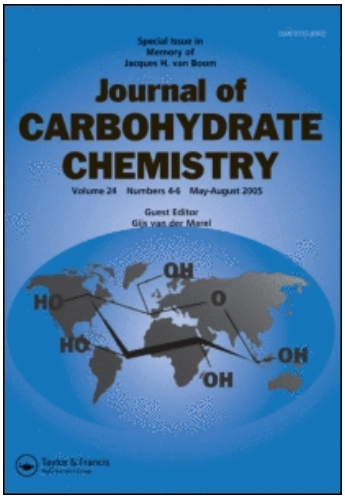

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Chemical and Immunochemical Studies on Lipopolysaccharides of Some *Yersinia* Species - A Review of Some Recent Investigations

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CHEMICAL AND IMMUNOCHEMICAL STUDIES ON LIPOPOLYSACCHARIDES OF SOME *YERSINIA* SPECIES - A REVIEW OF SOME RECENT INVESTIGATIONS

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ABSTRACT

The present paper revealed the results of some recent chemical and immunochemical studies of the lipopolysaccharides from various species and erologie variants of *Yersinia* genus as follows: *Y. pseudotuberculosis* IIC and VII; *Y. enterocolitica* 0:1, 2a, 3; 0:2a, 2b, 3; 0:3; 0:4, 32; 0:5; 0:5,27; 0:6,31; 0:7,8; 0:19,8; 0:8; *Y. frederiksenii* 0:16,29; *Y. intermedia* 0:4,33; *Y. aldovae*.

INTRODUCTION

Numerous species of *Yersinia* microorganisms (Enterobacteriaceae) are known at the present time. *Y. pseudotuberculosis* was found to be a pathogenic factor of the human and animal diseases namely pseudotuberculosis (PT). In 1959, a new form of PT termed as Far East Scarlet-like Fever (FESF) was discovered by V. A. Znamensky and G. P. Somov.[1] Serologic classification of this microorganism was made by W. Knapp,[2] H. H. Mollaret[3] and M. Tsubokura.[4] Serologic variants (serovars) IIC, VII, VIII were isolated by M. Tsubokura et al.[5] in 1984.

The serovar I was found to be of particular importance in human pathology. Thus, more than 90 per cent of FESF cases are caused by the I serovar.[1] Other cases are connected with serovar III and IV. Up to now, serovars II, V-VIII have not been recovered from the patients with FESF.

Y. enterocolitica[6] is known to be a causative factor of intestinal yersiniosis. The microorganism was first found in America in 1939 and 10 years later, it was discovered in Europe.[7] The World Health Organization states that yersiniosis is now registrated in more than 30 countries.

More than 34 serovars of the microorganism are known. In 1984, additional species were reported as follows: *Y. frederiksenii, Y. intermedia, Y. kristensenii* were distinguished from *Y. enterocolitica.*[7] Later,[8] the following species: *Y. aldovae, Y. ruckeri, Y. rohdei, Y. mollaretii* and *Y. bercoveiri* were discovered.

Numerous investigators have isolated various antigens from *Y. pseudotuberculosis.* In 1957, T. Sasaki[9] isolated antigens from non-typified strains of the microogranism. D.A.L. Davies[10] proved that O-somatic antigens of *Y. pseudotuberculosis* represented lipopolysaccharides (LPS). He has isolated LPS from five serovars of the S-form and from some R-forms. D.A.L. Davies[11] first obtained 3,6-dideoxyaldohexoses as immunodominants of the O-antigens. The structural features of O-specific polysaccharides of LPS from serovars I-VI have been elucidated by B. Lindberg et al.[12] (1972-1974) and Yu. S. Ovodov et al.[13] (1969-1983).

B. Lindberg[14] (1980) proposed the structural pattern of the main polysaccharide of O-specific side chains of LPS *Y. enterocolitica* 0:3 serovar. The data concerning the structural features of O-specific polysaccharide 0:9 and 0:5,27 serovars have been reported recently by M. B. Perry and co-workers.[15,16]

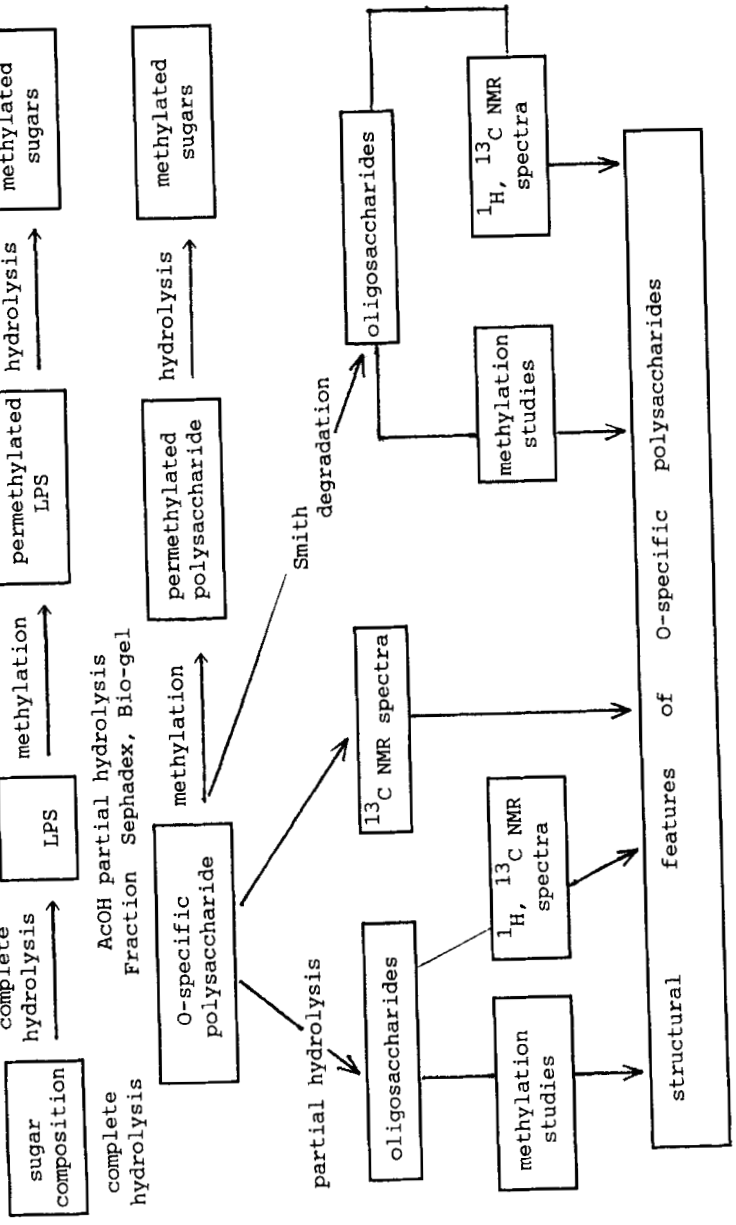
The present paper summarizes[17-32] chemical and immunochemical studies on LPS of *Yersinia* species as follows: *Y. pseudotuberculosis* serovars IIC, VII; *Y. enterocolitica* of various serovars; *Y. kristensenii, Y. frederiksenii, Y. intermedia,* and *Y. aldovae.*

RESULTS AND DISCUSSION

An outline of the *Yersinia* LPS studies is given in the Scheme 1. Starting with the LPS a complete acid hydrolysis was carried out to determine the sugar composition. Partial hydrolysis of LPS with dilute acetic acid afforded O-specific polysaccharides.[13] LPS and O-specific polysaccharides were also subjected to methylation studies.[33] It is noteworthy that O-specific polysaccharides of *Yersinia* LPS were stable as a rule to the action of dilute acetic acid, allowing elucidation of their structural features, using the various procedures (Scheme 1).

The yields of LPS from *Y. pseudotuberculosis* servovars IIC and VII were found to be 4.1 and 2.3% respectively. Both LPS samples showed serological activity giving a single precipitation band with the homologous antisera.

The repeating units of O-specific polysaccharides from *Y. pseudotuberculosis* IIC and VII serovars were shown to be branched pentasaccharides (Figures 1,2).[17,18] Abequose and colitose respectively were isolated as



Scheme I

Schematic route of *Yersinia* LPS studies

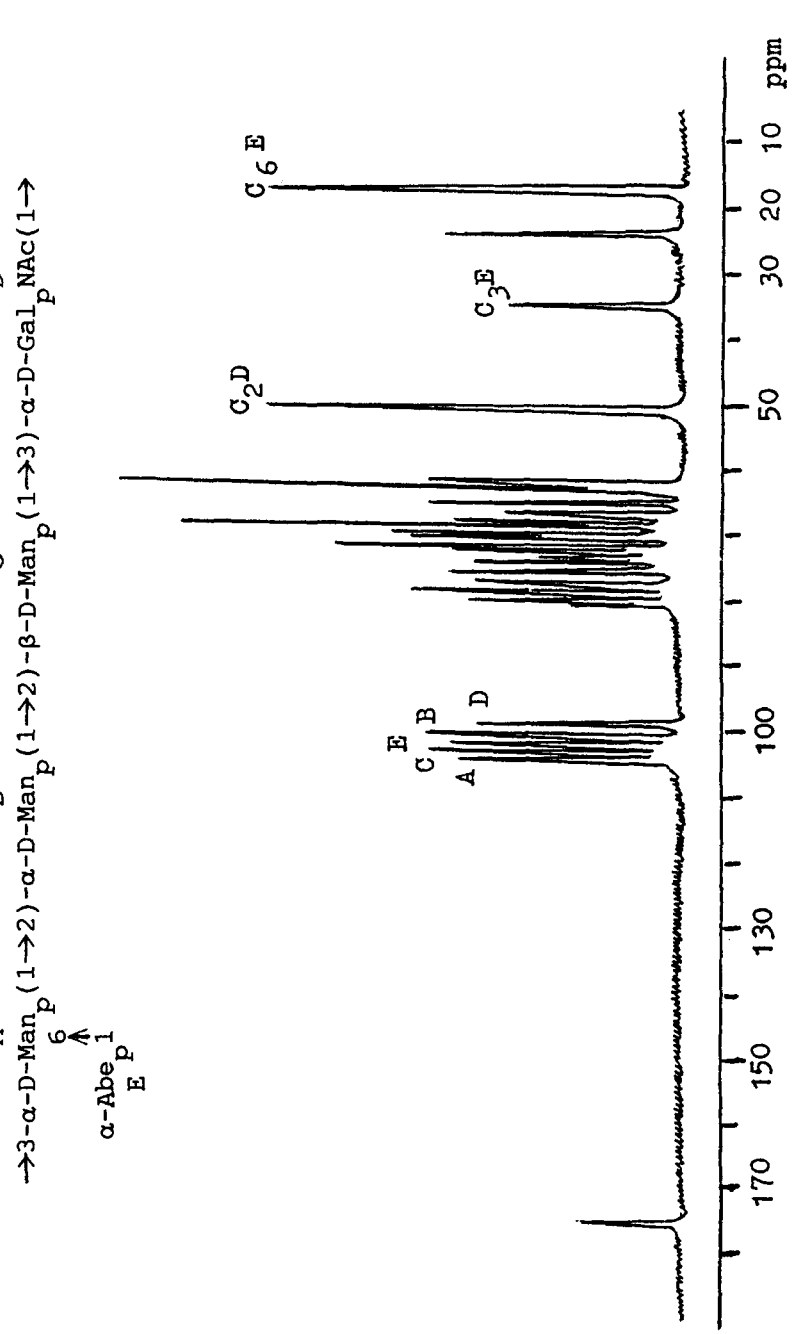


Fig. 1. ^{13}C NMR spectrum of O-specific polysaccharide *Y. pseudotuberculosis* IIC.[17] A (103.5), B (100.4), C (102.4), D(98.3), E (101.5) ppm; C2: D(50.2 ppm); C3: E (34.3 ppm); C6: E (16.6 ppm); 174.9 and 23.3 ppm acetyl group.

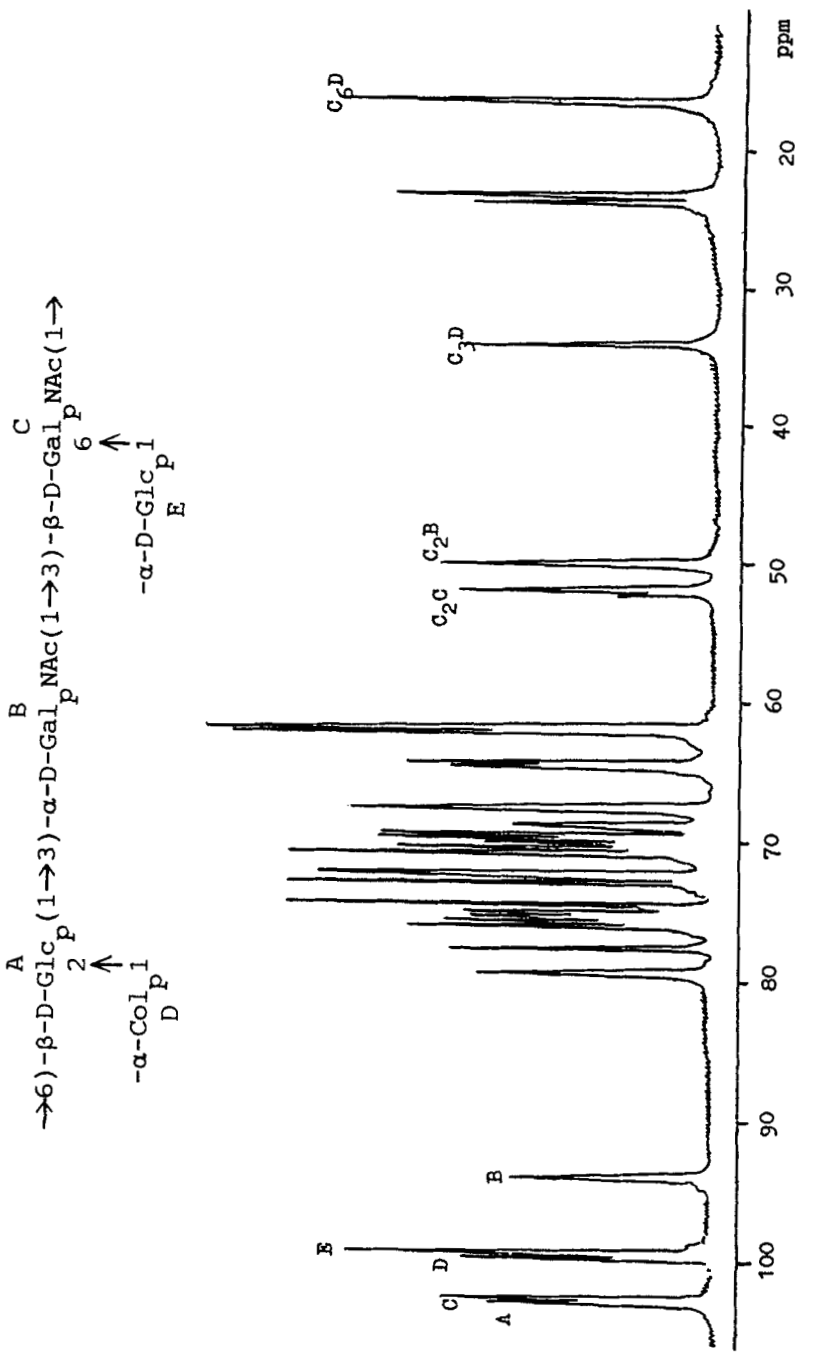
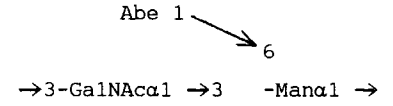


Fig. 2. ^{13}C NMR spectrum of O-specific polysaccharide *Y. pseudotuberculosis* VII.[18] C1: A (103.3), B (94.5), C(103.0), D (99.9), E (99.6) ppm; C2: B (50.1), C (52.0) ppm; C3: D (34.3 ppm); C6: D (16.7 ppm); 23.3 and 23.9 ppm acetyl group.

individual compounds and identified in the accord with their chromatographic behavior and with specific optical rotation data.[17,18]

O-Specific polysaccharide of IIC serovar was subjected to complete acid hydrolysis to afford abequose, mannose and galactosamine in the following relative amounts: 0.8:3:1. Five signals for anomeric C-atoms at 98.3 (α-D-GalNAc); 100.4 (2-α-D-Man); 101.5 (α-Abe); 102.4 (2-β-D-Man); 103.5 (3-α-D-Man) ppm were shown to be in ^{13}C NMR spectrum thus indicating the presence of a regular pentasaccharide repeating unit. In addition, signals of 6-deoxy-group of abequose (16.6 ppm), deoxy-group of abequose ring (34.3 ppm), acetyl group (23.3 and 174.9 ppm), and C-atom having an acetamide group (50.2 ppm), were identified in the spectrum. O-Specific polysaccharide was permethylated followed by complete hydrolysis to furnish 2,4-di-*O*-methylabequose, 3,4,6-tri-*O*-methyl-D-mannose, 2,4-di-*O*-methyl-D- mannose and 2-(*N*-methyl)-acetamido-2-deoxy-4,6-di-*O*-methyl-D-galactose. These data confirmed the structural pattern of the repeating unit. Partial hydrolysis of O-specific polysaccharide furnished a modified polysaccharide possessing a lower content of abequose. ^{13}C NMR spectral data of the material demonstrated that the abequose residue occupied a terminal position and was attached to an α-D-mannopyranose residue. O-Specific polysaccharide was subjected to a periodate oxidation. The material was analysed using ^{13}C NMR spectral data confirming the presence of the following fragment in the repeating unit:



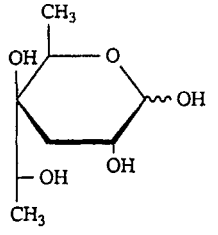
On the basis of data obtained, the structural pattern of the repeating unit of O-specific polysaccharide (Serovar IIC) was suggested as given in Figure 1. A similar approach was used for elucidation of structural features of O-specific polysaccharide from LPS *Y. pseudotuberculosis* serovar VII[18] (Figure 2).

O-Specific polysaccharides of LPS from *Y. enterocolitica* serovars 0:1, 2a, 3; 0:2a, 2b, 3 and 0:3[19] were found to be composed of 6-deoxy-L-altrose residues. Using ^{13}C NMR spectroscopy and methylation data, the structural features of backbones have been elucidated.[19] In addition, O-polysaccharide of serovar 0:2a, 2b, 3 was shown to contain an acetyl group at the C-3 position of some 1,2-linked sugar residues (Table 1).

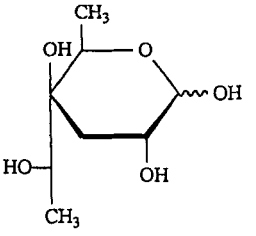
TABLE 1 O-Specific Polysaccharides of *Y. enterocolitica* Serovars 1 - 3

Serovar	Repeating unit
0:1,2a,3	→2)-β-6d-L-Alt$_p$(1→2)-β-6d-L-Alt$_p$(1→2)-β-6d-L-Alt$_p$(1→
0:2a,2b,3	→2)-β-6d-L-Alt$_p$(1→2)-β-6d-L-Alt$_p$(1→2)-β-6d-L-Alt$_p$(1→ 3 ⟍ ⟋ 3 OAc
0:3	→2)-β-6d-L-Alt$_p$(1→

LPS of *Y. enterocolitica* 0:4,32 and *Y. intermedia* 0:4,33 were found to be similar in relation to their structural features and serological behavior. They interacted with homologous and heterologous antisera to give a single precipitation band. Mild acid hydrolysis of LPS afforded yersiniose B represented as 3,6-dideoxy-4C-(1-hydroxethyl)-D-*xylo*-hexose.[20] Yersiniose



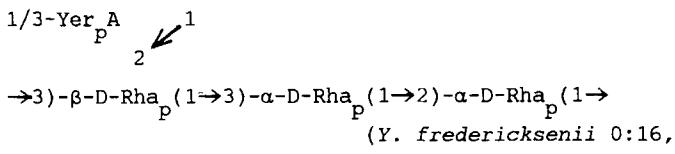
YERSINIOSE A



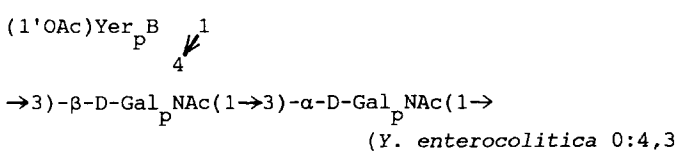
YERSINIOSE B

A (as a constituent of LPS from *Y. pseudotuberculosis* serovar VI[21] and *Y. frederiksenii* 0:16,29[22]) and yersiniose B (as a constituent of LPS from *Y. enterocolitica* 0:4,32[23] and *Y. intermedia* 0:4,33[24]) appeared to be a new group of 3,6-dideoxyhexoses isolated from LPS *Yersinia*.

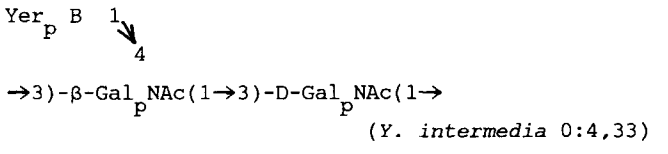
O-Specific polysaccharides of LPS from *Y. enterocolitica* 0:4,32[23] and *Y. intermedia* 0:4,33[24] were shown to be composed as trisaccharide repeating units:



(*Y. fredericksenii* 0:16,29)



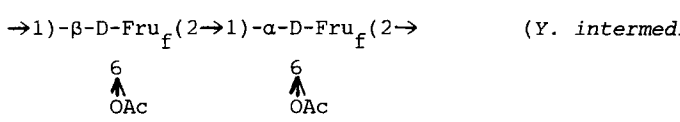
(*Y. enterocolitica* 0:4,32)



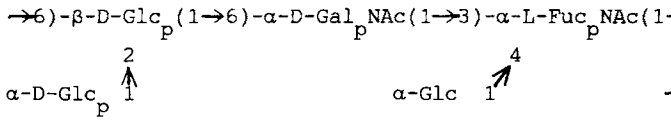
(*Y. intermedia* 0:4,33)

The structural patterns of O-specific polysaccharides from LPS of *Y. enterocolitica* serovars 0:5; 0:5,27;[25] 0:6,31;[26] 0:7,8;[27] and 0:19,8[27] are given in Table 2.

In addition, the structural features of O-specific polysaccharides from LPS of *Y. intermedia* (strain 680)[29] and *Y. kristensenii* 0:12,26[30] were elucidated in the same manner. The structural pattern of the repeating units are shown on the following page.



(*Y. intermedia*)



(*Y. kristensenii*)

In the case of *Y. aldovae*,[31] O-specific polysaccharide consisted of residues of D-gulcose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose and 3,6-dideoxy-(R)-3-hydroxybutyramido-D-galactose in relative amounts of 2:2:1:1, respectively. ^{13}C NMR spectral data indicated the presence of an O-acetyl group (21.1 ppm).

TABLE 2 O-Specific Polysaccharides of *Y. enterocolitica* Serovars 5-8.

Serovar	Repeating unit
0:5; 0:5,27	→3)-α-L-Rha$_p$(1→3)-α-L-Rha$_p$(1→3)-β-L-Rha$_p$(1→ 2 2 ↓ ↓ β-D-Xlu$_f$ β-D-Xlu$_f$
0:6,31	→2)-β-D-Gal$_p$(1→3)-α-6d-D-Gul$_p$(1→ 6d-Gul$_p$ 1 ↘ 4
0:7,8; 0:19,8	→2)-D-Man$_p$(1→3)-D-Gal$_p$(1→3)-D-Gal$_p$NAc(1→ 3 2 ↗ ↖ 6d-Gul$_p$ 1 L-Fuc$_p$ 1
0:8	→4)-D-Man$_p$(1→3)-D-Gal$_p$(1→3)-α-D-Gal$_p$NAc(1→ 3 2 ↗ ↑ 6d-Gul$_p$ 1 L-Fuc$_p$ 1

Solvolysis of the polysaccharide with anhydrous hydrogen fluoride furnished two pentasaccharides, one of which contained an O-acetyl group. The structural pattern of the oligosaccharide was elucidated using ^{1}H NMR-spectroscopy (Table 3) and the Nuclear Overhauser Effect (Table 4) as follows:

(A) (B) AcO (C) (D)

↘6

α-D-Glc$_p$NAc(1→4)-α-D-Gal$_p$NAc(1→3)-β-D-Glc$_p$NAc(1→2)-α-D-Glc$_p$F

3

↗

β-D-Glc$_p$ 1

(E)

Simultaneously, solvolysis was accompanied with the fluoridation of the glucose residue on the reducing end of oligosaccharide.

TABLE 3

^{1}H NMR Spectral Data for Pentasaccharide from O-Specific Polysaccharide

	Proton / Residue				
	A	B	C	D	E
H1	4.98	5.46	4.75	5.86	4.43
	$J_{1,2}$ = 3.0	$J_{1,2}$ = 4.0	$J_{1,2}$ = 8.0	$J_{1,F}$ = 53.0	$J_{1,2}$ = 8.5
H2	3.87	4.48	3.80	3.70	3.14
	$J_{1,2}$ = 3.0	$J_{2,3}$ = 9.0	$J_{2,3}$ = 9.0	$J_{1,2}$ = 3.5	$J_{2,3}$ = 9.0
H3	3.87	3.89	3.75	-	3.46
		$J_{3,4}$ = 3.0			$J_{3,4}$ = 9.1
H4	-	4.25	3.72	-	3.38

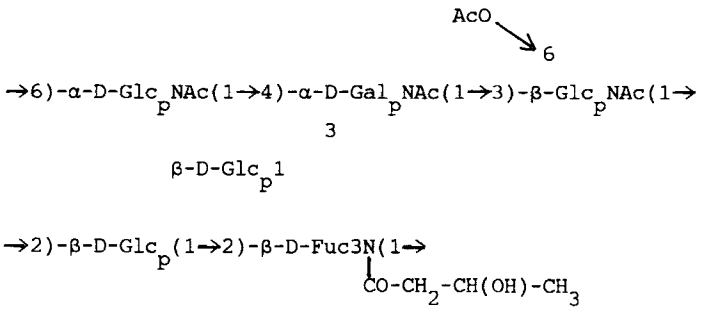
a. coupling constants are in Hz

TABLE 4

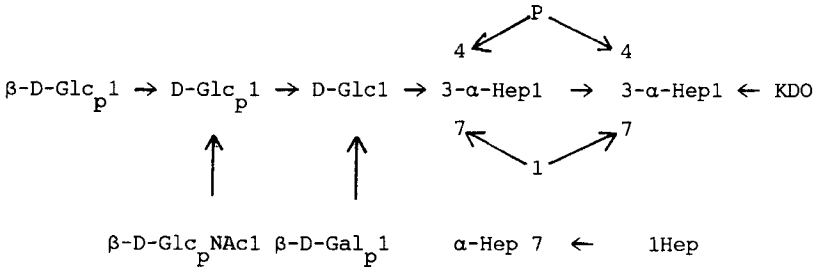
Nuclear Overhauser Effect Data of the Pentasaccharide

Irradiation proton	Observed proton of Units: A	B	C	D	E
H1 A	H2	H4			
H1 B		H2	H3		
H1 C			H2, H3, H4	H2	
H1 E	H3				H2, H3, H5

Methylation studies of the polysaccharide followed by ^{1}H and ^{13}C NMR spectral analysis of the polysaccharide and oligosaccharide allowed us to suggest the following structural features of the repeating unit:



A comparative study of the core of LPS from various serovars of *Y. pseudotuberculosis* showed that the core appeared to be a common one. The oligosaccharide of the core isolated from LPS of R-form of the microorganism has the following structure:[32]



EXPERIMENTAL

The strains of *Y. pseudotuberculosis* serovars IIC and VII were kindly provided by M. Tsubokura (Japan). The strains of other *Yersinia* species were kindly provided by H. H. Mollaret (France).

Organisms were grown at 20 °C in the nutrient media containing per liter a mixture of the following constituents: peptone (10 g); $(NH_4)_2SO_4$(2g); $MgSO_4.7H_2O$(0.2g); $CaCl_2$ (0.01 g); KH_2PO_4 (1.5 g); Na_2HPO_4 (6.5 g); $FeCl_3$ (0.0054 g); NaCl (50 g); glucose (5 g). Cells were separated by centrifugation and the materials were washed with water and dried with acetone.

Isolation of LPS was achieved using extraction of dry microbial cells with 45% aqueous phenol in accord with O. Westphal.[34]

O-Specific polysaccharides were obtained as described earlier.[17-32] LPS (100 mg of each) were treated with 1% acetic acid (10 mL) at 100 °C for 1 - 3 h. Lipid A was removed as a precipitate by ultracentrifugation at 105000 x g for 1 h. The polysaccharide fraction was subjected to molecular-sieve chromatography on Sephadex (G-50) or G-100) columns to afford O-specific polysaccharide and core oligosaccharide.

The usual procedures for complete and partial acid hydrolysis, methylation studies and Smith degradation were used.[17-32]

^{1}H and ^{13}C NMR spectra were performed on Bruker (WM-250) and Bruker Physics (HX-360) instruments in D_2O; chemical shifts are given from tetramethylsilane, internal standard was methanol. Optical rotations were determined on a Perkin-Elmer polarimeter (model 141) in water at 20 °C. The homogeneity of polysaccharides was examined using an analytical ultracentrifuge 3170 (MOM, Hungary). Solutions were lyophilized or concentrated *in vacuo*. Paper chromatography was carried out in the following solvent: butanol/pyridine/water (6:4:3 v/v/v), using Whatman 3MM and Filtrak (FN-3; FN-15) papers. Paper electrophoresis was performed in 0.025 M pyridine/acetate buffer (pH 5.3) for 6 h at 28 V/cm. Molecular-sieve chromatography was carried out in pyridine/acetic acid/water (10:4:986 v/v/v), using a column 6.5 x 1.8 cm with Sephadex G-50 and G-100.

GLC was run on a Pye-Unicam 104 chromatograph with a flame-ionization detector on a glass column (150 x 0.4 cm) containing 3% QF-1 on Gas-Chrom Q (100-200 mesh). The flow rate of the carrier gas (argon) and hydrogen was 60 mL/min. Sugars were analysed as alditol and aldononitrile acetates[35,36] at 175-225 °C, 5°/min. Methylated sugars were determined as alditol acetates [35] at 135-225 °5/min. GLC-MS was performed on LKB-9000 S (Sweden) instrument.

Antisera were obtained from rabbits immunized thrice with 10^9 bacterial cells per 1 mL, which were preheated at 56 °C for 30 min, 15 min, and 5 min, respectively. Supensions of the cells were injected into rabbits intravenously increasing portions 0.25, 0.5 and 1 ml, each day over three day period. The animals were bled during the week after the last injection. Immunoprecipitation on agar gel was performed according to Ouchterlony.[37]

CONCLUSION

LPS isolated from numerous species of the *Yersinia* genus were characterized by chemical and immunochemical methods. All LPS studies were found to possess serological activity.

O-Specific polysaccharides were obtained from all the LPS using mild acid hydrolysis. The structural features of the repeating units were elucidated with aid of the modern methods of carbohydrate chemistry. The data demonstrated that O-specific polysaccharides of *Yersinia* species differ considerably. Some of them were shown to be rather structurally simple, whereas the majority are characterized by a complicated structure composed of the various sugar residues. As usual, they possessed a linear backbone with single sugar residues as branches. The terminal sugars were found to be immunodominants of O-somatic antigens, and represented as a rule rare monosaccharides as follows: abequose, colitose, 6-deoxy-L-altropyranose, xylulose etc. Among them, a new group of unusual branch-chain 3,6-dideoxyhexoses termed as yersiniose A and B was discovered.[20,21]

The core oligosaccharides of *Y. pseudotuberculosis* LPS were isolated from R-forms of the microorganism. Their structures were clarified partially. The cores appeared to be common for *Y. pseudotuberculosis* LPS.

ACKNOWLEDGEMENT

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Fruitful assistance of Prof. Dr. A. S. Shashkov (Institute of Organic Chemistry, Moscow) in performing and interpretation of ^{1}H and ^{13}C NMR spectra is also acknowledged.

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