3. *Methylation and Periodate Oxidation Studies of the Alkali-stable Polysaccharide of Sugar-beet Pectin.*

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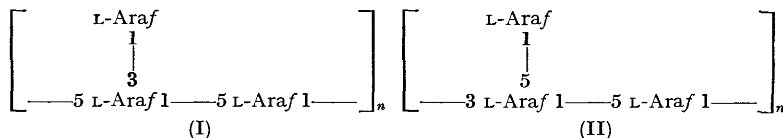
From the methylation experiments it is concluded that, in the soluble polysaccharide obtained by heating sugar-beet chips with lime-water, the araban component is intimately associated with a branched galactan which contains β-D-galactopyranosyl units linked through positions 1 and 4, with branching points arising from units linked through positions 1, 3, and 6; a minor product appeared to have a linear structure composed mainly of β-1,4-D-galactopyranosyl units.

Oxidation, reduction, and hydrolysis confirmed the branched-chain structure of the araban and galactan components and it is suggested provisionally that there are five L-arabinofuranosyl units per non-reducing end group in the araban.

THE polysaccharide obtained on treating sugar-beet chips with hot lime-water contains L-arabinofuranosyl units in the form of an araban,[1] in combination with units of D-galactose, L-rhamnose, and D-galacturonic acid in the approximate molecular proportions of 21 : 3 : 1 : 1·5 respectively.[2] Hirst and Jones,[1] in their examination of the hydrolysis of the methylated polysaccharide, estimated that approximately equimolecular proportions of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-L-arabinose, and 2-*O*-methyl-L-arabinose were present, which, considered together with the acid-lability of these pentose units, led them to suggest a branched structure (*e.g.*, I and II) for the araban.



The hydrolysis products have now been examined on paper chromatograms for the presence of galactose derivatives and small quantities of tetra-, tri-, and di-*O*-methyl derivatives of this sugar were detected amidst the predominant arabinose derivatives. A large quantity of the hydrolysate was therefore fractionated on a cellulose column,[3] benzene and benzene–ethanol being used successively as the mobile phase. In general, the separated products were either converted directly into crystalline derivatives or oxidised to the corresponding lactones and purified by paper chromatography. In addition to the above L-arabinose derivatives, there were identified small quantities of

[1] Hirst and Jones, *J.*, 1948, 2311.
[2] Andrews, Hough, Powell, and Woods, *J.*, 1959, 774.
[3] Hough, Jones, and Wadman, *J.*, 1949, 2511; 1950, 1702.

2,3,4,6-tetra, 2,3,6-tri-, and 2,4-di-*O*-methyl-D-galactose. Since only traces of galactose were liberated on heating a solution of the polysaccharide, adjusted to pH 2·0, at 80° for 6 hr., and no galactosyl-arabinose disaccharides were found,[2,4] it appears that the D-galactosyl units exist largely, if not entirely, in the pyranose form in combination one with another. Galactobiose, lactose, and *Strychnos nux vomica* galactan [5] were also slightly hydrolysed under these conditions. This evidence suggests that the galactan contains D-galactopyranosyl units joined by 1,4-linkages, with a number of branching points where the units are substituted at $C_{(1)}$, $C_{(3)}$, and $C_{(6)}$. Similar structural features were found in *Strychnos nux vomica* galactan,[5] whereas the arabogalactans of larch,[6] and snail galactan [7] differ in that their frameworks are constituted of 1,3-linked β-D-galactopyranose units.

An additional pectic galactan was detected during these methylation studies, thus illustrating the molecular complexity of the pectic substances. The above hydrolysis products were obtained by heating the methylated polysaccharide in solution in methanol–N-sulphuric acid (1 : 3 v/v), but a small quantity (*ca.* 1%) of insoluble material separated during the reaction. Hirst and Jones [1] made a similar observation. The insoluble product was acidic, gave analyses for a methylated hexose polymer, was insoluble in methanol, and had $[\alpha]_D$ $-11° \pm 2°$ (in chloroform). Although this product resisted hydrolysis by 5N-hydrochloric acid, formic acid was effective, and examination of the products on paper chromatograms showed the presence of a tri-*O*-methylhexose, which was indistinguishable from 2,3,6-tri-*O*-methylgalactose; traces of 2,3,4,6-tetra-*O*-methyl-galactose and hexuronic acid derivatives were also revealed. This methylated poly-saccharide clearly resembled the methylated pectic galactans of *Strychnos nux vomica* seeds ($[\alpha]_D$ $-6°$ in chloroform),[5] which was also insoluble in methanol, and of *Lupinus albus* ($[\alpha]_D$ $-12°$ in methanol); [8] both were difficult to hydrolyse. An unsuccessful attempt was made to procure further quantities of this methanol-insoluble derivative by methylation of another batch of the sugar-beet polysaccharide. It can only be assumed that the galactan was destroyed by the strongly alkaline conditions of the methylation (cf. Aspinall, Laidlaw, and Rashbrook [9]), and that in the former experiment a small quantity escaped destruction by prior methylation. On the other hand, the methylated galactan, which was intimately associated with the methylated araban, soluble in methanol, and readily hydrolysed, was detected in this second preparation.

Oxidations of the alkali-stable sugar-beet polysaccharide with sodium metaperiodate in unbuffered solution and at pH 3·5 gave similar results: rapid consumption in 20 hr. of about 0·68 mol. of periodate per anhydromonosaccharide unit (equivalent to 1 mole of periodate per 210 g. of polysaccharide), followed by slow over-oxidation. The acid produced was equivalent to 1 mole of formic acid per 1440 g. of polysaccharide. The branched structure (I or II) suggested for the araban component, which comprises some 75% of the alkali-stable polysaccharide, requires 0·66 mole of periodate per anhydro-pentose unit for complete oxidation. The araban would not, however, give rise to formic acid and consequently the acidity produced must be due to the oxidation of other mono-saccharide units. The oxidised polysaccharide was isolated from a larger-scale experiment, and the phenylhydrazone derivative was prepared. When the phenylhydrazone was heated at 95—100° for 4 hr. in ethanolic phenylhydrazine [10] and the solution was poured into an excess of alcohol, no precipitate was formed, showing that there was no fragment of high molecular weight that had been unattacked by periodate. Finan and O'Colla [11] have isolated 3-*O*-L-arabinofuranosylglycerosazone from this Barry degradation [10] and

[4] Hough and Powell, unpublished results.
[5] Andrews, Hough, and Jones, *J.*, 1954, 806.
[6] White, *J. Amer. Chem. Soc.*, 1941, **63**, 2871; Aspinall, Hirst, and Ramstad, *J.*, 1958, 593.
[7] Bell and Baldwin, *J.*, 1941, 125.
[8] Hirst, Jones, and Walder, *J.*, 1947, 1225.
[9] Aspinall, Laidlaw, and Rashbrook, *J.*, 1957, 4444.
[10] Barry, *Nature*, 1943, **152**, 537; Barry and Mitchell, *J.*, 1954, 4020.
[11] Finan and O'Colla, *Chem. and Ind.*, 1958, 493.

therefore the araban structure can only be represented by either (I) or (II). The phenylhydrazone of the oxidised polysaccharide was coupled with diazotised aniline in an attempt to prepare the *NN'*-diphenylformazan derivative.[12] In the case of the araban, only those units which originated from the non-reducing end-groups (L-Ara*f* 1–) should react and not those from the main chain (–5 L-Ara*f* 1–). However, the ultraviolet absorption spectrum of the product showed that the majority of the phenylhydrazone groups had been modified, presumably by coupling with diazotised aniline since the nitrogen contents of the derivatives suggested that one molecule each of phenylhydrazine and diazotised aniline had reacted with each oxidised pentose unit. Furthermore, the product did not give a dark blue colour with concentrated sulphuric acid, a property which is thought to be characteristic of *NN'*-diphenylformazans.[12] Mester [12] has described a formazan derivative of periodate-oxidised xylan, whereas no such product would have been expected.[13] Further information is clearly necessary regarding the products obtained from the reaction of oxy-polysaccharide phenylhydrazones with diazotised aromatic amines before this reaction can be used with confidence for structural information. Hydrolysis of the oxidised polysaccharide and its phenylhydrazone derivative, and subsequent estimations of the monosaccharides liberated, revealed that only 3·3—5·4% of arabinose and 1·0—1·4% of galactose were present in the hydrolysates, whereas on the branched araban structure (I or II) about one-third of arabinose should have remained unoxidised. Abdel-Aker, Hamilton, Montgomery, and Smith [14] have found that, in general, low yields of monosaccharides are obtained from oxy-polysaccharides unless reduction to the corresponding polyol is carried out before hydrolysis. When the oxidised sugar-beet polysaccharide was reduced with sodium borohydride and then hydrolysed, 14% of arabinose and 3·5% of galactose were found. Since the amount of arabinose remaining unoxidised by periodate is an indirect measure of the number of non-reducing end-groups attached to the araban, it appears that there are on the average five arabinose units per non-reducing end-group. Investigations of methylated arabans have suggested that one in every three arabinofuranosyl units represents a branching point, and consequently the estimate obtained by periodate oxidation studies must be held in reserve until further evidence is available. However, the araban structure may be subject to variation as the result of biological activity due either to seasonal changes or to differences during the life cycle of the plant. Since the galactose content of the polysaccharide fell from 10% to 3·5% on periodate oxidation, the galactan must also have a branched structure, one in every three galactose residues representing a branching point.

EXPERIMENTAL

Paper chromatography of the monosaccharides and their derivatives was carried out as described previously: [2] polyols, amides, and lactones were detected on paper chromatograms by periodate–*p*-anisidine hydrochloride,[15] ninhydrin, and hydroxylamine–ferric chloride,[16] respectively. Evaporations were under reduced pressure. Unless otherwise stated, optical rotations were observed at 25° for aqueous solutions. Products were dried under reduced pressure over phosphoric oxide.

Methylation.—The alkali-stable polysaccharide (25 g.; $[\alpha]_D$ −84°) (Found: sulphated ash, 5·0; N, 0·7%) was dissolved in water (150 ml.); dimethyl sulphate (150 ml.) and 40% sodium hydroxide solution (300 ml.) were added dropwise with stirring during 8 hr. with ice-cooling. After the mixture had been stirred overnight, the methylation was repeated. The mixture was then acidified with glacial acetic acid, dialysed against tap-water until free from sulphate ions, and evaporated. The residue was further methylated by four repetitions of the above procedure. The final sulphate-free solution was extracted continuously for 20 hr. with chloroform which was then evaporated to a syrup (22·3 g.) (Found: OMe, 37·2; sulphated ash, 8·3%).

[12] Mester, *Adv. Carbohydrate Chem.*, 1958, **13**, 105; *J. Amer. Chem. Soc.*, 1955, **77**, 5452.
[13] Barry and Mitchell, *Chem. and Ind.*, 1957, **35**, 1045.
[14] Abdel-Aker, Hamilton, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1952, **74**, 4970.
[15] Bragg and Hough, *J.*, 1958, 4050.
[16] Abdel-Aker and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.

This syrup was further methylated by three treatments with methyl iodide and silver oxide. The final product (20·5 g.) had $[\alpha]_D$ −103° ± 3° (*c* 0·7 in $CHCl_3$) (Found: OMe, 39·8; sulphated ash, 2·8%).

The methylated polysaccharide was fractionated by boiling it under reflux with mixtures (100 ml. each) of chloroform–light petroleum (b. p. 40—60°). The three main fractions were obtained with the tabulated properties.

Fraction	$CHCl_3$–petrol (v/v)	Weight (g.)	OMe (%)	Sulphated ash (%)	$[\alpha]_D$ in $CHCl_3$
1	15 : 85	0·8	39·3	0·9	−30·5
2	20 : 80	2·8	39·1	0·3	−111
3	25 : 75	15·8	38·7	0·4	−108·5

A solution of fraction 3 (1 g.) was heated in N-sulphuric acid (15 ml.) and methanol (5 ml.) under reflux at 95—100° and the optical rotation (1 dm. tube) was determined at intervals: +0·24° (11 hr.), +1·3° (15 hr.), and +1·75° (constant; 18 hr.). After removal of a small white precipitate, the hydrolysate was neutralised by Amberlite IR-4B resin (OH form), concentrated, and examined on paper chromatograms.

Another portion of fraction 3 (1 g.) was heated with methanolic hydrogen chloride (4% w/w; 20 ml.) at 95—100° for 16 hr. and then after the addition of N-hydrochloric acid (50 ml.) heated at 95—100° for a further 6 hr. The hydrolysate was neutralised with Amberlite IR-4B resin, concentrated, and examined on paper chromatograms. The two hydrolysates were similar in composition.

A larger hydrolysis of fraction 3 (10 g.) was carried out as above in N-sulphuric acid–methanol, and the white insoluble material (*A*) was isolated on the centrifuge, washed with methanol, and dried. This material (*A*; 120 mg.) had $[\alpha]_D$ −11° ± 2° (*c* 0·5 in $CHCl_3$) and was slightly acidic (Found: OMe, 43·7%). The clear supernatant liquid was neutralised by Amberlite IR-4B resin and concentrated to a syrup, fraction 3A.

The insoluble material *A* was stable to 5N-hydrochloric acid but was hydrolysed by heating it at 95—100° with anhydrous formic acid (2 ml.) for 16 hr. and, after addition of water (5 ml.), then for a further 6 hr. The hydrolysate was concentrated and examined on paper chromatograms. A high proportion of 2,3,6-tri-*O*-methylgalactose was thus revealed, together with a little 2,3,4,6-tetra-*O*-methylgalactose and slower-moving components, some of which were acidic to Bromophenol Blue.

A further quantity of fraction 3 (3 g.) was shaken with methanol, and the insoluble material (50 mg.) was collected on the centrifuge and thoroughly washed with methanol. Hydrolysis with formic acid and subsequent paper chromatography of this insoluble material {$[\alpha]_D$ −31° ± 3° (*c* 1·6 in $CHCl_3$)} suggested a similar composition to that obtained as above during acid-hydrolysis.

Methylation of a further quantity of the alkali-stable polysaccharide (35 g.) by the above method gave a methylated derivative (25 g.; OMe, 39·8%) which had $[\alpha]_D$ −110° ± 3° (*c* 1·1 in $CHCl_3$) and was completely soluble in methanol. When this product was hydrolysed in N-sulphuric acid–methanol (3 : 1 v/v) for 18 hr. no insoluble material separated.

The methylated sugars present in fraction 3A were separated efficiently and rapidly on a cellulose column (70 × 4 cm.) by using as the eluent benzene and subsequently benzene–ethanol mixtures.[3] The effluent emerged at a rate of *ca.* 2 ml. per min. and after examination on paper chromatograms was divided into three fractions. Benzene separated all of the tri-*O*-methylpentose, and the effluent (fraction i) was collected from 1½ to 2½ hr. after the mixture had been placed on the column. Benzene–ethanol (9 : 1 v/v) removed the di-*O*-methylpentose during 12—16 hr (fraction ii). After 20 hr., benzene–ethanol (1 : 1 v/v) was used as solvent, and mono-*O*-methylpentose was then detected in the effluent up to 28 hr. (fraction iii). Finally, ethanol was used to elute a small residue (100 mg.) containing traces of arabinose and methylated uronic acids. The fractions were evaporated to syrups and dried.

Fraction i (1·1 g.) had $[\alpha]_D$ +18° ± 3° (*c* 0·6) [Found: OMe, 50·5. Calc. for $C_5H_7O_2(OMe)_3$ and $C_6H_8O_2(OMe)_4$: OMe, 48·5 and 52·5% respectively]. Paper chromatography revealed two components (R_{Rh} 2·25 and 2·07) which were indistinguishable from 2,3,5-tri-*O*-methylarabinose and 2,3,4,6-tetra-*O*-methylgalactose. A solution of the syrup (120 mg.) in a mixture of ethanol (3 ml.) and aniline (50 mg.) was heated under reflux and then evaporated to give

crystals. After trituration with ether, recrystallisation from ethanol afforded *N*-phenyl-D-galactopyranosylamine tetramethyl ether (20 mg.), m. p. and mixed m. p. 188° [Found: OMe, 39·9. Calc. for $C_{12}H_{13}ON(OMe)_4$: OMe, 40·0%].

Another portion of the syrup (440 mg.) was oxidised with bromine-water for 7 days in the dark and, after neutralisation with silver carbonate and evaporation, a syrupy mixture of lactones (340 mg.) was obtained. Three components (R_F 0·88, 0·78, and 0·67) were detected on paper chromatograms and so the mixture was separated on sheet-paper chromatograms with benzene–ethanol–water as solvent. The faster-moving component (35 mg.) was 2,3,5-tri-*O*-methyl-L-arabinolactone contaminated with a little 2,3,4,6-tetra-*O*-methyl-D-galactonolactone and had $[\alpha]_D$ +16° (initial value) ⟶ −8° (60 hr.; *c* 0·6) [Found: OMe, 49·4. Calc. for $C_5H_7O_3(OMe)_3$: OMe, 48·9%]. The next component (11·1 mg.) crystallised and was purified by sublimation under reduced pressure. After recrystallisation from ether, the lactone had m. p. 100°, undepressed on admixture with 2,3,6-tri-*O*-methyl-D-galactonolactone [Found: OMe, 41·9. Calc. for $C_6H_7O_3(OMe)_3$: OMe, 42·3%]. The slowest-moving component (110 mg.) had $[\alpha]_D$ −35° (initial value) ⟶ −25·2° (7 days; final value; *c* 1·4) [Found: OMe, 35·6; equiv. wt., 173. Calc. for $C_5H_6O_3(OMe)_2$: OMe, 35·2%; equiv. wt., 176]. On treatment with methanolic ammonia, a crystalline amide was produced which co-chromatographed with 2,3-di-*O*-methylarabonamide and had m. p. and mixed m. p. 164° [Found: OMe, 31·9. Calc. for $C_5H_9O_3N(OMe)_2$: OMe, 32·1%].

Fraction ii (0·96 g.) had $[\alpha]_D$ +93·5° ± 1° (c 2·1) [Found: OMe, 36·2. Calc. for $C_5H_8O_3(OMe)_2$ and $C_6H_9O_3(OMe)_3$: OMe, 34·8 and 41·8% respectively]. Paper chromatography revealed at least two components which resembled 2,3-di-*O*-methylarabinose and 2,3,6-tri-*O*-methylgalactose. A portion of the syrupy mixture (70 mg.) was heated under reflux with ethanol (2 ml.) and aniline (35 mg.) for 1 hr. and after evaporation the product separated on a sheet-paper chromatogram using butan-1-ol–ethanol–water as solvent. After detection of the anilide on guide strips with *p*-anisidine hydrochloride, elution of the appropriate area of the chromatogram afforded *N*-phenyl-L-arabinosylamine 2,3-dimethyl ether (10 mg.), m. p. 137° [Found: OMe, 24·1; N, 5·4. Calc. for $C_{11}H_{13}O_2N(OMe)_2$: OMe, 24·5; N, 5·5%].

A further portion of the mixture of sugars (0·75 g.) was oxidised with bromine water as above; paper chromatography then revealed at least three lactones, which were also separated as above. The component with R_F 0·78 (11·6 mg.) crystallised and had m. p. 100°, unchanged on admixture with 2,3,6-tri-*O*-methyl-D-galactone-1 ⟶ 4-lactone [Found: OMe, 42·4. Calc. for $C_6H_7O_3(OMe)_3$: OMe, 42·3%]. The component with R_F 0·67 (150 mg.) had $[\alpha]_D$ −35° (initial value) ⟶ −25° (8 days; final value; *c* 1·4) [Found: OMe, 35·4; equiv. wt., 175. Calc. for $C_5H_6O_3(OMe)_2$: OMe, 35·2%; equiv. wt., 176]. After hydrolysis of the lactone with aqueous alkali, the sodium salt was oxidised with sodium metaperiodate, 0·96, 0·99, and 1·00 mol. being consumed per mol. of di-*O*-methylpentonic acid after 3, 6, and 24 hr. respectively: 0·93 mol. of formaldehyde was produced after 3 hr. When treated with methanolic ammonia, the lactone gave 2,3-di-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 164° [Found: OMe, 32·4. Calc. for $C_5H_9O_3N(OMe)_2$: OMe, 32·1%].

The third component (130 mg.) had $[\alpha]_D$ +78° ± 2° (*c* 1·2) and did not mutarotate (Found: OMe, 25·5%). It could not be related to any known pentono- or hexono-lactone.

Fraction iii (1 g.) had $[\alpha]_D$ +83° ± 3° (*c* 1·0) [Found: OMe, 23·0. Calc. for $C_5H_9O_4(OMe)$ and $C_6H_{10}O_4(OMe)_2$: OMe, 18·9 and 29·8% respectively]. A portion (0·1 g.) was oxidised with bromine-water, and the product examined on paper chromatograms. At least three lactones were detected. Another portion (0·1 g.) was shaken with anhydrous copper sulphate (0·1 g.) and dry acetone (10 ml.) for a week. After filtration and evaporation of the acetone solution, 3,4-*O*-isopropylidene-2-*O*-methyl-L-arabinose [17] was obtained, having m. p. 115° and $[\alpha]_D$ +108° ± 1° (*c* 0·6 in ethanol) [Found: C, 52·8; H, 7·6; OMe, 15·2. Calc. for $C_8H_{13}O_4(OMe)$: C, 52·9; H, 7·8; OMe, 15·2%]. A solution of the isopropylidene derivative (40 mg.) in ethanol (2 ml.) and aniline (18 mg.) was heated under reflux for ½ hr. Decolorisation with charcoal and concentration afforded a crystalline anilide (44 mg.), m. p. 142° [Found: C, 64·5; H, 7·4; N, 4·8; OMe, 12·1. Calc. for $C_{14}H_{18}O_3N(OMe)$: C, 64·6; H, 7·4; N, 5·0; OMe, 11·1%].

Another portion of fraction iii (0·4 g.) was heated under reflux with methanolic hydrogen chloride (1·5% w/w; 10 ml.) until the solution was non-reducing (*ca.* 10 hr.). The solution was neutralised with silver carbonate and concentrated to a syrup (264 mg.). A portion of this syrup (13·2 mg.) was dissolved in water (*ca.* 15 ml.), 0·3M-sodium metaperiodate (1 ml.) was

[17] Jones, Kent, and Stacey, *J.*, 1947, 1341.

added, and the whole was made up to 25 ml. with water. Aliquot samples (5 ml.) were withdrawn at intervals, and the consumption of periodate was determined; after 6 hr. a constant value of 0·68 mol. was consumed, on the assumption that a methyl mono-*O*-methylpentopyranoside had been oxidised. The remainder of the syrup (251 mg.) was similarly oxidised with sodium metaperiodate for 18 hr. Excess of periodate was then destroyed with ethylene glycol (0·2 ml.); the solution was evaporated to a syrup which was heated in N-sulphuric acid (5 ml.) at 95—100° for 7 hr. and neutralised with barium carbonate. After concentration to a small volume, the solution was passed into a squat charcoal column [18] and eluted first with water to remove inorganic ions and then with 5% ethanol. The eluate was concentrated to give syrupy 3(?)-*O*-methyl-L-arabinose (27 mg.) which had $[\alpha]_D$ +96° ± 4° (*c* 0·5) [Found: OMe, 18·5. Calc. for $C_5H_9O_4(OMe)$: OMe, 18·9%]. An attempt to prepare the phenylosazone of this sugar was unsuccessful and its conclusive identification awaits further investigation. Elution of the charcoal column with 10% ethanol afforded, after concentration, a syrup (6 mg.) which slowly crystallised. Recrystallisation from chloroform gave 2,4-di-*O*-methyl-D-galactose, m. p. and mixed m. p. 77°. An *X*-ray photograph and paper chromatograms of the di-*O*-methyl derivative were identical with those of an authentic specimen.[5] The syrup obtained by evaporation of the chloroform was examined on paper chromatograms and was observed to contain a substance that was indistinguishable from 3-*O*-methylrhamnose.

Periodate Oxidation of the Polysaccharide.—The de-ionised polysaccharide (201·8 mg.) was dissolved in water, 0·3M-sodium metaperiodate (10 ml.) added, and the whole made up to 100 ml. with water. Samples (5 ml.) were withdrawn in duplicate at various intervals of time for the determination of the consumption of periodate [19] and the acid liberated.[20] The polysaccharide (101·5 mg.) was also oxidised with 0·3M-sodium metaperiodate (5 ml.) in 0·2N-sodium acetate buffer (pH 3·6; 50 ml.). Blanks were run concurrently with each oxidation, and the reactions were all carried out in the dark and at room temperature. The results are summarised in the following Table:

Time (hr.)		0·5	10	20	50	75	95
Periodate uptake (ml. of 0·01N-thiosulphate)	unbuffered	0·8	7·2	9·3	9·8	10·2	10·3
	buffered	0·3	6·0	8·2	9·5	9·8	9·8
Acid liberated (ml. of 0·01N-alkali)		—	—	0·75	0·85	0·9	0·95

Extrapolation of the rate curves obtained for the periodate uptake under both unbuffered and buffered conditions showed a rapid uptake of 0·68 ± 0·04 mole per mole of anhydropentose after 20 hr., which corresponds to a consumption of 1 mole of periodate per 210 g. of polysaccharide. Similarly the extrapolated titre of 0·7 ml. of 0·01N-sodium hydroxide indicated that after 20 hr. 1 mole of formic acid was liberated per 1440 g. of polysaccharide.

The above procedure was repeated on a larger scale by dissolving the polysaccharide (5 g.) in water, adding 0·3M-sodium metaperiodate (250 ml.), and making the whole up to 2 l. with water. After 30 hr., sufficient ethylene glycol (*ca.* 6 ml.) was added to destroy the excess of periodate, and the solution was left for 30 min. The solution was then dialysed against tap water until free from iodate, as indicated by the acidified starch–iodide test, to give a solution *B* (2·1 l.) having α_D −0·18° (1 dm. tube).

A portion of solution *B* (100 ml.) was poured into an excess of ethanol, and the precipitated oxypolysaccharide collected in the centrifuge, washed with alcohol and ether, and dried. To another portion of solution *B* (1·6 l.) was added a solution of phenylhydrazone (10 ml.) in 10% acetic acid (25 ml.) with stirring, and the precipitated phenylhydrazone was collected in a sintered-glass crucible (porosity 4), thoroughly washed with dilute acetic acid and then water, and dried. The filtrate was optically inactive. The pale yellow solid (3·6 g.) had $[\alpha]_D$ −70° ± 6° [*c* 0·3 in pyridine–water (1 : 1 v/v)] (Found: N, 9·9%). The phenylhydrazone (1 g.) was dissolved in ice-cold pyridine (100 ml.), and a solution of diazotised aniline (20 ml.) added dropwise. The product (*NN*-diphenylformazan?) was isolated by pouring the solution into ice-cold water, and the precipitate was collected in a sintered-glass crucible (porosity 4) and purified by repeated precipitation from acetone solution by the addition of light petroleum (b. p. 60—80°), to give a reddish-brown solid (0·76 g.) which was dried (Found: N, 13·1%).

The remainder of solution *A* was evaporated to *ca.* 60 ml. and then made up to 100 ml. with

[18] Andrews, Hough, and Powell, *Chem. and Ind.*, 1956, 658.
[19] Neumüller and Vasseur, *Arkiv Kemi*, 1953, **5**, 235.
[20] Anderson, Greenwood, and Hirst, *J.*, 1955, 225.

water, to give a solution *C* with $[\alpha]_D$ $-0{\cdot}62°$ (1 dm. tube). Evaporation of an aliquot part (10 ml.) to dryness revealed that the solution *C* contained 0·768% of oxypolysaccharide which had $[\alpha]_D$ $-80{\cdot}8°$.

A quantitative estimate of the amount of sodium borohydride consumed by the oxypolysaccharide was made by Lindberg and Theander's method.[21] " AnalaR " boric acid (62 mg.) was dissolved in an aliquot part (10 ml.) of solution *C*, and a portion of this mixture (3 ml.) then reduced with sodium borohydride for 20 hr. The sample consumed the equivalent of 4·35 ml. of hydrogen at N.T.P. which, on the assumption that the original polysaccharide contained only pentose units, suggested that 1·1 mol. of hydrogen were consumed per oxypentose unit.

Sodium borohydride was added to solution *C* (40 ml.), which was then kept for 48 hr., acidified with dilute acetic acid, and dialysed until free from boric acid, as indicated by the quinalizarin test.[22] The solution was poured into a large excess of ethanol, and the precipitate was collected on the centrifuge, washed successively with alcohol and ether, and dried. The reduced oxypolysaccharide (260 mg.) had $[\alpha]_D$ $-31{\cdot}4° \pm 4°$ (*c* 0·9).

Acidic hydrolysates of the oxypolysaccharide, the phenylhydrazone derivative, the " diphenylformazan " derivative, and the reduced oxypolysaccharide were examined on paper chromatograms; arabinose, galactose, glycerol, and tetritol(s) were detected together with traces of rhamnose. Quantitative estimates of galactose and arabinose in each of the above hydrolysates, as determined by the benzidine method,[23] are shown in the annexed table.

	Sample (mg.)	Ribose added (mg.)	Found (mg.)			Yield (%)	
			Arab.	Gal.	Rib.	Arab.	Gal.
Oxypolysaccharide	80·7	11·8	1·05	0·30	4·04	3·3	1·0
Phenylhydrazone	100·0	32·3	1·33	0·34	7·00	5·4	1·4
Diphenylformazan(?)	72·6	22·8	0·51	0·12	3·46	4·1	1·0
Reduced oxypolysaccharide	50·0	23·3	1·16	0·28	3·40	14·0	3·5

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