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Studies of O-specific polysaccharide chains of *Pseudomonas solanacearum* lipopolysaccharides consisting of structurally different repeating units

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ABSTRACT

The structures of the O-antigenic polysaccharide chains of lipopolysaccharides of a number of *Pseudomonas solanacearum* strains were elucidated mainly with the help of methylation analysis and ^{13}C NMR spectroscopy, including a computer-assisted ^{13}C NMR-based analysis. Six structurally distinct but related polysaccharides were identified. They have a backbone which is built up of three L-rhamnopyranosyl residues and one 2-acetamido-2-deoxy-D-glucopyranosyl residue, and is unsubstituted or substituted with a residue of L-xylopyranose or L-rhamnopyranose as a monosaccharide side chain. The lipopolysaccharides of most of the strains contain polysaccharide chains consisting of at least two structurally different types of repeating units. Three of the polysaccharides are common to more than one strain.

INTRODUCTION

No classification scheme, based on serological specificity of outer-membrane lipopolysaccharides, has been elaborated for the phytopathogenic microorganism *Pseudomonas solanacearum*. In chemical studies of the lipopolysaccharides, a number of *P. solanacearum* strains have been found[1–4] to possess the identical O-specific polysaccharide **1**.

→ 3)-α-D-Glc*p*NAc-(1 → 2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 →

A B C D

1

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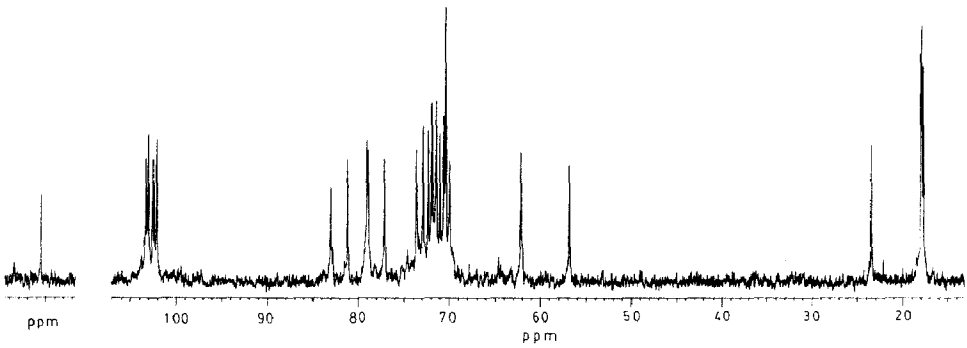


Fig. 1. ^{13}C NMR spectrum of the polysaccharide of strain ICMP 767.

We now report the structures of O-antigens of another group of strains of this microorganism, all of which also contain L-rhamnose and 2-acetamido-2-deoxy-D-glucose and some of which include additionally L-xylose. The results of a preliminary study of the polysaccharide of strain PDDCC 5712 have been reported[3].

RESULTS

The lipopolysaccharides were isolated by the phenol–water procedure[5] from *P. solanacearum* strains PDDCC 5712, ICMP 766, ICMP 767, ICMP 7944, ICMP 7955, ICMP 7959, ICMP 7960, ICMP 8066, ICMP 8072, and ICMP 8277. They were cleaved with dilute acetic acid to give O-specific polysaccharides isolated by gel-permeation chromatography on Sephadex G-50.

Acid hydrolysis of each of the polysaccharides showed the presence of L-rhamnose and 2-amino-2-deoxy-D-glucose. L-Xylose is a component of the polysaccharides of all the strains except ICMP 767, ICMP 7944, and ICMP 8277.

Strain ICMP 767.—Sugar analysis of the polysaccharide revealed the presence of L-rhamnose (75%), 2-amino-2-deoxy-D-glucose (22%), and D-glucose (5%).

The ^{13}C NMR spectrum (Fig. 1, Table I) indicated the presence of a tetrasaccharide repeating unit (there are four signals for anomeric carbons at 102.0–103.2 ppm), which includes three Rha residues (three signals for CH_3-C at 17.7–18.0 ppm) and one GlcNAc residue (C-2 at 56.8 ppm, C-6 at 62.1 ppm, CH_3CON at 23.4 ppm, CH_3CON at 175.5 ppm).

A computer-assisted approach[6] was applied to the structural analysis of this polysaccharide, based on the calculation of ^{13}C chemical shifts for all possible structures and searching for the structure with the best fit of the calculated and experimental data. The analysis revealed two structures that are characterised by the smallest S values (0.4 and 0.6, respectively), where S is the sum of the squared deviations for the chemical shifts of the corresponding signals per one sugar unit. These two structures differ only in the position of substitution of one of the rhamnose residues (unit C). All other structures had S value > 1 or a deviation

TABLE I

^{13}C NMR data (δ in ppm) [a]

Unit		C-1	C-2	C-3	C-4	C-5	C-6
The polysaccharide of strain ICMP 767							
Structure **2**							
→ 3)-β-D-Glc*p*NAc-(1 →	(A)	103.2	56.8	82.9	69.9	77.0	62.1
→ 3)-α-L-Rha*p*-(1 →	(B)	102.9	71.0	81.1	72.2	70.6	17.7
→ 2)-α-L-Rha*p*-(1 →	(C)	102.0	78.9	71.4	73.6	70.3	17.9
→ 3)-α-L-Rha*p*-(1 →	(D)	102.4	71.9	78.8	72.8	70.3	18.0
The polysaccharide of strain ICMP 8066							
Structure **3**							
→ 3)-β-D-Glc*p*NAc-(1 →	(A)	103.3	56.6	82.9	69.8	77.0	62.1
→ 3)-α-L-Rha*p*-(1 →	(B)	103.1	71.0	81.2	72.2	70.7	17.7
→ 2)-α-L-Rha*p*-(1 →	(C)	101.6	80.6	71.2	73.8	70.3	18.0
→ 3)-α-L-Rha*p*-(1 →	(D)	102.2	72.4	76.4	80.8	69.8	18.2
4 ↑ β-L-Xyl*p*-(1 →	(E)	104.9	74.7	77.2	70.3	66.3	
Oligosaccharide **4** [b]							
α-L-Rha*p*-(1 →	(D)	102.7	72.1	71.6	73.3	70.3	17.8
→ 3)-β-D-Glc*p*NAc-(1 →	(A)	103.4	57.0	82.7	69.8	77.2	62.0
→ 3)-α-L-Rha*p*-(1 →	(B)	100.4 (100.6)	71.4	81.4	72.3	70.4	17.8
→ 2)-Gro	(C)	62.8 (62.7)	79.6 (79.6)	62.0 (61.9)			
The polysaccharie of strain ICMP 7955							
Structure **6**							
→ 3)-α-D-Glc*p*NAc-(1 →	(A)	95.6	54.4	80.9	69.8	73.5	61.9
→ 3)-α-L-Rha*p*-(1 →	(B)	103.3	68.0	76.7	71.9	70.4	17.8
→ 2)-α-L-Rha*p*-(1 →	(C)	102.0	79.6	71.4	73.6	70.4	18.0
→ 3)-α-L-Rha*p*-(1 →	(D)	102.4	71.8	78.8	73.0	70.4	18.0
Oligosaccharide **7** [b]							
α-L-Rha*p*-(1 →	(D)	102.8	71.7	71.7	73.7	70.4	17.7
→ 3)-α-D-Glc*p*NAc-(1 →	(A)	95.7	54.6	80.7	69.6	73.3	61.7
→ 3)-α-L-Rha*p*-(1 →	(B)	100.8 (100.6)	68.3	76.6	72.2	70.4	17.0
→ 2)-Gro	(C)	62.8 (62.7)	79.7 (79.6)	62.0 (61.9)			
The polysaccharide of strain ICMP 8072							
Structure **1** [c]							
→ 3)-α-D-Glc*p*NAc-(1 →	(A)	97.5 (97.3)	54.4 (54.2)	80.6 (80.4)	69.8 (69.5)	73.7 (73.4)	62.0 (61.7)
→ 2)-α-L-Rha*p*-(1 →	(B)	100.7 (100.5)	77.6 (77.3)	70.9 (70.6)	73.7 (73.4)	70.7 (70.5)	18.0 (17.8)
→ 2)-α-L-Rha*p*-(1 →	(C)	102.0 (101.8)	79.6 (79.4)	71.4 (71.3)	73.7 (73.4)	70.4 (70.2)	18.0 (17.8)
→ 3)-α-L-Rha*p*-(1 →	(D)	102.2 (101.9)	71.9 (71.7)	79.0 (78.7)	72.9 (72.7)	70.4 (70.2)	17.8 (17.6)

TABLE 1 (continued)

Unit		C-1	C-2	C-3	C-4	C-5	C-6
Structure **8**							
→ 3)-α-D-Glc*p*NAc-(1 →	(A)	97.1	54.4	80.6	69.8	73.7	62.0
→ 2)-α-L-Rha*p*-(1 →	(B)	100.5	77.8	70.9	73.7	70.7	18.0
→ 2)-α-L-Rha*p*-(1 →	(C)	102.0	79.8	71.4	73.8	70.5	18.0
→ 3)-α-L-Rha*p*-(1 →	(D)	102.4	72.2	77.1	81.1	69.8	18.4
4 ↑							
β-L-Xyl*p*-(1 →	(E)	105.3	75.0	77.3	70.5	66.5	
The polysaccharide of strain ICMP 7944							
Structure **9**							
→ 3)-α-D-Glc*p*NAc-(1 →	(A)	97.2	54.4	79.2	69.5	72.9	61.7
→ 2)-α-L-Rha*p*-(1 →	(B)	100.4	78.8	75.6	73.6	70.5	18.0
3 ↑							
→ 2)-α-L-Rha*p*-(1 →	(C)	102.0	80.5	71.2	73.4	70.3	18.0
→ 3)-α-L-Rha*p*-(1 →	(D)	102.2	71.5	79.2	72.6	70.3	17.8
α-L-Rha*p*-(1 →	(E)	103.4	71.9	71.3	73.6	70.3	17.8

[a] Assignments of signals with differences in chemical shifts of < 0.5 ppm could be interchanged. Additional signals: NAc at 23.4–23.5 (CH_3) and 175.1–175.6 (CO) ppm. [b] The published data[11] are given in parentheses. [c] The published data[3] are given in parentheses.

for at least one signal of > 1 ppm and were, therefore, inconsistent with the experimental ^{13}C NMR spectrum.

In methylation analysis, 2,4-di-*O*-methylrhamnose, 3,4-di-*O*-methylrhamnose, and 2-deoxy-4,6-di-*O*-methyl-2-methylaminoglucose were identified by GLC–MS as alditol acetates, which allowed a choice between the two proposed structures in favour of structure **2** for the repeating unit.

→ 3)-β-D-Glc*p*NAc-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 →

A B C D

2 (*S* 0.4, strain ICMP 767)

Glucose found in the polysaccharide seems to be derived from the core: this sugar, together with rhamnose, is a typical component of this region of lipopolysaccharides of various pseudomonads[7].

Strains PDDCC 5712, ICMP 766, ICMP 7959, and ICMP 8066.—The polysaccharides of these four strains are practically identical by their monosaccharide composition and contain L-rhamnose (70% in strain PDDCC 5712), 2-amino-2-deoxy-D-glucose (17%), L-xylose (10%), and D-glucose (2.5%). The ^{13}C NMR spectra of the polysaccharides (Fig. 2) showed that they have the same structure and consist of two types of repeating units in the ratio ~ 3 : 1. The predominant unit is a pentasaccharide, which includes the same sugar constituents as **2** and additionally L-xylose. A more detailed study was performed with the polysaccharide from strain PDDCC 5712.

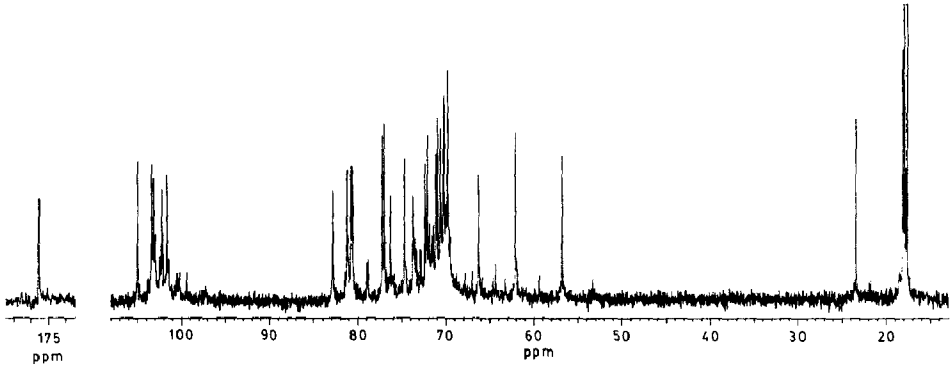
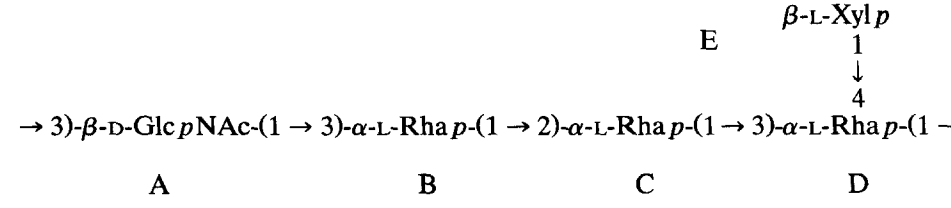


Fig. 2. ^{13}C NMR spectrum of the polysaccharide of strain ICMP 766.

Methylation analysis revealed 2,3,4-tri-*O*-methylxylose, 2,4-di-*O*-methylrhamnose, 3,4-di-*O*-methylrhamnose, 2-*O*-methylrhamnose, and 2-deoxy-4,6-di-*O*-methyl-2-methylaminoglucose. Hence, the polysaccharide is branched, one of the Rha residues is at the branching point and disubstituted at positions 3 and 4, the GlcNAc and the second Rha residue are 3-substituted, the third Rha residue is 2-substituted, and the Xyl is the lateral sugar residue.

These data allowed the conclusions that the main chain of this branched polysaccharide has the structure **2** and that one of the 3-substituted Rha residues is xylosylated at position 4. As judged by the presence in the ^{13}C NMR spectrum of signals at 104.9 and 66.3 ppm for C-1 and C-5, respectively, the Xyl residue (unit E) is β-pyranoid[8].

Comparison of the ^{13}C NMR chemical shifts for the linear (**2**) and branched repeating units (Table I) confirmed this conclusion and indicated that the substituted Rha residue is unit D (structure **3**). Indeed, the signal for C-4 of this unit is shifted downfield from 72.9 ppm in **2** to 80.8 ppm in **3** due to the α-effect of glycosylation, whereas the signals for C-3 and C-5 are shifted upfield from 78.9 and 70.4 ppm in **2** to 76.4 and 69.6 ppm in **3**, respectively, due to the β-effects of glycosylation[6]. The displacements of other signals are insignificant except for those of C-1,2 of unit C, which are close to the site of attachment of xylose.



3 (strains PDDCC 5712, ICMP 766, ICMP 7959, ICMP 8066)

In order to confirm further structure **3**, Smith degradation of the polysaccharide was carried out, affording an oligosaccharide containing rhamnose, 2-acetamido-

2-deoxyglucose, and glycerol in the ratios ~ 2:1:1. Methylation analysis indicated it to be linear and terminated with a Rha residue.

Only two structures (*S* values 0.4 and 0.6, respectively), evaluated for the oligosaccharide with the aid of the computer-assisted method[6,10], are consistent with both the methylation analysis and the ^{13}C NMR data. They differ in the sequence of the GlcNAc (unit A) and rhamnose (unit B) residues. The choice in favour of structure **4** was made by comparison of the ^{13}C NMR data for the oligosaccharide and the published data[11] for α-L-Rha*p*-(1 → 2)-Gro, which demonstrated a good fit of the chemical shifts for C-1 of the Rha residue (unit B) and C-1,2,3 of Gro (Table I).

α-L-Rha*p*-(1 → 3)-β-D-Glc*p*NAc-(1 → 3)-α-L-Rha*p*-(1 → 2)-Gro

D A B C

4 (*S* 0.4)

Another selective degradation of the polysaccharide was achieved by *N*-deacetylation with hydrazine hydrate in the presence of hydrazine sulphate[9] followed by deamination with nitrous acid and borohydride reduction. As a result, an oligosaccharide was obtained which was built-up of rhamnose, xylose, and 2,5-anhydromannitol (2,5anMan-ol). Methylation analysis proved the oligosaccharide to be branched with the Xyl and one of the Rha residues occupying the terminal ends, the second Rha residue substituted at position 2, and the third one substituted at positions 3 and 4. Thus, the deaminated and reduced oligosaccharide had structure **5** which, like structure **4**, is consistent with structure **3** for the polysaccharide.

E β-L-Xyl*p*
1
↓
4

α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-D-2,5anMan-ol

B C D A

5

Strain ICMP 7960.—Sugar analysis of the polysaccharide revealed the presence of L-rhamnose (74%), 2-amino-2-deoxy-D-glucose (20%), L-xylose (4%), and D-glucose (4%). The ^{13}C NMR spectrum (Fig. 3) contained two series of signals in the ratio of integral intensities ~ 3–4:1. The major series formed the spectrum of the polysaccharide from strain ICMP 767, which has structure **2**, whereas the minor series corresponded to the polysaccharide from strain PDDCC 5712 having structure **3**.

Methylation led to identification of the same sugars as in methylation of the polysaccharide of strain ICMP 767 (see above), with the main partially methylated monosaccharides derived from structure **2**, and, additionally, 2,3,4-tri-*O*-methyl-

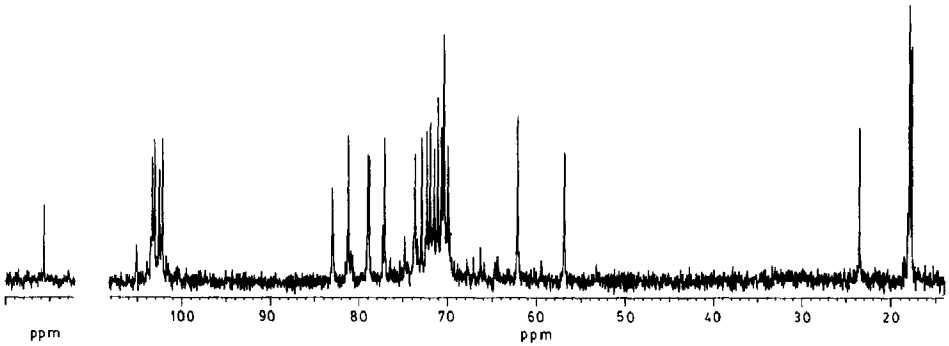


Fig. 3. ^{13}C NMR spectrum of the polysaccharide of strain ICMP 7960.

xylose and 2-*O*-methylrhamnose as the minor components originating from structure **3**. Smith degradation of the polysaccharide afforded the oligosaccharide **4**, which was identical to the Smith-degradation product of the polysaccharide of strain PDDCC 5712 (see above).

Therefore, the predominant repeating unit in strain ICMP 7960 has structure **2** and the minor one has structure **3**. Thus, this O-antigen belongs to the same structural type as that of the strains PDDCC 5712, ICMP 766, ICMP 7959, and ICMP 8066, and differs only in the degree of xylosylation.

Strain ICMP 7955.—The polysaccharide has practically the same sugar composition as that of strain 7960 (see above). The ^{13}C NMR spectrum (Fig. 4) indicated the presence of a predominant tetrasaccharide repeating unit, which constitutes not less than 70% of the total and contains one GlcNAc and three Rha residues.

The computer-assisted analysis[6] showed that the only structure consistent with

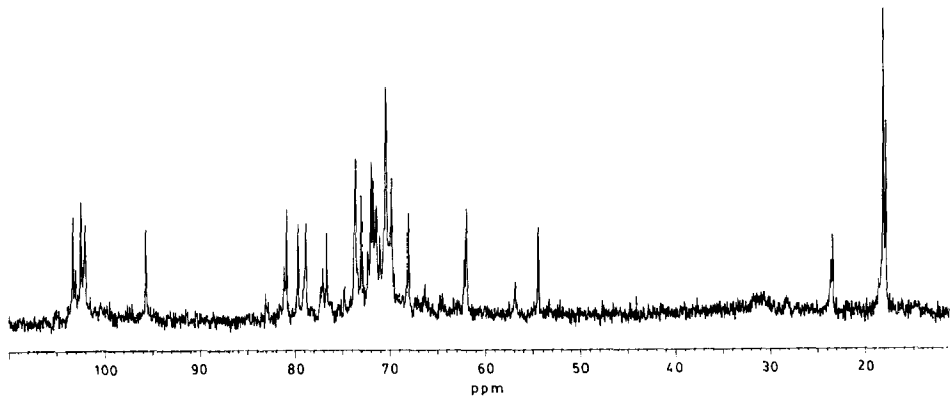


Fig. 4. ^{13}C NMR spectrum of the polysaccharide of strain ICMP 7955.

the ^{13}C NMR data for the predominant repeating unit is **6** (*S* 0.3, Table I); other structures have *S* values > 1 or a deviation of > 1 ppm for at least one signal.

→ 3)-α-D-Glc*p*NAc-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 →

A B C D

6 (*S* 0.3, strain ICMP 7955)

Structure **6** was confirmed by Smith degradation of the polysaccharide. The resulting oligosaccharide was structurally elucidated as the initial polysaccharide, only one structure (**7**) with the value *S* 0.9 being found consistent with the experimental ^{13}C NMR spectrum (Table I). This oligosaccharide differs from oligosaccharide **4** only in the configuration of the glycosidic linkage of the GlcNAc residue.

α-L-Rha*p*-(1 → 3)-α-D-Glc*p*NAc-(1 → 3)-α-L-Rha*p*-(1 → 2)-Gro

D A B C

7 (*S* 0.9)

The minor series of signals in the ^{13}C NMR spectrum of the polysaccharide definitely belong to the repeating unit **3**. This conclusion as well as structure **6** accord with the data from the methylation analysis of the polysaccharide, which resulted in identification of the same major and minor partially methylated sugars as in the methylation analysis of the polysaccharide from strain ICMP 7960 (see above).

The simultaneous presence in the polysaccharide from strain ICMP 7955 of the units of structure **2** and/or another minor repeating unit could not be excluded on the basis of the data obtained.

Strain ICMP 8072.—The sugar composition of the polysaccharide of this strain is very similar to that of strain PDDCC 5712 (see above). Judging from the ^{13}C NMR spectrum (Fig. 5), there are present several types of tetra- and penta-sac-

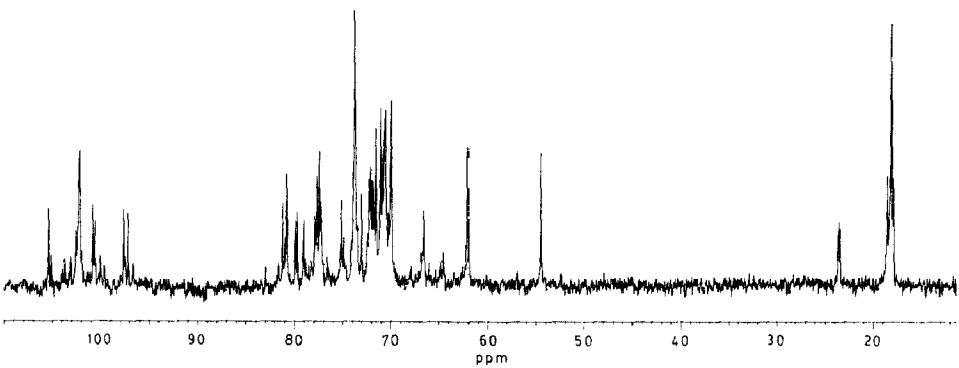
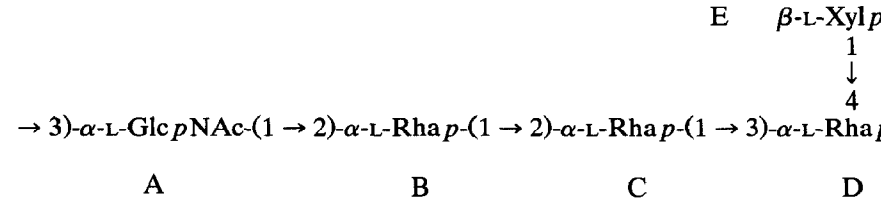


Fig. 5. ^{13}C NMR spectrum of the polysaccharide of strain ICMP 8072.

charide repeating units. The predominant tetrasaccharide repeating unit was identified as having structure **1** by comparison with the published[3] ^{13}C NMR data (Table I).

Methylation analysis led to the identification of 2,3,4-tri-*O*-methylxylose, 2,4-di-*O*-methylrhamnose, 3,4-di-*O*-methylrhamnose, 2-*O*-methylrhamnose, and 2-deoxy-4,6-di-*O*-methyl-2-methylaminoglucose as the major components and showed that, together with the repeating unit **1**, a branched repeating unit is present with the 3,4-disubstituted Rha residue at the branching point and the unsubstituted Xyl residue in the side chain. This result allowed the suggestion that, like strain PDDCC 5712, part of the linear repeating units **1** are xylosylated at position 4 of the only 3-substituted Rha residue (unit D, structure **8**).



8 (strain ICMP 8072)

This suggestion was confirmed by the assignment of the ^{13}C NMR signals for the repeating unit **8** (Table I), which was carried out by comparison with the corresponding data for **1** (for units A–C) and **3** (for units D and E). Similar changes of the ^{13}C chemical shifts for C-2,3,4 of unit D were observed when the spectra of **2** and **1** were compared with those of **3** and **8**, respectively.

As judged by the relative intensities of the signals of the two series in the ^{13}C NMR spectrum, the ratio of the repeating units **1** and **8** is ~ 55 : 45. According to this spectrum, a small amount (~ 10%) of the repeating units of structure **3** is also present and both the spectrum and the methylation analysis data (e.g., identification of 4-*O*-methylrhamnose as a minor component) point to the presence of another unidentified structure.

Strains ICMP 7944 and ICMP 8277.—The polysaccharides of these strains have identical ^{13}C NMR spectra (Fig. 6), which proved that, like the polysaccharide of strain ICMP 8072, there are present several structural types of repeating units, one of them having structure **1** and being slightly predominant. Further study was performed with the polysaccharide from strain ICMP 7944.

The polysaccharide consists of L-rhamnose (81%), 2-amino-2-deoxy-D-glucose (14%), and D-glucose (4%), xylose being absent. The following partially methylated monosaccharides were identified in methylation analysis: 2,4-di-*O*-methylrhamnose, 3,4-di-*O*-methylrhamnose, 4-*O*-methylrhamnose, and 2-deoxy-4,6-di-*O*-methyl-2-methylaminoglucose. According to these data, the second major repeating unit is branched with a Rha residue at the branching point (2,3-disubstituted) and another one occupying the terminal position of the side chain. This result

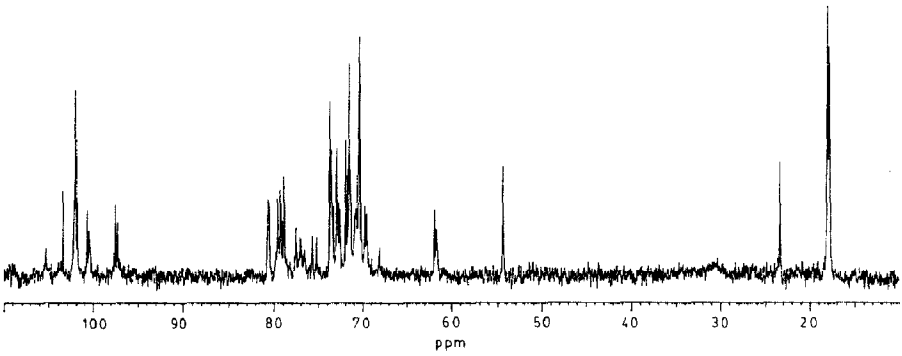
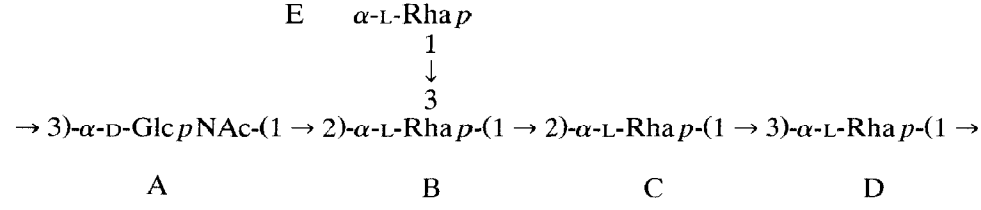


Fig. 6. ^{13}C NMR spectrum of the polysaccharide of strain ICMP 7944.

allowed the conclusion that one of the Rha residues in a part of the linear repeating units **1** is substituted by the fourth Rha residue (unit E).

Analysis of the ^{13}C NMR spectrum (Table I) showed that the signals for C-2,3,4 of one of the 2-substituted Rha residues (unit B) are shifted due to the α- and β-effects of glycosylation from 77.6, 70.9, and 73.7 ppm in **1** to 75.6, 79.2, and 72.6 ppm in the branched polysaccharide, whereas the signals of units A, C, and D did not markedly change their positions. Thus, unit B is substituted by the side chain at position 3 (structure **9**).



9 (strain ICMP 7944)

Structure **9** was confirmed by the computer-assisted analysis modified[12] for branched polysaccharides. It showed the best fit between the experimental ^{13}C NMR spectrum and that calculated for structure **9** ($S = 1.0$). Moreover, **9** is the only structure with a monosaccharide side chain consistent with the experimental ^{13}C NMR data.

The ratio of the repeating units of the two major types **1** and **9** is ~6:4. A minor series of signals present in the ^{13}C NMR spectrum of the polysaccharide, in particular, the unassigned signal at 105 ppm, belong to an unidentified minor type of repeating unit.

DISCUSSION

The O-specific polysaccharides of the group of strains of *P. solanacearum* being studied are built-up of L-rhamnose and 2-acetamido-2-deoxy-D-glucose and some

TABLE II

Distribution of different types of repeating units in the O-antigens of *P. solanacearum*

ICMP strain	Types of repeating units	Approximate ratio(s)
6523, 6524, 7859, 7956, 8110, 8202 [a]	**1**	–
767	**2**	–
PDDCC 5712, 766, 7959, 8066	**2, 3**	25:75
7960	**2, 3**	75:25
7955	**6, (3+2)**	70:30
8072	**8, 1, (3+2)**	40:50:10
7944, 8277	**9, 1**	40:60

[a] Data from refs 3 and 4.

of them also contain L-xylose. Interestingly, the last-named sugar is a rare component of bacterial polysaccharides; it has been found[13] hitherto only in the lipopolysaccharide of *Pseudomonas maltophila* NCTC 10257.

Each of the *P. solanacearum* strains is characterised by an O-antigen consisting of more than one type of repeating unit. Three of them represent a linear tetrasaccharide built-up of one D-GlcNAc and three L-Rha residues. Their structures differ in the configuration of the glycosidic linkage of the GlcNAc residue (α in **1** and **6** or β in **2**) and the position of its attachment to the Rha residue (2 in **1** or 3 in **2** and **6**). Three other repeating units (structures, **3**, **8**, and **9**) are branched pentasaccharides, which have **1** or **2** as the main chain and L-Xyl (in **3** and **8**) or L-Rha (in **9**) as the side chain.

The distribution of the O-antigen structures among the *P. solanacearum* strains is summarised in Table II. One can see that structures **6**, **8**, and **9** are unique, whereas each of structures **1**, **2**, and **3** is common for more than one O-antigen. Such a feature, which may complicate serotyping of strains, is characteristic also for some other microorganisms, in particular for *Pseudomonas cepacia*[14], *Pseudomonas pseudomallei*[15], and *Serratia marcescens* (ref 16 and refs therein).

Structure **1**, which is present in a number of *P. solanacearum* strains, has been identified[17] also in the O-antigen of *S. marcescens* O22 and differs from the O-antigen of *Shigella flexneri* variant Y (ref 18) only by the anomeric configuration of the GlcNAc residue. Interestingly, other serotypes of *S. flexneri* contain additionally D-glucose, which is attached to a part of the repeating units as a side chain[18]. It seems to be true also for the O-antigens of *P. solanacearum*, in which the branched structures arise from nonstoichiometric xylosylation or rhamnosylation of the linear structures. It is unclear, however, whether the linear and branched repeating units are parts of the same polysaccharide chain or whether each of them forms a separate polysaccharide chain.

EXPERIMENTAL

General methods.—^{13}C NMR spectra were recorded with a Bruker AM-300 instrument for solutions in D_2O at 60°C (internal acetone, δ 31.4 ppm).

GLC was performed with a Hewlett–Packard 5890 instrument equipped with a flame-ionisation detector and a glass capillary column (0.2 mm × 25 m) coated with OV-1. GLC–MS was performed with a Varian MAT 311 instrument under the same chromatographic conditions as in GLC. Gel-permeation chromatography was performed on a column (3.5 × 70 cm) of Sephadex G-50 in a pyridine–acetate buffer (pH 5.5) with monitoring by the phenol–H_2SO_4 reaction, or a column (80 × 1.6 cm) of TSK HW 40 (S) in water with monitoring with a Knauer differential refractometer.

Growth conditions and isolation of the polysaccharides.—Strains of *P. solanacearum* were grown at 28°C for 36–40 h on a synthetic medium N (ref 19) on a rotary shaker (240 rpm), the culture suspension was centrifuged, and the cells were dried with acetone and ether. Isolation of the lipopolysaccharides and the O-specific polysaccharides was performed as described[5,20].

Sugar analysis.—Samples were hydrolysed with 2 M CF_3CO_2H in sealed ampoules at 120°C for 2 h. Monosaccharides were identified by GLC as alditol acetates. Absolute configurations of L-rhamnose and L-xylose were determined by GLC of the corresponding (−)-2-octyl glycosides[21]. The absolute configuration of 2-acetamido-2-deoxy-D-glucose was proved by analysis of the glycosylation effects in the ^{13}C NMR spectra of the polysaccharides taking into account the known regularities[22,23].

Methylation analysis.—Methylation was performed according to the Hakomori procedure[24] and the products were recovered using a Sep-Pak cartridge[25]. Partially methylated monosaccharides were identified by GLC–MS as alditol acetates[26].

Smith degradation.—The polysaccharide of strain PDDCC 5712 (20 mg) was oxidised with 0.1 M $NaIO_4$ (1 mL) for 48 h at room temperature in the dark, and the product was reduced with an excess of $NaBH_4$, desalted by gel-permeation chromatography on TSK HW 40, and hydrolysed with aq 1% acetic acid (100°C, 2 h) to give the oligosaccharide **4** (8 mg), which was isolated by gel-permeation chromatography on TSK HW 40.

N-*Deacetylation–deamination.*—The polysaccharide of strain PDDCC 5712 (20 mg) was dried over P_2O_5 *in vacuo*, then dissolved in anhydrous hydrazine (1 mL) that contained hydrazine sulphate[9] (50 mg). The solution was heated in a sealed tube for 20 h at 105°C, then concentrated, and the resulting *N*-deacetylated polysaccharide was desalted by gel-permeation chromatography on TSK HW 40. The product was dissolved in water (1 mL), and aq 5% sodium nitrite (1.5 mL) and aq 33% acetic acid (1.5 mL) were added. The mixture was kept for 1 h at 20°C, deionised by treatment with KU-2 (H^+) resin, and concentrated, the product was reduced conventionally with $NaBH_4$, and oligosaccharide **5** (5 mg) was isolated by gel-permeation chromatography on TSK HW 40.

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