539. *The Extracellular Mannan of* Penicillium charlesii *G. Smith.*

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Growth of *Penicillium charlesii* G. Smith on a medium containing D-glucose gave an extracellular galactan and mannan. Selective hydrolysis of the galactan component with 0·015N-sulphuric acid led to the isolation of the mannan. It was electrophoretically homogeneous and contained eight D-mannopyranosyl units. Hydrolysis of the methylated mannan gave 2,3,4,6-tetra-*O*-methyl-D-mannose (2 parts), 3,4,6-tri-*O*-methyl-D-mannose (5 parts), and 3,4-di-*O*-methyl-D-mannose (1 part). Hence the mannose units are joined together by 1,2-linkages, with a branching point at $C_{(6)}$ of one of these units. Periodate oxidation confirmed these structural features and suggested that there are four D-mannopyranosyl units on either side of the 1,6-linkage, as in (I).

Periodate oxidation methods [1,2] have been developed for investigating, on a semimicro-scale, the interglycosidic units present in oligosaccharides, with a view to their subsequent application to polysaccharides, in particular those isolated in small yield by microbiological procedures. These methods have now been applied, in conjunction with the methylation method, to the mannan [3] of low molecular weight produced in the culture medium of

[1] Hough and Perry, *Chem. and Ind.*, 1956, 769.
[2] Hough, Perry, and Woods, *Chem. and Ind.*, 1957, 1100.
[3] Clutterbuck, Haworth, Raistrick, Smith, and Stacey, *Biochem. J.*, 1934, **28**, 94; Haworth, Raistrick, and Stacey, *ibid.*, 1935, **29**, 612; Stacey and Barker, "Polysaccharides of Micro-organisms," Oxford Univ. Press, London, 1960, p. 178.

Penicillium charlesii. The mannan was originally thought to be composed of eight or nine D-mannopyranosyl units joined exclusively by 1,6-linkages, but, in a re-interpretation of the methylation evidence,[3] Stacey [4] suggested a branched-chain structure containing 1,2- and 1,6-linkages.

Penicillium charlesii was grown as a surface culture on a 5% solution of D-glucose containing mineral salts. The polysaccharide material was isolated in 12% yield based on the glucose supplied. The preparation passed through a regenerated cellulose membrane, thus indicating it to be of low molecular weight. Quantitative hypoiodite oxidation [5] suggested an average molecular weight of 1540. Tiselius electrophoresis showed that the preparation contained at least two components, in agreement with the previous detection of a galactan and a mannan. Selective hydrolysis of the galactan component with 0·015N-sulphuric acid at 100° caused the optical rotation to rise from −28° to +75° in 4 hr. with concomitant release of D-galactose. The addition of ethanol to the concentrated hydrolysate gave the mannan ($[\alpha]_D$ +55°). Examination of the mannan in the Tiselius electrophoresis apparatus showed single ascending and descending peaks which were found to be enantiographic at both pH 9·3 and pH 7·0, thus suggesting that it was homogeneous. When the mannan was heated with 0·015N-sulphuric acid at 100° for 6 hr., the optical rotation of the solution remained constant and neither mono- nor oligosaccharide was detected. The optical-rotation evidence indicates that the original preparation contained about 60% of a polymer of low molecular weight, composed of D-galactofuranosyl units,[6] with $[\alpha]_D$ −86°.

Exhaustive methylation of the mannan gave a product whose molecular weight, determined by a modification of Barger's isothermic distillation technique,[7] was found to be 1600 ± 65, corresponding to a composition of eight mannose units. Examination of a hydrolysate of the methylated mannan on paper chromatograms suggested the presence of tetra-, tri-, and di-*O*-methylmannose derivatives which, after separation on a cellulose column,[8] were identified as 2,3,4,6-tetra-, 3,4,6-tri-, and 3,4-di-*O*-methyl-D-mannose. The molecular ratios of these derivatives were determined by quantitative paper chromatography,[9] the benzidine method [10] being used for their colorimetric assay, and the results were in good agreement with those obtained from the yields in the cellulose column separation (Table 1).

TABLE 1.

Molecular ratios of the methylated sugars obtained from the methylated mannan.

Determination	Di-*O*-methyl-mannose	Tri-*O*-methyl-mannose	Tetra-*O*-methyl mannose
Quantitative paper chromatography	1	5·4	1·86
Cellulose column (wt. of fraction)	1	5·9	2·04

It was therefore established that the mannan contained two chains of 1,2-linked D-mannopyranosyl units joined together by a 1,6-linkage as in (I) which is one of several branched structures consistent with the methylation evidence. Periodate oxidations were then carried out in order to verify these results and to obtain further structural information.

D-Man*p* 1—2 D-Man*p* 1—2 D-Man*p* 1—2 D-Man*p*
1
|
6 (I)
D-Man*p* 1—2 D-Man*p* 1—2 Man*p* 1—2 D-Man*p*

[4] Stacey, *J.*, 1947, 853.
[5] Hirst, Hough, and Jones, *J.*, 1949, 928; Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.
[6] Haworth, Raistrick, and Stacey, *Biochem. J.*, 1937, **31**, 640; O'Dea, *Chem. and Ind.*, 1953, 1338; Gorin and Spencer, *Canad. J. Chem.*, 1959, **37**, 499.
[7] Barger, *J.*, 1904, 85, 286; Niederl, Kasanof, Kisch, and Subba Rao, *Mikrochem.*, 1949, **34**, 132.
[8] Hough, Jones, and Wadman, *J.*, 1949, 2511.
[9] Flood, Hirst, and Jones, *J.*, 1948, 1679.
[10] Jones and Pridham, *Biochem. J.*, 1954, **58**, 288.

Reduction of the reducing group of the mannan with borohydride and subsequent periodate oxidation [2] at pH 3·6 rapidly released 0·12 mole of formaldehyde per 162 g. of mannan. Only the 2-*O*-substituted mannitol end group will give rise to formaldehyde [2] (1 mole) and hence the molecular weight of the mannan was found to be 1360, in close agreement with the value of 1270 ± 50 obtained from the molecular weight of the methylated mannan. The value (1507) obtained by the hypoiodite technique is likely to be a little high since mannose derivatives are usually incompletely oxidised.[5]

Extrapolation of the results obtained when the mannan was oxidised with periodate under unbuffered conditions showed a rapid uptake of 1·1 moles of periodate with the formation of 0·275 mole of formic acid per mannose unit, followed by slow over-oxidation. No mannose could be detected in a hydrolysate of the oxidised mannan. A mannan of structure (I) would reduce 1·25 mole of periodate and give 0·25 mole of formic acid (from the non-reducing end groups) per mannose unit. Hence these results support the suggested structure (I).

Periodate oxidation of the mannan at pH 8 in phosphate buffer [1] gave 0·39 mole of formaldehyde per mannose unit, corresponding to the release of 3·12 moles of formaldehyde from a mannan composed of eight units. Whilst the exact position of the 1,6-linkage cannot be decided, this result shows that there are four mannose units on either side of this branching point, as in (I), the formaldehyde arising from $C_{(6)}$ of those mannose units that can be progressively oxidised from the reducing end group *via* malondialdehyde derivatives.[1] Whilst this structure (I) can be reconciled with the evidence of Haworth, Raistrick, and Stacey,[3] it differs from that suggested later by Stacey.[4] Differences in structure between the mannans could arise by spontaneous mutation in the course of culture.

Other mannans of microbial origin have received structural investigation. The extracellular mannan produced by *Saccharomyces rouxii* has been shown to contain D-mannopyranosyl units joined by 1,6- and α-1,2-linkages.[11] Yeast mannan has a highly branched structure with 1,2-, 1,6-, and 1,3-linkages.[12] The mannan produced by *Bacillus polymyxa*,[13] which resembles that from *Bacillus diphtheriae*,[14] is considered to have a linear structure made up of 1,3- and 1,6-linkages. On the other hand, the mannan isolated from *Bacillus anthracis* is made up essentially of 1,4-linkages.[15]

Experimental

Paper chromatography was carried out as described previously.[16]

Production of the Extracellular Polysaccharides.—*Penicillium charlesii* G. Smith (Commonwealth Mycological Culture Collection, No. 40,232) was kept in sub-culture on Czapek–Dox agar slopes. The mould was grown in the dark at 25° as a surface culture on a medium (pH 4·0) of the following composition: D-glucose (75 g.), tartaric acid (4 g.), ammonium tartrate (4 g.), potassium carbonate (0·6 g.), diammonium hydrogen phosphate (0·6 g.), magnesium carbonate (0·4 g.), ammonium sulphate (0·25 g.), ferrous sulphate hydrate (0·07 g.), zinc sulphate hydrate (0·07 g.), and water (1·5 l.).

During growth the pH of the medium was observed to fall from 4 to a constant value of 2·1 after 16 days. The mycelium felts were green on the upper surface and brown on the reverse side. After 28 days' incubation, the brown liquor (30 l.) was decanted, filtered through cotton wool, and de-ionised by passage through columns (5 × 50 cm.) of Amberlite IR-120(H) and Amberlite IR-4B(OH) ion-exchange resins. The neutral effluent was concentrated under reduced pressure at $<30°$ in a recycling, climbing-film evaporator, to about 500 ml. Attempted dialysis of a small portion of the concentrate through a re-generated cellulose

[11] Gorin and Perlin, *Canad. J. Chem.*, 1956, **34**, 1796; 1957, **35**, 262.
[12] Haworth, Hirst, and Isherwood, *J.*, 1937, 784; Haworth, Heath, and Peat, *J.*, 1941, 833; Fischer, Kohtes, and Fellig, *Helv. Chim. Acta*, 1951, **34**, 1132.
[13] Murphy, Bishop, and Adams, *Canad. J. Biochem. Physiol.*, 1956, **34**, 1271.
[14] Orlova, *Biokhimiya*, 1950, **15**, 362; 1953, **18**, 683.
[15] Cave-Browne-Cave, Fry, El Khadem, and Rydon, *J.*, 1954, 3866.
[16] Andrews, Hough, Powell, and Woods, *J.*, 1959, 774.

membrane resulted in the loss of the polysaccharides. Consequently the main bulk of the concentrate was poured with stirring into ethanol (2·8 l.) to precipitate the polysaccharides. The supernatant liquor was poured off, the residual gummy mass dissolved in a little warm water, and ethanol (1·5 l.) added with stirring. The precipitated material was isolated by centrifugation and then reprecipitated twice in the same way. The product was washed with ethanol and dried under reduced pressure at 40° to give a fine white powder (17 g.) which had $[\alpha]_D^{25}$ −28° (*c* 1·5 in water) (Found: N, 1·5%; sulphated ash, nil).

Some Properties of the Polysaccharide Preparation.—The polysaccharide gave no colour with iodine and slowly reduced Fehling's solution on prolonged boiling. Three samples of the preparation (*ca.* 20 mg.) were oxidised at pH 11·4 with hypoiodite for 20 hr. and were found to consume iodine equivalent to molecular weights of 1520, 1550, and 1555.

Qualitative examination [17] of 1% solutions of the preparation dissolved in 0·05M-sodium borate in the Tiselius electrophoresis apparatus showed two distinct and widely separated peaks.

Another portion of the preparation (0·75 g.) was heated with 0·015N-sulphuric acid (75 ml.) at 95—100° and the change in the optical rotation followed: $[\alpha]_D^{25}$ −28° (initial), +12° (30 min.), +32° (60 min.), +54° (120 min.), +63° (180 min.), +75° (240 min. constant value). The hydrolysate was neutralised with barium carbonate, the filtered solution concentrated to *ca.* 5 ml., and ethanol (50 ml.) added. The precipitated material was collected on the centrifuge and the alcohol supernatant was concentrated to near dryness. Paper-chromatographic examination of the concentrate revealed the presence of galactose only. When kept, the concentrate gave crystals of D-galactose (201 mg.), $[\alpha]_D^{25}$ +80° (*c* 2·2 in water, equil.), m. p. and mixed m. p. 164°.

The alcohol-precipitated material was re-precipitated three times from aqueous solution by alcohol and then dried under reduced pressure, to give a white hygroscopic powder (192 mg.), $[\alpha]_D^{25}$ +55° (*c* 1·0 in water). The material was examined in the Tiselius electrophoresis apparatus as a 1% solution in either 0·05M-sodium borate (pH 9·3) or 0·07M-phosphate buffer (pH 7·0) and in each case the single ascending and descending peaks were found to be enantiographic, suggesting that the material was homogeneous. Hydrolysis of this polysaccharide (20 mg.) with 2N-sulphuric acid (8 ml.) at 100° for 6 hr., followed by neutralisation with barium carbonate and paper chromatography of the concentrated neutral solution, revealed a single spot having a mobility and giving a colour with the *p*-anisidine hydrochloride spray identical with those of mannose; no galactose could be detected.

Isolation of the Mannan.—The original mixture of polysaccharides (14 g.) was heated at 95—100° with 0·015N-sulphuric acid (500 ml.) until the optical rotation became constant (300 min.). The cooled hydrolysate was neutralised with barium carbonate, then filtered, and the filtrate was concentrated to about 15 ml. Ethanol (300 ml.) was added with vigorous stirring and the precipitated material was collected on the centrifuge and reprecipitated three times with alcohol from aqueous solution. The final precipitate was washed with ethanol and dried under reduced pressure, to yield a white powder (4·97 g.). Paper chromatography of the concentrated alcoholic liquors revealed galactose; no spot due to mannose could be detected.

Some Properties of the Mannan.—The polysaccharide was readily soluble in water to give a neutral solution, which gave no colour with iodine. It gave a copper complex with concentrated Fehling's solution and slowly reduced dilute Fehling's solution on prolonged boiling. The material had $[\alpha]_D^{25}$ +57° (*c* 1·5 in water) (Found: C, 44·3; H, 6·2; N, nil; sulphated ash, nil. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2%).

Two samples of the polysaccharide (*ca.* 20 mg.) were oxidised with alkaline hypoiodite [5] and consumed iodine equivalent to molecular weights 1495 and 1520.

The mannan (30 mg.) was heated at 100° with 0·015N-sulphuric acid (4 ml.) in a sealed glass polarimeter-tube for 6 hr. No change in the optical rotation of the solution was observed and no oligosaccharide or mannose could be detected chromatographically in the neutralised solution.

The mannan (50 mg.) was heated at 95—100° with 2N-sulphuric acid (5 ml.) and the change in its optical rotation followed: $[\alpha]_D^{25}$ +57° (initial), +41° (10 min.), +29·5° (20 min.), +23° (35 min.), +17° (50 min.), +16° (70 min.), +15·8° (90 min., constant value).

Paper chromatography of the neutralised hydrolysate revealed only mannose. The concentrated hydrolysate in moist methanol gave crystals of D-mannose (29 mg.), $[\alpha]_D^{25}$ +14·5° (*c* 1·2 in water; equil.), m. p. and mixed m. p. 132°.

[17] Northcote, *Biochem. J.*, 1954, **58**, 353.

Electrophoresis [17] of 1% solutions of the mannan in either 0·05M-sodium borate or 0·05M-sodium phosphate buffer (pH 7·0) gave a single enantiographic peak. Mixtures containing 0·5% of the mannan and 0·5% of the original mixed polysaccharide fraction in borate buffer were similarly examined. The faster-moving mannan peak was enlarged and was accompanied by the slower-moving peak due to the galactan.

Exhaustive Methylation of the Mannan.—To the mannan (3·1 g.) in water (10 ml.) was added, simultaneously and dropwise, 20% w/v sodium hydroxide solution (20 ml.) and dimethyl sulphate (10 ml.), with stirring and ice-cooling, at a rate such that the mixture remained slightly alkaline throughout. After 8 hr., 40% w/v sodium hydroxide solution (200 ml.) and dimethyl sulphate (95 ml.) were added dropwise during 8 hr. and stirring was continued for 24 hr. The mixture was then heated on a boiling-water bath for 30 min. to destroy any excess of dimethyl sulphate, and the cooled solution was carefully neutralised with 2N-sulphuric acid. The partially methylated mannan was extracted with chloroform (7 × 100 ml.) and the dried (Na_2SO_4) extract was concentrated to a syrup (3·3 g.). This syrup was remethylated with 40% w/v sodium hydroxide solution (200 ml.) and dimethyl sulphate (95 ml.) as described above, and the product was isolated by extraction with chloroform. After removal of the chloroform from the dried (Na_2SO_4) extract, methylation was continued by heating the product under reflux with methyl iodide (25 ml.) containing methanol (0·5 ml.), while silver oxide (8 g.) was added in portions (1 g. each) during 9 hr. The methylated material was extracted in the usual way and was again heated under reflux with methyl iodide (20 ml.) and silver oxide (6 × 1 g.). Extraction of the product with chloroform afforded a syrup (2·6 g.), which had $[\alpha]_D^{25}$ −91° (*c* 2·2 in chloroform) [Found: OMe, 43·7. Calc. for $C_6H_7O_2(OMe)_3$: OMe, 45·6%].

Further methylation of the material failed to increase the methoxyl content.

Investigation of the Methylated Mannan.—The methylated mannan (2·4 g.) was heated at 100° with 98% formic acid (100 ml.) for 10 hr. After removal of the formic acid by distillation, the residue was further hydrolysed by N-sulphuric acid (30 ml.) at 100° for 3 hr. The hydrolysate was neutralised with barium carbonate, filtered, and passed through mixed Amberlite IR-120(H) and IR-4B(OH) resins (2 × 3 cm.) and concentrated to a syrup (2·1 g.).

Paper chromatography of the hydrolysate revealed spots at R_G 0·96 (corresponding in position with 2,3,4,6-tetra-*O*-methylmannose), R_G 0·79, and R_G 0·56.

The mixture of methylated sugars (1·9 g.) was fractionated on a cellulose column (3 × 40 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3 v/v) as the mobile phase. Evaporation of appropriate parts of the effluent gave three fractions.

Fraction I. The syrup (411 mg.) was chromatographically indistinguishable from 2,3,4,6-tetra-*O*-methylmannose and had $[\alpha]_D^{25}$ +3° (*c* 0·9 in water) (Found: OMe, 51·8. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). A portion of the syrup (110 mg.) was heated under reflux with aniline (60 mg.) in ethanol (4 ml.) to give, on cooling, crystalline 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine, which on recrystallisation from ethanol had m. p. and mixed m. p. 142°, $[\alpha]_D^{25}$ −80° ⟶ −7° (*c* 0·5 in MeOH) (Found: C, 62·2; H, 7·6; N, 5·0. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·0; N, 4·5%).

Fraction II. The syrup (1·12 g.) was chromatographically the same as 3,4,6-tri-*O*-methylmannose and had $[\alpha]_D^{25}$ +10° (*c* 2·3 in water). The fraction crystallised (420 mg.) from ether–light petroleum (b. p. 100—120°) and had 106°, undepressed on admixture with authentic 3,4,6-tri-*O*-methyl-D-mannose, and $[\alpha]_D^{25}$ +9° (*c* 1·0 in water) (Found: OMe, 41·9. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). A portion of the residual syrup (50 mg.) was oxidised in 0·3M-sodium metaperiodate solution (5 ml.) at room temperature for 18 hr. and examination of an ether extract on paper chromatograms revealed two spots which co-chromatographed with 2,3,5-tri-*O*-methylarabinose and its formyl ester.[18] Another portion of syrup (0·1 g.) was oxidised with bromine water in the usual way, to give 3,4,6-tri-*O*-methyl-D-mannonolactone (78 mg.) which on recrystallisation from ether–light petroleum (b. p. 100—120°) had m. p. and mixed m. p. 98° and $[\alpha]_D^{25}$ +160° ⟶ +110° (*c* 1·2 in water) (Found: OMe, 41·8. Calc. for $C_9H_{16}O_6$: OMe, 42·3%).

Paper chromatography of the prepared lactone revealed a single spot at R_G 0·84, identical in its rate of movement with that of 3,4,6-tri-*O*-methyl-D-mannonolactone (2,3,6-tri-*O*-methyl-D-mannono-1 ⟶ 4-lactone had R_G 0·92).

Fraction III. This fraction (178 mg.), examined by paper chromatography gave a single spot at R_G 0·56 corresponding to 3,4-di-*O*-methylmannose. The syrup was moistened with

[18] Hemming and Ollis, *Chem. and Ind.*, 1953, 85.

methanol, seeded with crystals of 3,4-di-*O*-methyl-D-mannose, and set aside for 3 weeks. The whole fraction crystallised and, after recrystallisation from acetone–ether, had m. p. 114°, undepressed on admixture with authentic 3,4-di-*O*-methyl-D-mannose, and $[\alpha]_D^{25}$ +2° (*c* 2·1 in water) (Found: OMe, 29·0. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). A portion of the fraction (40 mg.) was oxidised with bromine in the usual way, to give 3,4-di-*O*-methyl-D-mannonolactone, m. p. and mixed m. p. 157—159°, $[\alpha]_D^{25}$ +173° ⟶ +130° (*c* 0·3 in water).

A portion of the original hydrolysate (*ca.* 30 mg.) was separated on paper chromatograms; [9] filter papers were used that had been previously washed with 1% aqueous ammonia. The separated sugars were analysed in triplicate by the benzidine method.[10] The results are recorded in Table 2.

TABLE 2.

Molecular ratios of the methylated sugars.

Determination	Di-*O*-methyl mannose	Tri-*O*-methyl mannose	Tetra-*O*-methyl mannose
1	1	5·3	1·84
2	1	5·7	1·81
3	1	5·2	1·94

A modification [7] of Barger's capillary technique was used to determine the molecular weight of the methylated mannan. Standard solutions of sucrose octa-acetate in ethyl acetate (2—8 × 10^{-3}M) were compared with 1% w/v solutions of the methylated mannan. The standard and the unknown solutions were contained separately in tubes of 1 mm. internal diameter, enclosed in close-fitting, sealed, glass outer tubes. The tubes, mounted on glass slides, were kept at 22° and, at intervals, meniscus movements were determined by means of a travelling microscope reading to 0·01 mm. The methylated mannan solution was found to be isopiestic with solutions of sucrose octa-acetate between 6 × 10^{-3}M and 6·5 × 10^{-3}M, indicating the methylated mannan to have a molecular weight between 1666 and 1538.

Periodate Oxidation of the Mannan.—The mannan (511 mg.) was dissolved in water (450 ml.), 0·3M-sodium metaperiodate (40 ml.) added, and the total volume adjusted to 500 ml. with water. A blank solution containing no mannan was prepared at the same time. Oxidation was allowed to proceed in the dark at 20°, and samples (10 ml.) were withdrawn at intervals and analysed for formic acid [19] and periodate [20] (Table 3).

TABLE 3.

Periodate oxidation of the mannan.

Time of oxidn. (hr.) ...	3	6	12	24	36	48	60	72	84	96
Mole of IO_4 consumed per 162 g. of mannan	0·52	0·64	0·88	1·05	1·14	1·18	1·20	1·21	1·23	1·25
Mole of HCO_2H released per 162 g. of mannan	0·169	0·201	0·255	0·310	0·330	0·345	0·365	0·380	0·400	0·420

A solution of the mannan (240 mg.) in water (250 ml.) containing 0·3M-sodium metaperiodate (20 ml.) was kept in the dark for 52 hr. at 20°. A 3% solution of lead acetate was added until no more precipitation occurred, and the insoluble lead periodate was removed by centrifugation. The clear supernatant liquid was passed down a column of mixed Amberlite IR-120(H) and IR-4B(OH) ion-exchange resins, and the neutral effluent was concentrated. The syrup was hydrolysed by 2N-sulphuric acid for 8 hr. at 95—100° and, after neutralisation with barium carbonate, the solution was concentrated and examined by paper chromatography, but no mannose could be detected.

To a solution of the mannan (*ca.* 6—8 mg., accurately weighed) in 0·06M-phosphate buffer containing 0·1% of *p*-hydroxybenzaldehyde (pH 8·0; 19 ml.) was added 0·3M-sodium metaperiodate (5 ml.), and the total volume was adjusted to 25 ml. with phosphate buffer. Samples (1 ml.) were withdrawn at intervals and neutralised by 20% lead dithionate solution (1 ml.), and, after removal of the lead periodate, the formaldehyde contents of the clear supernatant liquids were determined by the chromotropic acid method (Table 4).

[19] Halsall, Hirst, and Jones, *J.*, 1947, 1427.
[20] Malaprade, *Bull. Soc. chim. France*, 1934, **1**, 833.

TABLE 4.

Periodate oxidation of the mannan at pH 8·0.

Time of oxidation (hr.)	2·5	10	25	45	70	85
Mole of CH_2O released per 162 g. of mannan	0·15	0·25	0·32	0·37	0·39	0·39

The mannan (*ca.* 20 mg., accurately weighed) was dissolved in water (5 ml.), and sodium borohydride (25 mg.) was added. After 24 hr. at room temperature, the mixture was slightly acidified with 2N-sulphuric acid (0·5 ml.) and kept for 3 hr. to ensure complete destruction of the excess of borohydride. 0·1M-Sodium acetate–acetic acid buffer (pH 3·6), containing 0·01% of *p*-hydroxybenzaldehyde (16 ml.), was added, followed by 0·3M-sodium metaperiodate (3 ml.), and the total volume was adjusted to 25 ml. Samples (1 ml. each) were withdrawn and analysed for formaldehyde.[21] The values (mole) found per 162 g. of mannan were: 0·104 (15 min.); 0·118 (35 min.); 0·120 (60 min.); 0·119 (90 min.).

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[21] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.