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HIGH SELECTIVITY IN THE PARTIAL DEGRADATION OF AN EXTRACELLULAR POLYSACCHARIDE OF *Rhizobium japonicum* WITH LIQUID HYDROGEN FLUORIDE: A N.M.R.-SPECTROSCOPIC STUDY

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ABSTRACT

The extracellular polysaccharide of *Rhizobium japonicum* consists of D-glucosyl, D-mannosyl, D-galactosyl, 4-*O*-acetyl-D-galactosyluronic acid residues, and 4-*O*-methyl-D-galactosyl groups. When subjected to treatment with liquid hydrogen fluoride at −40°, the polymer affords a pentasaccharide (**4**) and a higher homolog (**2**) of it, representative of both the major repeating sequence and minor structural variants that differ in degrees of *O*-methylation and *O*-acetylation. The structures of **2** and **4**, and hence that of the polymer, were determined primarily by ^{1}H- and ^{13}C-n.m.r. spectroscopy and methylation analysis. Another product of the degradation reaction, both at −40 and −23°, was the trisaccharide *O*-β-D-glucopyranosyl-(1→3)-*O*-(4-*O*-acetyl-α-D-galactopyranosyluronic acid)-(1→3)-D-mannose which, under controlled conditions, was isolated as the α-glucosyl fluoride. The results show that, in the *Rhizobium* polysaccharide, the α-D-glucopyranosyl residue is by far the most highly prone to hydrofluorinolysis, followed in susceptibility by the α-D-mannopyranosyl residue and α-D-galactopyranosyl group.

INTRODUCTION

An extracellular polysaccharide[1] of *Rhizobium japonicum* strain 311b 138 may play an important role in the recognition that takes place between the bacteria and soybeans in the establishment of a nitrogen-fixing symbiosis[2,3]. Structure **1** was proposed[1] as the major, fundamental repeating-unit of the polymer, on the basis of chemical, enzymic, and n.m.r.-spectroscopic evidence. However, specimens of the polymer isolated from cultures of different ages, as well as from different

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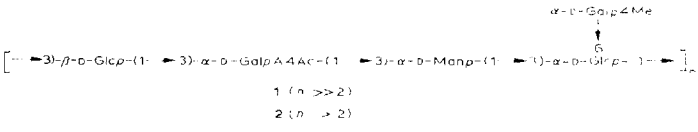
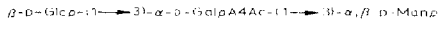
strains of *R. japonicum*, vary in the extent of acetylation[1,4], and methylation[4] at O-4, of the D-galactosyl groups of **1**. There is also a variation in the infectivity of the bacteria with culture age, which correlates very well[5] with the degree of methylation of the polysaccharide that the bacteria produces.



1 ($n \gg 2$)
2 ($n > 2$)



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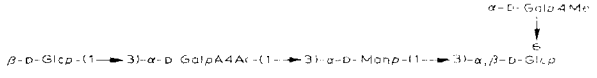

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When treated with liquid hydrogen fluoride at −23°, the polymer gave[1] a high yield of trisaccharide **3**, together with monosaccharides. It has now been found that, by performing the degradation reaction at −40°, larger oligosaccharides are produced in substantial yield. As shown here by a comparison of their ^{1}H- and ^{13}C-n.m.r. spectra with those of **3**, as well as by corroborative chemical analysis, these oligosaccharides include a pentasaccharide (**4**) and a higher homolog (**2**) that are representative of the entire repeating-unit (**1**) and minor variants of it.

RESULTS AND DISCUSSION

Preparation and chemical characterization of oligosaccharides. — As reported previously[1], trisaccharide **3** was prepared by immersing the polymer (**1**) in liquid HF for 15 min at −23°; chemical characterization of the trisaccharide was also described in detail.

In the present study, the intact polysaccharide was treated with liquid HF for 30 min at −40°, and the reaction was then quenched by the use of a slurry of calcium carbonate, Dry Ice, and dichloromethane. On being chromatographed on Bio-Gel P-2, the water-soluble products that were isolated gave the elution profile shown in Fig. 1.

Fractions were pooled as indicated in Table I, and their sugar compositions were determined. Minor monosaccharide fractions consisted mostly of 4-*O*-methyl-D-galactose (in tubes 42–46), and D-glucose *plus* D-galactose and a small proportion

TABLE I

NEUTRAL-SUGAR ANALYSIS OF OLIGOSACCHARIDES PRODUCED[a] FROM THE EXTRACELLULAR POLYSACCHARIDE OF *R. japonicum* 311b 138 IN HF AT −40°

Sugar	*Native polysaccharide*[b]	*Tube numbers*				
		22–31[b]	*32–36*[b]	*37–39*[b]	*42–46*[c]	*47–50*[d]
D-Mannose	1.0	**1.0**	**1.0**	**1.0**	0.09	0.11
D-Galactose	0.34	0.3	0.21	0.14	0.09	0.24
4-*O*-Methyl-D-galactose	0.66	0.48	0.64	0.11	**1.0**	0.0
D-Glucose	2.18	1.88	1.93	2.14	0.21	**1.0**

[a]Galacturonic acid does not produce a volatile derivative as the alditol acetate. [b]Molar ratio relative to mannose. [c]Molar ratio relative to 4-*O*-methylgalactose. [d]Molar ratio relative to glucose.

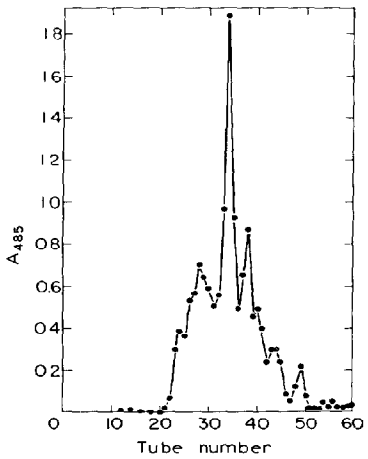

Fig. 1. Chromatography on Bio-Gel P-2 of the oligosaccharides produced by treatment of the extracellular polysaccharide of *R. japonicum* 311b 138 in liquid HF for 30 min at −40°.

of D-mannose (in tubes 47–50). Their formation indicated that, as in the reaction at −23°, the α-D-mannosyl-D-glucose and the α-D-galactosyl-D-glucose linkages are much more labile than the β-D-glucosyl and α-D-galactosyluronic linkages.

Assuming that the positions of bonds between the sugar residues did not rearrange in the HF, and that the constitution of the parent polysaccharide is that proposed earlier (namely, **1**), only a few structures are possible for each oligosaccharide. The oligosaccharide contained in tubes 37–39, which spanned the elution volume of a tetrasaccharide, had the sugar composition (see Table I) expected for

TABLE II

METHYLATION ANALYSIS OF THE PENTASACCHARIDE FRACTION

Glycosyl residue	*Position of O-methyl groups*	*Deduced glycosidic linkage*	*Molar ratio relative to galactose*
D-Glucose	2,3,4,6	terminal	0.9
D-Galactose	2,3,4,6	terminal	**1.0**
D-Mannose	2,4,6	3-*O*-	0.9
D-Glucose	2,4,6	3-*O*-	0.28
D-Glucose	2,4	3,6-di-*O*-	0.71

a tetrasaccharide containing D-glucose, D-mannose, and D-galacturonic acid in the ratios of 2:1:1, admixed with minor contaminants. Application of this fraction to a column of Bio-Rad AG-1 X-2, and elution with a formic acid gradient, permitted complete removal of the D-galactose and *O*-methyl-D-galactose present, to give pure tetrasaccharide.

The oligosaccharide contained in tubes 32–36 had a sugar composition almost identical to that of the intact polymer, the only difference being a slightly *higher* ratio of *O*-methyl-D-galactose to D-galactose. According to its elution volume and sugar composition (see Table I), this material was representative of the pentasaccharide repeating-unit of the polysaccharide. Methylation analysis of the fraction (see Table II) showed that all of the linkages of the polysaccharide were represented in their appropriate ratios, allowing for the conversion of most of the 3-*O*-linked D-glucosyl residues into terminal D-glucosyl groups. Therefore, the pentasaccharide must have been formed by cleavage of the glycosidic bond between the branched D-glucosyl residue and the 3-*O*-linked D-glucosyl residue of the backbone of the polysaccharide, and must have structure **4**. The fact that some of the 3-*O*-linked D-glucosyl residues remained intact indicated that the pentasaccharide fraction was contaminated with larger oligomers, as well as with the tetrasaccharide. Prior to analysis by n.m.r. spectroscopy (see later), these contaminants were removed by adsorption of the pentasaccharide fraction onto Bio-Rad AG-1 X-2, and elution with a formic acid gradient.

The pooled material from tubes 22–31 had almost the same sugar composition as the intact polymer, aside from a *lower* ratio of *O*-methyl-D-galactose to D-galactose. Judging from the elution volume required with the P-2 column, this fraction contained oligomers greater than pentasaccharide, and, as the composition was almost that of the repeating unit, the fraction was probably composed mainly of dimers and trimers of the repeating unit. In some instances, as indicated by the low contents of D-galactose and *O*-methyl-D-galactose, side branches may have been missing. The difference observed between the pentasaccharide and higher oligomers in their ratios of D-galactose to *O*-methyl-D-galactose may be indicative

of an influence of *O*-methyl and *O*-acetyl groups on the lability of nearby glycosidic linkages.

In an effort to obtain higher recoveries of oligosaccharides from the HF reaction, a new method was developed for quenching the reaction. That is, instead of neutralizing the HF with calcium carbonate, as had been done formerly[1], the reaction mixture was forced into 10–12 volumes of cooled, anhydrous ether. As HF forms a complex with the ether, further reaction with the sugars was thereby prevented. At the concentrations used, monosaccharides are soluble in the HF–ether complex, whereas the oligosaccharides are precipitated. Thus, when the rhizobial polysaccharide was treated in HF at −23°, and the products were recovered in this way, up to 70% of the theoretical yield of trisaccharide **3** was recovered as a solid; chromatography of the precipitate on Bio-Gel P-2 showed that it was almost pure. As shown later, this material was not the free trisaccharide (**3**) but, rather, the α-glycosyl fluoride thereof, the solvolysis product to be expected. Subsequent addition of calcium carbonate to the HF–ether complex, and evaporation of the ether, permitted the extraction, with water, of the monosaccharides from the calcium carbonate–fluoride residue.

^1H-N.m.r. spectra. — Close structural relationship between oligosaccharides

TABLE III

IDENTITY OF ^{1}H RESONANCES IN THE SPECTRA OF **3**, **4**, AND **2** (Fig. 2)

Compound	*Spectrum No.*	*Signal No.*	*δ*	*Spacing (H,H) (in Hz)*	*Proton*	*Glycosyl residue[a], glycose residue[b], or glycosyl group[c]*
3	2a	1	5.80	3.4 (4,3)	H-4	α-Gal*p*A 4Ac[a]
		2	5.36	4.0 (1,2)	H-1	α-Gal*p*A 4Ac[a]
		3	5.15	≤1 (1,2)	H-1	α-Man*p*[b]
		4	4.90	≤1 (1,2)	H-1	β-Man*p*[b]
		5	4.68	8.0 (1,2)	H-1	β-Glc*p*[c]
		6	4.48	≤1 (5,4)	H-5	α-Gal*p*A 4Ac[a]
		7	4.30	3.4 (3,4); 10 (3,2)	H-3	α-Gal*p*A 4Ac[a]
		8	4.06	4.0 (2,1); 10.0 (2,3)	H-2	α-Gal*p*A 4Ac[a]
		9	3.76	5.0 (6′,5); 12.0 (6′,6)	H-6′	β-Glc*p*[c]
		10	3.50	8.8 (3,2); 8.8 (3,4)	H-3	β-Glc*p*[c]
		11	3.28	8.0 (2,1); 9.2 (2,3)	H-2	β-Glc*p*[c]
		12	2.12	singlet	OAc	α-Gal*p*A 4Ac[a]
4	2b	13	5.21	≤1 (1,2)	H-1	α-Man*p*[a]
		14	5.24	3.4 (1,2)	H-1	α-Glc*p*[b]
		15	4.77	8.0 (1,2)	H-1	β-Glc*p*[b]
		16	4.97	~3.5 (1,2)	H-1	α-Gal*p* 4Me[c]
		17	3.53	singlet	OMe	α-Gal*p* 4Me[c]
2	2c	18	5.32	broad	H-1	α-Glc*p*[a]

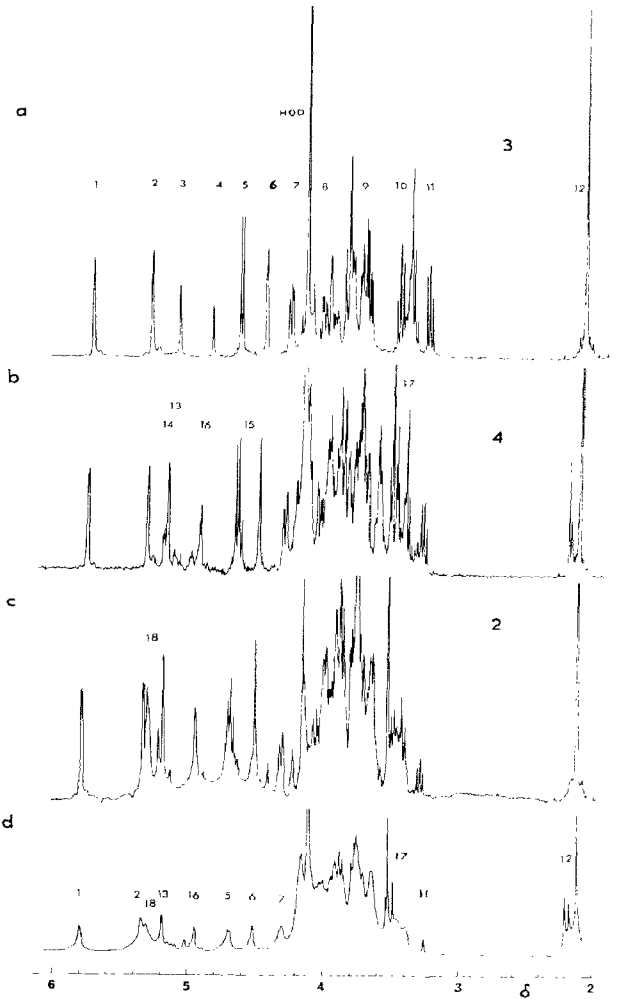

Fig. 2. ^{1}H-N.m.r. spectra (400 MHz) of oligosaccharides **3**, **4**, and **2** (a, b, and c, respectively), and (d) of the extracellular polysaccharide of *R. japonicum* from which they were prepared. (Solvent, D_2O; temp. 70°. Signal assignments are listed in Table III.)

2–**4** and the polysaccharide are clearly evident from their 400-MHz, ^{1}H-n.m.r. spectra, presented in Fig. 2, and the corresponding chemical-shift and spin–spin coupling parameters, listed in Table III.

As only partial data for the trisaccharide (**3**) were reported earlier, a fuller description of its spectrum is given here. With the knowledge that this compound consists of D-glucose, D-mannose, and D-galacturonic acid residues, most of the signals in its spectrum (see Fig. 2a) were readily identified. The D-mannose (reducing-end) residue is represented by the two signals (Nos. 3 and 4) characteristic in chemical shifts and spacings (Table III) of H-1 of α and β anomers of the sugar. Signals that were utilized to demonstrate the presence of a β-D-glucopyranosyl group in **3** are the wide, H-1 doublet (No. 5), and the doublets of doublets (Nos. 9, 10, and 11) attributable to H-6′, -3, and -2, respectively.

All five resonances of the D-galactopyranosyluronic acid residue are well resolved (see Fig. 2a). Homonuclear decoupling showed that signals 1, 2, 7, and 8 account for the four-spin system of H-1 to -4, but could not differentiate between the H-1 and H-4 doublets, due to the absence of coupling with H-5. However, the less-shielded proton (signal No. 1) must be H-4, because heteronuclear decoupling showed (see later) that it is not attached to an anomeric ^{13}C atom. The coupling of 4.0 Hz between H-1 (signal No. 2) and H-2 (signal No. 8), as well as the chemical shift[6] of H-1, shows that the anomeric configiration in this residue is α. Furthermore, the far-downfield location of the H-4 doublet (No. 1) may be confidently attributed to deshielding by the *O*-acetyl group known to be present (signal No. 12); this was confirmed when, on *O*-deacetylation, the signal disappeared and a new one appeared 1.2 p.p.m. upfield. Consequently, the n.m.r. data support the evidence from methylation analysis that the glycosyluronic acid residue bears a 4-*O*-acetyl substituent. Although the H-5 signal (No. 6) exhibits no splitting, its identity was evident from its marked sensitivity to pH: *i.e.*, acidification resulted in a downfield shift of 0.3 p.p.m., characteristic[7] for H-5 of a glycuronic acid.

Most of the resonance signals identified in Fig. 2a have close counterparts in the spectrum of pentasaccharide **4** (see Fig. 2b). In this instance, as the D-mannose is not situated at the reducing end, its anomeric proton produces only one, typically narrow, signal (No. 13), the chemical shift of which (see Table III) is consistent with an α-*manno* configuration. Chemical evidence showing that the reducing-end unit is a D-glucose residue is substantiated by the presence of two signals (Nos. 14 and 15) characteristic of an equilibrated mixture of α- and β-glucopyranose. Signals 5, 10, and 11, ascribable to H-1, -3, and -2 of the β-glucopyranosyl group, and signals 1, 2, 6, 7, 8, and 12, accounting for all protons of the 4-*O*-acetyl-α-D-galactopyranosyluronic residue, are virtually identical, in chemical shift and splitting, to the corresponding signals in Fig. 2a.

The only other major signal in the anomeric region, a quasidoublet (No. 16), accounts for H-1 of the 4-*O*-methyl-D-galactosyl constituent and, because of its spacing of ~3.5 Hz, indicates that the group is the α anomer. However, the integral for this signal is only ~65% of those for nearby signals 1, 2, 13, and 6. Similarly,

the sharp singlet (signal No. 17), which must be due to the 4-*O*-methyl substituent, is weaker than the main *O*-acetyl signal (No. 12). Consequently, only about two-thirds of the specimen giving spectrum 2b may be accommodated by formula **4**. Also, the relatively low intensity of signal 16, and other signs of structural heterogeneity evident in the form of groups of minor *O*-acetyl singlets (near δ 2.2) and of minor resonances between δ 5.2 and 5.0, indicate that there are several modifications of **4**, depending mainly upon the pattern of substitution of the α-galactopyranosyl residue by *O*-methyl or *O*-acetyl groups, or both.

A further indication of this situation comes from the spectrum (see Fig. 2c) of oligosaccharide **2**. Only one type of *O*-acetyl group is detected here (signal No. 12), corresponding to the main ester substituent in **4**, and the minor signals at δ 5.2–5.0 in Fig. 2b are absent as well, whereas the H-1 signal (No. 16) is now relatively more prominent and more evidently a doublet. In addition to peaks No. 1–17 that appear (see Table III) to be common to Figs. 2b and 2c, the latter spectrum contains a signal (No. 18) that is most readily attributed to H-1 of a fifth type of residue. Accordingly, this signal can account for the second D-glucose present in the parent polysaccharide, and its chemical shift and narrow width indicate that the residue is an α-glucopyranoside.

The low chromatographic mobility of **2** indicates that the molecular weight of the material is larger than that of pentasaccharide **4**. Moreover, although its ^{1}H-n.m.r. spectrum suggests that it may be represented primarily by structure **2**, it is not a single species. Thus, there are two sets of signals in Fig. 2c accounting for reducing-end units, namely, signals 14 and 15, which suggest the presence of an α,β-glucose unit (as in Fig. 2b), whereas signals 3 and 4 correspond to an α,β-mannose unit (as in Fig. 2a). Minor, unidentified signals are also observed at δ 4.65, 4.45, and 4.30. In its general appearance, however, the spectrum of **2** is strikingly similar to that of the intact polysaccharide (see Fig. 2d).

Many resonances in the latter spectrum are readily identified by comparison with the preceding series of oligosaccharide spectra. Signals 1, 2, 6, 7, and 12 are attributable to H-4, H-1, H-5, H-3, and the *O*-acetyl group, respectively, of the 4-*O*-acetyl-α-D-galactopyranosyluronic acid residue of **1**. The β-D-glucopyranosyl residue is represented by its H-1 and H-2 signals (Nos. 5 and 11), the 4-*O*-methyl-α-D-galactopyranosyl residue by its H-1 and *O*-methyl signals (Nos. 16 and 17), whereas the α-D-mannopyranosyl and α-D-glucopyranosyl residues are represented by their anomeric-proton signals (Nos. 13 and 18, respectively). Other close analogies are found with respect to Fig. 2b, in the form of several minor *O*-acetyl (δ ~2.2) and other (δ 5.2–5.1) resonances which, as already noted, appear to be related to the 4-*O*-methyl-α-D-galactopyranosyl residue (or an unmethylated form of it).

^{13}C-N.m.r. spectra. — In full accord with the repeating sequence (**1**) formulated, the ^{13}C-n.m.r. spectrum of the polysaccharide (see Fig. 3) contains a total of five resonances in the anomeric region. Of these, only the peak farthest downfield (δ 104.6) has a chemical shift characteristic[8] of an equatorial, anomeric C–O bond,

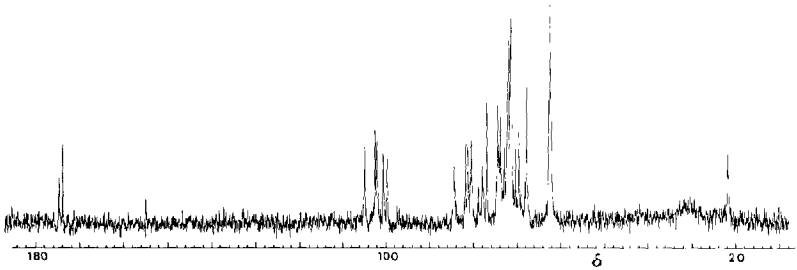

Fig. 3. ^{13}C-N.m.r. spectrum (100 MHz) of the extracellular polysaccharide of *R. japonicum* in D_2O at 25°. (Signal assignments are listed in Table IV.)

and hence, it accounts for the β-D-glucopyranosyl residue (see Table IV). Selective ^{1}H-decoupling on the basis of the ^{1}H-n.m.r. data in Fig. 2d confirmed this assignment, and also showed that the peak at δ 102.3 is that of C-1 of the α-D-mannopyranosyl residue, and the peak at δ 99.6, that of C-1 of the α-D-galactopyranosyl group. Overlap of the other two H-1 signals in Fig. 2d prevented identification of the corresponding, ^{13}C-1 resonances by heteronuclear decoupling. However, reference to the ^{13}C-n.m.r. spectrum of trisaccharide **3** (see Table IV) indicated that the signal at δ 101.8 is that of C-1 of the 4-*O*-acetyl-α-D-galactopyranosyluronic acid residue; the remaining peak, therefore, is due to C-1 of the α-D-glucopyranosyl residue.

Other signals of particular value in checking the validity of structure **1** are those produced by the carbon atoms engaged in glycosidic bonding. Secondary carbon atoms of this class are readily recognized, because, through selective deshielding, they commonly resonate in the region of δ 80, or below. Of this group, C-3 of the β-D-glucopyranosyl residue undoubtedly produces the signal farthest downfield, according to data[9] on 3-linked β-glucans. The signals at δ 80.5 and 77.9 are attributed to the linkage carbon atoms of the α-D-mannopyranosyl and the 4-*O*-acetyl-α-D-galactopyranosyluronic acid residues, respectively, by reference to the spectrum of trisaccharide **3** (see Table IV). Hence, the other two principal peaks in this region must be those of C-3 of the α-D-glucopyranosyl residue, as well as of the carbon atom (C-4) of the α-D-galactopyranosyl residue bearing the methoxyl substituent. Consistent with the glycosidic bond through O-6 of the α-D-glucopyranosyl residue in **1** is the two-carbon signal at δ 67, which may reasonably be attributed to overlapping resonances of a glycosylically bonded C-6 and a strongly deshielded, secondary carbon atom, probably C-4 of the α-D-mannopyranosyl residue. Another two-carbon signal, at δ 75, appears to accommodate assignments of C-5 for both the β-D-glucopyranosyl and α-D-mannopyranosyl residues.

The presence of a number of relatively weak, ^{13}C resonances in the spectrum confirms, as already seen from the ^{1}H-n.m.r. data, that minor structural arrange-

TABLE IV

IDENTITY OF ^{13}C RESONANCES IN THE SPECTRUM OF THE POLYSACCHARIDE (FIG. 3), AND CORRESPONDING SIGNALS IN THE 100-MHz, ^{13}C-N.M.R. SPECTRUM OF TRISACCHARIDE **3**

δ (for the polysaccharide)	*Carbon atom*	*Glycosyl residue or group*	*δ (for* **3***)*
174.6	CO(Ac)	α-Gal*p*A 4Ac	175.4
173.9	C-6	α-Gal*p*A	174.2
104.6	C-1	β-Glc*p*	104.9
102.3	C-1	α-Man*p*	
101.8	C-1	α-Gal*p*A	102.1
100.5	C-1	α-Glc*p*	
99.6	C-1	α-Gal*p*	
84.4	C-3	β-Glc*p*	
81.7	C-3	α-Glc*p*	
81.2	C-4	α-Gal*p*	
80.5	C-3	α-Man*p*	80.2
77.9	C-3	α-Gal*p*A	78.0
76.9	C-5	β-Gal*p*	77.4
			77.2
74.1	C-5	α-Man*p*	74.1
72.2	C-4	α-Gal*p*A	72.2
71.9	C-5	α-Gal*p*A	72.0
69.4	C-2	α-Gal*p*A	69.4
67.5	C-6	α-Glc*p*	67.6
67.5	C-4	α-Man*p*	
62.6	C-6	α-Gal*p*	
62.4	C-6	α-Man*p*	62.4
62.1	C-6	β-Glc*p*	62.2
61.9	CH_3(OMe)	α-Gal*p* 4Me	
21.9	CH_3(Ac)	α-Gal*p*A 4Ac	21.9

ments are yet to be identified in the extracellular polysaccharide elaborated by this strain of *Rhizobium*.

Detection of a glycosyl fluoride. — Although the use of liquid hydrogen fluoride for degradation of the polysaccharide is expected to give glycosyl fluorides as products, the spectra of oligosaccharides **2**–**4** (see Fig. 2) clearly show that these compounds are derivatives of free aldoses. Consequently, the fluoride generated initially must have been hydrolyzed during isolation of the products. However, a sample of trisaccharide **3** obtained by quenching the reaction with ether, as noted earlier, was found to have been preserved largely as a glycosyl fluoride. This is seen on comparing the ^1H-n.m.r. spectra of **3** depicted in Figs. 4a and 4b, recorded at 200 MHz. The former depicts the sample shortly after being dissolved in D_2O; the solution was slightly acidic, as shown by the downfield location (δ 4.68) of the H-5 signal of the α-galactosyluronic acid residue. By contrast, Fig. 4b is that of the solution 2 h following neutralization (the H-5 signal is shifted, accordingly, to δ 4.53).

The H-1 resonances of the α,β-mannose residue of **3** are relatively much weaker in Fig. 4a than in 4b, although Fig. 4a also contains two narrow peaks, at

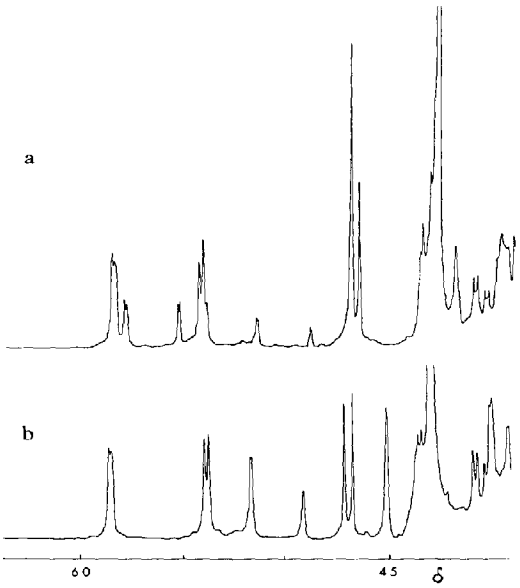

Fig. 4. ^{1}H-N.m.r. spectra (200 MHz) of oligosaccharide **3** (upper) in the form of the α-glycosyl fluoride, and (lower) after hydrolysis of the fluoride (see corresponding 400-MHz spectrum in Fig. 2a). (Solvent, D_2O; temp. 70°.)

δ 5.71 and 5.47, that are not observed in Fig. 4b, nor in any of the spectra in Fig. 2. It is proposed that these two, unique, signals are actually components of a single resonance produced by H-1 of an α-mannopyranosyl fluoride residue. Thus, the chemical shift (δ 5.59) and spacings ($J_{1,2}$ 1.8, $J_{1,F}$ 48.0 Hz) are essentially those reported[10] for 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl fluoride in $CDCl_3$ (δ 5.6; $J_{1,2}$ 1.7, $J_{1,F}$ 48.6 Hz). Consequently, hydrolysis of the fluoride restored the H-1 signals of the α,β-mannose residue to their appropriate intensities: this is evident in Fig. 4b, which corresponds to the downfield portion of the spectrum of trisaccharide **3** in Fig. 2a.

EXPERIMENTAL

General. — The extracellular polysaccharide of *R. japonicum* 3I1b 138 was isolated from stationary-phase cultures 12 days after inoculation, using the culture conditions and procedure already reported[4]. Methylation analysis was performed

as described previously[1]. For ion-exchange chromatography, a column (10 × 0.9 cm) of Bio-Rad AG-1 X-2 was employed, using a 0.0–1.0M linear gradient of formic acid solution (total volume, 150 mL). N.m.r. spectra (^1H and ^{13}C) were recorded with D_2O as the solvent (following prior H–D exchange for ^1H-n.m.r. spectra), usually at 70°, with a Bruker WH-400 spectrometer or a Varian XL-200 spectrometer; ^1H-chemical shifts (δ) were measured with respect to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS).

Partial degradation of the polysaccharide with hydrogen fluoride. — The polysaccharide was partially degraded by treatment with liquid hydrogen fluoride as already described[1], unless noted otherwise; for the treatment at −40°, this temperature was maintained with a mixture of Dry Ice and chlorobenzene.

An alternative method for quenching the reaction was used in some experiments. That is, instead of neutralizing the hydrogen fluoride with calcium carbonate in the cold, as previously, the hydrogen fluoride solution was forced through a coil at −73° into a Teflon vessel containing a slurry of Dry Ice in ethyl ether. Oligosaccharide material that was precipitated was collected on a Teflon filter, washed with ether, and dried under diminished pressure.

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