The Galactan of Strychnos nux-vomica *Seeds.*

By P. Andrews, L. Hough, and J. K. N. Jones

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Strychnos nux-vomica seeds contain a mixture of polysaccharides which, in all, are composed of galactose, mannose, xylose, and arabinose units (roughly 5 : 2 : 1 : 1 w/w). A galactan has been isolated from the seeds: methylation and periodate studies reveal that its structure consists of chains of β-D-galactopyranose units, the majority being linked through $C_{(1)}$ and $C_{(4)}$, but with a small number of branching points arising from units linked through $C_{(1)}$, $C_{(3)}$, and $C_{(6)}$.

The polysaccharide material in *Strychnos nux-vomica* seed is reported (Bourquelot and Laurent, *Compt. rend.*, 1900, **130**, 1411) to consist of galactose and mannose in the proportions 4 : 1. A galactomannan of this composition would be of great interest since, of those examined hitherto, only one contains more galactose than mannose (cf. Andrews, Hough, and Jones, *J.*, 1952, 2744). Further interest arises from the fact that galactomannans have only been isolated from seeds of the family *Leguminosae*, whereas *Strychnos* belongs to the *Loganiaceae*.

The heterogeneity of the polysaccharide material in *Strychnos nux-vomica* seed was shown by the fact that successive extractions of the seed with hot water and with aqueous alkalis gave polysaccharide fractions which differed widely in composition. In addition to galactose and mannose, xylose and arabinose were found, the relative total amounts of the four sugars being roughly 5 : 2 : 1 : 1 w/w respectively. Addition of Fehling's solution to aqueous solutions of the various fractions resulted in complete precipitation of the mannose- and xylose-containing polysaccharides as copper complexes, with partial precipitation of those containing galactose and arabinose. Evidently the seed contains a mixture of polysaccharides which is probably composed of a galactan, a mannan, a xylan, and an araban; neither the galactan nor the araban forms insoluble copper complexes, but is, to some extent, coprecipitated with the insoluble mannan– and xylan–copper complexes. It was not possible to obtain any one polysaccharide in a pure state from some of the fractions isolated from the seed, by fractional precipitation and fractional extraction with aqueous ethanol or fractional precipitation with Fehling's solution. However, small quantities of galactan were isolated from polysaccharide fractions (composed of galactose, mannose, and small amounts of arabinose) which had been freshly precipitated by alcohol, by extracting them with cold Fehling's solution. Some galactan dissolved in this reagent and was recovered from the solution after removal of salts by dialysis and on ion-exchange resins. On hydrolysis the products from each of several experiments yielded, in addition to galactose, small and differing amounts of arabinose (from about 5% to $< 1\%$), depending on the proportions of arabinose in the original polysaccharide mixtures.

The galactan dissolved easily in water; the solution had $[\alpha]_D$ +69° and after seven hours at 100° in N-sulphuric acid hydrolysis was complete, indicating that in the polysaccharide the D-galactose units exist largely in the pyranose form and are joined by β-links. It is interesting that "galactocarolose" (Haworth Raistrick, and Stacey, *Biochem. J.*, 1937, **31**, 640), which is composed of D-galactofuranose units, had $[\alpha]_{5780}$ −84° in water and was hydrolysed in three hours at 100° in 0·01N-sulphuric acid.

The *Strychnos* galactan was methylated by several treatments with methyl sulphate and sodium hydroxide in the usual way to a methoxyl content of 41·6%, but it resisted further methylation (a fully methylated galactan should have a methoxyl content of 45·6%). This material was easily soluble in chloroform and formic acid, but insoluble in acetone, methanol, methyl iodide, or dioxan, solvents which dissolve many methylated polysaccharides. Its optical rotation ($[\alpha]_D$ −6° in chloroform) suggested the presence of a large proportion of β-links. It is noteworthy that Hirst, Jones, and Walder (*J.*, 1947, 1225) observed a similar resistance to methylation by the galactan of *Lupinus albus*.

Since the methylated product was insoluble in methanol, fission to reducing sugars was accomplished by successive treatments with formic acid and N-sulphuric acid. The mono-

saccharide derivatives so produced were identified as 2 : 3 : 4 : 6-tetra-, 2 : 3 : 6-tri-, 2 : 6-di-, and 2 : 4-di-*O*-methyl-D-galactose, in the molecular proportions of about 1 : 24 : 3 : 1 respectively.

The isolation of a di-*O*-methylgalactose fraction suggests that the polysaccharide has a branched chain structure, but a large proportion of this di-*O*-methyl sugar fraction undoubtedly arises from incomplete methylation. Thus, of the two di-*O*-methylgalactoses isolated, the 2 : 4-isomer, but probably not the 2 : 6-isomer, was derived from the galactose units from which the points of branching originate, for the following reasons. (i) Hydrolysis of an incompletely methylated polysaccharide composed of chains of galactose units linked through $C_{(1)}$ and $C_{(4)}$ should yield some 2 : 6(and/or 3 : 6)-di-*O*-methylgalactose, but only the merest trace, if any, of the 2 : 4-isomer, since the latter can only be derived from the non-reducing end groups. The same considerations apply to the products that may be formed by partial demethylation during hydrolysis of such a polysaccharide. (ii) A careful examination of the tri-*O*-methyl-sugar fraction showed that it consisted solely of 2 : 3 : 6-tri-*O*-methyl-D-galactose, and therefore contained no tri-*O*-methyl-sugar which by loss of one methoxy-group (*i.e.*, through demethylation or incomplete methylation) could give 2 : 4-di-*O*-methyl-D-galactose. (iii) The relative molecular proportions of the 2 : 4-di- and the 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactoses were *ca.* 1 : 1, whereas in the case of the 2 : 6-isomer the ratio was *ca.* 3 : 1. Hence, the 2 : 4-isomer probably represents all the points of branching, and the 2 : 6-isomer is an artefact.

The possibility remains that the origin of the 2 : 4-di-*O*-methyl-D-galactose lies in the fact that the sample of galactan used for methylation studies contained a few per cent. of a galactoaraban. However, no arabinose derivatives were detected amongst the hydrolysis products of the methylated galactan, indicating that the methylated arabinose-containing polysaccharide was removed during the fractionation.

About thirty galactose residues (average) per non-reducing end-group are present in the methylated galactan, whereas periodate oxidation of the galactan yielded one molecule of formic acid per seventeen hexose units. These figures indicate (cf. Andrews, Hough, and Jones, *J.*, 1953, 1186) that the galactan has a branched structure, but in the absence of any value for its molecular weight a reliable estimate of the average number of points of branching cannot yet be made.

Clearly, the structure of the *Strychnos nux-vomica* galactan is not yet fully established, but on the basis of the evidence presented above it is suggested that it consists of chains of β-D-galactopyranose units linked in the main through $C_{(1)}$ and $C_{(4)}$, with about 3% of the units existing as non-reducing end groups and a similar proportion of the units linked through $C_{(1)}$, $C_{(3)}$, and $C_{(6)}$, giving the galactan a branched structure. Similarly, the galactan of *Lupinus albus* seeds, investigated by Hirst, Jones, and Walder (*loc. cit.*), consists of 1→4′-linked β-D-galactopyranose units.

Experimental

Chromatography was carried out on Whatman No. 1 filter paper sheets by the descending method (Partridge, *Biochem. J.*, 1948, **42**, 238), with the following solvent systems: (*a*) *n*-butanol–ethanol–water (40 : 11 : 19 v/v), (*b*) *n*-butanol–pyridine–water (10 : 3 : 3 v/v), (*c*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4 v/v). Sugars were detected on the chromatograms with ammoniacal silver nitrate (Partridge, *loc. cit.*) or *p*-anisidine hydrochloride. R_G and R_{Rh} values of sugars and their derivatives are the rates of movement on the paper chromatogram relative to tetra-*O*-methylglucose and to rhamnose respectively, with solvent (*a*) as mobile phase.

Solutions were concentrated under reduced pressure. Optical rotations were determined at 20°.

Preliminary Fractionations.—*Strychnos nux-vomica* seed was provided as a coarse brown powder of which nearly 20% (w/w) was polysaccharide that could be extracted with hot water and a further 8% (w/w) polysaccharide that was soluble in hot aqueous sodium hydroxide. The seed polysaccharides contained galactose, mannose, xylose, and arabinose in roughly 5 : 2 : 1 : 1 proportions.

Fractional extraction of the polysaccharides from the seed (90 g.) with solvents (700 c.c. portions) at 70° gave fractions of widely differing compositions (Table). Fehling's solution (35 c.c. of A; 35 c.c. of B) was added to each extract, and four polysaccharide fractions (I—IV)

were isolated in the usual way (Andrews, Hough, and Jones, *loc. cit.*) from the precipitated copper complexes. The combined mother-liquors from which the copper complexes of fractions I and II were precipitated, were dialysed in Cellophane casing for 48 hr. against tap-water and then poured into alcohol (2 vols.), causing precipitation of further polysaccharide (fraction V). The mother-liquors which had yielded fractions III and IV were similarly treated but yielded very little material.

Fractionation of Strychnos nux-vomica *polysaccharides.*

Fraction	Solvent	Yield (%)	N (%)	Sulphated ash (%)	Composition * Gal.	Mann.	Arab.	Xyl.
I	Water (2 portions)	7	0·2	2·7	2	1	Trace	Trace
II	Water (5 portions)	2	<0·4	2·3	5	1	,,	,,
III	5% NaOH	5·5	<0·3	nil	1	1	*ca.* 5%	,,
IV	20% NaOH	2·5	<0·1	3·0	1	3	Trace	4
V	Water	9	0·9	5·7	3	Nil	1	Nil

* Approximate proportions estimated by relative spot sizes on paper chromatograms (cf. Andrews, Hough, and Jones, *locc. cit.*).

Precipitation and extraction procedures, with ethanol–water mixtures, afforded only a partial fractionation of fraction I and no detectable fractionation of fraction V. Attempts to separate the mannan as its insoluble copper complex from solutions of fractions I and II were likewise unsuccessful, the precipitates containing both galactose and mannose.

Preparation of the Galactan.—Galactan was extracted from fraction II (2 g.), freshly precipitated from its aqueous solution with alcohol, by stirring it in a macerator with Fehling's solution (25 c.c. of A, 25 c.c. of B, 200 c.c. of water) for 5 min. Stirring for longer periods effected no further extraction. The resultant slurry was centrifuged, and the residue (A) was retained; the clear supernatant liquor was neutralised with acetic acid, dialysed to remove most of the salts, and then passed through columns of Amberlite IR-120 and IR-400 ion-exchange resins. The clear, colourless solution so obtained was concentrated to a small volume and poured into absolute ethanol (3 vols.). The precipitated galactan was isolated on the centrifuge, washed successively with absolute ethanol, acetone, and ether, and dried under reduced pressure over silica gel (yield, 0·1 g.). Copper was removed from the material (A) by twice dissolving it in dilute acetic acid (pH 5—6) and precipitating it with alcohol. It was again extracted with Fehling's solution, and the extract, after working up as above, yielded more galactan (0·3 g. from three experiments). A further quantity of purified galactan (*ca.* 1·5 g.) was obtained by the same procedure from a polysaccharide fraction (6 g.), containing galactose and mannose (3 : 1) and a little arabinose (of the order of 2—3%), prepared as were fractions I and II. When this procedure was applied to polysaccharide mixtures composed of galactose, mannose, and higher proportions of arabinose units, the isolated galactan was contaminated with considerable amounts of araban.

Properties and Hydrolysis of the Galactan.—The purified galactan is a white, slightly hygroscopic powder, easily soluble in water to a clear, colourless solution, $[\alpha]_D$ +69° ± 2° (*c*, 1·0 in H_2O) [Found: C, 44·0; H, 6·3; N, 0·3; sulphated ash, 0·5. Calc. for $(C_6H_{10}O_5)_n$: C, 44·4; H, 6·3%].

The rate of hydrolysis of the galactan (110·7 mg.) in N-sulphuric acid (10 c.c.) at 100° was determined by following the change in optical rotation of the solution: $[\alpha]_D$ +68° (initial value) ---► +73° (2 hr.) ---► +78° (4 hr.) ---► +87° (7 hr.) ---► +89° (9 hr.; final value). Estimates of the amount of reducing sugar in the galactan hydrolysate by the alkaline hypoiodite method (Hirst, Hough, and Jones, *J.*, 1949, 928; Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289) (with 0·1 c.c. of hydrolysate and 2 c.c. of 0·1N-iodine at pH 11·4; oxidation for 3 hr.) gave values of 121·2 and 120·2 mg. (calc. as hexose). Complete hydrolysis of the galactan should give 123·2 mg. of galactose, and $[\alpha]_D$ +89°. D-Galactose was found to have $[\alpha]_D$ +78 ± 2° (*c*, 1·1 in N-H_2SO_4).

The concentrated hydrolysate, after neutralisation in the cold with barium hydroxide and treatment with Amberlite IR-120 ion-exchange resin, showed on the paper chromatogram, besides galactose, traces (1—2%) of arabinose; uronic acids were not detected. D-Galactose, m. p. and mixed m. p. 164—165°, $[\alpha]_D$ +79° (equil. value; *c*, 1·0 in H_2O), was crystallised from the hydrolysate and recrystallised from methanol.

When the galactan was heated at 100° in 0·01N-sulphuric acid for 4 hr., only a trace of galactose was liberated, but several oligosaccharides were detected on the paper chromatogram. Hydrolysis of the galactan was also incomplete after 8 hr. at 100° in 0·1N-sulphuric acid, the solution still containing considerable amounts of oligosaccharides and reducing material of higher molecular weight which remained on the base-line of the paper chromatogram.

The galactan was also hydrolysed to reducing sugars by the commercial enzyme preparation, Pectinol 10M.

Periodate Oxidation of the Galactan.—The galactan was oxidised with potassium metaperiodate (Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27), and the formic acid yield determined. To the galactan (278·3 mg.) in water (20 c.c.) were added potassium chloride (3 g.) and sodium metaperiodate (0·2N; 30 c.c.). The mixture was kept in the dark and occasionally shaken. At intervals, portions (5 c.c.) were withdrawn, ethylene glycol (1 c.c.) was added, and the formic acid titrated with 0·01N-sodium hydroxide (methyl-red screened with methylene-blue) [Found (c.c. of alkali, corr. for blank): 0·76 (70 hr.); 1·03 (174 hr.); 1·02 (239 hr.); 1·00 (282 hr.)]. The titre of 1·03 c.c. of 0·01N-alkali corresponds to the production, from the galactan, of one molecule of formic acid per 17 galactose units.

Methylation of the Galactan.—The galactan (2·5 g.; containing about 5% of araban) was dissolved in 2N-sodium hydroxide (100 c.c.), the mixture cooled in ice, and methyl sulphate (10 c.c.) added dropwise during 1 hr. Then sodium hydroxide (10 g.) and methyl sulphate (12 c.c., dropwise) were again added, followed by twice the quantity of the same reagents. After the reaction was over, the mixture was neutralised with acetic acid and dialysed against tap-water for 48 hr. The resultant clear solution was concentrated to *ca.* 100 c.c., and methylation continued by the alternate addition of sodium hydroxide (40 g.) and methyl sulphate (95 c.c.). After six methylations, with intermediate dialysis and concentration of the mixture, the crude methylated galactan was isolated by filtration of the hot, neutralised reaction mixture. The crude product was somewhat purified by dissolution in chloroform, filtration, and evaporation to dryness, giving a crisp, buff-coloured solid (2·0 g.) (Found: OMe, 39·6; sulphated ash, 4·5%). This material was insoluble in cold and hot water, aqueous sodium hydroxide, acetone, methanol, dioxan, methyl iodide, and liquid ammonia. Attempts to raise its methoxyl content by treatment of small portions with (*a*) sodium hydroxide and methyl sulphate, (*b*) sodium and methyl iodide in liquid ammonia, and (*c*) silver oxide and methyl iodide, were unsuccessful.

The methylated galactan (1·5 g.) was fractionated by boiling it with light petroleum (b. p. 40—60°) and with mixtures of chloroform and light petroleum (b. p. 40—60°) (2 extractions for 30 min. with 30 c.c. of each mixture). The largest fraction so obtained (0·9 g.) {Found: OMe, 41·6; sulphated ash, 2·2%; $[\alpha]_D$ $-6° \pm 2°$ (*c*, 1·1 in $CHCl_3$)} was soluble in a 4 : 1 (v/v) mixture, but insoluble in a 7 : 3 mixture. The next largest fraction (0·16 g.) {Found: OMe, 41·5%; $[\alpha]_D$ $-4° \pm 2°$ (*c*, 1·1 in $CHCl_3$)} was soluble in a 7 : 3 mixture, but insoluble in a 6 : 4 mixture.

Scission of the Methylated Galactan.—The material was insoluble in methanol, but soluble in anhydrous formic acid, and was therefore hydrolysed as follows: the largest fraction (0·86 g.) was dissolved in formic acid (9 c.c.), water (1 c.c.) was added, and the solution heated at 100° for 3 hr. It was then concentrated, the resultant thin syrup dissolved in N-sulphuric acid (*ca.* 15 mg. of insoluble inorganic material was filtered off), and the solution heated at 100° for 12 hr. It was cooled, neutralised with barium carbonate, filtered, treated with small amounts of Amberlite IR-120 and IR-4B resins, and concentrated to a syrup (0·80 g.) of reducing methyl sugars.

Examination of the Methyl Sugar Mixture.—Paper chromatographic examination of the mixture indicated that it consisted mainly of tri-*O*-methylgalactose (R_G 0·75) which gave a dark-red spot with *p*-anisidine hydrochloride. Small amounts of tetra-*O*-methylgalactose (R_G 0·94) and two di-*O*-methylgalactoses (R_G 0·48, pink-brown spot, and R_G 0·43, dark brown spot) were also present.

The methyl-sugar mixture (640 mg.) was fractionated on a hydrocellulose column (24 × 3 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with benzene–ethanol mixtures, nearly saturated with water, as the mobile phase. Elution of the sugars was commenced with benzene and ethanol in the proportions 5 : 1, and this was changed in stages to 3 : 1 as the separation proceeded. The following fractions were obtained.

Fraction (1) (29 mg.) was a syrup with n_D^{20} 1·4570 and $[\alpha]_D$ +101° (*c*, 1·0 in H_2O). It consisted mainly of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (R_G 0·94), but contained also *ca.* 10% of tri-*O*-methylgalactose (R_G 0·75). When boiled under reflux with aniline (12 mg.) in ethanol (3 c.c.), the fraction yielded *N*-phenyl-D-galactopyranosylamine tetra-*O*-methyl ether (20 mg.), which had m. p. and mixed m. p. 190° and $[\alpha]_D$ +38° (equil. value; *c*, 0·4 in acetone) after recrystallisation from ethanol.

Fraction (2) (510 mg.) [Found: OMe, 42·3. Calc. for tri-*O*-methylhexose, $C_6H_9O_3(OMe)_3$: OMe, 41·8%] was a syrup with $[\alpha]_D$ +90° (*c*, 1·2 in H_2O) which gave only one spot (R_G 0·75) on the paper chromatogram. It was indistinguishable in position and colour reaction (dark-red spot with *p*-anisidine hydrochloride) from 2 : 3 : 6- and 2 : 4 : 6-, but unlike 2 : 3 : 4-tri-*O*-

methylgalactose; the last had R_G 0·71 and gave a much browner spot. Accordingly, a portion of the syrup (300 mg.) was oxidised with bromine water, and the lactone (270 mg.), isolated in the usual way, crystallised spontaneously. This product gave one spot on the paper chromatogram (butanol–ethanol–water solvent, and ammoniacal silver nitrate spray; cf. Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859), R_G 1·00, corresponding to 2 : 3 : 6-tri-*O*-methylgalacto-γ-lactone. Under the same conditions, 2 : 4 : 6-tri-*O*-methylgalactolactone gave a long streak on the paper chromatogram, and not a discrete spot.

A portion of the crude lactone was hydrolysed by titration with 0·1N-sodium hydroxide (Found: equiv., 222. Calc. for $C_9H_{16}O_6$: equiv., 220). The sodium salt from 124 mg. of lactone was oxidised with sodium metaperiodate (0·4N; 10 c.c.), and, after oxidation for 3 hr., saturated sodium hydrogen carbonate solution (20 c.c.) was added to the reaction mixture, followed by excess of potassium iodide. The liberated iodine was titrated with 0·1N-sodium thiosulphate (Found: 12·43 c.c. 0·1N-sodium metaperiodate consumed). The result corresponds to the reduction of 1·10 moles of periodate per mole of sodium salt.

The remainder of the crude lactone was recrystallised from ether–light petroleum (b. p. 60—80°) and characterised as 2 : 3 : 6-tri-*O*-methyl-D-galacto-γ-lactone, with m. p. and mixed m. p. 97—98° and $[\alpha]_D$ −38° (init. value; *c*, 1·0 in H_2O) [Found: OMe, 42·1. Calc. for $C_6H_7O_3(OMe)_3$: OMe, 42·3%]. The derived 2 : 3 : 6-tri-*O*-methyl-D-galactonamide was recrystallised from chloroform–light petroleum (b. p. 60—80°), and had m. p. and mixed m. p. 129—130°.

Fraction (3) (65 mg.) [Found: OMe, 29·7. Calc. for di-*O*-methylhexose, $C_6H_{10}O_4(OMe)_2$: OMe, 29·8%] was a syrup, with $[\alpha]_D$ +85° (*c*, 1·1 in H_2O) and R_{Rh} 1·53, which gave a pink-brown spot with *p*-anisidine hydrochloride. On the paper chromatogram, 2 : 4-, 2 : 6-, 3 : 4-, and 4 : 6-di-*O*-methylgalactoses have R_{Rh} values 1·36, 1·53, 1·18, and 1·34 respectively, and show light brown, pink-brown, brown, and dark brown colours with *p*-anisidine hydrochloride.

This fraction crystallised when seeded with the 2 : 6-isomer; recrystallisation from chloroform–light petroleum afforded 2 : 6-di-*O*-methyl-β-D-galactose (27 mg.) as colourless flakes, $[\alpha]_D$ +48° (init. value, *c*, 0·4 in H_2O) ---► +84° (equil. value), m. p. 119—120°, undepressed on admixture with an authentic specimen, m. p. 118° (Dewar and Percival, *J.*, 1947, 1622, give m. p. 119—120°).

Fraction (4) (20 mg.) was a syrup, consisting mainly of a compound indistinguishable from 2 : 4-di-*O*-methylgalactose on the paper chromatogram, but containing also a little of the 2 : 6-isomer. It was induced to crystallise by adding chloroform and scratching the syrup; the crystals (14 mg.) had m. p. 95—96°, raised to 98—99° by recrystallisation from chloroform–light petroleum (yield, 9 mg.), $[\alpha]_D$ +130° (init. value; *c*, 0·3 in H_2O) ---► +85° (equil. value), and $[\alpha]_D$ +50° (init. value; *c*, 0·3 in 2% w/w MeOH–HCl) ---► +80° ± 20° (18 hr.) ---► +90° ±10° (50 hr.) ---► +120° ± 20° (113 hr.) [Found: OMe, 27·7; loss of weight at 100°, 9·1. Calc. for $C_6H_{10}O_4(OMe)_2,H_2O$: OMe, 27·4; H_2O, 8·0%]. Smith (*J.*, 1939, 1724) gives m. p. 103° and $[\alpha]_D$ +122° (10 min.) ---► +86° (equil. value; in H_2O), and Baldwin and Bell (*J.*, 1938, 1461) give m. p. 100—103° for 2 : 4-di-*O*-methyl-α-D-galactose monohydrate. Accordingly, a specimen of 2 : 4-di-*O*-methyl-D-galactose was recrystallised from chloroform–light petroleum, the solution being seeded with the above crystals; the crystals so obtained had m. p. 100—102°, and m. p. 99—101° in admixture with the crystals, m. p. 98—99°.

Quantitative Analysis of the Methyl-sugar Mixture.—The molecular proportions of tetra-, tri-, and di-*O*-methylhexoses were estimated by the alkaline hypoiodite method (Hirst, Hough, and Jones, *loc. cit.*; Chanda *et al.*, *loc. cit.*). The sugar mixture (*ca.* 25 mg.) was separated on the paper chromatogram by using solvent (*c*), and the separated sugars were eluted from the appropriate sections of the papers by Soxhlet-extraction with water (6—8 c.c.). For the estimations, we used 1 c.c. of 0·1N-iodine and 2 c.c. of phosphate buffer (pH 11·4) for the tetra-, double these amounts for the di-, and five times the amounts for the tri-*O*-methylhexoses. The oxidations were allowed to proceed for 3 hr., then 2N-sulphuric acid (vol. equal to that of buffer solution) was added, and the liberated iodine titrated with 0·01N-sodium thiosulphate [Found (results quoted as c.c. of 0·01N-iodine consumed): " tetra," 0·59, 0·41; " tri," 13·26, 10·80; " di," 2·49, 1·80]. These results correspond to tetra : tri : di molecular ratios of 1·0 : 22·5 : 4·2, and 1·0 : 26 : 4·4, corresponding to an average chain length of 30 ± 3 hexose units.

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THE UNIVERSITY, BRISTOL.
[Present address (J. K. N.):
QUEEN'S UNIVERSITY, KINGSTON, ONTARIO.]

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