238. *Mannose-containing Polysaccharides. Part III.* The Polysaccharides in the Seeds of* Iris ochroleuca *and* I. sibirica.

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The polysaccharides isolated from *I. ochroleuca* and *I. sibirica* seeds were found to be very similar. Each was composed of D-glucose and D-mannose in approximately equal amounts, and D-galactose (*ca.* 3%). Examination of the methylated polysaccharides showed that they were composed of chains of glucose and mannose units linked through $C_{(1)}$ and $C_{(4)}$. The galactopyranose residues were linked through $C_{(1)}$ only.

The reserve polysaccharide in the rhizomes of some species of iris is starch, whilst in others it is a fructosan (irisin) (Colin and Augem, *Compt. rend.*, 1927, **185**, 475; Augem, *Rev. gén. botan.*, 1928, **40**, 456, 537, 591; *Chem. Abs.*, 1929, **23**, 634; Schulbach, Knoop, and Liu, *Annalen*, 1933, **504**, 30), which may vary in composition from species to species (Colin and Augem, *Bull. Soc. Chim. biol.*, 1928, **10**, 489). In addition, the endosperms of the ripe seeds of three species (*I. pseudacorus, I. germanica, and I. foetidissima*) are said (*idem, ibid.*, p. 822; *Chem. Abs.*, 1928, **22**, 3682; Augem, *loc. cit.*) to contain a mannoaraban, consisting of mannose 82% and arabinose 18%. These sugars were identified as mannose phenylhydrazone and arabinosazone, m. p. 147° (lit., m. p. 160°), respectively, and their proportions were deduced from the optical rotation ($[\alpha]_D$ +31°) of iris endosperm hydrolysates; arabinose was also determined colorimetrically (as pentose). Other sugars were not detected. It was of interest to investigate further the possible occurrence of this material, since no polysaccharide composed entirely of these two sugars has yet been examined in detail. An investigation of the seeds of two species of iris (*I. ochroleuca* and *I. sibirica*) was therefore undertaken, but no mannoaraban could be detected, the seeds containing very little arabinose.

The iris seed consists of a hard horny endosperm, 2—3 mm. in diameter, covered by a brown wrinkled testa. Extraction of the endosperms of the two species of iris with 10% aqueous sodium hydroxide yielded thick mucilaginous solutions from which the polysaccharides were precipitated with alcohol. Like other neutral mannose-containing polysac-

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charides so far examined, they formed insoluble copper complexes, thus providing a method of purification. The two polysaccharides showed a striking resemblance to one another; *e.g.*, both contained D-glucose and D-mannose in the main (*ca.* 1 : 1; 97%) and a little (3%) D-galactose, and the optical rotations of the polysaccharides and of their acetyl and methyl derivatives were similar. Several precipitations of the *I. sibirica* polysaccharide as its copper complex caused no appreciable change in its composition, and as starch was absent it is likely that the material is one polysaccharide compounded of the three hexoses.

An examination of the methyl sugars produced by fission of the methylated polysaccharides also suggested that the polysaccharides were similar in structure. The trimethyl fraction accounted for about 93% (on a molecular basis) of the total methyl sugar mixture in both cases, and consisted of equal parts of 2 : 3 : 6-trimethyl D-glucose and 2 : 3 : 6-trimethyl D-mannose. Such a mixture would have $[\alpha]_D$ +32°, and approximately this value was found for both the trimethyl fractions. The tetramethyl fractions formed 3·7% (*I. ochroleuca*) and 3·2% (*I. sibirica*) of the total methyl sugars, and consisted largely of 2 : 3 : 4 : 6-tetramethyl D-galactose, thus accounting for all the galactose found in the original polysaccharides. Very small amounts of tetramethyl glucose and tetramethyl mannose were also detected. This evidence suggests that D-galactose forms the majority of the end-groups in the polysaccharides, but that there are also a small number of glucose and mannose end-groups. Whether these represent end-groups present in the original polysaccharides, or whether they were produced during isolation of the polysaccharides is unknown. Small amounts (*ca.* 3%) of dimethyl sugars were present, but none was identified: probably they arose in part from incomplete methylation of the polysaccharides, but they may also represent points of branching.

The possibility that the polysaccharides are mixtures is compatible with the evidence available. Thus, the galactose end-group may have arisen from the presence of a small amount of galactomannan (cf. Parts I and II), which would also give an insoluble copper complex; in this case the iris polysaccharides were mixtures of glucomannan with a small proportion of galactomannan. However, in view of the similarity, especially in composition, between the two iris polysaccharides, we tend to the view that they are not mixtures. On the above evidence and the assumption that all the sugar residues are in the pyranose form, the polysaccharides appear to consist of chains of D-glucose and D-mannose units linked glycosidically, in a sequence as yet undetermined, through positions 1 and 4, and terminated at the non-reducing ends largely by D-galactopyranose residues, a small number being terminated by glucopyranose and mannopyranose units.

The amount of end-group as determined by the methylation procedure corresponds to repeating units of 25 and 30 hexose residues for the *I. ochroleuca* and *I. sibirica* polysaccharides respectively, whilst one molecule of formic acid is produced from 19 and 12 hexose residues respectively when the polysaccharides are oxidised with potassium metaperiodate. In the case of a linear polysaccharide composed of x sugar units, including a reducing end-group, the methylation results will give a repeating unit of x sugar units, whereas on oxidation of the unmethylated polysaccharide with periodate, one molecule of formic acid will be produced per $x/3$ sugar units. If the polysaccharide of x units possesses n points of branching, these figures are $x/(n + 1)$ and $x/(n + 3)$ respectively. The values become more nearly equal, the greater the degree of branching of the polysaccharide. Consideration of the results with the two iris polysaccharides suggests that the *I. ochroleuca* polysaccharide is of the branched-chain type (with, apparently, an average of five or six branches per molecule and a degree of polymerisation of 150—175 hexose units), whereas the *I. sibirica* polysaccharide may be linear, or branched very infrequently, and have a smaller molecular weight. However, since the original polysaccharides were isolated by extraction with warm 10% sodium hydroxide solution, and the effect of this procedure on the reducing ends of the polysaccharide molecules is unknown, the exact significance of the periodate results is uncertain. This is especially true with the *I. sibirica* polysaccharide because of the difficulty of determining the end-point of this oxidation. In this case, the conditions of oxidation were altered to obtain a solution of the polysaccharide. It is unlikely that at the pH of the oxidation (*ca.* 5), complex formation due to the presence of sulphate ions would interfere with the normal course of the oxidation [cf. however, Bell,

Palmer, and Thomas (*J.*, 1949, 1536), who found differences from the normal reaction when the oxidation was carried out in the presence of sulphate and under alkaline conditions]. The estimates of the degree of polymerisation of the two iris polysaccharides, by their reducing power, indicate that the *I. ochroleuca* polysaccharide has a considerably greater molecular weight than the other, but the values obtained bear no clear relation to the methylation and periodate results, and in any case they must be considered unreliable (cf. Lansky, Kooi, and Schoch, *J. Amer. Chem. Soc.*, 1949, **71**, 4066) because they vary with the conditions used [but compare the results of Chanda, Hirst, Jones, and Percival (*J.*, 1950, 1289) with esparto xylan] and because of the uncertain nature of the reducing end-groups of the polysaccharides. The possibility of a difference in the molecular weights of the two polysaccharides may be supported by the fact that, although they are both soluble in dilute alkali, the *I. ochroleuca* polysaccharide is precipitated when the solution is neutralised, whilst the other is not.

It is possible that some of the formic acid produced by periodate oxidation of the polysaccharides arises from hexose units linked through $C_{(1)}$ and $C_{(6)}$ only. Calculation shows that if all the formic acid arises from such sugar units, the maximum amount of 2 : 3 : 4-trimethyl hexose obtained by hydrolysis of the methylated polysaccharides would be 5% and 8·5% for the *I. ochroleuca* and *I. sibirica* polysaccharides respectively. However, no evidence for any trimethyl hexoses other than the 2 : 3 : 6-derivatives of glucose and mannose was obtained by chromatography of the eluates from the hydrocellulose column, or otherwise. It is evident that an exact determination of the degree of branching of these polysaccharides cannot yet be made by chemical means, and that molecular-weight determinations by physical means will be required.

To summarize, the polysaccharides isolated from the two species of iris seeds resemble one another closely, but may not be identical. They differ from the galactomannans (Parts I and II) in that they do not possess a highly branched structure. However, in both types of polysaccharide all the galactose units are present as end groups, and the methylated polysaccharides yield 2 : 3 : 6-trimethyl D-mannose as one of the products of hydrolysis.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper by the descending method (Partridge, *Biochem. J.*, 1948, **42**, 238). The following solvent systems were used, (*c*) being used for methylated sugars only : (*a*) *n*-butanol–ethanol–water (40 : 11 : 19; v/v) (*b*) ethyl acetate–acetic acid–water (9 : 2 : 2; v/v); (*c*) benzene–ethanol–water (169 : 47 : 15; v/v; top layer clarified with ethanol). For further details see Part II (*loc. cit.*). Ammoniacal silver nitrate and *p*-anisidine hydrochloride sprays were used.

Hydrolyses were performed with N-sulphuric acid at 95—100°, unless otherwise stated, and solutions were concentrated under reduced pressure. Optical rotations were determined at 18°. The analyses were carried out by Mr. W. Eno, Bristol.

Extraction of the Polysaccharides.—The whole seeds were reduced to coarse powders by milling, whereupon most of the broken seed-coats could be removed by flotation in water. Chromatography (solvent *b*) showed that the sugars produced by hydrolysis of the endosperm for 16 hours were mainly glucose and mannose, in approximately equal amounts, with much smaller amounts of galactose, arabinose, and xylose.

The endosperms were unaffected by boiling water, but with hot aqueous alkali they formed a gelatinous mass which partly dissolved to give a viscous brown solution. A quantity of each polysaccharide was prepared as follows : The milled seed (60 g.) was extracted for 3 hours at 50° with 2% sodium sulphite solution (2 × 1 l.) and then with water at 70—80° until the extracts were nearly colourless. The aqueous layer was decanted each time, thus removing most of the broken seed-coats. The residual endosperms were then extracted with sodium hydroxide solution (10% w/v; 3 × 800 c.c.) at *ca.* 50°, with frequent stirring. The viscous supernatant extracts were isolated on the centrifuge, combined, and poured into alcohol (2 vols.). The precipitate, which contained protein, was washed with aqueous alcohol until the washings were colourless, and dissolved in warm N-sodium hydroxide, from which solution the polysaccharide copper complex was precipitated by the slow addition, with continuous stirring, of Fehling's solution (50 c.c.). After regeneration with ice-cold 0·5N-hydrochloric acid (cf. Part II), the polysaccharide was twice precipitated with alcohol from its solution in dilute alkali, then washed

with alcohol, acetone, and ether, and dried under reduced pressure. The two polysaccharides so obtained had the following properties.

Polysaccharide from	Yield (%) from whole seed	$[\alpha]_D$ (in 2N-NaOH)	N (%)	Sulphated ash (%)
I. ochroleuca	20	$-25° \pm 2°$ (*c*, 0·7)	0·1	3·1
I. sibirica	18	$-26° \pm 3°$ (*c*, 0·7)	0·3	4·7

The *I. sibirica* polysaccharide (material A) was four times precipitated from alkaline solution with Fehling's solution, the polysaccharide being regenerated after each precipitation with ice-cold 0·5N-hydrochloric acid. The product (B) (Found : sulphated ash, 1·1%) was used in the following experiments.

The polysaccharides were both buff-coloured powders, which swelled, but failed to dissolve, in water. They formed solutions in dilute alkali which, after neutralisation with sulphuric acid, did not give a blue colour with iodine solution. The *I. ochroleuca* polysaccharide was slowly precipitated in a gelatinous form when its solution in alkali was neutralised.

Hydrolysis of the Polysaccharides.—Heating the polysaccharides at 100° with dilute acid resulted in their incomplete hydrolysis. More complete hydrolysis was effected with formic acid : the polysaccharide (0·5 g.) was dissolved in anhydrous formic acid (30 c.c.), water (5 c.c.) was added, and the solution heated at 100° for 12 hours. It was then concentrated to a syrup, which was heated with acid (5 c.c.) for 1 hour to hydrolyse any formyl esters, neutralised (Amberlite IR-4B ion-exchange resin), and concentrated.

The resultant sugar mixtures were chromatographed on hydrocellulose (22 × 2 cm.) as described by Hough, Jones, and Wadman (*J.*, 1949, 2511), with *n*-butanol half saturated with water as the mobile phase. The same sugars were isolated in each case ($[\alpha]_D$'s are equil. values in water) : From *I. ochroleuca* polysaccharide, D-glucose, m. p. 146°, $[\alpha]_D$ +51·6°, D-mannose, m. p. 132°, $[\alpha]_D$ +14·5°, and galactose 1-methyl-1-phenylhydrazone, m. p. 185°; from *I. sibirica* polysaccharide, D-glucose, m. p. 146°, $[\alpha]_D$ +51°, D-mannose, m. p. 132°, $[\alpha]_D$ +14°, and galactose 1-methyl-1-phenylhydrazone, m. p. 186° (mixed m. p.s of all these compounds with authentic specimens showed no depression). The galactose derivatives (later identified as D, see below) were each isolated from mixtures of galactose and glucose; after being warmed with dilute hydrochloric acid, each yielded a sugar which was indistinguishable from galactose on the paper chromatogram.

For quantitative analyses, the polysaccharides (*ca.* 50 mg.) were hydrolysed with 90% formic acid (3 c.c.) as above; ribose was added to the hydrolysates, which after dilution with water to 30 c.c. were concentrated to small volumes and any formyl esters hydrolysed with acid. After neutralisation (Amberlite IR-4B resin), the solutions were concentrated to syrups, which were separated into their components by partition chromatography on sheets of filter-paper by elution with solvent system (*a*) for *ca.* 90 hours. After extraction from the papers, the sugars were estimated by the periodate oxidation method of Hirst and Jones (*J.*, 1949, 1659).

To the hydrolysate of the *I. ochroleuca* polysaccharide (48·0 mg.) was added ribose (20·0 mg.) (Found : galactose + glucose, 1·84, 1·19; mannose, 1·835, 1·18; ribose, 1·55, 0·975 mg.). If complete recovery of the ribose is assumed, these results indicate that hydrolysis of the polysaccharide yielded : galactose + glucose, 21·4, 22·0, and mannose, 21·3, 21·9 mg. (calc. as $C_6H_{10}O_5$). The ratio (galactose + glucose) : mannose is thus very close to 1 : 1. In another experiment, the chromatograms were irrigated with solvent (*a*) for 8 days, in order to separate the galactose and glucose, during which time the ribose was lost off the bottom of the paper (Found : galactose, 0·195, 0·15; glucose, 2·75, 2·04; mannose, 2·99, 2·22 mg.). The corresponding ratios of these three sugars are 1·0 : 14·1 : 15·3 and 1·0 : 13·6 : 14·8.

To hydrolysates of the *I. sibirica* polysaccharide [(A) 56·2, and (B) 60·4 mg.] was added ribose (17·2 and 21·4 mg. respectively) [Found for (A) : galactose, 0·23, 0·17; glucose, 4·69, 3·74; mannose, 4·82, 4·02; ribose 2·92, 2·31 mg. For (B) : galactose, 0·26, 0·19; glucose 5·75, 4·05; mannose, 5·86, 4·36; ribose, 4·23, 3·04 mg.]. Again on the assumption of complete recovery of the ribose, these results correspond to the production of : From (A) : galactose, 1·22, 1·14; glucose, 24·9, 25·0; mannose, 25·5, 26·9 mg. (total recovery, 92, 94%), and from (B) : galactose, 1·18, 1·21; glucose, 26·2, 25·7; mannose, 26·7, 27·6 mg. (total recovery 90, 90%) (sugars calc. as $C_6H_{10}O_5$). The figures represent average galactose : glucose : mannose ratios of, for (A) : 1·0 : 21·2 : 22·3; for (B), 1·0 : 21·7 : 22·7.

Acetyl and Methyl Derivatives.—For acetylation, the polysaccharides (0·5 g.) were each dissolved in cold anhydrous formic acid (20 c.c.), and the viscous solutions added dropwise to ice-cold mixtures of pyridine (100 c.c.) and acetic anhydride (50 c.c.). When reaction had

ceased, the precipitates were filtered off and reacetylated at 100° with pyridine (50 c.c.) and acetic anhydride (50 c.c.). The materials dissolved slowly, and after 2 hours the solutions were poured on ice. The precipitates were soluble in acetone only after another similar acetylation (yield, *ca.* 0·4 g. in each case).

For methylation, the *I. ochroleuca* polysaccharide (4 g.) was dissolved in sodium hydroxide solution (40% w/v; 120 c.c.), and methyl sulphate (100 c.c.) added dropwise with continuous stirring and cooling. Next morning more sodium hydroxide (200 c.c.) and methyl sulphate (200 c.c.) were added. When the reaction had ceased, the solution was neutralised with acetic acid, dialysed to remove the inorganic material, and concentrated to *ca.* 50 c.c. Acetone (50 c.c.) was added, and the methylation continued by the alternate additions (3 times) of sodium hydroxide (40 g.) and methyl sulphate (90 c.c.) at room temperature. Inorganic material was then removed by dialysis, and the resulting clear aqueous solution extracted with chloroform. Evaporation of the chloroform left the methylated polysaccharide (2·8 g.), which was dissolved in methyl iodide (25 c.c.), and the solution boiled under reflux overnight with silver oxide (10 g.). The isolated product (2·7 g.; OMe, 42·2%) was further treated with Purdie's reagent. A portion (2·2 g.) of the product was fractionated by extraction with boiling mixtures of chloroform and light petroleum (b. p. 40—60°): the main fraction, a crisp yellow solid (1·86 g.), was soluble in 80%, but insoluble in 90% light petroleum.

The *I. sibirica* polysaccharide (5·5 g.) was methylated similarly. After eight methylations the chloroform-soluble product (4·7 g.) was fractionated as above, giving mainly one fraction (4·2 g.), soluble in 80%, but insoluble in 90%, light petroleum (b. p. 40—60°).

The acetyl and methyl derivatives are compared in the following Table.

	Acetyl derivative		Methyl derivative	
Polysaccharide from	Acetyl (%)	$[\alpha]_D$ (in $COMe_2$)	OMe (%)	$[\alpha]_D$ (in $CHCl_3$)
I. ochroleuca	42·5	−14° (*c*, 1·0)	43·3	−9° (*c*, 1·1)
I. sibirica	43·1	−13° (*c*, 1·2)	43·5	−11° (*c*, 1·4)

Hydrolysis of the Methylated Polysaccharides.—A solution of the methylated *I. ochroleuca* polysaccharide (0·97 g.) in methanolic hydrogen chloride (2% w/w; 30 c.c.) was boiled under reflux for 12 hours, neutralised with a slurry of silver carbonate in methanol, and filtered, and the filtrate concentrated to a syrup. The methylglycosides were hydrolysed at 100° in N-hydrochloric acid (30 c.c.) for 16 hours. After neutralisation (silver carbonate) the solution was filtered and hydrogen sulphide passed in, to remove silver ions; since the sulphide so formed could not be filtered off, the solution was concentrated to a small volume and acetone (20 c.c.) added. The precipitate was filtered off and washed with hot acetone, and the filtrate and washings were combined and concentrated to a syrup (0·93 g.).

Similarly, the methylated *I. sibirica* polysaccharide (1·54 g.) yielded a syrupy mixture of reducing sugars (1·44 g.) with $[\alpha]_D$ +30° ± 3° (*c*, 1·0 in H_2O).

Separation of the Methylated Sugars.—Paper chromatographic examination indicated that each mixture consisted mainly of trimethyl hexose, which gave only one spot (R_G 0·89, solvent *a*), and was indistinguishable from both 2 : 3 : 6-trimethyl glucose and 2 : 3 : 6-trimethyl mannose. The small amount of end group corresponded to 2 : 3 : 4 : 6-tetramethyl galactose (R_G 0·96), whilst the dimethyl hexose gave an elongated spot and appeared to be a mixture.

The methyl sugar mixtures were fractionated by partition chromatography on cellulose (28 × 4 cm.), with solvent (*c*) as the mobile phase (for details, see Part II, *loc. cit.*). Evaporation of appropriate parts of the eluate gave three syrupy fractions in each case, which were purified by dissolution in water, acetone, and ether (or acetone–ether), with intermediate filtration and evaporation, and dried.

The *I. ochroleuca* mixture of methyl sugars (0·84 g.) gave the following fractions:

Fraction (1) (39 mg.) (Found: OMe, 50·8. Calc. for tetramethyl hexose, $C_{10}H_{20}O_6$: OMe, 52·5%) gave two spots of unequal intensity on the chromatogram (R_G 0·95 and 1·00). These materials were separated on a sheet-paper chromatogram, by using solvent (*c*) and there were obtained:

Fraction (1*a*) (28 mg.) (R_G 0·95), with $[\alpha]_D$ +100° (*c*, 0·6 in H_2O). When boiled with aniline in alcohol it gave *N*-phenyl-D-galactopyranosylamine tetramethyl ether (10 mg.), m. p. and mixed m. p. 189°, $[\alpha]_D$ +37° (equil. value, *c*, 0·5 in $COMe_2$).

Fraction (1*b*) (4 mg.) (R_G 1·00). Partial demethylation was achieved with hydrobromic acid (48% w/w; 1 c.c.) at 100° for 10 min. (Hough, Jones, and Wadman, *J.*, 1950, 1702). The products included both glucose and mannose (by paper chromatography), indicating that the fraction consisted of tetramethyl glucose and tetramethyl mannose.

Fraction (2) (710 mg.) (Found: OMe, 41·7. Calc. for trimethyl hexose: OMe, 41·9%) had

$[\alpha]_D$ +34° (*c*, 1·2 in H_2O) and gave only one spot (R_G 0·89) on the chromatogram. A portion (*ca.* 0·3 g.) was dissolved in ether, and the solution seeded with 2 : 3 : 6-trimethyl D-glucose. After 7 days at 0° 30 mg. of this sugar had crystallised, which after recrystallisation from ether had m. p. and mixed m. p. 120°, and $[\alpha]_D$ +95° (initial)→+70° (equil. value; *c*, 0·7 in H_2O) $[\alpha]_D$ −40° (equil. value; *c*, 0·5 in 1% methanolic hydrogen chloride) (Found: OMe, 41·1. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The mixture from which this sugar crystallised was oxidised with bromine water in the usual way. Attempts to crystallise 2 : 3 : 6-trimethyl D-mannonolactone from the resultant syrup were unsuccessful. Accordingly it was boiled with an alcoholic solution of phenylhydrazine, whereupon crystalline 2 : 3 : 6-trimethyl D-mannonic acid phenylhydrazide (52 mg.) was produced, which after recrystallisation from ethanol, had m. p. and mixed m. p. 132°, $[\alpha]_D$ −18° (*c*, 0·7 in H_2O) (Found: N, 8·4. Calc. for $C_{15}H_{24}O_6N_2$: N, 8·5%). The residual mixture of phenylhydrazides gave another small crop of the above phenylhydrazide, but then could not be induced to crystallise further.

Fraction (3) (42 mg.) (Found: OMe, 28·3. Calc. for dimethyl hexose: OMe, 29·8%), when examined on the paper chromatogram, appeared to contain at least three compounds which were incompletely separated from each other. Comparison with authentic samples indicated that the 2 : 3-dimethyl derivatives of glucose and mannose might be present in the fraction, and demethylation of a portion yielded both these parent sugars. No crystalline derivatives could be isolated from this fraction.

The *I. sibirica* methyl sugar mixture (1·14 g.) gave the following fractions:

Fraction (1′) (38 mg.) (Found: OMe, 50·9%) had $[\alpha]_D$ +97° (*c*, 0·8 in H_2O). It contained only *ca.* 5% of material with R_G 1·0; the remainder had R_G 0·95. When boiled with alcoholic aniline it yielded *N*-phenyl-D-galactopyranosylamine tetramethyl ether (20 mg.), m. p. and mixed m. p. 188°, $[\alpha]_D$ +38° (equil. value; *c*, 0·6 in $COMe_2$).

Fraction (2′) (960 mg.) (Found: OMe, 40·7%) had $[\alpha]_D$ +32° (*c*, 4·4 in H_2O). A portion (*ca.* 20 mg.) was oxidised in 0·2M-sodium metaperiodate solution (5 c.c.) for 18 hours; the product, isolated by continuous extraction with ether, contained no 2 : 3 : 5-trimethyl arabinose (by paper chromatography), indicating that this fraction did not contain the 3 : 4 : 6-trimethyl derivatives of glucose and mannose. A portion (*ca.* 350 mg.), dissolved in ether, gave, when seeded, 2 : 3 : 6-trimethyl D-glucose (34 mg.); after recrystallisation from ether it had m. p. and mixed m. p. 122° and $[\alpha]_D$ +91° (initial value)→+69° (equil. value; *c*, 0·6 in H_2O) [Found: OMe, 41·3. Calc. for $C_6H_9O_3(OMe)_3$: OMe, 41·9%]. A further portion of the syrup (230 mg.) was oxidised with bromine water, and the syrupy lactones boiled with alcoholic phenylhydrazine. Crystalline 2 : 3 : 6-trimethyl D-mannonic acid phenylhydrazide (50 mg. in two crops), m. p. and mixed m. p. 132°, $[\alpha]_D$ −17° (*c*, 0·6 in H_2O), but no other crystalline material was isolated from the reaction mixture.

Fraction (3′) (20 mg.) (Found: OMe, 28·8%) had $[\alpha]_D$ +24° (*c*, 0·4 in H_2O); it gave an elongated spot on the chromatogram, and consisted of derivatives of glucose and mannose, since both these sugars were produced on demethylation with hydrobromic acid; it was not further examined.

Quantitative Examination of the Methyl Sugar Mixtures.—The relative amounts of tetra-, tri-, and di-methyl sugars were estimated, after chromatographic separation on filter-paper with solvent (*c*), by the alkaline hypoiodite method (Hirst, Hough, and Jones, *J.*, 1949, 928; Jones, *J.*, 1950, 3292). 1 c.c. of 0·1N-iodine was used for the tetra- and di-, and 5 c.c. for the tri-methyl fractions; oxidation was allowed to proceed for 17 hours and the excess of iodine was titrated, after acidification, with 0·01N-sodium thiosulphate. The results are given in the following Table.

Polysaccharide from	Consumption of 0·01N-I_2 (c.c.)			Corresponding mol. ratios		
	"Tetra"	"Tri"	"Di"	"Tetra"	"Tri"	"Di"
I. ochroleuca	0·68, 0·77	15·7, 20·0	0·90, 1·06	1·0, 1·0	23, 26	1·3, 1·4
I. sibirica	1·06, 0·82	28·3, 26·8	0·92, 0·62	1·0, 1·0	27, 33	0·9, 0·8

Periodate Oxidation.—The *I. ochroleuca* polysaccharide was oxidised with potassium periodate, as described by Brown, Halsall, Hirst, and Jones (*J.*, 1948, 28). The finely powdered material (0·485 g.) was heated with water (25 c.c.) at 70—80° for 5 hours; this softened it, but it showed no signs of dissolving. The suspension was cooled, and water (50 c.c.), potassium chloride (3 g.), and 0·36N-sodium metaperiodate (25 c.c.) were added, bringing the pH to *ca.* 5. The mixture was kept in the dark, and frequently shaken. At intervals, portions (5 c.c.) of the clear supernatant liquid were withdrawn, ethylene glycol (2 c.c.) added, and the formic acid titrated with 0·0109N-sodium hydroxide: a control experiment, containing no polysaccharide, was run concurrently [Found (titres of 0·0109N-alkali corrected for blank): 0·47 (96 hr.), 0·56 (147 hr.),

0·63 (240 hr.), 0·74 (342 hr.), 0·75 c.c. (504 hr.)]. The reaction was apparently complete after 342 hours, but none of the polysaccharide had dissolved. The total yield of formic acid, including that withdrawn for estimation, was 14·4 c.c. of 0·0109N, which corresponds approximately to the formation of one molecule of formic acid from every 19 hexose units.

Oxidation of the *I. sibirica* polysaccharide was carried out as follows. In order to obtain a solution, it (0·186 g.) was dissolved in potassium hydroxide solution (*ca.* 0·1N; 15 c.c.) and the solution brought to pH 7 with 0·1N-sulphuric acid. Potassium sulphate (2 g.) and 0·36N-sodium metaperiodate (18·8 c.c.) were added, giving a total volume of 50 c.c. and a final pH of *ca.* 5. The mixture was kept in the dark, and frequently shaken. At intervals, portions (5 c.c.) of the clear solution were withdrawn, and the formic acid content was determined in the usual way [Found (titres of 0·01N-alkali): 1·32 (190 hr.), 1·53 (284 hr.), 1·80 c.c. (452 hr.)]. Extrapolation to zero time gives a titre of 0·95 c.c., corresponding to a yield of one molecule of formic acid from every 12 hexose residues.

The oxidised polysaccharides were recovered and hydrolysed, and the products examined on the paper chromatogram. The *I. ochroleuca* polysaccharide gave traces of glucose and mannose, and the *I. sibirica* polysaccharide gave traces of one sugar, possibly glucose.

Reducing Power of the Polysaccharides.—In order to remove any traces of alcohol from them, water (30 c.c.) was added to each polysaccharide (*ca.* 1 g.), and the mixtures were evaporated to dryness (thrice). The materials were then dried at 100°/20 mm. for 5 hours. Reducing power was estimated by the alkaline hypoiodite method (Chanda *et al.*, *loc. cit.*). The polysaccharides (50—100 mg.) were treated as follows: (*a*) the sample was dissolved in 2N-sodium hydroxide (5 c.c.), and 0·1N-iodine (1 or 2 c.c.) added; mixtures were kept in the dark for 17 and 41 hours respectively, then acidified with 2N-sulphuric acid (10 c.c.); (*b*) the sample was dissolved in 2N-sodium hydroxide (2 c.c.), and 2N-sulphuric acid (1·8 c.c.), sodium hydroxide–phosphate buffer (pH 11·4) (Ingles and Israel, *J.*, 1948, 810) (4 c.c.), and 0·1N-iodine (2 c.c.) were added; after 17 hours, the mixtures were acidified with 2N-sulphuric acid (5 c.c.). In all cases, the liberated iodine was titrated with 0·01N-sodium thiosulphate. The results corresponded to the presence of one reducing group per (*a*) 20—24 and (*b*) 55—65 hexose residue in the *I. ochroleuca* polysaccharide, and per (*a*) 13—14 and (*b*) 22—23 hexose residues in the *I. sibirica* polysaccharide.

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