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THE STRUCTURE OF THE ACIDIC POLYSACCHARIDE SECRETED BY *Rhizobium phaseoli* STRAIN 127 K87[†,*]

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ABSTRACT

The acidic polysaccharide secreted by *Rhizobium phaseoli* strain 127 K87 was analyzed and found to have the structure shown on page 170. Each ofthe glycosyl residues was determined to be in the D configuration and in the pyranoid ring-form. The repeating unit of 11 glycosyl residues and 1 pyruvic acetal group is the largest yet found in any bacterial polysaccharide. The structures of the acidic polysaccharides secreted by four strains of *R. phaseoli,* namely, 127 K36, 127 K38, 127 K44, and 127 K87, have now been determined. The polysaccharides of all four strains have been found to be structurally distinct, although they possess certain structural features in common.

INTRODUCTION

Investigations previously reported[1–3] demonstrated that the acidic polysaccharides secreted by three strains of *Rhizobium phaseoli* (127 K36, 127 K38, and 127 K44) have structures that differ from each other, although they share certain structural features in common. The repeating units of these polysaccharides vary in size, having 8, 10, and 9 glycosyl residues, respectively. We here describe structural analysis of the acidic polysaccharide secreted by a fourth strain of *R. phaseoli* (127 K87), a polysaccharide whose structure differs from all three *R. phaseoli* polysaccharides previously studied.

[†]Host–Symbiont Interactions. Part XIII. For Part XII, see ref. 1.

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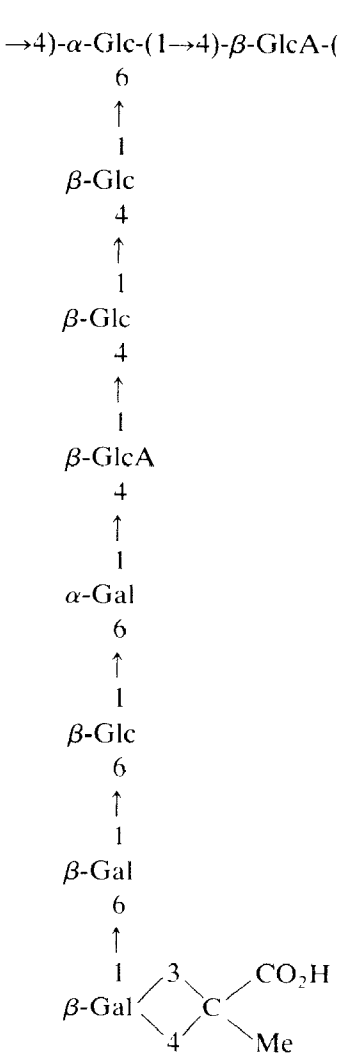

EXPERIMENTAL

Rhizobium phaseoli strain 127 K87 was obtained from Dr. J. Burton, Nitragin Company, Milwaukee, WI. Experiments were performed to confirm that strain 127 K87 is capable of nodulating bean plants. The acidic polysaccharide secreted by strain 127 K87 was purified, and structurally characterized, by procedures described previously[1,2,4], or referred to in the Results section

RESULTS AND DISCUSSION

Composition of the polysaccharide. — The glycosyl composition was determined as described[2]. Galactosyl, glucosyluronic acid, and glucosyl residues are pre-

TABLE I

GLYCOSYL-LINKAGE COMPOSITION OF THE ACIDIC POLYSACCHARIDE SECRETED BY *Rhizobium phaseoli* STRAIN 127 K87[a]

Glycosyl residues	*Positions of O-methyl groups*	*R.t.*[b]	*Sample*[a]	
			A (mol %)	*B*
Glucosyl	2,3,6	0.69	36	30
Glucosyl	2,3,4	0.72	13	9
Galactosyl	2,3,4	0.76	15	18
Galactosyl	2,6	0.77	12	9
Glucosyl	2,3	0.84	14	34

[a]Sample A was *O*-methylated and hydrolyzed, and the resulting, partially *O*-methylated aldoses were reduced ($NaBD_4$), and the alditols acetylated. Sample B was *O*-methylated, carboxyl-reduced ($LiAlD_4$), and hydrolyzed, and the resulting, partially *O*-methylated aldoses were reduced ($NaBD_4$), and the alditols acetylated. [b]Retention time relative to *myo*-inositol hexaacetate on a 10-m, SP-2100 capillary column, programmed from 150 to 240° at 4°/min.

sent in this polysaccharide in the ratios of ~1:1:2. All of the glycosyl constituents were shown to be in the D configuration[2,5].

Determination of pyruvic and acetic acid substituents by colorimetric methods[6,7] gave values of 4.0 and 6.8%, respectively. After the size of the repeating unit had been defined (see later), these values were calculated to be equivalent to 0.94 mol of pyruvic acid and 3.2 mol of acetic acid per repeating unit.

Glycosyl-linkage composition of the polysaccharide. — The polysaccharide was successively methylated, hydrolyzed, reduced, and acetylated[2], and found to contain (1→4)-linked, (1→6)-linked, and 4,6-di-*O*-substituted glucosyl residues, and (1→6)-linked and 3,4-di-*O*-substituted galactosyl residues (see Table I, column A). Carboxyl reduction [2,8] of the glucosyluronic acid residues of the *O*-methylated polysaccharide led to an increased proportion of 4,6-di-*O*-substituted glucosyl residues (Table I, column B). These data suggest a 10- or 11-glycosyl-residue repeating-unit composed of three (1→4)-linked glucosyl, one (1→6)-linked glucosyl, two (1→6)-linked galactosyl, one 3,4-di-*O*-substituted galactosyl, one 4,6-di-*O*-substituted glucosyl, and two, or three, (1→4)-linked glucosyluronic acid residues. The size of the repeating unit was rigorously shown to be 11, and the number of (1→4)-linked glucosyluronic acid residues to be 3, by the experiments to be described.

Base-catalyzed degradation of glucosyluronic acid residues. — The *O*-methylated polysaccharide was degraded by base-catalyzed elimination reactions[3,9]. The procedure included *O*-ethylation of hydroxyl groups freed by the elimination reactions. The products of the reactions were separated by 3.5-MPa liquid chromatography (l.c.) using a refractive-index monitor. Two major, carbohydrate-containing peaks were detected. The peaks yielded identical electron impact (e.i.) spectra when analyzed by mass spectrometry (m.s.) using a direct-inlet probe. The

TABLE II

DIAGNOSTIC IONS FROM THE E.I. MASS SPECTRUM OF THE PARTIALLY *O*-METHYLATED, PARTIALLY *O*-ETHYLATED TETRASACCHARIDE METHYL GLYCOSIDE OBTAINED AFTER BASE-CATALYZED DEGRADATION OF THE GLUCOSYLURONIC ACID RESIDUES OF THE PER-*O*-METHYLATED POLYSACCHARIDE SECRETED BY *Rhizobium phaseoli* STRAIN 127 K87[a]

	Diagnostic fragment-ions[b]
b′ a Et→4Glc→4Glc→OMe 6 ↑ b Glc 4 ↑ c Glc 4 ↑ Et	201 (100, cA_2 + $b'A_2$), 233 (39.0, cA_1 + bA_1), 373 (0.8, cbA_3), 405 (0.7, cbA_2), 437 (2.0, cbA_1 + $b'aJ_2$), 497 (3.6, $b'aJ_1$) 641 (0.2, $bab'J_2$ + $cbaJ_2$) 701 (2.0, $bab'J_1$ + $cbaJ_1$)

[a]This tetrasaccharide methyl glycoside is derived from the same portion of the polysaccharide that is labeled fragment [n] in Fig. 3. [b]Nomenclature described in ref. 13 (% of base peak and fragment-ion designations are in parentheses).

mass spectrum of the two peaks is consistent with that expected of the methyl α- and β-glycosides of the tetrasaccharide shown in Table II. The formation of a methyl glycoside by this procedure has been previously discussed[3].

Further analysis of one of the l.c.-purified, tetrasaccharide methyl glycosides by gas–liquid chromatography (g.l.c.)–e.i.-m.s. established the essential purity of the polysaccharide degradation-product, showing a single g.l.c. peak eluted with a retention time consistent with that of a tetrasaccharide fragment (R.t. 20.45 min). Diagnostic fragment-ions of the e.i.-mass spectrum are given in Table II.

An aliquot of the l.c.-purified tetrasaccharide methyl glycoside was hydrolyzed with 2M trifluoroacetic acid (TFA) for 1 h at 120°, the sugars were reduced ($NaBD_4$), the alditols acetylated, and the partially *O*-methylated, partially *O*-ethylated alditol acetate constituents analyzed by g.l.c. and g.l.c.–m.s. This procedure yielded the partially *O*-alkylated alditol acetates resulting from two terminal, originally (1→4)-linked, glucosyl residues, one internal (1→4)-linked glucosyl residue, and one 4,6-di-*O*-substituted glucosyl residue. The tetrasaccharide structure presented in Table II was thus established, except for one ambiguity. The data presented are consistent with both the structure presented in Table II and another structure that differs by having the glucosyl residues that are attached to O-4 and O-6 of the branched glucosyl residue reversed, so that glucosyl residue b′ is attached to O-6 and glucosyl residue b to O-4. The authenticity of the structure presented in Table II was established by the isolation and characterization of trisaccharide oligosaccharides [h] and [j] (see data given and Fig. 3).

Partial hydrolysis of the carboxyl-reduced, O-methylated polysaccharide, and separation and analysis of the oligomeric fragments after reduction and O-ethyla-

TABLE III

G L C RETENTION-TIMES, AND DIAGNOSTIC IONS OBTAINED BY E I -M S , OF PARTIALLY *O*-METHYLATED, PARTIALLY *O*-ETHYLATED DISACCHARIDE-ALDITOLS DERIVED FROM THE ACIDIC POLYSACCHARIDE SECRETED BY *Rhizobium phaseoli* STRAIN 127 K87

Oligosaccharides	*Fragment*[a]	*R t.*[b]	*Electron-impact mass-spectral fragment-ions*[c]							
			aJ_2	aJ_1	bA_1	bA_2	*Additional fragments*			
Et→4Glc→4Glc→	[a]	8 82	264 (71.8)	324 (15.6)	233 (17.9)	201 (100)	468 (0.4)	453 (0.4)	409 (1.9)	
Et→6Gal→4GlcA→	[b]	9.07	280 (100)	340 (27.2)	233 (63 5)	201[d] (84.0)	469 (1 3)	468 (1.3)	425 (1.3)	410 (0.6)
Et→4Glc→4GlcA→ 6 ↑ Et	[c]	10 98	280 (84.1)	340 (19.2)	247 (18.3)	215 100	483 (0.9)	482 (0 9)	439 (0.6)	424 (0.9)
Et→4GlcA→4Glc→	[d]	11.17	264 (100)	324 (15.0)	249 (20 5)	217 (82.9)	425 (2 2)			
Et→4GlcA→4GlcA→	[e]	11.23	280 (97.1)	340 (22 6)	249 (23.0)	217 (100)	485 (0.3)	484 (1.6)	441 (2.9)	426 (0.5)
Et→4Glc→6Glc→ 4 ↑ Et	[f]	12.13	278 (70.0)	338 (17.1)	233 (15.5)	201 (100)	206 (35.8)	174 (89.5)		
Et→4Gal→6Gal→ 3 ↑ Et	[g]	12.23	264 (100)	338 (1.8)	247 (18.8)	201 (40)	192 (5.0)	379 (2.0)	467 (0.7)	423 (0.1)

[a]See Fig. 1. [b]Retention time(in min) on a capillary column (30 m × 0.25 mm) of fused silica–SE-30. [c]Figures in parentheses show peak intensity relative to base peak (= 100). [d]The intensity of *m/z* 187 is <8% of that of *m/z* 201, indicating that the nonreducing (terminal)hexosyl group is (1→6)-linked[13].

TABLE IV

G.l.c. RETENTION-TIMES, AND DIAGNOSTIC IONS OBTAINED BY E.I.-M.S., OF PARTIALLY *O*-METHYLATED PARTIALLY *O*-ETHYLATED TRISACCHARIDE-ALDITOLS DERIVED FROM THE ACIDIC POLYSACCHARIDE SECRETED BY *Rhizobium phaseoli* STRAIN 127 K87

Oligosaccharides	*Fragment*[a]	*R.t.*[b]	*Electron-impact mass-spectral fragment-ions*[c]									
			aJ_2	aJ_1	abJ_2	abJ_1	cbA_1	cbA_2	cA_1	cA_2	*Additional fragments*	
(i) *Released by formolysis*												
Et→4Glc→4Glc→6Glc→ 4 ↑ Et	[h]	17.42	278 (12.0)	338 (0.3)	482 (14.2)	542 (4.4)	437 (0.3)	405 (0.3)	233 (15.3)	201 (100)	206 (2.0)	174 (1.3)
Et→4GlcA→4GlcA→4Glc→	[i]	17.53	264 (100)	324 (17.4)	484 (0.7)	544 (1.1)	469 (0.9)	437 (0.7)	249 (6.9)	217 (59.9)	646 (0.2)	645 (0.4)
Et→4GlcA→4Glc→4Glc→ 6 ↑ Et	[j]	17.77	278 (86.0)	338 (13.2)	482 (1.4)	542 (1.1)	453 (1.1)	421 (0.7)	249 (10.3)	217 (100)	643 (0.4)	
Et→4GlcA→4Glc→4Glc→	[k]	17.80	264 (100)	324 (15.0)	468 (0.5)	528 (1.2)	453 (1.0)	421 (0.5)	249 (7.5)	217 (69.9)	629 (0.2)	
(ii) *Released by hydrolysis (2M TFA)*												
Et→6Gal→6Glc→6Gal→	[l]	15.53	264 (100)	324 (0.8)	468 (0.4)	528 (5.7)	437 (0.8)	405 (0.4)	233 (37.8)	201 (30.6)	657 (0.8)	569 0.4

[a] [b] [c] See footnotes *a–c* to Table III

TABLE V

G.L.C. RETENTION-TIMES, AND DIAGNOSTIC IONS OBTAINED BY E.I.-M.S., OF PARTIALLY *O*-METHYLATED, PARTIALLY *O*-ETHYLATED TETRASACCHARIDE-ALDITOLS DERIVED FROM THE ACIDIC POLYSACCHARIDE SECRETED BY *Rhizobium phaseoli* STRAIN 127 K87

Oligosaccharide	*Fragment*[b]	*R.t.*[c]	*Electron-impact mass-spectral fragment-ions*[a]								
			aJ_2	aJ_1	abJ_2	abJ_1	dcA_1	dcA_2	dA_1	dA_2	$abcJ_1$
Et→4Glc→4GlcA→4GlcA→4Glc→ 6 ↑ Et	[q]	22.30	264 (100)	324 (6.0)	484 (0.8)	544 (0.6)	467 (0.7)	435 (0.2)	247 (7.4)	215 (96.7)	764 (0.7)
			abJ_2	abJ_1	cbA_1	cbA_2	cA_1	cA_2	$b'A_1$		
c b a Et→4GlcA→4Glc→4Glc→ 6 ↑ Et→4Glc b′	[m]	22.25	482 (10)	542 (6.6)	453 (4.1)	421 (2.5)	249 (5.5)	217 (100)	233 (9.6)		

[a–c] See footnotes *a–c* to Table III.

tion. — The polysaccharide was per-*O*-methylated, and the methyl-esterified carboxyl groups of the glucosyluronic acid residues were reduced with lithium aluminum deuteride, as previously described[3]. The product was treated with 88% formic acid, for 90 min at 80°, and the mixture of partially *O*-methylated oligosaccharides produced was converted into partially *O*-methylated, partially *O*-ethylated oligosaccharide-alditols[4]; these were fractionated by l.c. on a Brownlee Spheri-5 RP-18 column, using a mass spectrometer as the detector, as previously described[2]. The locations of the partially per-*O*-alkylated oligosaccharide-alditols in the l.c. fractions were determined by reconstructed, selected-ion chromatograms of the (M + 1) ions of all theoretically possible di-, tri-, and tetra-saccharide-alditols. The possible (M + 1) ions were calculated from the glycosyl-linkage composition (see Table I). Many of the fractions collected after l.c. on the Spheri-5 column were still complex mixtures of per-*O*-alkylated oligosaccharide-alditols. These fractions were combined, and rechromatographed by l.c. on a Whatman Partisil 5 ODS C-18 column, using a refractive index monitor as the detector. The purified fractions were then analyzed by g.l.c.–e.i.-m.s.

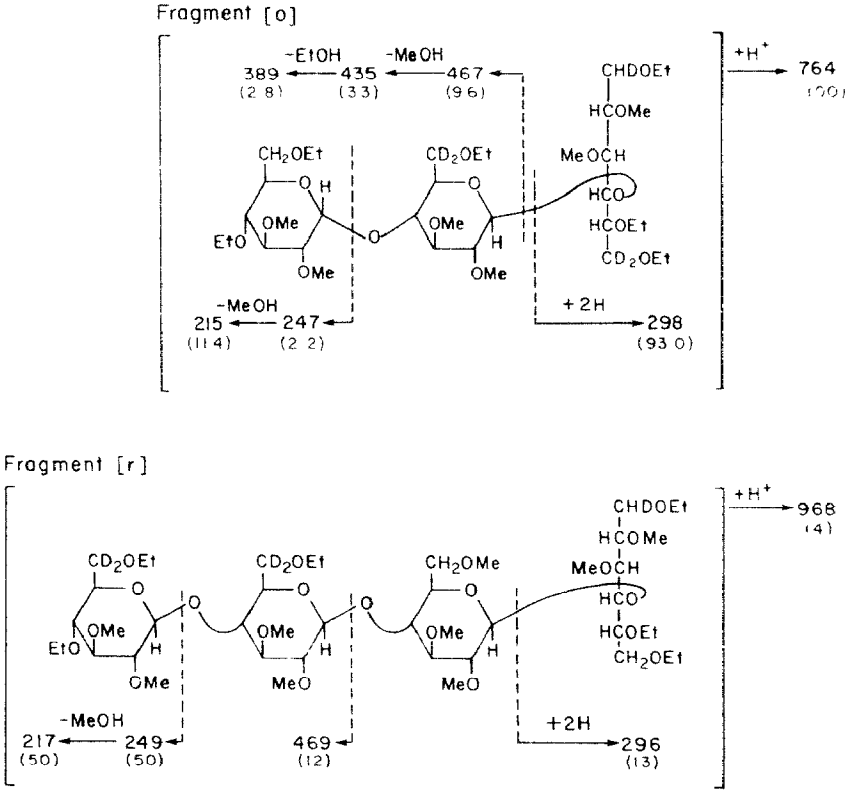

Fig. 1. Cleavage pattern obtained by e.i.-m.s. of partially *O*-methylated, partially *O*-ethylated trisaccharide-alditol fragment [o] and tetrasaccharide-alditol fragment [r] (see Fig. 3). [The figures in parentheses indicate the intensity of the ions relative to that of the base peak. The base peak of the partially *O*-methylated, partially *O*-ethylated tetrasaccharide [r] was m/z 265, its origin is unknown.]

Seven per-*O*-alkylated disaccharide-alditols (see Table III), four per-*O*-alkylated trisaccharide-alditols (see Table IV), and two per-*O*-alkylated tetrasaccharide-alditols (see Table V) were thus identified. In addition to these per-*O*-alkylated oligosaccharide-alditols, another per-*O*-alkylated trisaccharide-alditol [o] and per-*O*-alkylated tetrasaccharide-alditol [q] were identified during l.c.–m.s. analysis, but not further analyzed by g.l.c.–m.s., because the c.i. mass spectra of these two per-*O*-alkylated oligosaccharide-alditols were sufficiently distinctive to allow the fragments to be identified (see Fig. 1). It should be noted that, as a result of deuterio-reduction before *O*-ethylation, each D-glucosyluronic residue was converted into a 6,6-dideuterio-D-glucosyl residue having an ethyl group on O-6. In many of the per-*O*-alkylated oligosaccharide-alditols (see Tables III, IV, and V), (1→4)-linked glucosyl residues could not be distinguished from (1→6)-linked glucosyl or galactosyl residues. However, the isolation and characterization of fragments [p] and [l] (see later) clearly established the location of the two (1→6)-linked galactosyl residues and the one (1→6)-linked glucosyl residue known to be present in the polysaccharide (see Table I). Thus, the (1→6)-linked galactosyl, (1→6)-linked glucosyl, and (1→4)-linked glucosyl residues could all be unambiguously assigned, as given in Tables III, IV, and V.

No fragments containing the acid-labile, glycosyl linkages of the β-(1→6)-linked galactosyl residues were produced under the aforegoing hydrolysis conditions with 88% formic acid for 90 min at 80°. However, fragments containing these linkages were isolated from the carboxyl-reduced, *O*-methylated polysaccharide after hydrolysis with 2M trifluoroacetic acid (TFA) for 90 min at 80°. The mixture of partially *O*-methylated fragments produced by the milder hydrolysis was reduced with $NaBD_4$, the alditols *O*-ethylated, and the products analyzed by l.c.–m.s., using a Brownlee Spheri-5 RP-18 column. Reconstructed, selected-ion chromatograms were used to locate two partially *O*-methylated, partially *O*-ethylated oligosaccharide-alditols containing the (1→6)-linked galactosyl residues, and a pentasaccharide fragment having the originally 3,4-di-*O*-substituted galactosyl residue at the nonreducing end and the alditol derivative of a (1→4)-linked, carboxyl-reduced glucosyluronic acid residue at the reducing end.

The trisaccharide fragment, consisting of three (1→6)-linked hexosyl residues, had an (M + 1) ion at *m/z* 718, and was eluted from the l.c. column with a retention time of 18 min. Further analysis of this fragment by g.l.c.–e.i.-m.s. showed that its fragment-ions were consistent with a sequence of three glycosyl residues (fragment [l], Table IV). A sample of trisaccharide fragment [l] was hydrolyzed with 2M TFA for 1 h at 120°, the sugars were reduced, the alditols converted into acetates, and these analyzed by g.l.c.–e.i.-m.s. This process resulted in the formation of a 6-*O*-acetyl-1,5-di-*O*-ethyl-2,3,4-tri-*O*-methylhexitol, a 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol, and a 1,5-di-*O*-acetyl-6-*O*-ethyl-2,3,4-tri-*O*-methylhexitol. Thus, the trisaccharide fragment contained three (1→6)-linked hexosyl residues. The results of the glycosyl-linkage analysis of the polysaccharide (see Table I) required that two of the residues be galactosyl, and the third, a glucosyl

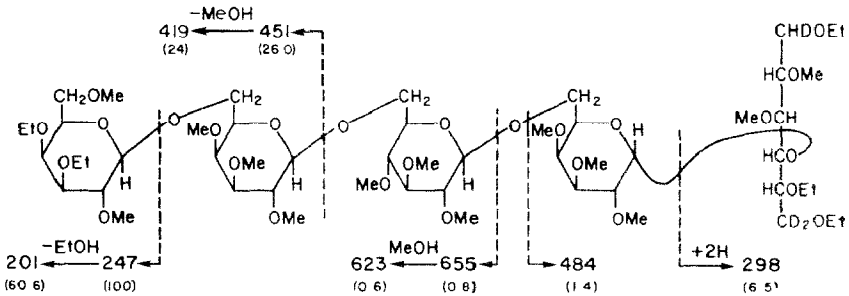

Fig. 2. Cleavage pattern obtained by c.i.-m.s. of partially *O*-methylated, partially *O*-ethylated pentasaccharide-alditol fragment [p] (see Fig. 3). [The figures in parentheses indicate the intensity of the ions relative to that of the base peak.]

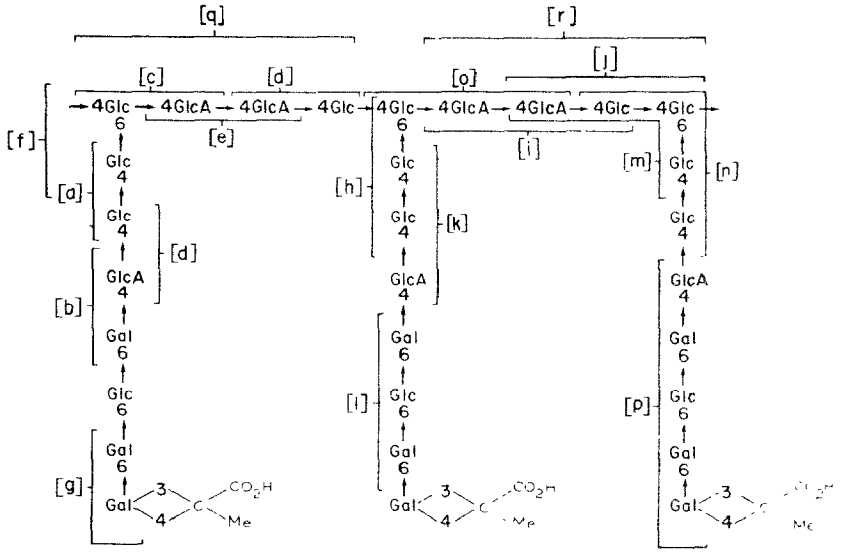

Fig. 3. Summary of the structurally characterized oligosaccharides derived from the acidic polysaccharide secreted by *R. phaseoli* strain 127 K87. [The glycosyl sequences of these fragments, together with the glycosyl-linkage composition, define the illustrated repeating-unit of the polysaccharide.]

residue. Comparison of the retention time of the 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol (0.72 relative to *myo*-inositol) with those of the appropriate, partially *O*-methylated, partially *O*-acetylated glucitol and galactitol standards established that this derivative was 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol. The two other (1→6)-linked glycosyl residues of fragment [l] are, thus, both galactosyl residues, and the sequence of fragment [l] is defined.

The (M + 1) ions of the per-*O*-alkylated pentasaccharide-alditols could not be located, because the mass spectrometer used could not detect masses greater than *m/z* 1000. However, the desired pentasaccharide fragment was known to have a 3,4-di-*O*-substituted galactosyl group at the nonreducing terminus, and a (1→4)-linked, carboxyl-reduced glucuronic acid residue at the alditol terminus. Selected-

ion chromatograms reconstructed to detect these terminal residues (*m/z* 247 and 298, respectively) in the c.i.-mass spectrum of the l.c. effluent were obtained. A per-*O*-alkylated fragment was detected, having a retention time of 29.2 min (reasonable for the derivative of a pentasaccharide fragment), which yielded the correct c.i.-m.s. fragment-ions (see Fig. 2). This component was further analyzed by fast-atom-bombardment, mass spectrometry, which yielded a strong (M + H) ion at *m/z* 1156. This (M + H) ion identified the molecule as a pentasaccharide that, in the polysaccharide, had a "branched" hexosyl residue at the nonreducing end, a (1→4)-linked, carboxyl-reduced glycuronic acid residue at the reducing end, and three internal hexosyl residues.

Sequential hydrolysis, reduction, and acetylation of this partially *O*-methylated, partially *O*-ethylated pentasaccharide-alditol, and analysis of the derived per-*O*-alkylated alditol acetates, showed that the pentasaccharide is composed of a galactosyl group, that was originally 3,4-di-*O*-substituted, at the nonreducing terminal end, two (1→6)-linked galactosyl residues, one (1→6)-linked glucosyl residue, and one (1→4)-linked glucuronic residue at the alditol terminus. The se-

TABLE VI

^{1}H-N M.R CHEMICAL SHIFTS AND COUPLING CONSTANTS OF THE ANOMERIC PROTONS OF SELECTED PER-*O*-ALKYLATED OLIGOSACCHARIDE-ALDITOLS AND THE PER-*O*-ALKYLATED TETRASACCHARIDE METHYL GLUCOSIDE

Material	*Fragment*	*Chemical shift (δ)*[a]	*Coupling constant $J_{1,2}$ (Hz)*	*Assigned anomeric configuration*
Et→6Gal→4GlcA→	[b]	5.27	2.0	one α
Et→4Gal→6Gal→	[g]	4.24	not resolved	one β
3 ↑ Et				
Et→4GlcA→4GlcA→4Glc	[i]	4.37	8.4	one β
		4.45	8.4	one β
Et→4Glc→4GlcA→4GlcA→	[o]	5.62	4.0	one α
6 ↑ Et		4.52	8.4	one β
Et→6Gal→6Glc→6Gal→	[l]	4.26	9.0	one β
		4.29	8.0	one β
Et→4Gal→6Gal→6Glc→6Gal→4GlcA→	[p]	5 25	2.0	one α
3 ↑ Et		4.26	not resolved	three β
Et→4Glc→4Glc→6Glc→OMe	[n]	4.8	2.7	methyl glucoside α
4 ↑ Et→4Glc		4.22	not resolved	three β

[a]Relative to the signal for internal chloroform at δ 7.26.

quence of the three internal (1→6)-linked hexosyl residues is defined by fragment [l], and, therefore, the structure of the pentasaccharide fragment was established.

The repeating unit of the polysaccharide. — Shown in Fig. 3 are the fragments identified among the products obtained by base-catalyzed degradation of the glycosyluronic acid, and by partial hydrolysis of the *O*-methylated, carboxyl-reduced polysaccharide. The repeating unit of eleven glycosyl residues that is depicted in Fig. 3 is defined by the structures of all of the per-*O*-alkylated oligosaccharide-alditols, the base-catalyzed degradation-products, and the glycosyl-linkage analysis (see Table II). No fragments were found that had glycosyl sequences contradictory to the structure shown in Fig. 3.

Determination of the anomeric configurations of the glycosyl linkages by ^{1}H-n.m.r. spectroscopy. — The ^{1}H-n.m.r. spectrum of the methylated polysaccharide showed two α-anomeric signals (at δ 5.44 and 5.59, insufficiently resolved to permit measurement of the coupling constants) and multiple, unresolved β signals. Six of the per-*O*-alkylated oligosaccharide-alditols ([b], [g], [i], [l], [o], and [p]) isolated after partial hydrolysis and derivatization of the *O*-methylated polysaccharide, and the per-*O*-alkylated tetrasaccharide methyl glucoside [n] obtained after base-catalyzed degradation and *O*-ethylation of the *O*-methylated polysaccharide, were examined by ^{1}H-n.m.r. spectroscopy (see Table VI) in order to determine the position of the two β-anomeric, glycosidic linkages.

The ^{1}H-n.m.r. spectrum of fragment [b] clearly showed that the (1→6)-linked galactosyl residue attached to a glucosyluronic acid residue in the side chain is α-linked. This assignment was confirmed by the ^{1}H-n.m.r. spectra of fragments [p], [l], and [g].

Fragment [o] exhibited one α and one β signal, and fragment [i] possessed two β-linked glycosyl residues. Therefore, the α signal of fragment [o] could be assigned to the anomeric linkage between the branch-point, 4,6-di-*O*-substituted glucosyl and the (1→4)-linked glucosyluronic acid residue. Thus, the two α-anomeric linkages were located.

All of the remaining anomeric linkages in the repeating unit were, with one exception, represented in the various fragments listed in Table VI, and all of these were confirmed to be in the β configuration. The one linkage not accounted for was that of the glucosyluronic acid residue in the side chain. However, l.c.-m.s. analysis confirmed that this linkage is in the β-anomeric configuration. Fragment [d] (see Fig. 3) occurs both in the side chain and the backbone chain. If the side-chain fragment [d] were in the α-anomeric configuration, it would have been separated during liquid chromatography from the β-linked fragment [d] in the backbone chain[8]. Reconstructed, selected-ion chromatography performed on the l.c.-mass spectra clearly showed that fragment [d] was eluted in only a single position, confirming the β assignment.

DISCUSSION

The results of this investigation have established that the acidic polysaccharide secreted by *R. phaseoli* strain 127 K87 has an undecasaccharide repeating-unit, the largest yet to be reported for any bacterial polysaccharide. The acidic polysaccharides secreted by four strains of *R. phaseoli* (127 K36, 127 K38, 127 K44, and 127 K87) have now been examined, and each has been found to be structurally different from the others[1–3] (see Fig. 4). How many structurally unique, acidic polysaccharides may be secreted by other strains of *R. phaseoli* is still unknown.

The four *R. phaseoli* polysaccharides are structurally identical with respect to the backbone chain and the first two residues of the side chain, but differ in the remaining glycosyl sequences of their side chains. The polysaccharides also differ in the size of their repeating units; the repeating units respectively contain 8, 9, 10, and 11 residues.

The extracellular polysaccharides secreted by *R. meliloti* show similar characteristics. Two strains of this organism were found to secrete identical acidic polysaccharides[10,11], but the polysaccharide of a third strain was shown to differ from that of the first two[12]. As with the *R. phaseoli* polysaccharides, the polysaccharides secreted by the *R. meliloti* strains are structurally identical to each other with respect to the backbone chain and the first two residues of the side chain; the

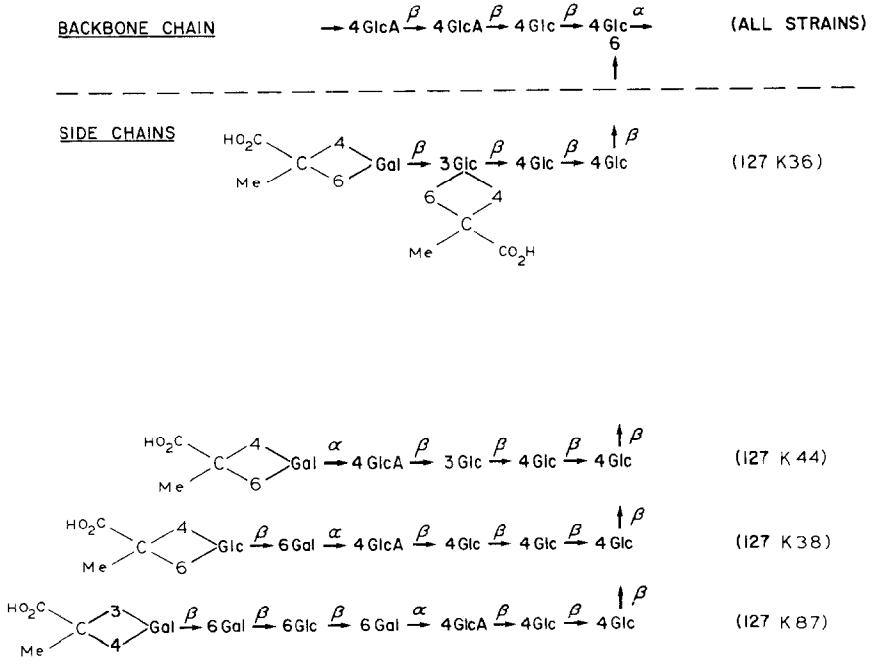

Fig. 4. The glycosyl-residue sequence, location of pyruvic acetal groups, and anomeric configuration of the glycosyl residues of the repeating units of the acidic polysaccharides secreted by four strains of *R. phaseoli*

differences are located at the terminal residues of the side chains. The backbone chains of the *R. meliloti* polysaccharides differ structurally from those of the *R. phaseoli* polysaccharides.

One of the *R. phaseoli* polysaccharides, that of strain 127 K36, is identical, with respect to glycosyl sequences, pyruvic acetal substitution, and anomeric configuration, to the acidic polysaccharides secreted by two strains of *R. leguminosarum* and two strains of *R. trifolii*[2-8]. The differences between the polysaccharides secreted by a single *Rhizobium* species and the identical glycosyl sequences in the polysaccharides secreted by strains of three different *Rhizobium* species raise interesting questions regarding the role of these polysaccharides in symbiotic specificity. If it is proposed that the recognition process between bean plants and the four strains of *R. phaseoli* involves some common structural features present in all of the acidic polysaccharides of the four strains, the same mechanism must be capable of rejecting the apparently identical polysaccharides of *R. trifolii* strains NA30 and 0403 and *R. leguminosarum* strains 128c53 and 128c63. The latter strains do not nodulate bean plants, nor does *R. phaseoli* strain 127 K36 nodulate pea or clover plants[2]. Polysaccharides that appear to be identical may, in fact, be structurally unique, being distinguished by possessing different, base-labile *O*-acyl substituents, or similar *O*-acyl substituents located at different positions, in them. All of these polysaccharides were shown, by ^{1}H-n.m.r. analysis, to contain two, or three, acetyl groups per repeating unit (data not presented), as well as other *O*-acyl groups, but the locations of the *O*-acyl groups have not yet been determined.

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