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# THE HEX-5-ENOSE DEGRADATION: CLEAVAGE OF MODIFIED GLYCOSIDURONIC ACID LINKAGES IN METHYLATED *Khaya ivorensis* GUM*

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ABSTRACT

Reduction of uronic acid residues in permethylated polysaccharide A from *Khaya ivorensis* gum, followed by transformation of the resulting hexose into 6-deoxy-6-iodohexose residues, affords a modified polysaccharide which undergoes depolymerization on treatment with zinc dust. The main products after reduction with sodium borohydride are hexenitol-terminated oligosaccharides whose structures have been established on the basis of n.m.r. and mass-spectral data and compositional analysis as members of a series of *O*-α-L-rhamnopyranosyl-(1→3)-1,2-dideoxyhex-1-enitol derivatives, with and without β-D-galactopyranosyl substituents. The major portion of the polysaccharide may be formulated as a backbone of alternating 4-linked α-D-galacturonic acid and 2-linked α-L-rhamnopyranose residues with side chains of β-D-galactopyranosyl or 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-β-D-galactopyranosyl units attached to O-4 of rhamnose residues.

## INTRODUCTION

Exudate gums from West African trees of the *Khaya* genus contain mixtures of partially acetylated polysaccharides[1]. For gums of three species, *K. grandifoliola*, *K. senegalensis*, and *K. ivorensis*, *O*-deacetylation followed by fractionation yields, as the major polysaccharide component, a rhamnogalacturonan carrying, as side chains, terminal D-galactopyranosyl and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactopyranosyl groups. In previous studies, the anomeric configurations of L-rhamnose and D-galactose residues were not established, and the attachment of the side chains to O-4 of rhamnose residues was inferred rather than directly proven. The selective cleavage of 6-deoxy-6-halohexopyranosides, which takes place on treatment with zinc dust to furnish 5,6-dideoxyhex-5-enoses[2], proceeds satisfactorily from residues of this type introduced into permethylated polysaccharides[3,4]. The hex-5-enose degradation is presently the only chemical degradation[5] suitable for the selective fragmentation of galacturonans and yields hexenose-

*Dedicated to Professor Bengt Lindberg.

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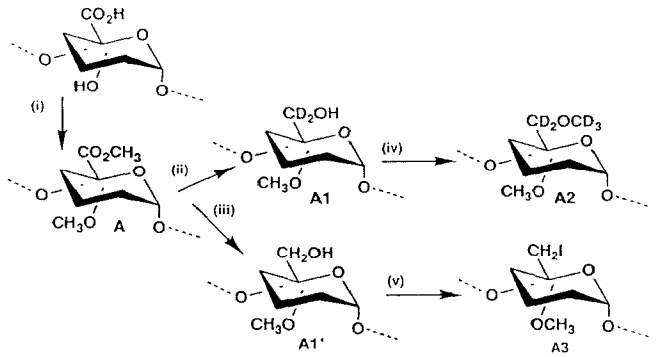
terminated oligosaccharides bearing unaltered neutral glycose residues. We have recently used this procedure[4] to examine aspects of structure of two exudate gums from *Sterculia* species, to which the *Khaya* polysaccharides show similarities in rhamnogalacturonan inner structure but definite differences in outer chain units. Whereas *Khaya* glycans contain units of the aldobiouronic acid 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactopyranose, the *Sterculia* glycans contain β-D-glucuronic acid residues linked directly to D-galacturonic acid residues in the rhamnogalacturonan core[1]. We now report the application of the hex-5-enose degradation to a derivative of the major glycan component, polysaccharide A, of *K. ivorensis* gum[6].

## RESULTS AND DISCUSSION

Scheme 1 summarizes reactions of uronic acid residues leading to the generation of 6-deoxy-6-iodohexose residues for selective fragmentation, together with reactions used to monitor the completeness of structural modifications. Permethylated polysaccharide was prepared by the Hakomori procedure[7]. For analytical purposes, a sample was reduced with lithium aluminum deuteride to give methylated glycan A1 and this in turn was further alkylated with trideuteriomethyl iodide to give methylated glycan A2. These methylated glycans were hydrolyzed and analysis of the derived partially methylated alditol acetates[7] by g.l.c.–m.s. (Table I) confirmed the identities of sugars formed from uronic acid residues by the presence of fragment ions containing $CD_2OCD_3$ groups. For studies on a preparative scale, methylated glycan A was treated with diazomethane to ensure completeness of esterification of uronic acid residues and then reduced with "superhydride"[8] to give methylated glycan A1′. Reaction of this reduced methylated glycan with *N*-iodosuccinimide and triphenylphosphine afforded iodinated methylated glycan A3. Treatment of the modified glycan with tributylstannane[9] followed by hydrolysis and methylated sugar analysis (Table I) showed, through the disappearance of uronic acid-derived hexose and the appearance of the corresponding 6-deoxyhexose derivatives, that iodination had proceeded satisfactorily.




Scheme 1. Transformations of uronic acid residues leading to the generation of 6-deoxy-6-iodohexose residues in methylated glycan: (*i*) methylation, $CH_3I/^-CH_2SOCH_3$; (*ii*) reduction, $LiAlD_4$; (*iii*) reduction, "superhydride"; (*iv*) trideuteriomethylation, $CD_3I/^-CH_2SOCH_3$; (*v*) iodination, $NIS/PPh_3$.

TABLE I

SUGAR ANALYSES OF DERIVATIVES OF METHYLATED GLYCAN A

| *Sugars*[b] | *Polysaccharide derivatives* | | |
|---|---|---|---|
| | *A1* | *A2* | *A3* |
| 2,3,5-$Me_3$Ara | 3.3[c] | 3.5[e] | 7.7[k] |
| 2,3,4-$Me_3$Rha | | | |
| 2,3,4-$Me_3$ 6-deoxyGlc | | | |
| 3,4-$Me_2$Rha | 7.1 | 7.0 | 9.2[b] |
| 3,4-$Me_2$6-deoxyGlc | | | |
| 2,3-$Me_2$Rha | 1.0 | 2.2[f] | 0.8 |
| 2,3,4,6-$Me_4$Glc | | 6.2[g] | |
| 2,3-$Me_2$Fuc | | | 25.1 |
| 2,3,4,6-$Me_4$Gal | 9.7 | 13.5[h] | 9.2 |
| 3 MeRha | 18.3 | 16.6 | 17.6 |
| Rha | 3.3 | 5.0[i] | 3.5 |
| 3,4,6-$Me_3$Glc | | | |
| 2,3,6-$Me_3$Gal | 19.3[c,d] | 35.5[j] | 14.4[m] |
| 2,3,4-$Me_3$Glc | | | |
| 2,3,4-$Me_3$Gal | 2.8 | 2.5 | 2.3 |
| 3,4-$Me_2$Glc | 2.5 | | |
| 2,3-$Me_2$Gal | 25.9 | | 2.1 |

[a]A1, Carboxyl-reduced ($LiAlD_4$) methylated polysaccharide A from *Khaya ivorensis* gum; A2, trideuteriomethylated glycan from A1; A3, iodinated methylated glycan obtained from carboxyl-reduced ($LiAlH_4$) methylated glycan A1′ and then treated with tributylstannane. [b]Sugars characterized by conversion into partially methylated alditol acetates for quantitative analysis by g.l.c. on column *A* (isothermal, 190°) and g.l.c.–m.s. on column *A* or *C*. [c]Derivatives of bracketed sugars are not separated under the conditions used for quantitative analysis, but m.s. fragment ions showed the presence of both components. [d]The presence of both sugars was also inferred from transformations occurring during the formation of methylated glycans A2 and A3. [e]A small proportion of 2,3,4-$Me_3$Rha carried a $CD_3$ group at O-2. [f]O-2 carries both $CH_3$ and $CD_3$ groups. [g]This sugar originates from 2,3,4-$Me_3$GlcA since relevant fragment ions show >95% of label in $CD_2OCD_3$ groups. [h]Fragment ions showed that ~25% of the sugar carried a $CD_3$ group at O-4. [i]3,4,6-$Me_3$Glc originates from 3,4-$Me_2$GlcA since relevant fragment ions show >95% of label in $CD_2OCD_3$ groups. [j]G.l.c.–m.s. showed that ~75% of the sugar arises from 2,3-$Me_2$GalA with incorporation of label in $CD_2OCD_3$ groups, and that no fragment ions that could have arisen from unchanged 2,3,4-$Me_3$Glc could be detected. [k]The derivative of 2,3,4-$Me_3$ 6-deoxyGlc is inseparable from that of 2,3,4-$Me_3$Rha, but the presence of the former sugar may be inferred from the increase in the amount of this fraction and the disappearance of 2,3,4-$Me_3$Glc. [l]Derivatives of these two sugars were separated on a column of DB-WAX. [m]G.l.c.–m.s. showed the absence of a derivative of 2,3,4-$Me_3$Glc.

Table I (with major constituents underlined) provides quantitative confirmation of earlier qualitative characterization of sugars formed on hydrolysis of carboxyl-reduced methylated glycan A1. Uronic acid units are present mainly as terminal glucuronic acid and 4-linked galacturonic acid residues, and furnish, respectively, 2,3,4-tri-*O*-methylglucose and 2,3-di-*O*-methylgalactose, 2,3,4,6*-tetra-*O*-methylglucose and 2,3,6*-tri-*O*-methylgalactose, and 6-deoxy-2,3,4-tri-*O*-methylglucose

*Designates trideuteriomethyl substituents.

and 2,3-di-*O*-methylfucose from methylated glycans A1, A2, and A3. The main neutral sugar constituents, 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose, and 3,4-di- and 3-*O*-methylrhamnose, remained largely unaltered during the structural modifications.

Iodinated methylated glycan A3 was heated with zinc dust in 95% ethanol and the whole reaction mixture was reduced with sodium borohydride. Separate samples of the reaction products were trideuteriomethylated and acetylated, and the resulting mixtures of derivatives were examined by g.l.c.–m.s. Analysis of the fully alkylated oligosaccharides (Table II) showed the presence of major disaccharide (**3c**) and trisaccharide (**4c** and **5c**) fractions, and a tetrasaccharide fraction (**6a** and **6b**) in smaller amount, all carrying terminal dideoxyhexenitol residues, and, as minor components, two disaccharide fractions (**7a** and **7b**, and **8a** and **8b**). With the exception of the tetrasaccharide fraction, each component in the mixture had a retention time identical to that of a compound formed in similar manner from *Sterculia* gums. Structures **3**–**8**, which are shown together with *m/z* values for significant fragment ions (including *J* ions in parenthesis) in their mass spectra, may be assigned to the oligosaccharides and their derivatives. It may be noted that all peralkylated oligosaccharide fractions other than **3c** had non-uniform distributions of *O*-trideuteriomethyl substituents in the galactose residues. Evidence on this point for the trisaccharide fraction was obtained from g.l.c.–m.s. analysis of the mixture of acetylated derivatives in which the major disaccharide (**3b**) and two major trisaccharide (**4b** and **5b**) components, each with a uniform distribution of

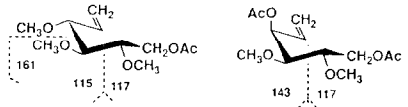
TABLE II

G.L.C.–M.S. ANALYSIS OF DERIVATIVES OF OLIGOSACCHARIDE PRODUCTS FROM HEX-5-ENOSE DEGRADATION OF IODINATED METHYLATED GLYCAN A3

| *Fraction* | *Retention time (min)*[a] | *Fragment ions* (m/z) *from terminal units* | *Compound* | *Relative proportion*[b] |
|---|---|---|---|---|
| *After trideuteriomethylation* | | | | |
| 1 | 2.1 | 192 | **3c** | 25 |
| 2 | 4.7 | 219,222 | **7a** | 1.5 |
| | | | **7b** | 1.5 |
| 3 | 5.2 | 219,222 | **8a** | 1 |
| | | | **8b** | 1 |
| 4 | 11.4 | 219,222 | **4c** | 31 |
| | | | **5c** | 31 |
| 5 | 17.5 | 219,222 | **6a** | 4 |
| | | | **6b** | 4 |
| *After acetylation* | | | | |
| 6 | 4.6 | 217 | **3b** | |
| 7 | 13.0 | 222 | **4b** | |
| 8 | 13.6 | 247 | **5b** | |

[a]Retention times (min) are for column *B* [150° (2 min), 8°/min→280°, hold]. [b]The relative proportions of compounds whose trideuteriomethylated derivatives differed only in isotopic labelling are based on the abundances of relevant fragment ions.

*O*-acetyl substituents, were present. Tetrasaccharide and the minor disaccharides corresponding to **6–8**, but bearing *O*-acetyl in place of *O*-trideuteriomethyl groups, were not detected in this mixture, but at a lower column temperature, 1,2-dideoxy-tri- and -di-*O*-methylhex-1-enitols (**1** and **2**) were detected.

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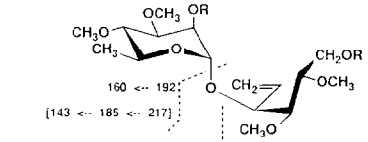
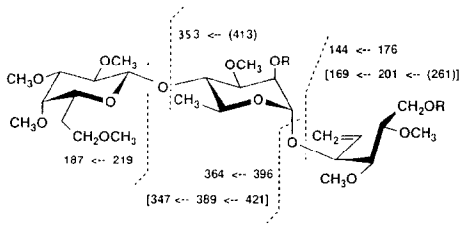
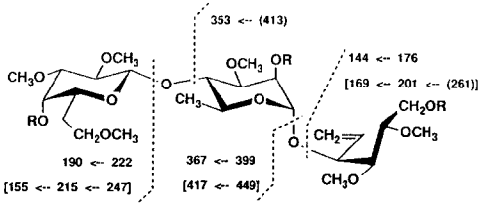


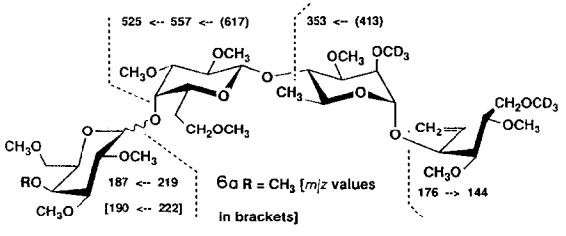

3a R = H; 3b R = Ac [*m/z* values in brackets]; 3c R = $CD_3$




4a R = H; 4b R = Ac [*m/z* values in brackets];
4c R = $CD_3$ (*m/z* values shown)

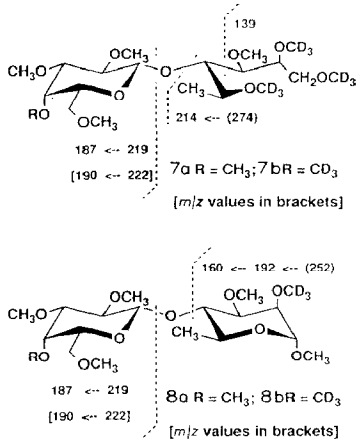



5a R = H; 5b R = Ac [*m/z* values in brackets];
5c R = $CD_3$ (*m/z* values shown)




6a R = $CH_3$ [*m/z* values in brackets]

6b R = $CD_3$




Preparative-scale chromatography of the original mixture of oligosaccharides afforded the three major components **3a, 4a,** and **5a**. The provisional structural assignments were confirmed and extended on the basis of (*i*) $^1$H-n.m.r. spectra for assignments of anomeric configurations, (*ii*) g.l.c.–m.s. of the trideuteriomethylated derivatives for identification of structural units and confirmation of linkage positions in the acyclic 1,2-dideoxyhexenitol termini, and (*iii*) where necessary to locate positions of trideuteriomethyl substituents, characterization of the glycosyl residues after hydrolysis, and conversion into partially methylated alditol acetates for g.l.c.–m.s. The components of the tetrasaccharide mixture may be assigned structures **6a** and **6b** as homologues of trisaccharides **4a** and **5a** with more extended side chains. Disaccharide-alditols **7a** and **7b** probably arise as minor products from inadvertent breakdown from units furnishing trisaccharides **4a** and **5a**, since the acid lability of 4-*O*-glycosylhex-5-enoses under the conditions of the zinc degradation has been observed previously[4,10].

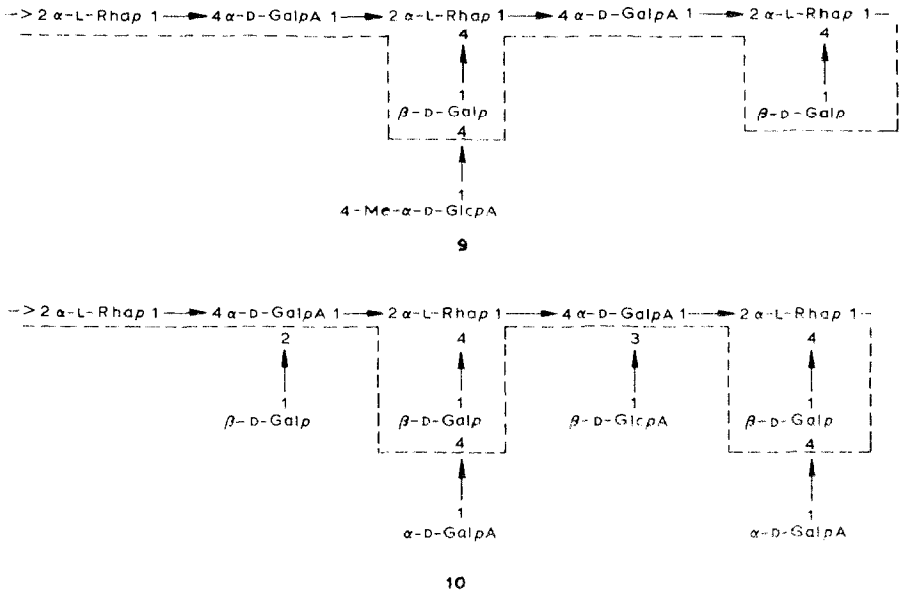
Oligosaccharides **3**–**6** as the main degradation products are all derivatives of 1,2-dideoxy-3-*O*-α-L-rhamnopyranosyl-L-*lyxo*-hex-1-enitol*. Since most acid residues in internal chains are those of D-galacturonic acid, it is assumed, without further proof, that each glycosylated hexenitol has the L-*lyxo* configuration. The disaccharide **3a** arises from unbranched chains, whereas the two trisaccharides containing β-D-galactopyranose residues arise from branch points and differ only in that **4a** carries a terminal residue whilst **5a** bears a 4-linked β-D-galactopyranose residue from which the glycosyl substituent has been removed. It may be concluded that the substituent removed is that from terminal 4-*O*-methyl-D-glucuronic acid residues which afford the aldobiouronic acid 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactopyranose on partial hydrolysis of the parent glycan[6]. The formation, albeit in relatively small proportions, of the tetrasaccharides **6a** and **6b** points to the presence of some longer side chains.

*Systematic nomenclature requires that reduction of 5,6-dideoxyhex-5-enoses gives 1,2-dideoxyhex-1-enitols with consequent changes in configurational prefixes and the numbering of substituents.

The formation of minor degradation products giving rise to disaccharides **8a** and **8b** requires special comment since these compounds probably represent artifactual reducing termini in the rhamnogalacturonan chain, resulting from limited depolymerization during the methylation of the parent glycan. The formation of **8b** in experiments on *Sterculia* gums[4] was attributed to base-catalyzed β-elimination from esterified galacturonic acid residues with exposure of reducing rhamnose residues for conversion into methyl glycosides. In these studies, loss of outer-chain galacturonic, but not of terminal glucuronic, acid residues was observed. These observations were consistent with the assumption that the base-catalyzed β-elimination of 4-*O*-substituents from uronic esters has some E2 character and occurs more readily with, but is not restricted to, galacturonates having leaving groups in antiperiplanar orientation. In the present studies, analyses of methylated glycan derivatives together with the identification of the tri-*O*-methylhexenitol **1** among the degradation products indicate that most of the 4-*O*-methyl-D-glucuronic acid end-groups were retained during methylation of the glycan.

The present results permit the definition of further aspects of structure of polysaccharide A as the main glycan component of *K. ivorensis* gum. Much of the main chain can be accommodated as a rhamnogalacturonan with alternating 2-linked α-L-rhamnopyranose and 4-linked α-D-galacturonic acid residues as in the *Sterculia* gums[4], the plant mucilages of the Malvaceae family[11], and rhamnogalacturonan I from the cell walls of many dicots[12]. As in rhamnogalacturonan I, branching in the *Khaya* polysaccharide has only been demonstrated through O-4 of rhamnose residues, whereas the *Sterculia* and Malvaceae polysaccharides have side chains attached also through O-2 and/or O-3 of galacturonic acid residues. In both the *Khaya* and the *Sterculia* polysaccharides, β-D-galactopyranose residues are attached to O-4 of rhamnose residues, and this degree of homology is shown in Scheme 2 with partial structures **9** and **10** for the two glycans. In the *Sterculia* gums, the galactose residues are glycosylated at O-4, probably by α-D-galacturonic acid residues. In the *Khaya* polysaccharide, approximately equal proportions are present as terminal single-unit side chains and as 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactosyl units. Less frequently, an additional galactose residue is attached, with or without a terminal 4-*O*-methyl-α-D-glucuronic acid residue.

Previous results[6] established that the *Khaya* glycan preparation also contained contiguous 4-linked α-D-galacturonic acid residues as shown by the isolation of a galacturonobiose on partial acid hydrolysis. The presence of some such units in the methylated glycan was also indicated by the characterization of the di-*O*-methylhexenitol derivative **2** from the hexenose degradation. Quantitative analyses of the methylated glycan derivatives, however, suggested that these residues were present only in small proportion, in that the proportions of 2,3-di-*O*-methylgalactose or derived units were only in slight excess over those of the rhamnose derivatives required for chains with alternating rhamnose and galacturonic acid residues. An estimate of the proportion of uronic acid residues[13] in methylated glycan A




Scheme 2. Partial structures for glycan A (**9**, with omission of longer side chains) from *Khaya ivorensis* gum and polysaccharides (**10**) from *Sterculia* gums, showing the extent of homology in rhamnogalacturonan chains and β-D-galactopyranose residues linked to O-4 of α-L-rhamnopyranose residues within designated area, together with sites of attachment of side chains (not in defined sequence).

gave a value of ~34%, a figure in reasonable agreement with that estimated from quantitative analysis of those sugars in the methylated glycan derivatives (Table I) which were formed from units of 2,3,4-tri- and 3,4-di-*O*-methylglucuronic acid, and 2,3-di-*O*-methylgalacturonic acid. Uronic acid constituents are therefore present in methylated glycan A in considerably lower amount than the ~50% proportion in the parent polysaccharide. The most probable explanation of this observation is that homogalacturonan regions in the parent glycan have been substantially degraded during methylation. The present evidence does not distinguish between the presence in the native gum of the branched rhamnogalacturonan and a homogalacturonan as two distinct polysaccharides or as two domains within a single polymer. Likewise, the new results throw no fresh light on the structural role of the minor constituents in the methylated glycans. The failure to detect arabinofuranosyl end-groups or 6-linked galactopyranose residues in any of the hexenose degradation products would suggest that these units remain combined in polymeric form as constituents of a neutral glycan domain.

## EXPERIMENTAL

*General methods.* — The sample of polysaccharide from *Khaya ivorensis* gum was that used in the previous investigation[6]. The equivalent weight by titration of 338 corresponded to a uronic acid content of 48% and colorimetric assay as galac-

turonic acid by the 3-hydroxybiphenyl method[13] gave a value of 53%. Methylated glycans were hydrolyzed with 2M trifluoroacetic acid at 120° for 1 h[14] and the resulting sugars were determined as partially methylated alditol acetates by g.l.c.–m.s.[7]. $^1$H-N.m.r. spectra were recorded with a Bruker AM 300 spectrometer for solutions in $CDCl_3$ with tetramethylsilane as internal standard. G.l.c. was performed with a Perkin–Elmer Sigma 3B chromatograph, using fused-silica columns (Chromatographic Specialties Limited) *A*, a 15-m wide-bore capillary of DB-225 or *B*, a 2-m narrow-bore capillary of DB-5. For g.l.c.–m.s., columns *B* for oligosaccharides or *C* (30-m wide-bore capillary of DB-WAX) for partially methylated alditol acetates were attached by a jet separator to a VG Micromass 16F mass spectrometer, which was operated with an inlet temperature of 250°, an ionization potential of 70 eV, and an ion-source temperature of ~250°.

*Methylated glycan A and derivatives.* — Polysaccharide A (510 mg) from *K. ivorensis* was methylated by the Hakomori procedure as described by Jansson *et al.*[7]. The methylated glycan was purified by chromatography on Sephadex LH-20 with dichloromethane–methanol (2:1) as eluant, to give a product that was treated with diazomethane in dichloromethane to ensure complete esterification and furnished methylated glycan A (260 mg), whose i.r. spectrum showed a strong absorption at ~1740 $cm^{-1}$ with no change on treatment with triethylamine (absence of ionizable carboxyl groups), and no significant absorption at ~3500 $cm^{-1}$ for non-etherified hydroxyl groups. Quantitative estimation of uronic acid residues[13], using methyl 3,4-di-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methyl-α-D-galactopyranosyluronic acid)-L-rhamnopyranoside dihydrate[15] as a reference compound for methylated uronic acid residues, gave a value of ~34%.

Methylated glycan A was reduced with lithium aluminum deuteride in boiling dry oxolane for 4 h to give methylated glycan A1, a portion of which was trideuteriomethylated to give methylated glycan A2. Samples of these methylated glycans were analyzed (Table I). The main portion (230 mg) of methylated glycan A in dry oxolane (10 mL) was boiled with M lithium triethylborohydride in oxolane (10 mL) for 4 h. Acetic acid was added dropwise to the cooled solution to destroy excess of hydride, the solution was concentrated, and a solution of the residue in ethanol–water (1:1) was passed through Dowex 50W-X8 ($H^+$) resin to remove lithium ions. The eluate was concentrated, and a solution of the dry residue in dichloromethane was filtered and then chromatographed on Sephadex LH-20 in dichloromethane–methanol (2:1) to give chromatographically immobile (benzene–methanol, 6:1) methylated glycan A1′ (140 mg), which contained no detectable uronic acid residues[13] and showed no i.r. absorption at ~1740 $cm^{-1}$.

A solution of methylated glycan A1′ (130 mg) in dry pyridine (20 mL) containing freshly prepared *N*-iodosuccinimide (450 mg) and triphenylphosphine (525 mg) was heated for 16 h at 60°. Methanol (1 mL) was added to the cooled solution to quench the reaction, the mixture was concentrated, the residue was dissolved in dichloromethane, and the solution was washed with aqueous sodium thiosulfate, dried, and concentrated. The residue was purified by passage through a column of

Sephadex LH-20, using dichloromethane–methanol (2:1) as eluant to ensure complete removal of triphenylphosphine oxide, and afforded iodinated methylated glycan A3 (118 mg). A solution of the sample of methylated glycan A3 in dry oxolane was boiled with tributylstannane[9,16] in the presence of a trace quantity of 2,2′-azobis(isobutyronitrile) as radical initiator. Hydrolysis of the resulting 6-deoxymethylated glycan and analysis of sugars as derived alditol acetates (Table I) showed that a high degree of iodination had been achieved in the preparation of the methylated glycan, as judged by >92% conversion of former 2,3-di-*O*-methylgalactose residues into those of 2,3-di-*O*-methylfucose.

*Zinc degradation of iodinated methylated glycan A3.* — Iodinated methylated glycan A3 (110 mg) was heated in boiling ethanol–water (19:1, 8 mL) with freshly activated zinc dust[17] (300 mg) for 6 h. T.l.c. in benzene–methanol (6:1) showed the formation of mobile components. The cooled reaction mixture was filtered, diluted with water, reduced with sodium borohydride, and processed to give the corresponding mixture of hexenitol-terminated derivatives (97 mg). A sample of the mixture was trideuteriomethylated and analysis of the products by g.l.c.–m.s. showed fractions 1–5 (Table II). A second sample of the hexenitol-terminated derivatives was acetylated and analysis of the products under the same conditions showed fractions 6–8 (Table II). Analysis of the acetylated derivatives on column *A* (130°, isothermal) showed the presence of two derivatives which may be assigned structures **1** and **2**.

The mixture (10 mg) of hexenitol-terminated oligosaccharides was separated in batches (2 mg each) by h.p.l.c., using a Waters Associates analytical Partisil column (4.6 × 250 mm, 10-$\mu$m silica) with benzene containing 6% of methanol as eluant, to give chromatographically pure samples of oligosaccharides **3a, 4a,** and **5a**. The $^1$H-n.m.r. spectra contained signals for individual anomeric protons together with those at $\delta$ 5.86–5.99 (m, 1 H, C*H*=CH$_2$), 5.22–5.35 (m, 2 H, CH=C*H*$_2$), 3.37–3.59 (requisite s, 3 H each, OMe), and 1.22–1.23 (d, 3 H, $J_{5',6'}$ 6.2 Hz, H-6,6,6 of Rha). The oligosaccharides afforded the corresponding trideuteriomethylated derivatives **3c, 4c,** and **5c**, whose mass spectra showed uniform incorporation of isotopic labelling. Hydrolysis of these derivatives gave glycoses whose labelled and unlabelled methyl ether substituents were defined by g.l.c.–m.s. of partially methylated alditol acetates. Disaccharide **3a** had an anomeric proton resonance at $\delta$ 4.97 (d, $J_{1',2'}$ 1.63 Hz, H-1 of $\alpha$-Rha) and gave 2*,3,4-tri-*O*-methylrhamnose on hydrolysis of **3c**. Trisaccharide **4a** had anomeric proton resonances at $\delta$ 4.98 (d, $J_{1',2'}$ 1.63 Hz, H-1 of $\alpha$-Rha) and 4.51 (d, $J_{1'',2''}$ 7.59 Hz, H-1 of $\beta$-Gal), and gave 2*,3-di-*O*-methylrhamnose and 2,3,4,6-tetra-*O*-methylgalactose on hydrolysis of **4c**. Trisaccharide **5a** had anomeric proton resonances at $\delta$ 4.98 (d, $J_{1',2'}$ 1.63 Hz, H-1 of $\alpha$-Rha) and 4.58 (d, $J_{1'',2''}$ 7.48 Hz, H-1 of $\beta$-Gal), and gave 2*,3-di-*O*-methylrhamnose and 2,3,4*,6-tetra-*O*-methylgalactose on hydrolysis of **5c**.

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REFERENCES

1 G. O. ASPINALL, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 333–379.
2 B. BERNET AND A. VASELLA, *Helv. Chim. Acta*, 62 (1979) 1990–2016, 2400–2410, 2411–2431.
3 G. O. ASPINALL AND V. PUVANESARAJAH, *Can. J. Chem.*, 62 (1984) 2736–2739.
4 G. O. ASPINALL, L. KHONDO, AND B. A. WILLIAMS, *Can. J. Chem.*, 65 (1987) 2069–2076.
5 G. O. ASPINALL, *Acc. Chem. Res.*, 20 (1987) 114–120.
6 G. O. ASPINALL AND A. K. BHATTACHARJEE, *J. Chem. Soc., C*, (1970) 361–365.
7 P.-E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–75.
8 W. S. YORK, A. G. DARVILL, M. MCNEIL, T. T. STEVENSON, AND P. ALBERSHEIM, *Methods Enzymol.*, 118 (1986) 1–40.
9 A. KLEMER, B. BRANDT, U. HOFMEISTER, AND E. R. RUTER, *Justus Liebigs Ann. Chem.*, (1983) 1920–1929.
10 G. O. ASPINALL, D. CHATTERJEE, AND L. KHONDO, *Can. J. Chem.*, 62 (1984) 2728–2735.
11 M. TOMODA, R. GONDA, N. SHIMUZU, S. AKIYAMA, AND H. ARAI, *Chem. Pharm. Bull.*, 33 (1985) 4320–4325, and papers there cited.
12 J. M. LAU, M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 168 (1987) 245–274.
13 N. BLUMENKRANTZ AND G. ASBOE-HANSEN, *Anal. Biochem.*, 54 (1973) 484–489.
14 P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH, AND A. KARR, *Carbohydr. Res.*, 5 (1967) 340–345.
15 G. O. ASPINALL AND R. S. FANSHAWE, *J. Chem. Soc.*, (1961) 4215–4225.
16 G. O. ASPINALL, R. C. CARPENTER, AND L. KHONDO, *Carbohydr. Res.*, 165 (1987) 281–298.
17 R. L. FRANK AND P. V. SMITH, *Org. Synth. Coll. Vol.*, 3 (1955) 410–412.