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THE STRUCTURE OF THE ANTIGENIC LIPOPOLYSACCHARIDE O-CHAINS PRODUCED BY *Salmonella urbana* AND *Salmonella godesberg**

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ABSTRACT

The lipopolysaccharides of *Salmonella urbana* and *Salmonella godesberg*, which belong in group N (O:30) of the Kauffmann–White system, were shown by SDS-PAGE electrophoresis, glycose analysis, periodate oxidation, methylation, and ^{1}H- and ^{13}C-n.m.r. analyses to have identical O-chains composed of repeating, branched pentasaccharide units having the structure:

$$\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-GalNAc}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-PerNAc}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Fuc}p\text{-}(1\rightarrow)_n$$
$$\begin{array}{c} 4 \\ \uparrow \\ 1 \\ \beta\text{-D-Glc}p \end{array}$$

The serological cross-reactivity of *S. urbana* and *S. godesberg* with *Brucella abortus* and *Yersinia enterocolitica* (O:9) can now be related to the presence of a 1,2-glycosylated *N*-acyl derivative of 4-amino-4,6-dideoxy-α-D-mannopyranosyl residues in their respective lipopolysaccharide O-chains.

INTRODUCTION

The clinically important serological cross-reactivity between the antigenic lipopolysaccharides (LPS) of *Brucella abortus* and *Yersinia enterocolitica* O:9 (refs. 1–4) has recently been explained by the finding that the O-chains of their respective LPS are identical, being homopolymers of 1,2-linked 4,6-dideoxy-4-formamido-α-D-mannopyranose (D-perosamine) residues[5–7]. Similar serological cross-reactivities have been observed between the O-antigens of *B. abortus* and *Y. enterocolitica* O:9 and their antisera, with strains of *Escherichia coli* and with *Salmonella* strains of the Kauffmann–White group N (O:30)[8–11]. We have shown that the LPS of *E.*

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coli O:157 (ref. 12) and of *S. landau* O:30 (ref. 13), which show such serological cross-reactivities, have LPS O-chains composed of tetrasaccharide repeating-units containing D-glucose, L-fucose, 2-acetamido-2-deoxy-D-galactose, and 4-acetamido-4,6-dideoxy-D-mannose (1:1:1:1) and it is speculated that the glycosidically 1,2-substituted 4-acetamido-4,6-dideoxy-α-D-mannopyranosyl residues form part of the epitope responsible for the serological specificity and cross reactions.

There have been reports[10,14] of serological cross-reactions of *Brucella* antigen with the LPS of *S. urbana* O:30. However, a structure proposed for the O-chain of *S. urbana* and *S. godesberg*[15], both of which belong in the Kauffmann–White group N, fails to reveal a possible reason for cross reactivity with either the *B. abortus* or *Y. enterocolitica* O:9 LPS, and for this reason the structures of the O-chains of the LPS produced by *S. urbana* were reinvestigated.

It was found that the O-chains of the LPS of both *S. urbana* and *S. godesberg* were identical, being composed of a repeating pentasaccharide unit containing D-glucose, L-fucose, 2-acetamido-2-deoxy-D-galactose, and 4-acetamido-4,6-dideoxy-D-mannose (2:1:1:1). Physical and chemical structural analyses indicated that the latter aminoglycose component was the structural unit in common with the *B. abortus* and *Y. enterocolitica* O:9 antigens.

RESULTS AND DISCUSSION

Cells of *S. urbana* and *S. godesberg* (300 g, wet weight), after saline washing and enzymic digestion[16], were extracted by the hot aqueous phenol method[17] and the dialyzed aqueous phase, after concentration and ultracentrifugation (105,000*g*), gave essentially pure LPS (~2.5 g). The phenol phase gave an R-type LPS (~150 mg) which was not examined further. SDS–PAGE analysis[18] gave a typical separation pattern of a S-type LPS[19] in which the spacing of the bands was indicative of an O-chain having a repeating pentasaccharide unit.

Partial hydrolysis of both the *S. urbana* and *S. godesberg* aqueous-phase LPS (1 g) with hot 0.33M acetic acid gave an insoluble lipid A (0.16 g). Gel filtration on Sephadex G-50 of the water-soluble products gave a polysaccharide fraction (O-chain, 0.36 g) eluting at the void volume of the column, a minor fraction (K_{av} 0.64, 0.09 g) of core oligosaccharide, and a fraction (K_{av} 1.00, 0.14 g) identified as containing 3-deoxy-D-*manno*-octulosonic acid[20] and phosphate.

Polysaccharide O-chain. — In separate analyses, the O-chains from *S. urbana* and *S. godesberg* gave the same physical and chemical results and were identical in all respects. In all subsequent discussions the recorded data refer only to the O-chain of *S. urbana*.

The O-chain had $[\alpha]_D$ +21.2° (*c* 5.1, water). *Anal.* Found: C, 40.02, H, 5.81, N, 1.75; ash, 0.60%. On quantitative analysis[12] the O-chain was found to have the composition D-glucose (36.1%), L-fucose (15.1%), 2-acetamido-2-deoxy-D-galactose (21.4%), and 4-acetamido-4,6-dideoxy-D-mannose (17.3%), indicating a respective molar ratio of 2:1:1:1. The configuration and identities of the glycoses

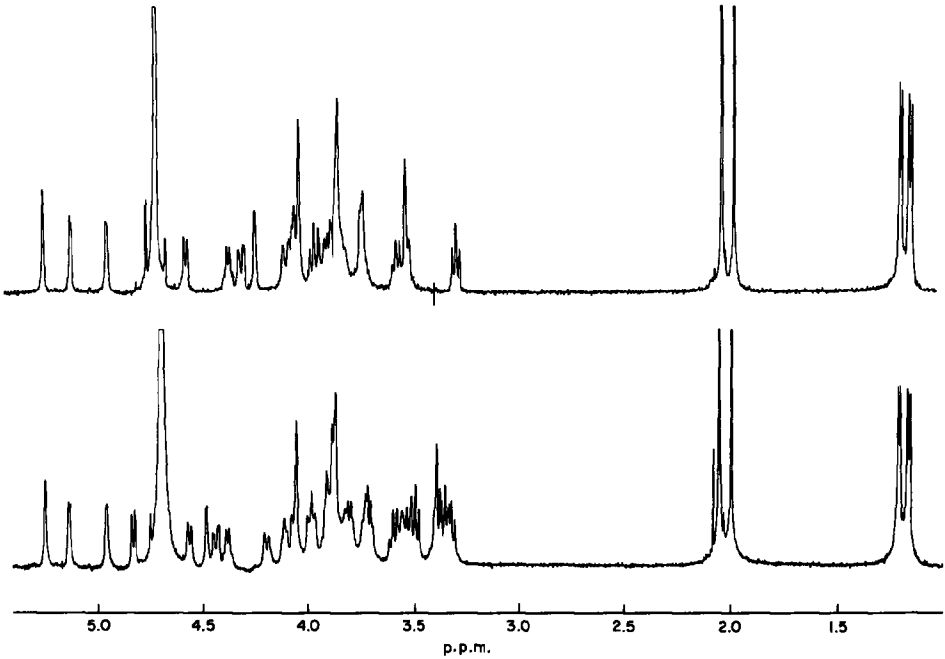


Fig. 1. Resolution-enhanced ^{1}H-n.m.r. spectra of (a) the O-polysaccharide of *S. urbana* at 305K and (b) the O-polysaccharide of *S. landau* at 305K.

were established by their isolation and characterization as previously described[12].

The ^{1}H-n.m.r. spectrum of the O-chain showed a similarity to the spectrum of the O-chain of the LPS of *S. landau*[13] (Fig. 1), which is composed of a repeating tetrasaccharide unit containing the same four glycose residues. However, as expected from the composition analyses, the *S. urbana* O-chain showed five clear signals for anomeric protons, as compared to the four anomeric-proton signals observed in the spectrum of the *S. landau* O-chain.

Assignments of the proton resonances of the O-chain polysaccharide were made from a COSY experiment[21] (Fig. 2A, B) and, following the convention adopted in the elucidation of the *S. landau* O-chain structure[13], each anomeric signal was assigned an arbitrary designation *a–e*. As the proton spectra of the *S. landau* and *S. urbana* O-chains bore a close similarity to each other, the new anomeric resonance at 4.83 p.p.m. in the *S. urbana* spectrum was assigned the designation *e*, whilst the other anomeric signals were labelled *a–d* in order of decreasing chemical shifts. Connectivities were traced via cross-peaks to each anomeric and 6-deoxy resonance, which provide convenient windows to begin spectral analyses. The majority of the proton resonances were assigned in this manner (Table I). These assignments were corroborated by a ^{13}C–^{1}H shift-correlated experiment[22] (Fig. 3), which also served to establish the ^{13}C assignments (Table II). Comparison of the ^{1}H and ^{13}C resonances of the *S. landau* and *s. urbana*

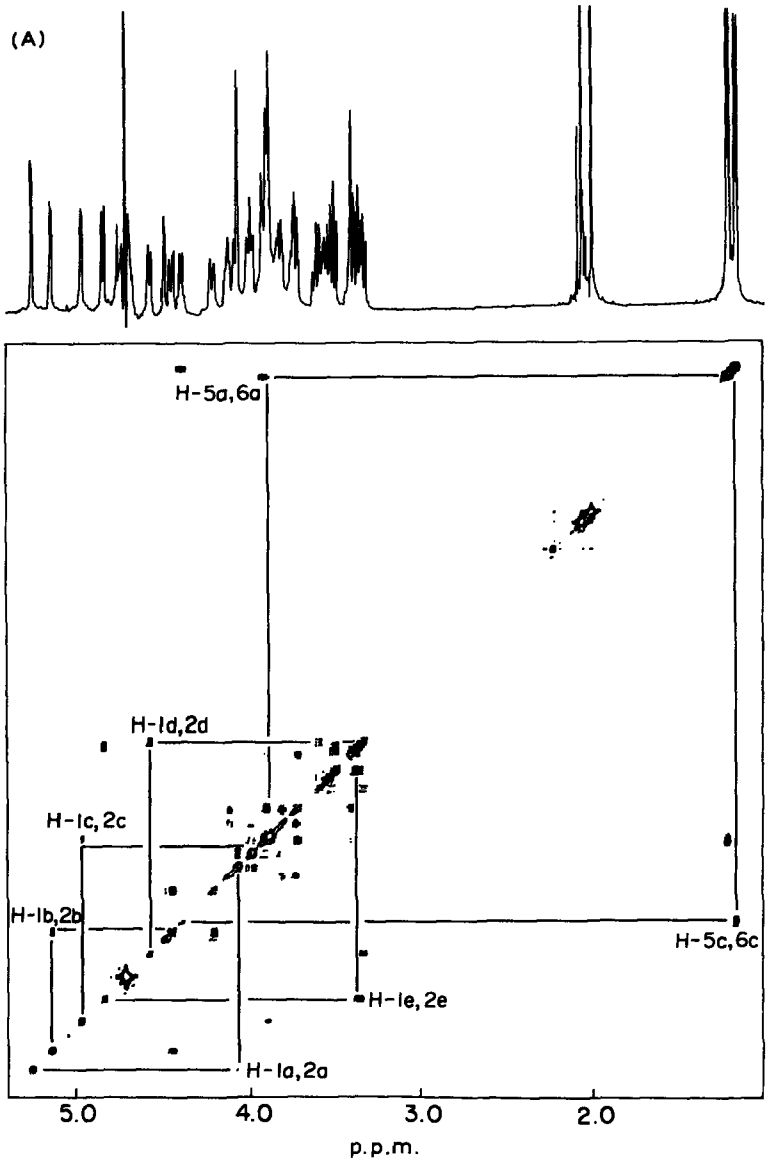


Fig. 2(A) COSY contour-plot of the complete spectrum (5.40–0.90 p.p.m.) of *S. urbana* O-polysaccharide, recorded at 305K. The resolution-enhanced one-dimensional projections are displayed along the F_2 axis. The anomeric resonances are labelled (a–e) and the connectivities are labelled in accordance with this system.

O-chains (Tables I and II) show a striking similarity in chemical shifts and coupling constant for nearly all of the resonances, with the principal deviation occurring for the H-4b (+0.26 p.p.m.) and the C-4b (+6.9 p.p.m.) signals. These features suggest that the linear sequence found for the *S. landau* O-chain — →2)-α-D-PerNAc*p*-(1→3)-α-L-Fuc*p*-(1→4)-β-D-Glc*p*-(1→3)-α-D-GalNAc*p*-(1→ — was main-

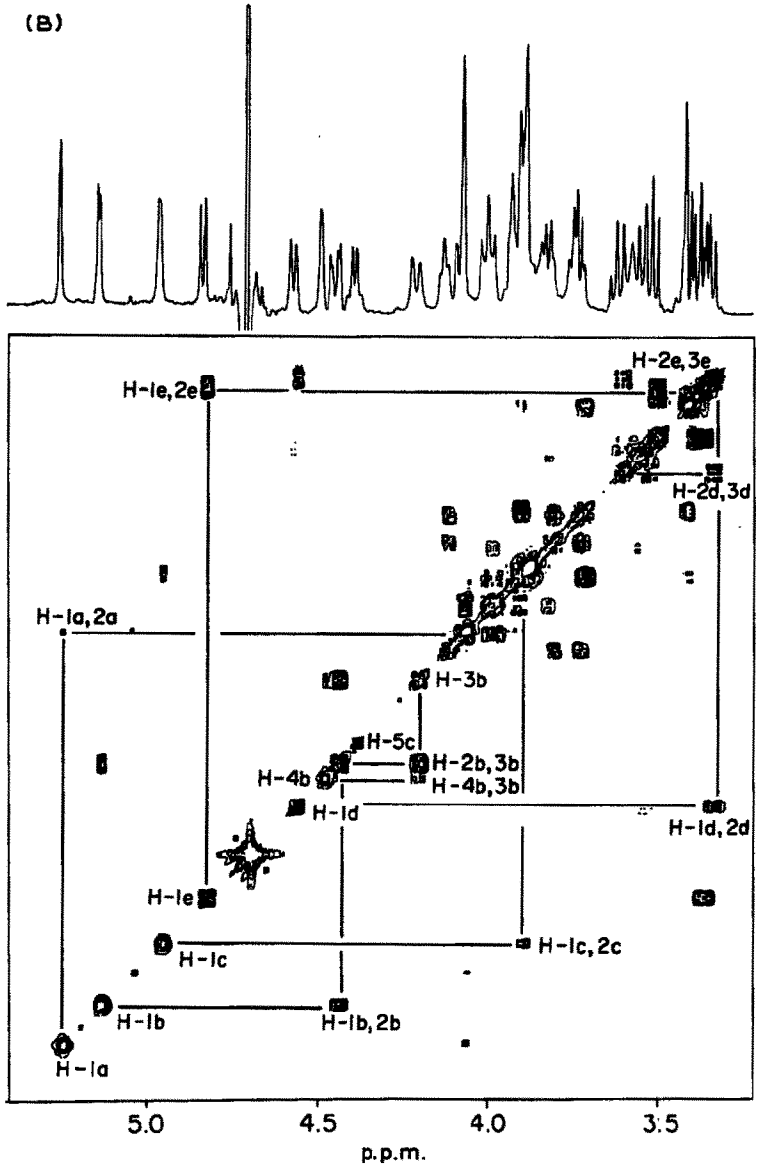


Fig. 2(B) Expanded COSY contour-plot of the ring proton region (5.40–3.20 p.p.m.) of *S. urbana* O-polysaccharide, recorded at 305K.

tained in the *S. urbana* O-chain and that the second β-D-glucopyranosyl group occurred as a nonreducing endgroup at O-4 of the 2-acetamido-2-deoxy-α-D-galactopyranosyl residues, which form the branch points. As all of the anomeric signals were well separated, it was possible to determine the monosaccharide sequence by one-dimensional n.O.e. difference spectroscopy[23]. Irradiation of each

TABLE I

FIRST-ORDER PROTON CHEMICAL SHIFTS[a] (δ) AND COUPLING CONSTANTS (Hz) OF *S. urbana* AND *S. landau* *O*-POLYSACCHARIDES

Proton	*Hexopyranosyl residues*								
	Residue a α-D-*PerNAcp*		*Residue b* α-D-*GalNAcp*		*Residue c* α-L-*Fucp*		*Residue d* β-D-*Glcp*		*Residue e* β-D-*Glcp*
	S. urbana	*S. landau*	*S. urbana*	*S. landau*	*S. urbana*	*S. landau*	*S. urbana*	*S. landau*	*S. urbana*
H-1	5.25 (~1)	5.21 (~1)	5.13 (3.7)	5.08 (3.6)	4.96 (2.5)	4.92 (2.4)	4.56 (7.7)	4.55 (7.9)	4.83 (7.7)
H-2	4.06 (~4)	4.02 (4.8)	4.43 (10.2)	4.29 (10.7)	3.87 m[b]	3.86 m[b]	3.33 (8.5)	3.27 (8.9)	3.35 (8.6)
H-3	4.07(9.8)	4.02 (10.3)	4.19 (~3)	4.06 (~3)	3.84 (9.0)	3.83 m[b]	3.60 (9.0)	3.56 (8.5)	3.50 (9.5)
H-4	3.98 (10.0)	3.94 (10.0)	4.48 (~1)	4.22 (~1)	3.82 (~1)	3.83 (~1)	3.52 (9.0)	3.51 (8.5)	3.38
H-5	3.89 (6.5)	3.85 (6.2)	4.11 (6.0)	4.04 (6.6)	4.38 (6.0)	4.34 (6.4)	3.55	3.51	3.40
H-6	1.16	1.17	3.79 3.72 (12.2)	3.72	1.21	1.12	3.88 (5.0) 3.72 (11.0)	3.82 (6.1) 3.89 (2.5,11.1)	3.90
CH_3CONH	2.00	1.95	2.06	2.01	—	—	—	—	—

[a]Chemical shifts determined at 305K relative to internal acetone (2.225 p.p.m.). [b]Unresolved multiplet.

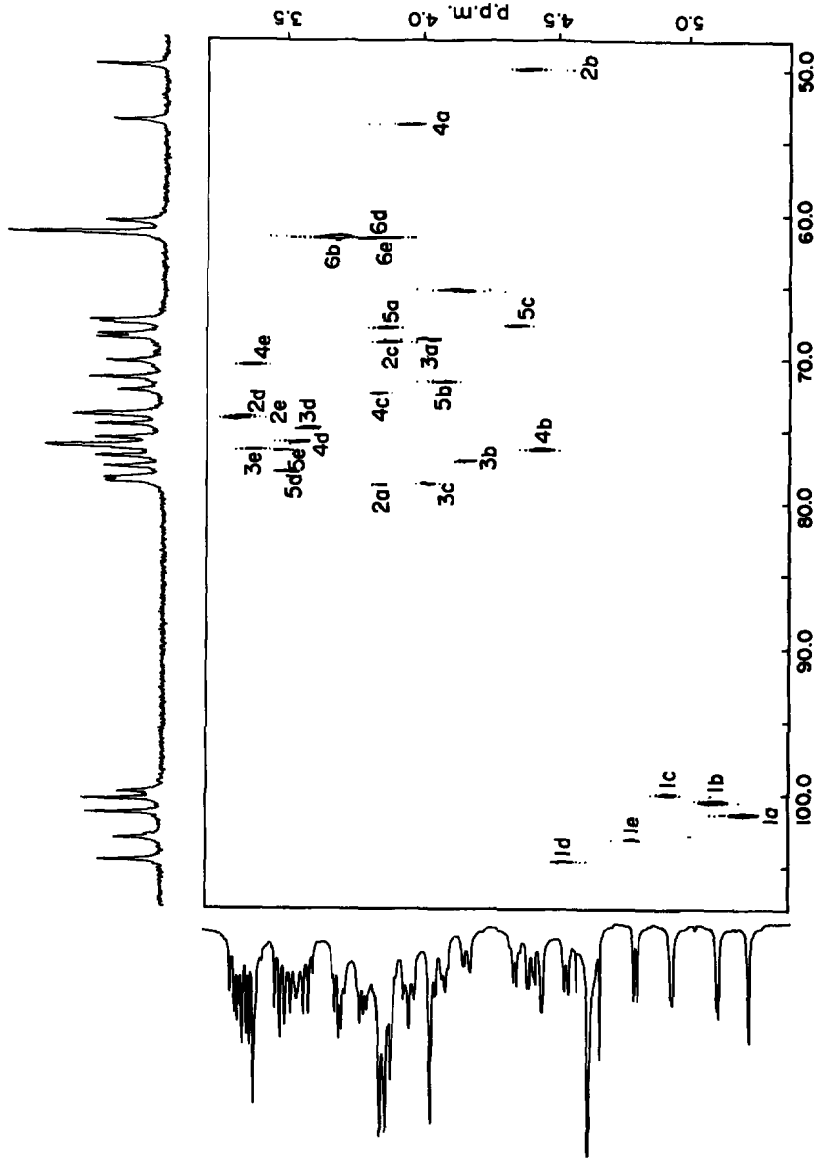


Fig. 3. Heteronuclear ^{1}H–^{13}C shift-correlation map for the spectral region F_2 (108.0–48.0 p.p.m.) and F_1 (5.4–3.2 p.p.m.). The respective ^{13}C and ^{1}H one-dimensional projections are displayed along the F_2 and F_1 axes and the correlated resonances are labelled (*a*–*e*).

TABLE II

CARBON-13 CHEMICAL SHIFTS (δ) AND ANOMERIC $^1J_{C,H}$ COUPLING CONSTANTS (Hz) OF *S. urbana* AND *S. landau* *O*-POLYSACCHARIDES[a]

Carbon atom	*Hexopyranosyl residues*								
	Residue a α-D-*PerNAcp*		*Residue b* α-D-*GalNAcp*		*Residue c* α-L-*Fucp*		*Residue d* β-D-*Glcp*		*Residue e* β-D-*Glcp*
	S. urbana	*S. landau*	*S. urbana*	*S. landau*	*S. urbana*	*S. landau*	*S. urbana*	*S. landau*	*S. urbana*
C-1	101.5 (175)	101.7 (172)	100.6 (172)	100.8 (174)	100.1 (172)	100.2 (170)	104.8 (165)	104.7 (163)	103.3 (164)
C-2	78.8	78.9	50.1	49.8	69.0	69.1	74.2	74.3	74.3
C-3	68.7	68.8	77.1	78.0	78.9	79.1	74.9	75.1	76.4
C-4	53.9	54.0	76.3	69.4	72.5	72.6	75.8	75.9	70.5
C-5	67.9	68.0	71.7	72.1	67.7	67.8	77.8	77.9	76.4
C-6	17.6	17.7	61.7	62.1	15.9	16.0	60.9	60.7	61.6
CH_3COHN	175.4[b]	175.5	175.5[b]	175.7	—	—	—	—	—
CH_3CONH	22.8[b]	22.9	22.9[b]	23.0	—	—	—	—	—

[a]Chemical shifts were measured at 310K and are expressed relative to internal 1,4-dioxane (67.4 p.p.m.). [b]Resonances whose assignments may be reversed.

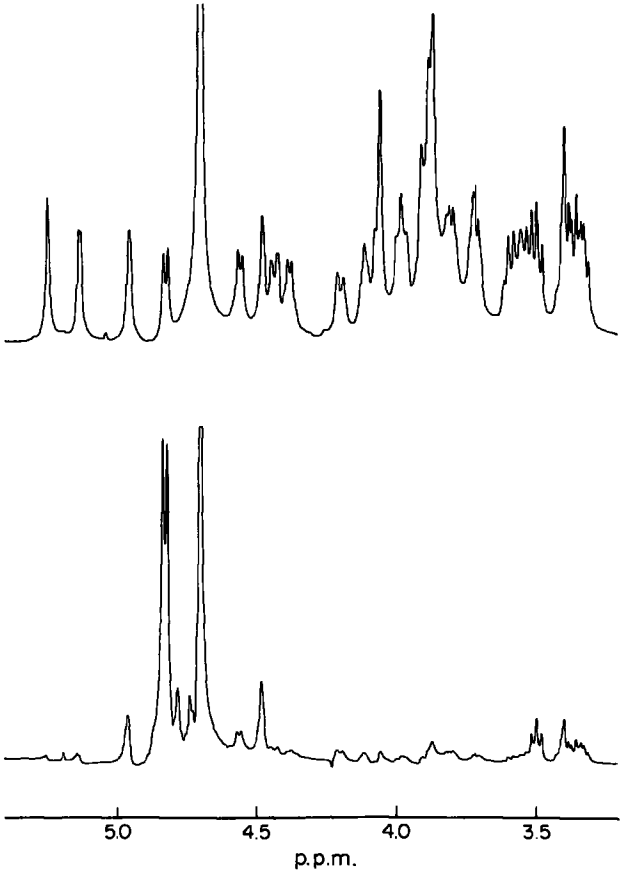
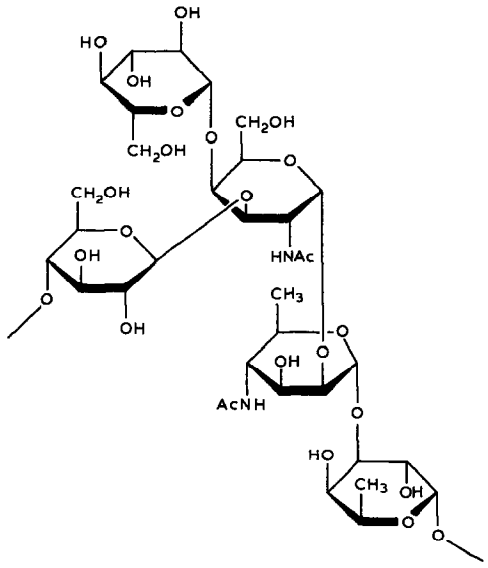


Fig. 4. One-dimensional n.O.e. difference-spectrum (b) obtained by subtraction of the spectrum, obtained for one-resonance irradiation of the H-1e resonance, from the off-resonance spectrum (a).

anomeric resonance *a–d* gave results identical to those reported for the *S. landau* O-chain analysis[13] and establish both the linkage sequence *a*→*c*→*d*→*b* and the positions of the linkages, which were in agreement with the linear structure determined for the repeating unit of *S. landau* O-chain. Irradiation of the new H-1e resonance gave a difference spectrum (Fig. 4) that clearly shows a strong n.O.e. to the protons H-4b, H-3e, and H-5e. As the n.O.e. was measured in a manner that emphasises the initial build-up and not the steady-state n.O.e. (in which spin-diffusion effects may be evident), the linkage of residue *e* is to O-4b and this β-D-glucopyranosyl unit is the branch-point substituent. Thus, based on the three experiments, COSY, heteronuclear ^{1}H–^{13}C shift-correlation, and truncated-drive n.O.e., the structure of the *S. urbana* and *S. godesberg* O-antigens is established as a polymer of a repeating, branched pentasaccharide unit having the structure:



(a) (e) (b) (a) (c)

→4)-β-D-Glc-(1→3)-[β-D-Glc-(1→4)]-α-D-GalNAc-(1→2)-α-D-PerNAc-(1→3)-α-L-Fuc-(1

Further proof of the foregoing structure was obtained from chemical analyses. G.l.c.–m.s. analyses of the hydrolyzed, reduced ($NaBD_4$), and acetylated products of the methylated O-chain were identified and quantified as recorded in Table III. The results are consistent with the position of the linkages assigned from the n.m.r. analyses.

Periodate oxidation of the O-chain resulted in the complete oxidation of both the D-glucopyranosyl residues present in the repeating unit. Smith-type hydrolysis[24] of the reduced ($NaBH_4$), periodate-oxidized O-chain gave a single oligosaccharide which, from ^{13}C- and ^{1}H-n.m.r. analyses, periodate oxidation, methylation, optical rotation studies (identical to those previously performed on the O-chain of *E. coli* O:157 (ref. 12)), positively identified the oligosaccharide as having the structure α-D-GalNAc*p*-(1→2)-α-D-PerNAc*p*-(1→3)-α-L-Fuc*p*-(1→2)-D-erythritol, the expected product resulting from oxidation of the D-glucopyranosyl residues.

While the methylation and periodate-oxidation evidence are consistent with the proposed structure for the *S. urbana* O-chain, they would also be consistent with an alternative structure in which the substituents at O-3 and O-4 of the 2-acetamido-2-deoxy-α-D-galactopyranosyl branch-point were reversed. The correctness of the proposed structure was confirmed by the fact that limited periodate oxidation of the O-chain with 1.3 mol of periodate per pentasaccharide unit (18 h, 20°) followed by reduction ($NaBH_4$) and Smith-type mild hydrolysis gave a product which, after gel filtration on Sephadex G-50, afforded a degraded polysaccharide of lower molecular weight, resulting from some cleavage (~6%) of the D-glucose residues in the main chain. The degraded polysaccharide (~50% yield), which was

TABLE III

G.L.C.–M.S. METHYLATION ANALYSIS OF THE *O*-CHAIN OF *S. urbana* LPS AND ITS LIMITED PERIODATE-OXIDATION PRODUCT

Derivative	T_{GM}	*Molar ratio*	
		Original O-*chain*	*Limited periodate-oxidized* O-*chain*
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol-*1-d*	1.00[a]	1.00	0.01
1,3,5-Tri-*O*-acetyl-2,4-di-*O*-methyl-L-fucitol-*1-d*	1.09[a]	0.94	0.96
1,4,5-Tri-*O*-acetyl-2,3,6-œtri-*O*-methyl-D-glucitol-*1-d*	2.04[a]	1.06	1.00
1,2,5-Tri-*O*-acetyl-4,6-dideoxy-3-*O*-methyl-4-(*N*-methylacetamido)-D-mannitol-*1-d*	2.38[b]	0.87	0.82
3,5-Di-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-galactitol-*1-d*	2.61[b]	—	0.78
3,4,5-Tri-*O*-acetyl-2-deoxy-6-*O*-methyl-2-(*N*-methylacetamido)-D-galactitol-*1-d*	3.31[b]	0.91	0.03

[a]G.l.c. program A. [b]G.l.c. program B.

selected as the fraction having a molecular weight >4,500, was essentially identical to the O-chain of *S. landau* LPS[13] as revealed by the identity of the ^{1}H- and ^{13}C-n.m.r. spectra with those of the *S. landau* spectra. The methylation analysis of the methylated, degraded polysaccharide (Table III) gave the *O*-methylglycose derivatives expected of a structure identical to the O-chain of *S. landau*, the essential absence of 2,3,4,6-tetra-*O*-methyl-D-glucose in the hydrolysis products indicating that the limited periodate oxidation had resulted in selective removal of the single β-D-glucopyranosyl residue at the branch point. The identification of 2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylamino)-D-galactose indicates that the aminoglycose is linked at O-3 in the degraded polysaccharide, and at the same time confirms that the D-glucopyranosyl residue removed was originally linked at O-4 at the branch point, thus confirming the structural conclusions drawn from the n.m.r. analyses.

The results obtained thus far suggest that there are at least two basic structures of O-chains in *Salmonella* species classified in the Kauffmann–White group N (O:30). One structure is represented by the repeating tetrasaccharide unit found in the *S. landau*[13] and the other by the repeating pentasaccharide unit found in the *S. urbana* and *S. godesberg* LPS. In either case, the serological cross-reactivity of both LPS O-chains with *B. abortus* and *Y. enterocolitica* (O:9) antigens can be related to the common occurrence of 1,2-linked *N*-acyl derivatives of 4-amino-4,6-dideoxy-α-D-mannopyranosyl residues in their linear chains. Although we were unable to remove the side β-D-glucopyranosyl residues with β-D-glucosidase, preliminary results indicate that the two types of O-chains of *Salmonella* group N may be differentiated serologically on the basis of the epitope containing the β-D-glucopyranosyl residue in one chain and not in the other, thus allowing a sub-classification of this group.

EXPERIMENTAL

Production of lipopolysaccharide. — Cultures of *S. urbana* (LCDC S-451, NRCC 4151) supplied by Dr. H. Lior, LCDC Health & Welfare Canada, Ottawa, Canada, and *S. godesberg* O:30 (NRCC 4157) supplied by Dr. M. J. Corbel, Central Veterinary Lab., Weybridge, U.K. were grown in a fermenter (28 L, Microfirm, New Brunswick Scientific) using a medium of 3.7% (w/v) brain heart infusion (Difco) at 37°, 200 r.p.m. and aeration at 25 L/min for 18 h. The collected cells were washed with 2% (w/v) saline, digested with lysozyme, ribonuclease, and deoxyribonuclease[16] and subsequently extracted by the hot aqueous phenol method[17]. LPS were recovered from the dialyzed, separated phenol and water layers by repeated ultracentrifugation at 105,000*g* (12 h at 4°) until judged pure by the carbocyanine dye assay[25].

Polysaccharide O-chain. — LPS (1 g) in 2% (v/v) acetic acid (300 mL) was heated for 2 h in a boiling-water bath and the precipitated lipid A was removed by low-speed centrifugation. The lyophilized centrifugate was fractionated on a column (2.6 × 100 cm) of Sephadex G-50 using pyridinium acetate (0.05M, pH 4.7)

as the eluant and 10-mL fractions were monitored for neutral glycose, aminoglycose, and 3-deoxyoctulosonate.

Analytical methods. — Quantitative methods used were (*a*) the phenol–sulfuric acid method for neutral glycoses[26], (*b*) the modified Elson–Morgan method for aminoglycoses[27], and (*c*) the periodate oxidation–thiobarbituric acid method for deoxyoctulosonate[28].

Glycoses were determined by g.l.c. of their derived alditol acetates[29], using *myo*-inositol as an internal standard. Oligo- and poly-saccharide samples (0.5 mg) were hydrolyzed in sealed glass tubes with M sulfuric acid (1 mL) for 15 h at 100°, followed by neutralization ($BaCO_3$) to obtain the released glycoses. Samples containing 4-amino-4,6-dideoxy-D-mannose or its derivatives (0.5 mg) were analyzed following cleavage with anhydrous hydrofluoric acid (0.5 mL) for 4 h at 20° and removal of the HF in a stream of dry nitrogen.

G.l.c. employed a Hewlett–Packard model 5710A gas chromatograph fitted with a hydrogen-flame detector and a model 3380A electronic integrator. The following conditions were used: program A, glass column (2 mm × 180 cm) packed with 3% (w/w) SP2340 on 80–100 mesh Supelcoport; temperature program 200° (delay 2 min) to 240° at 1°/min; program B, glass column (2 mm × 180 cm) packed with 3% (w/w) OV17 on 80–100 mesh Chromosorb W; temperature program 180–270° at 6°/min. Development was with dry nitrogen at 20–30 mL/min and retention times are quoted relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (T_{GM}).

A Hewlett–Packard 5985B g.l.c.–m.s. system was used for g.l.c.–m.s. employing the g.l.c. programs A and B and an ionization potential of 70 eV. The identity of each glycose was established by direct comparison of its retention time and mass spectrum with those of a laboratory reference specimen.

Gel filtration was performed on columns of Sephadex G-50 (2 × 80 cm) or Sephadex G-15 (2.5 × 80 cm) (Pharmacia Fine Chemicals). The gel-filtration properties of the eluted materials are expressed in terms of their distribution coefficients K_{av}; $K_{av} = (V_e - V_0)/(V_t - V_0)$, where V_e is the elution volume of the specific material, V_0 is the void volume of the system, and V_t is the total volume of the system.

Methylation analyses. — Polysaccharide samples (2–3 mg) were methylated with sodium methylsulfinylmethanide and methyl iodide in dimethyl sulfoxide according to the Hakomori procedure[30] and the products were purified by dialysis against distilled water. Oligosaccharides methylated by the same method were recovered from the mixture by partition between chloroform and water.

Methylated products were hydrolyzed with 90% formic acid (1 mL) for 2 h at 100° and, following evaporation, the residues were further hydrolyzed with 0.5M sulfuric acid (0.5 mL) for 12 h at 100°. Samples containing aminoglycoses were also hydrolyzed with anhydrous HF as already described. The methylated glycoses released, in water (2 mL), were reduced during 12 h by the addition of sodium borodeuteride (12 mg). Following acidification with acetic acid and evaporation

dry methanol (5 × 8 mL) was distilled from the residue to remove borate. The product was acetylated by treatment with acetic anhydride (1 mL) for 2 h at 115° and the resulting derivatives were analyzed directly by g.l.c.

Periodate oxidation. — Full periodate oxidations were made in aqueous solution in the dark at 4° with an approximately four-fold excess of sodium meta-periodate. The excess of periodate was decomposed by the addition of ethylene glycol the oxidized material was reduced by treatment with sodium borohydride according to previously established procedures[24]. The products were hydrolyzed with 2% acetic acid for 2 h at 100° to effect the required cleavage, to yield oligo-saccharides or degraded polysaccharide.

General methods. — Concentrations were made under diminished pressure and below 40°. Optical rotations were determined with a Perkin–Elmer 243 polarimeter and 10-cm microtubes.

Nuclear magnetic resonance. — Two solutions of the polysaccharide in D_2O (10 mg/400 μL and 80 mg/500 μL) were prepared for ^1H and ^{13}C measurements, respectively. All spectra were recorded in the pulsed Fourier-transform mode, with quadrature detection on a Bruker AM-500 spectrometer equipped with a 16-bit digitizer. Either a selective or dual $^1H/^{13}C$ 5-mm probehead was used.

Proton spectra (500 MHz) were recorded at 305 and 310K using spectral widths of 2.5 kHz, a $\pi/2$ pulse (9 μs for selective and 28 μs for the dual probehead), a 16K data-set for a digital resolution of 0.3 Hz/point, an acquisition time of 3.2 s, and a relaxation delay of 3.0 s. Resolution enhancement prior to Fourier transformation was achieved in one of two ways; either the Lorentz-to-Gauss line-shape transformation[31] was used with typical values for line broadening of −1.0 Hz and a Gaussian broadening factor of 0.4, or a shifted sine-bell filtering function was used for maximum resolution enhancement with optimum signal-to-noise. Chemical shifts are expressed relative to internal acetone (1%, 2.225 p.p.m.). In order to optimize delay periods for 2-D experiments, proton T_1 values were determined by the nonselective inversion-recovery method[32] and were ~1.5 s for the hexose ring protons. Truncated-driven n.O.e. difference spectra were obtained with the pulsed sequence[23] $t_1(\omega) - (\pi/2) - T_{ac} - T_d$. A low-power radiofrequency pulse, typically 35 dB below 0.2 watt, was applied to the anomeric resonance for 200 ms, followed by a $\pi/2$ observation pulse. The acquisition time T_{ac} and relaxation delay T_d were chosen such that their sum did not exceed $4 \times T_1$. Eight scans with irradiation on each of the five anomeric resonances and eight scans for an off-resonance reference spectrum were acquired in six separate files. The entire sequence was cycled to achieve a satisfactory signal-to-noise ratio.

Carbon-13 spectra (125 MHz) were recorded at 310K for a 25-kHz spectral width using a $\pi/2$ pulse (8.5 μs) and a 32K data-set. In order to minimize heating effects, gated broadband decoupling of 1 watt was used to establishe n.O.e. build-up during a delay period of 0.35 s, followed by ~1 watt composite pulse decoupling (WALTZ)[33] during a 0.33-s acquisition time. DEPT experiments[34] were performed for a 12.5-kHz spectral width, using a $3\pi/2$ proton pulse to distinguish

CH and CH_2 resonances and for enhanced signal-to-noise during measurement of the anomeric $^1J_{C,H}$ coupling constants. The delay between pulses $(2J)^{-1}$ was set at 3.4 ms. Chemical shifts are expressed relative to internal 1,4-dioxane (1%, 67.4 p.p.m.). Heteronuclear $^1J_{C,H}$ coupling constants for the anomeric positions were measured by gated decoupling[35].

Two-dimensional n.m.r. experiments were recorded by means of Bruker DISNMRP software. Homonuclear shift-correlated COSY[21] experiments were performed at 305K without suppression of the HOD resonance. The data were obtained using a matrix $(t_1 \times t_2)$ of 512 × 1024 points that were zero-filled to 2048 × 4096 points. After resolution enhancement in both dimensions by a non-shifted sine-bell window function, the doubly transformed data were processed to give magnitude spectra. The spectral width of the matrix was 2500 Hz in both dimensions, which resulted in a digital resolution of 1.2 Hz/point. The $\pi/2$ pulse was 9.0 μs, and a minimum delay of 1.5 s was used between transients for each value of the incrementable delay t_1. The number of transients per F.i.d. was 64 for the COSY experiment.

Heteronuclear shift-correlated spectra[22] were recorded on a 500-μL sample (80 mg of polysaccharide) in a 5-mm tube using a spectral width in F_2 of 12820 Hz (102.0 p.p.m.) and 2600 Hz (5.20 p.p.m.) in F_1. The data were obtained using a matrix $(t_1 \times t_2)$ of 256 × 2048 points that were zero-filled to 512 × 4096 points and doubly transformed to give a power spectrum. The $\pi/2$ pulse widths were 8.5 μs for ^{13}C and 30.0 μs for ^1H, and the fixed delays τ_1 and τ_2 were selected for all multiplicities [$\tau_1 = (2J)^{-1}$ 3.4 ms, $\tau_2 = (4J)$ 1.7 ms]. Digital resolution in F_2 was 3.8 Hz/point and in F_1 1.1 Hz/point. A recycle delay of approximately one proton T_1 was employed, 180 transients per f.i.d. were collected, and composite decoupling of ~1 watt was applied during acquisition in order to minimize heating effects. Gaussian window-functions were used in both dimensions, a line broadening of 3 Hz was employed for the F_2 domain, whilst the F_1 domain was optimized by the Lorentz-to-Gauss transformation employing a line broadening of −3.0 Hz and a Gaussian broadening-factor of 0.3. The total acquisition time was 14 h.

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