mannose (+14°), and the similarly high $[\alpha]_D$ of the octa-acetate, suggested an α-linkage. The $[\alpha]_D$ of the mannan (+89°) also leads one to suspect a high content of α-linkages. Ness, Fletcher, and Hudson [11] found, moreover, that condensation of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide with methanol occurred with retention of configuration. The conclusion that the disaccharide linkage has the α-configuration has been confirmed by reports appearing since the completion of this work. Jones and Nicholson [12] obtained two 1,6-linked disaccharides as products of the acid-reversion of mannose: one of these had $[\alpha]_D$ +52°. The presence of the 1,6-link was established by methylation analysis and by periodate oxidation. This was confirmed by lead tetra-acetate oxidation,[13] and the configuration of the link established by examining the optical properties of the periodate-oxidised disaccharide.[14] The second 1,6-linked disaccharide, having $[\alpha]_D$ −12·4°, was assigned the β-configuration.[12] Gorin and Perlin [15] recently re-examined the disaccharide synthesised by the method of Talley *et al.*[10] and they too conclude that the glycosidic bond has the α-configuration.

The yeast-mannan hydrolysate contained a second disaccharide (27 mg.) which gave only glucose on hydrolysis and, on oxidation with periodate, yielded 4·75 mol. of formic acid. It had the same R_F and M_G values as gentiobiose or isomaltose. Its $[\alpha]_D$ was +22°, suggesting it was a mixture of the two glucose oligosaccharides with gentiobiose ($[\alpha]_D$ +9·6°) predominating over isomaltose ($[\alpha]_D$ +122°). It seemed that this material was to be associated with the glucan impurity and, indeed, gentiobiose is one of the products of the partial hydrolysis of yeast glucan.[3]

Only one trisaccharide (483 mg.) was isolated from the mannan hydrolysate. This formed a crystalline acetate. On the basis of the following evidence it was assigned the constitution *O*-α-D-mannopyranosyl-(1 → 6)-*O*-α-D-mannopyranosyl-(1 → 6)-D-mannose. (i) It had the same M_G value as the 1,6-linked disaccharide. (ii) Only this disaccharide and mannose were found in the products of partial acid hydrolysis of the trisaccharide. (iii) The difference in molecular rotation of the tri- and the di-saccharide acetate was the same as between the rotations of the disaccharide acetate and β-mannose penta-acetate. Finally, a tetrasaccharide (284 mg.) was obtained (from the mannan) which gave only the previously mentioned mannose tri- and di-saccharide and mannose on partial hydrolysis and seemed therefore to contain only 1,6-bonds.

The oligosaccharides larger than the tetrasaccharide (6 g.) were collected together, partly hydrolysed with acid, and refractionated. A di-, tri-, and tetra-saccharide were obtained, each having the same $[\alpha]_D$ and R_F value as the corresponding mannose oligosaccharide previously isolated. There were no glucose oligosaccharides. In addition a penta-saccharide was isolated. When partly hydrolysed with acid the tri-, tetra-, and penta-saccharide each gave mannose and one disaccharide, the latter migrating on paper chromatography with the α-1,6-linked disaccharide. That the four oligosaccharides contained the same repeating α-1,6-linkage was confirmed by the linear relations obtained

[10] Talley, Reynolds, and Evans, *J. Amer. Chem. Soc.*, 1943, **65**, 575.
[11] Ness, Fletcher, and Hudson, *J. Amer. Chem. Soc.*, 1950, **72**, 2200.
[12] Jones and Nicholson, *J.*, 1958, 27.
[13] Charlson and Perlin, *Canad. J. Chem.*, 1956, **34**, 1200.
[14] Charlson and Perlin, *Canad. J. Chem.*, 1956, **34**, 1804.
[15] Gorin and Perlin, *Canad. J. Chem.*, 1959, **37**, 1930.

when the R_M values and the $[M]_D$ values were plotted against the degree of polymerisation.[16] The oligosaccharides larger than the pentasaccharide were collected together and examined by periodate oxidation. The limiting values for consumption of periodate and liberation of formic acid were 1·85 and 1·05 moles, respectively, per mannose unit. These values are near enough to those expected for a 1,6-linked aldohexose polymer (2 and 1 mole respectively) to suggest that the higher oligosaccharides, like the di- to penta-saccharides, contained mainly, if not entirely, 1,6-bonds.

The behaviour of the original mannan on oxidation by periodate was markedly different. In this case the limiting periodate consumption was about 1·3 moles and the acid liberated 0·35 mole per mannose unit. Clearly the polysaccharide contains a linkage or linkages other than the 1,6-bond. It seems, however, that under the conditions of hydrolysis most of these other bonds were preferentially split, giving rise, at the particular degree of hydrolysis chosen, to a series of oligosaccharides mainly containing the α-1,6-link. This is to be expected if, as in the case of glucose, the 1,6-link is the strongest of the possible glycosidic bonds. We conclude that the mannan contains sequences of α-1,6-bonds and it is possible that these form a backbone to the molecule, with side chains containing other links radiating from the main chain. The methylation analyses [5,6] agree with the presence of 1,6-links but, in addition, indicate that the molecule is highly branched.

Consideration of the products of partial acid-hydrolysis of polysaccharides must include the possibility that an oligosaccharide isolated from the hydrolysate may be the product of acid-reversion rather than a structural fragment of the polysaccharide. To determine the extent to which mannose polymerises under our conditions an approximately equivalent weight of the monosaccharide (36 g.) was treated in the same way (cf. ref. 8). Fractionation of the product on charcoal gave lævomannosan, identified as the crystalline acetate, in about two-thirds the amount obtained from the mannan. Jones and Nicholson,[12] in their studies of mannose reversion, also obtained evidence for the presence of lævomannosan. Material having the same R_F value as the α-1,6-linked disaccharide was also obtained. It is possible that this was a mixture of the α- and β-1,6-linked disaccharides, the $[\alpha]_D$ suggesting a ratio 2·7 : 1. The weight of the whole fraction was 182 mg., compared with 380 mg. of the α-1,6-linked disaccharide from the mannan, and a further 220 mg. when the mannan-derived oligosaccharides were re-hydrolysed for a short time (see Experimental section). No tri- or higher oligo-saccharide was found in the reversion products, nor did the mannan hydrolysate appear to contain any appreciable amount of the β-1,6-linked disaccharide. It seems reasonable to conclude that the di- and higher oligo-saccharides in the mannan hydrolysate came mainly from the polysaccharide and were not acid-reversion products. Jones and Nicholson [12] also obtained a 9% yield of the α-1,6-linked disaccharide from yeast mannan but did not regard the evidence as necessarily proving the presence of these bonds in the mannan.

The nature of the other linkage(s) in the mannan was not investigated in detail. A hydrolysate (mannan II; 19·6 g.; see Experimental section) in which the degree of apparent conversion into mannose was much lower (29%) was fractionated and a disaccharide (550 mg.) having R_F and M_G values different from those of the α-1,6-linked sugar was isolated. This was strongly reactive towards the benzidine–trichoroacetic acid spray for reducing sugars [17] but only weakly reactive towards alkaline silver nitrate.[18] This behaviour was later recognised to be characteristic of 1,2-linked disaccharides [18,19] and it is possible that this was 2-*O*-α-D-mannopyranosyl-D-mannose. It had $[\alpha]_D$ +48·6°, to be compared with $[\alpha]_D$ +40° for the α-1,2-disaccharide isolated by Gorin and Perlin [21] from the slime mannan produced by *Saccharomyces rouxii*.

[16] Cf. Whelan, Bailey, and Roberts, *J.*, 1953, 1293.
[17] Bacon and Edelman, *Biochem. J.*, 1951, **48**, 114.
[18] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[19] Feingold, Avigad, and Hestrin, *Biochem. J.*, 1956, **64**, 351.
[20] Haq and Whelan, *Nature*, 1956, **178**, 1222.
[21] Gorin and Perlin, *Canad. J. Chem.*, 1956 ,**34**, 1796.

Experimental

General Methods.—These are described in Parts I [2] and II [3] of this series and by Peat, Whelan, and Roberts.[22] Solutions for optical rotation were in a 4 dm. tube. Whatman no. 3 MM paper was used for preparative partition chromatography. Charcoal–Celite chromatography was performed with B.D.H. "Activated charcoal for decolorizing purposes" and Celite no. 535 (Johns-Manville Co. Ltd.). All oligo- and poly-saccharide concentrations were calculated on the weight of mannose released on acid-hydrolysis in 1·5N-sulphuric acid at 100° for 3—6 hr., the Somogyi reagent [23] being used to measure the liberated mannose. A correction was made for loss of mannose owing to destruction by acid.[24] Measured with chromatographically pure mannose this was 2% after 4 hours' and 3% after 6 hours' heating. To develop full copper-reducing power mannose required 50 minutes' heating with Somogyi reagent.

Isolation of Yeast Mannan.—Baker's yeast (10 lb.; Distillers Co. Ltd.) was crumbled, stirred into 19 mM-citrate buffer (2·2 l.; pH 7·0), heated in an autoclave at 140° for 2 hr., then centrifuged and the gelatinous solid was again autoclaved with 5 l. of water. The combined extracts were concentrated under reduced pressure to 2·5 l. and made up to N in acetic acid. A brown gelatinous solid was removed on the centrifuge and washed with N-acetic acid, and the washings were added to the mannan solution which was next neutralised with 6N-sodium hydroxide and concentrated to 2 l. Ethanol (4 l.) was added and the precipitated mannan washed twice with 60% aqueous ethanol. The centrifuged solid was dissolved in water (2 l.), and a brown residue removed on the centrifuge. The cloudy supernatant solution was made alkaline with sodium hydroxide, and Fehling's solution ($CuSO_4,5H_2O$ 3·5%, Rochelle salt 1·7%, sodium hydroxide 5%) was added until the supernatant solution above the precipitated grey-blue mannan complex was deep blue and no further precipitation occurred. The complex was separated and washed several times on the centrifuge with warm water (40°). Then the complex was suspended in water (670 ml.), and concentrated hydrochloric acid was added slowly with stirring to dissolve the complex. The solution was made slightly acid and filtered through sintered-glass into ethanol (3 vol.). The white precipitate was washed with ethanol, redissolved in water (1·4 l.), and precipitated with ethanol (3 vol.). The precipitate was dissolved in water (460 ml.), and acetic acid (139 ml.) was added, followed by charcoal (5 g.). After being stirred for a few minutes the solution was centrifuged and poured into ethanol (1·85 l.). The precipitate was collected on the centrifuge and treated twice more by the same procedure. Finally the polysaccharide was dehydrated under ethanol and dried *in vacuo* over phosphoric oxide, to give 45 g. of white powder (mannan I).

In a second preparation of the mannan from 10 lb. of yeast, the copper-complex formation was repeated three times but the final precipitation from acetic acid was omitted. The yield was 24·5 g. (mannan II). Both this and the first sample gave mannose as the main component of an acid-hydrolysate, with a trace of glucose.

The first preparation was dried *in vacuo* and hydrolysed in 1·5N-sulphuric acid for 4 and for 6 hr. The reducing powers of the neutralised hydrolysates, corrected for acid-destruction of mannose (see above), corresponded to polymannose contents in the mannan of 90·0% and 89·5%, respectively. $[\alpha]_D$ of the polysaccharide, based on the carbohydrate content, was +89° (*c* 0·23).

Partial Acid-hydrolysis of Mannan I.—A small-scale hydrolysis of mannan I in 0·33N-sulphuric acid at 100° was performed to measure the rate of hydrolysis. The reducing powers at 4, 6·16, 8, and 9·5 hr. corresponded to apparent conversions into mannose of 46, 61, 68, and 74%, respectively.

Air-dried mannan I (43 g.) was heated in 0·33N-sulphuric acid (4·2 l.) in a boiling-water bath for 8 hr. After cooling, a portion (0·2 ml.) was removed for measurement of reducing power and a further portion (1 ml.) was heated in 1·5N-sulphuric acid (14·3 ml.) for 4 hr. in order to measure the total mannan content of the solution. This was 33·7 g. and the apparent conversion into mannose was 51%. The digest was again heated for 2 hr., the conversion being then 58%, and after a further 2 hr., at 67% conversion, the digest was cooled, neutralised with sodium hydroxide, and concentrated to about 500 ml.

Fractionation of Partly Hydrolysed Mannan I.—The mannan hydrolysate was adsorbed on

[22] Peat, Whelan, and Roberts, *J.*, 1957, 3916.
[23] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.
[24] Cf. Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224.

charcoal–Celite (1 : 1 by wt.; 6 × 103 cm.) and was eluted with water. Fractions (200 ml. each) were collected and the optical rotations measured. After 36 fractions had been collected the column was eluted with 7·5% aqueous ethanol, and after fraction 76 with 15% ethanol. The fractions were combined as experience suggested and examined by paper chromatography. Fractions 52—54 (740 mg.) contained a non-reducing sugar with $R_{mannose}$ 1·50, and another sugar migrating less rapidly than mannose. By refractionation on charcoal–Celite (4 × 64 cm.) the non-reducing substance (400 mg.) was isolated. Fractions 55—60 were refractionated on charcoal–Celite (3·5 × 80 cm.). 4% Ethanol eluted more of the non-reducing substance (103 mg.), and 6% ethanol gave two separate fractions, the first *A* ($R_{mannose}$ 0·50) weighing 332 mg. and the second 280 mg. This second fraction was separated by chromatography on thick paper into three fractions *B* ($R_{mannose}$ 0·39; 27 mg.), *C* ($R_{mannose}$ 0·22; 136 mg.) and *D* ($R_{mannose}$ 0·10; 70 mg.). Further quantities of *A*, *C*, and *D* were obtained by refractionation of fractions 61—68 (990 mg.) and 69—86 (1·00 g.) on thick paper, bringing the total yields of *A*, *C*, and *D* to 380, 483, and 284 mg., respectively.

Identification of the Monosaccharides.—Fractions 21—35 (see above) contained two substances, a major component migrating with mannose and a minor component migrating with glucose. A portion (150 mg.) was fractionated on thick paper, and the major component (120 mg.) was reduced with sodium borohydride and acetylated with sodium acetate–acetic anhydride to yield mannitol hexa-acetate (229 mg., 80%), m. p. and mixed m. p. 124—125°.

Identification of 6-O-α-D-*Mannopyranosyl*-D-*mannose.*—Fraction *A* had $[\alpha]_D$ +58° in water (*c* 0·51), the concentration being determined by acid-hydrolysis to mannose, this being the only sugar detected in the hydrolysate. On oxidation in 0·185M-sodium metaperiodate at room temperature formic acid was liberated as follows: 14 hr., 4·39 mol.; 20·2 hr., 4·58; 37·7 hr., 4·65; 62·25 hr., 4·78; 72 hr., 4·78. Acetylation of the fraction (91 mg.) with anhydrous pyridine–acetic anhydride at 4° for 4 days gave an acetate (162 mg., 90%) which after three crystallisations had $[\alpha]_D$ +23·6° (*c* 0·036 in $CHCl_3$) and m. p. 151° (unchanged on admixture with synthetic disaccharide acetate, prepared as follows).

α-D-Mannopyranosyl bromide [25] (17 g.) in alcohol-free chloroform (80 ml.) was added with stirring during 1 hr. to a mixture of 1,2,3,4-tetra-*O*-acetyl-β-D-mannose [26] (16 g.), silver oxide (15 g.), calcium sulphate (50 g.; heated at 240° for 2 hr.), and iodine (2 g.) in chloroform (100 ml.).[27] After being stirred for 24 hr. the mixture was filtered through Celite and evaporated to dryness. The resulting syrup was dissolved in the minimum volume of ethanol and adsorbed on charcoal–Celite (5 × 80 cm.). An ethanol–water gradient was applied to the column, ethanol being fed into a mixing chamber containing water (15 l.) which led to the top of the column. Fractions (500 ml.) were collected, combined in pairs, evaporated to dryness, extracted with chloroform, and again recovered. Inspection of the weights of the fractions suggested that the disaccharide could be in fractions 31—38 (3 g.). These were combined and crystallised twice from ethanol. The crystals had m. p. 148—149°, $[\alpha]_D$ +22·2° (*c* 0·12 in $CHCl_3$). Talley *et al.*[10] give m. p. 152—153°, $[\alpha]_D$ +19·6°.

Examination of Fractions B, C, *and* D.—Fraction *B* gave only glucose when hydrolysed with acid (evidence of paper chromatography). It had $[\alpha]_D$ +22° (*c* 0·032 in H_2O). On oxidation with periodate as for fraction *A*, the yields of formic acid after 62·5 and 87 hr. were 4·50 and 4·75 mol., respectively. The sugar had the same R_F and M_G values as gentiobiose and isomaltose.

Fraction *C* was partly hydrolysed with acid and examined by paper chromatography. Apart from unchanged material the hydrolysate contained substances with the R_F values of mannose and fraction *A*. The sugar had $[\alpha]_D$ +68° (*c* 0·42 in H_2O). On acetylation with pyridine–acetic anhydride at 4° an acetate was obtained which after three crystallisations from ethanol had m. p. 162—163° and $[\alpha]_D$ +43·7° (*c* 0·09 in $CHCl_3$).

Fraction *D* ($[\alpha]$ +70°) was partly hydrolysed with acid, and substances migrating with fractions *C*, *A*, and mannose were then seen on a paper chromatogram.

Partial Hydrolysis and Fractionation of Mannose Oligosaccharides.—Fractions 87—108 obtained in the separation of the partly hydrolysed mannan (see above) were found by paper chromatography to consist of sugars with $R_{mannose} < 0·1$. Partial acid-hydrolysis of a small portion gave substances migrating with mannose and fractions *A*, *C*, and *D*. Fraction *B* and

[25] Brauns, *J. Res. Nat. Bur. Stand.*, 1931, **7**, 573.
[26] Reynolds and Evans, *J. Amer. Chem. Soc.*, 1940, **62**, 66.
[27] Evans, Reynolds, and Talley, *Adv. Carbohydrate Chem.*, 1951, **6**, 27.

glucose were absent. The whole fraction (6 g.) was heated in 0·33N-sulphuric acid (550 ml.) in a boiling-water bath for 3·25 hr. The cooled hydrolysate was neutralised with 6N-sodium hydroxide and fractionated on charcoal–Celite (4·5 × 80 cm.). The column was eluted with water (1·4 l.) and then by gradient elution, 15% aqueous ethanol being fed into water (10 l.) in the mixing chamber, and 100 ml. fractions being collected. Mannose was removed by water, and chromatographically pure fraction *A* (220 mg.) was found in fractions 46—53. The next series of fractions contained fractions *C*, *D*, and a substance having $R_{mannose}$ 0·0 (fraction *E*). The substances had not been separated sufficiently well, and so fractions 55—91 were combined and 600 mg. of the total of 803 mg. were separated on thick filter paper into fractions *C* (165 mg.), *D* (130 mg.), and *E* (45 mg.). Fraction *A* had $[\alpha]_D$ +59°, *C* +69°, *D* +68°, and *E* +67·5° in water.

Periodate Oxidation of Mannan I and Mannan Oligosaccharides.—Mannan I was dissolved in water, the solution clarified by passing through a Seitz filter, and its concentration (472 mg./25 ml.) determined by acid-hydrolysis for 6 hr. with measurement of the mannose liberated. On periodate oxidation the following data were obtained (results are in moles per mannose unit):

Time (hr.)	4·25	22·75	47·5	74·75	
Periodate uptake	1·07	1·30	1·37	1·28	
Time (hr.)	5·0	18·75	28·5	42·5	65
Formic acid liberated	0·22	0·30	0·32	0·33	0·35

Fractions 87—108 (200 mg.) were dissolved in water (10 ml.), and the amount of oligosaccharide, calculated as $C_6H_{10}O_5$, was determined by acid-hydrolysis as 182 mg. When oxidised with periodate the results were as follows (moles per mannose unit):

Time (hr.)	4	8·7	23·5	49·25	71·75	95·7	—	—
Periodate uptake	1·52	1·66	1·79	1·75	1·85	1·85	—	—
Time (hr.)	4	9·5	24	33·5	47·25	72·5	119·5	143·5
Formic acid liberated	0·59	0·74	0·86	0·88	0·91	0·96	1·03	1·05

Acid-reversion of Mannose.—Mannose (36 g.) was heated in 0·33N-sulphuric acid (4 l.) for 10 hr. in a boiling-water bath. After cooling and neutralisation (sodium hydroxide), the solution was fractionated on charcoal–Celite (7 × 150 cm.). Fractions of 200 ml. each were collected, the column being eluted with water (5·2 l.), 10% ethanol (5·4 l.), and 50% ethanol (1·8 l.). Fractions 11—22 displayed positive optical rotation, and paper chromatography showed only mannose to be present. Fractions 32—44 had negative rotation and chromatography showed five sugars (*P*—*T*), that with the greatest R_F value (*P*, $R_{mannose}$ 1·50) being non-reducing. This was followed, in order of decreasing R_F value, by a trace of a reducing sugar (*Q*), mannose (*R*), and two spots (*S*, *T*) moving close to each other and in the position occupied by the α-1,6-linked dimannose (fraction *A*). The solution of fractions 32—44 was treated with barium hydroxide–zinc sulphate [28] to remove substances interfering with measurement of reducing power [15] and then diluted to 50 ml. The mannose content, determined by acid-hydrolysis, was 589 mg. (as monosaccharide). A 10-ml. portion of the solution was fractionated on thick filter paper into three zones, *P*, *Q* + *R*, and *S* + *T*, the mannose contents of which were determined by elution and acid-hydrolysis. Elution of an untreated sheet of filter paper gave no optically active material and a negligible reducing power, equivalent to 0·28 mg. of mannose per 343 sq. cm. of paper. In terms of the whole 50 ml. sample the amounts of sugar, as mannose, were *P* 366 mg., *Q* + *R* 16 mg., *S* + *T* 192 mg., a recovery of 97·4%. The yield of *P*, as 1,6-anhydromannose (see below), was 329 mg. and of *S* + *T*, as disaccharide, was 182 mg. Fraction *P* had $[\alpha]_D$ −126° in water. By fractionation of the remainder of the reversion products 177 mg. of *P* were obtained which formed a crystalline acetate (253 mg.; pyridine–acetic anhydride), m. p. 81° and $[\alpha]_D$ −121° (in $CHCl_3$). 1,6-Anhydromannose has $[\alpha]_D$ −127·6°, and its tri-*O*-acetate has m. p. 90—91° and $[\alpha]_D$ −123·6°.[28] Fraction *Q* + *R* had $[\alpha]_D$ +8·8° in water, and *S* + *T* +39·3° in water.

We thank the Department of Scientific and Industrial Research for a maintenance grant (to T. E. E., 1952—1955), and Mrs. O. Owen and Mr. M. Jones for assistance in the acid-reversion experiments.

UNIVERSITY COLLEGE OF NORTH WALES,
BANGOR.

[*Received, June 22nd,* 1960.]

[28] Somogyi, *J. Biol. Chem.*, 1945, **160**, 69.
[29] Knauf, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1941, **63**, 1447.