*Carbohydrate Polymers* **18** (1992) 51–57

# The O1 antigen of *Escherichia coli:* structural characterization of the O1A1-specific polysaccharide

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(Received 13 March 1991; revised version received 10 June 1991; accepted 12 July 1991)

The O-specific polysaccharide moiety of the O1A1 antigen (lipopolysaccharide) from *E. coli* 01:K1 consists of L-rhamnose, *N*-acetyl-D-glucosamine and *N*-acetyl-D-mannosamine in the molar ratio of 3:1:1. By using fragmentation procedures, methylation analysis, and NMR spectroscopy, the O1A1 polysaccharide was found to have the structure

3)-α-L-Rhap-(1→3)-β-L-Rhap-(1$_{1}$→4)-β-D-GlcpNAc-(1$_{3}$→3)α-L-Rhap-(1$_{3}$→
2
↑
1
β-D-ManpNAc

## INTRODUCTION

*Escherichia coli* O1 strains, frequently expressing the K1 capsular antigen, are causes of extra-intestinal infections in humans (Robbins *et al.*, 1974; Sarff *et al.*, 1975; Myerowitz *et al.*, 1977; Nimmich & Zingler, 1984). In a clonal analysis of extra-intestinal *E.coli*, two O1:K1 clones and several O1 strains without the K1 antigen (O1:non-K1) have been described (Nimmich & Zingler, 1984; Achtman *et al.*, 1983; Kusecek *et al.*, 1984). Whereas no differences between the lipopolysaccharides (LPS) from two O1:K1 strains could be found in the chemical analyses, certain differences in their SDS-PAGE pattern led to a distinction into O1A and O1A1 LPS (Moll *et al.*, 1986). The LPS of the O1:non-K1 strains differed from that of the O1:K1 clones and also between each other in all analyses, and they were described as O1B and O1C (Achtman *et al.*, 1983; Kusecek *et al.*, 1984; Moll *et al.*, 1986). Since strains with the O1A/O1A1 antigen are prevalent among virulent *E. coli* O1, we elucidated the primary structure of the O1A1-specific polysaccharide.

## MATERIALS AND METHODS

### Bacteria and cultivation

*E.coli* 21450 (O1:K1) was used, which had been previously described as A109 (Achtman *et al.*, 1983). The bacteria were grown to the stationary phase (about 5 h) in 14-liter batch cultures at 37°C with an aeration of 1 liter/min in a medium containing, per liter, tryptone (7·5 g), tryptose (7·5 g), yeast extract (10 g), glucose (10 g), NaCl (3 g), $Na_2HPO_4 \times 12H_2O$ (8 g), $MgSO_4 \times 7H_2O$ (0·2 g), and polyethylene glycol (0·3 g). Glucose and magnesium sulfate were sterilized separately. At the end of the cultivation, the bacteria were killed with phenol (1% final concentration) and harvested by centrifugation.

### Isolation of the O1A1 polysaccharide

The lipopolysaccharide was obtained from the bacteria with 45% aqueous phenol at 65°C and was purified as described (Westphal & Jann, 1965). The polysaccharide was obtained from the lipopolysaccharide by hydrolysis in 1% acetic acid at 100°C for 90 min and was purified as described (Gupta *et al.*, 1984; Jann *et al.*, 1975).

### NMR spectroscopy

$^{1}H$- and $^{13}C$-NMR spectra were recorded with a Bruker AM-300 spectrometer in $D_2O$ at 60°C by using acetone, $\delta_H 2{\cdot}23, \delta_C 31{\cdot}45$, as the internal standard. Homonuclear 2D COSY spectra, H-relayed H,H-COSY spectra (one- and two-step), and heteronuclear $^{13}C/^{1}H$-COSY spectra

Carbohydrate Polymers 0144-8617/92/$05.00

were obtained by using standard Bruker software for ASPECT 3000 (COSYHG, COSYRCT, COSYRCT2, and XHCORRD, respectively). N.O.e. experiments were performed in the Truncated Driven (TOE) mode (Wagner & Wüthrich, 1979) with the Bruker NOEMULT program. The relaxation delay D1 was 1 s, the irradiation time of every component of multiplets (D2) was 0·1 s, and the total pre-irradiation time of whole multiplets was 1·0–1·2 s.

### Analytical methods

Gas-chromatography–mass-spectrometry (g.l.c.–m.s.) analyses were run on a Hewlett-Packard 59585 and a Finnigan MAT 1020B automatic system at 70 eV on a CB CP-SIL 5 (25 m × 0·25 mm) column, with helium as the carrier gas. Chemical ionization (c.i.)-m.s. was done with ammonia as the reactant gas.

Rhamnose was determined as alditol acetate and also with the cysteine-sulfuric acid reagent (Kabat & Mayer, 1971). The amino sugars were identified and determined as their alditol acetates by g.l.c. on Poly A103 at 220°C (Niedermeier & Tomana, 1974). The absolute configuration of rhamnose was determined with the L-Rha specific rhamnose isomerase (Wilson & Ajl, 1957). L-Rhamnose isomerase was a gift of Dr W. D. Fessner, of the University of Freiburg.

### Methylation analysis

The polysaccharide and oligosaccharides were methylated with dimethyl sulfoxide–potassium hydride–methyl iodide, as described earlier (Phillips & Frazer, 1981). To establish the linkage to *N*-acetylglucosamine, the hydrolysis procedure of Stellner *et al.* (1973) was used. The mixture of methylated sugars was analysed, after reduction and acetylation, by g.l.c.–m.s.

### Periodate oxidation, *N*-deacetylation, and deaminating fragmentation

These methods have been described in detail elsewhere (Gupta *et al.*, 1984).

### Smith degradation

The periodate-oxidized and borohydride-reduced polysaccharide (35 mg) was hydrolyzed (20°C, 4 days) in 3·5 ml 0·25M trifluoroacetic acid (Janson *et al.*, 1984). After lyophilization, the material was dissolved in a minimum amount of water and chromatographed on a (1·5 × 40 cm) column of Biogel P-2 with water as eluant.

## RESULTS AND DISCUSSION

### Characterization of the polysaccharide

The polysaccharide preparation, as obtained after chromatography on Sephadex G-50 ($K_d$ = 0·95), was free of protein, nucleic acid, and lipid A. It consisted of rhamnose, *N*-acetylglucosamine, and *N*-acetylmannosamine in the molar ratio of 3:1:1, as evidenced by chemical analysis and NMR spectroscopy. The presence of *N*-acetylmannosamine had been overlooked previously (Moll *et al.*, 1986). Oxidation with sodium metaperiodate destroyed the *N*-acetylmannosamine and left the other constituents intact. This indicated that in the polysaccharide the *N*-acetylmannosamine is either 6-linked or terminal.

### Methylation analysis of the polysaccharide

The polysaccharide, before and after Smith degradation (Goldstein *et al.*, 1965), was methylated (Phillips & Frazer, 1981) and hydrolyzed with a method facilitating the analysis of partially methylated amino sugars (Stellner *et al.*, 1973). After reduction and acetylation, the partially methylated alditols were analysed with g.l.c.–m.s. Table 1 shows that the polysaccharide consisted of 3-linked rhamnose, 2,3-linked rhamnose, 4-linked *N*-acetylglucosamine, and terminal *N*-acetylmannosamine. Thus the terminal *N*-acetylmannosamine was removed by Smith degradation, and the results of subsequent methylation showed that this amino sugar was linked to C-2 of a rhamnose unit.

**Table 1. Results of methylation analyses of the O1A1 polysaccharide before (PS) and after Smith degradation ($PS_{sm}$)**

| Partially methylated alditol acetates (as identified (with g.l.c.–m.s.) | Interpretation | Molar ratios in polysaccharide PS | $PS_{sm}$ |
|---|---|---|---|
| 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylrhamnitol | 3-Rha | 2 | 3 |
| 1,2,3,5-tetra-*O*-acetyl-4-*O*-methylrhamnitol | 2,3-Rha | 1 | — |
| 1,4,5-tri-*O*-acetyl-3,6-di-*O*-methyl-2-*N*-methylacetamidohexitol | 4-GlcNAC[a] | 1 | 1 |
| 1,5-di-*O*-acetyl-3,4,6-tri-*O*-methyl-2-*N*-methylacetamidohexitol | t-ManNAC[a] | 1 | — |

[a]These assignments were made on the basis of periodate sensitivity of ManNAc and periodate resistance of GlcNAc in the polysaccharide.

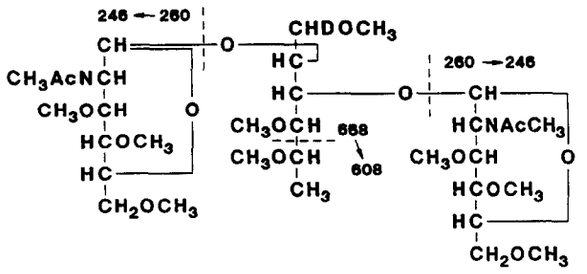


**Fig. 1.** Structure and mass-spectrometric-fragmentation pattern of the reduced and methylated trisaccharide, obtained from the O1A1 polysaccharide by partial acid hydrolysis.

## Chemical degradations of the polysaccharide and analysis of the products

Hydrolysis of the polysaccharide in 0·5M trifluoroacetic acid (100°C, 40 min) yielded oligosaccharides that were isolated by chromatography on Biogel P-2. Their methylation analysis showed that one oligosaccharide was GlcpNac-(1,3)-Rhap-(1,3)-Rhap, the second was Rhap-(1,3)-Rhap-(GlcpNAc), and the third was ManpNAc-(1,2)-[(1,3)-GlcpNAc]-Rhap. Whereas the first two trisaccharides are part of the main chain, the last one represents the branching of the polysaccharide. Its structure and fragmentation pattern are shown in Fig. 1.

Treatment of the polysaccharide with hydrazine/hydrazine sulfate (110°, 48 h) resulted in the complete removal of the *N*-acetyl groups from both *N*-acetylhexosamines. One part of the de-*N*-acetylated polysaccharide was subjected to periodate oxidation, which resulted in the oxidative destruction of both hexosamines. Subsequent reduction with sodium borohydride and hydrolysis in 0·5M trifluoroacetic acid (20°C, 4 days) yielded a main fragment of which methylation revealed the structure shown in Fig. 2. Another part of the *N*-deacetylated polysaccharide was subjected to deaminating fragmentation with nitrous acid. Methylation analysis of the purified major product showed it to be Rha-(1,3)-Rha-(1,3)-Rha-O-(2,5 anhydromannitol).

These results indicate that the O1A1 polysaccharide consists of the backbone 3)-Rhap-(1,3)-Rhap-(1,4)-GlcpNAc-(1,3)-Rhap-(1,3). One of the rhamnose units is substituted with ManpNAc.

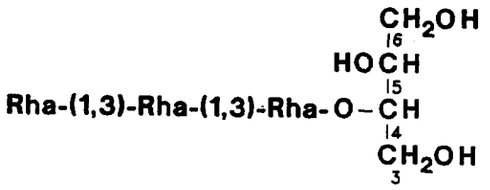


**Fig. 2.** Structure of the fragment obtained from the *N*-deacetylated O1A1 polysaccharide by oxidation with sodium metaperiodate, reduction with sodium borohydride, and acid hydrolysis.

## NMR analysis of the polysaccharide

The structure of the O1A1 polysaccharide was supported by $^{1}H$- and $^{13}C$-NMR spectroscopy, including nuclear Overhauser enhancement and two-dimensional homo- and heteronuclear COSY experiments. The $^{1}H$-NMR spectrum (Fig. 3) was interpreted (Table 2) by using 2D COSY, one- and two-step H-relayed H,HCOSY spectra (Jeener *et al.*, 1979; Dabrowski, 1987). The visual multiplicities and coupling constants were determined from homonuclear spin-decoupling experiments in a modified differential mode (Kocharova *et al.*, 1988).

A series of NOE experiments with pre-irradiation of the anomeric protons (Table 3) revealed the sequence and anomeric configuration of the sugar residues. The substitution pattern was elucidated with additional experiments with pre-irradiation of H-2 and H-3 protons of the individual sugars. This is also shown in Table 3. The signal assignments in the $^{13}C$-NMR spectra (Fig. 4 and Table 4) were accomplished by using a 2D heteronuclear COSY spectrum (Fig. 5). The glycosylation effects in the $^{13}C$-NMR spectrum were calculated, and the relative absolute configurations of the sugar residues were determined according to Shashkov *et al.* (1988). All rhamnose residues proved to be of the same absolute configuration, and both amino sugars have the opposite configuration.

The results of our studies show that the O1A1 polysaccharide of *E.coli* O1:K1 has the structure:

3)-α-L-Rhap-(1,3)-β-L-Rhap-(1,4)-β-D-GlcpNAc-(1,3)-α-L-Rhap-(1,
2
1
β-D-ManpNAc

In $^{13}C$-NMR spectroscopy and methylation analysis, we did not find differences between the polysaccharide reported here and that from the O1A LPS of *E.coli* O1:K1 strain 21451 (A110 in Moll *et al.*, 1986). It is possible that the differences in SDS-PAGE reported for the O1A and O1A1 LPS result from differences in the respective core oligosaccharides.

## ACKNOWLEDGEMENT

We thank Mr Dietmar Borowiak for running the g.l.c.-m.s.

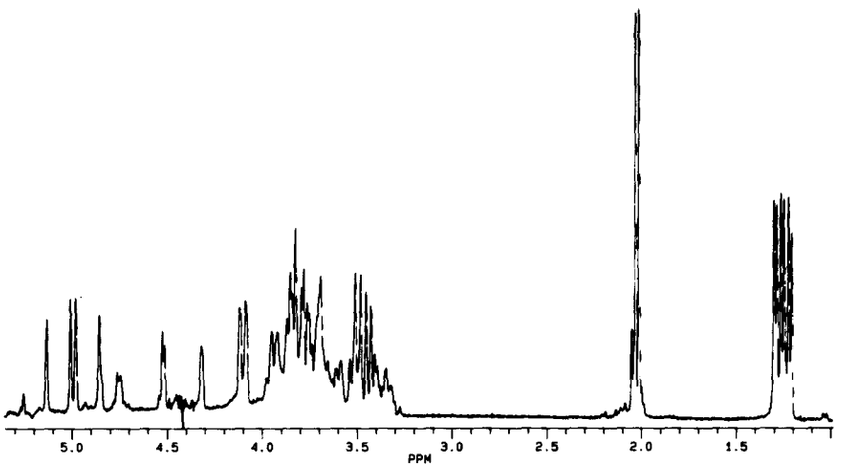


**Fig. 3.** 300-MHz $^1$H-NMR spectrum ($\delta$1·0–5·3) of the O1A1 polysaccharide, recorded in $D_2O$ (60°C) with acetone ($\delta$2·25) as internal standard.

**Table 2. Data of the $^1$H NMR spectrum of the O1A1 polysaccharide (60°C, $D_2O$, acetone $\delta$2·225)**

| Residue | Proton | Chemical shift | Visual multiplicity[a] | Coupling constant |
|---|---|---|---|---|
| | | ppm | | Hz |
| →3)Rhapα (A) | H—1 | 4·96 | bs | $J_{12}$ <2 |
| | H—2 | 4·07 | bd | $J_{23}$ 3 |
| | H—3 | 3·85 | dd | $J_{34}$ 10 |
| | H—4 | 3·49 | t | $J_{45}$ 10 |
| | H—5 | 3·86[b] | | $J_{56}$ 6 |
| | H—6 | 1·28 | d | |
| →3)Rhapβ (B) | H—1 | 4·85 | bs | $J_{12}$ <2 |
| | H—2 | 4·10 | bd | $J_{23}$ 3 |
| | H—3 | 3·58 | dd | $J_{34}$ 9 |
| | H—4 | 3·43 | t | $J_{45}$ 9 |
| | H—5 | 3·73[b] | | $J_{56}$ 6 |
| | H—6 | 1·20 | d | |
| →4)GlcpNAcβ (C) | H—1 | 4·73 | d | $J_{12}$ 7 |
| | H—2+H—3+H—4 | 3·60–3·71 | m | |
| | H—5 | 3·46–3·52[b] | m | |
| | H—6+H—6′ | 3·7–3·9[b] | | |
| →3)Rhapα (D) | H—1 | 5·12 | bs | $J_{12}$ <2 |
| 2 | H—2 | 4·30 | bd | $J_{23}$ 4 |
| ↑ | H—3 | 3·92 | dd | $J_{34}$ 10 |
| | H—4 | 3·41 | t | $J_{45}$ 10 |
| | H—5 | 3·81[b] | | $J_{56}$ 6 |
| | H—6 | 1·24 | d | |
| ManpNAcβ (E) | H—1 | 5·00 | bs | $J_{12}$ <2 |
| | H—2 | 4·50 | bd | $J_{23}$ 4 |
| | H—3 | 3·76 | dd | $J_{34}$ 10 |
| | H—4 | 3·46 | t | $J_{45}$ 10 |
| | H—5 | 3·32 | m | |
| | H—6,6′ | 3·8–3·9[b] | | |

[a] *b* – broadened, *s* – singlet, *d* – doublet, *t* – triplet, *m* – multiplet; [b] from two-dimensional $^1$H/$^{13}$C-spectrum.

(A) (B) (C) (D)
→3)α-L-Rhap-(1→3)-β-L-Rhap-(→4)-β-D-GlcpNAc-(1→3)-α-L-Rhap-(1→
2
↑
β-D-ManpNAc-1
(E)

**Table 3. NOE — data for polysaccharide O1A**

| NOE-signals in differential spectra | Pre-irradiated protons H—1(A) | H—1(B) | H—1(C) | H—1(D) | H—1(E) | H—2(D) | H—3(D) |
|---|---|---|---|---|---|---|---|
| H—2(A) | + | | | | | | |
| H—3(A) | | | | + | | | |
| H—2(B) | | + | | | | | |
| H—3(B) | + | + | | | | | |
| H—5(B) | | + | | | | | |
| H—1(C) | | | | | | | + |
| H—2,3,4(C) | | + | + | | | | |
| H—5(C) | | | + | | | | |
| H—1(D) | | | | | | + | + |
| H—2(D) | | | +[a] | + | + | | + |
| H—3(D) | | | + | | +[a] | | |
| H—1(E) | | | | | | + | |
| H—2(E) | | | | | + | | |
| H—3(E) | | | | | + | | |
| H—5(E) | | | | | + | | |

[a]Small signal due to spin-diffusion.

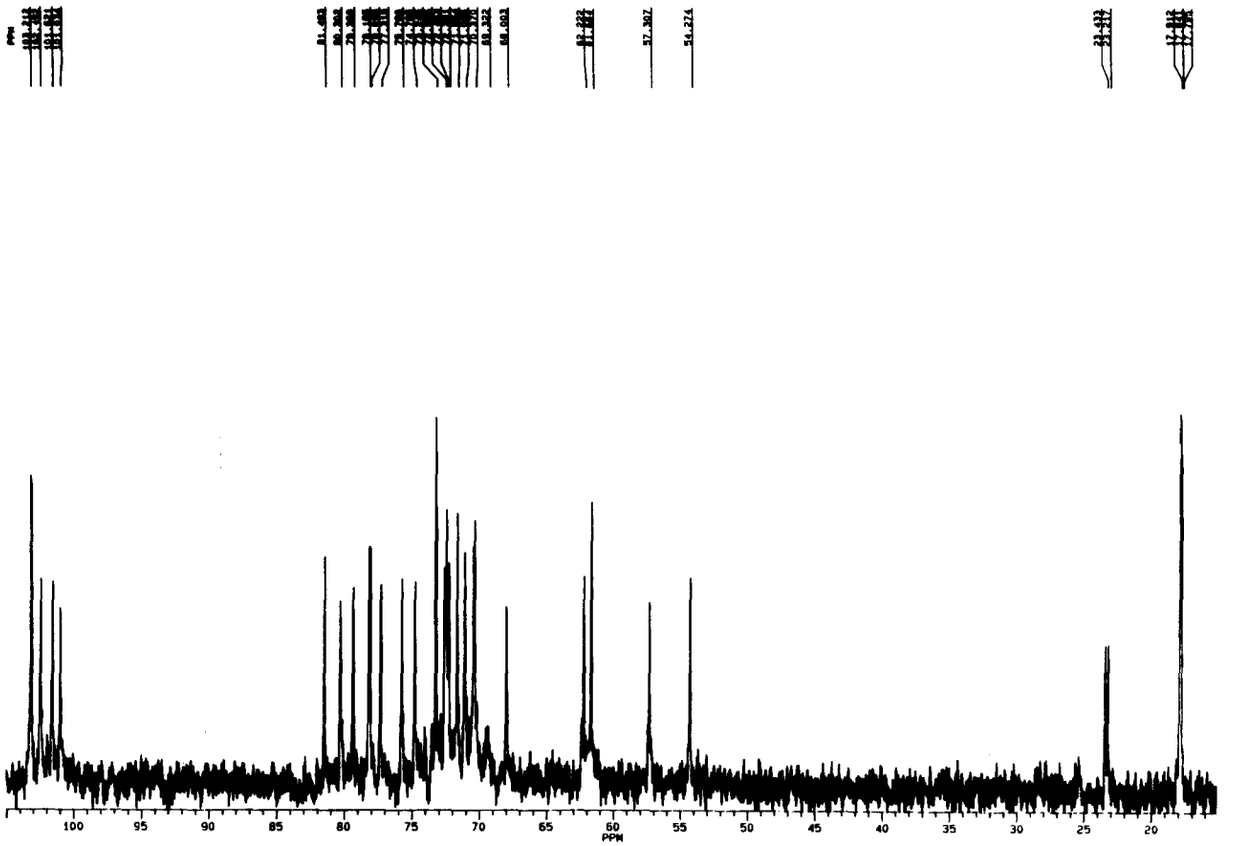


**Fig. 4.** 75-MHz $^{1}$C-NMR spectrum ($\delta$16–104) of the O1A1 polysaccharide, recorded in $D_2O$ (60°C) with acetone ($\delta$31·45) as internal standard.

**Table 4. Data for $^{13}C$ NMR spectrum of polysaccharide O1A1 (glycosylation effects in parentheses)**

| Residue | | Chemical shifts, ppm | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C—1 | C—2 | C—3 | C—4 | C—5 | C—6 | NHCO | $CH_3$ |
| →3)Rhapα | (A) | 103·2 (+8·0) | 71·1 (−1·0) | 79·35 (+8·0) | 72·6 (−0·9) | 70·4 | 17·9[a] | | |
| →3)Rhapβ | (B) | 101·6 (+6·9) | 71·7 (−0·9) | 81·5 (+7·5) | 72·3 (−0·8) | 73·25 | 17·85[a] | | |
| →4)GlcpNAc | (C) | 103·15 (+7·0) | 57·3 | 74·8 (−0·3) | 78·2 (+7·0) | 75·8 (−1·4) | 61·7 | 175·8[b] | 23·4[c] |
| →3)Rhap<br>2<br>↑ | (D) | 102·5 (+7·3) | 78·1 (+6·0) | 80·3 (+9·0) | 72·45 (−1·0) | 70·45 | 17·8[a] | | |
| ManpNAc | (E) | 101·05 (+6·8) | 54·3 | 73·25 | 69·3 | 77·3 | 62·2 | 176·4[b] | 23·2[c] |

[a–c]Assignments could be interchanged.

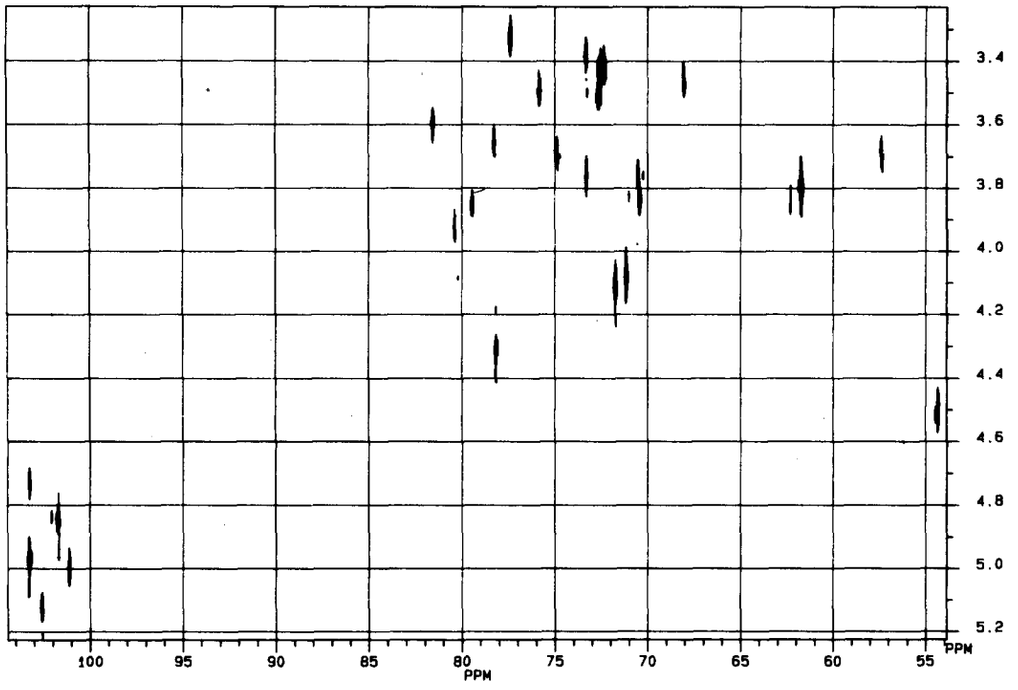


**Fig. 5.** 2D $^{13}C/^{1}H$ COSY spectrum of the O1A1 polysaccharide.

## REFERENCES

Achtman, M., Mercer, A., Kusecek, B., Pohl, A., Heuzenroeder, M., Aaronson, W., Sutten, A. & Silver, R. P. (1983). Six widespread bacterial clones among *Escherichia coli* K1 isolates. *Infect. Immun.*, **39**, 315–35.

Dabrowski, J. (1987). Application of two-dimensional NMR methods in the structural analysis of oligosaccharides and other complex carbohydrates. In: *Methods in Stereochemical Analysis, Two-dimensional NMR Spectroscopy: Application for Chemists and Biochemists*, vol. 9, ed. W. R. Croasmun & R. M. K. Carlson. VCH, New York, pp. 349–86.

Goldstein, I. J., Hay, G. W., Lewis, B. A. & Smith, F. (1965). Controlled degradation of polysaccharides: general procedures. *Meth. Carbohydr. Chem.*, **5**, 357–61.

Gupta, D. S., Jann, B. & Jann, K. (1984). *Escherichia coli* O18ac antigen: structure of the O-specific polysaccharide moiety. *Infect. Immun.*, **45**, 203–9.

Jann, B., Reske, K., & Jann, K. (1975). Heterogeneity of lipopolysaccharides. Analysis of polysaccharide chain length by sodium dodecyl-sulfate-polyacrylamide gel electrophoresis. *Eur. J. Biochem.*, **60**, 239–46.

Jansson, P.-E., Lindberg, B., Ogunlesi, M., Svenson, S. B. & Wrangsell, G. (1984). Structural studies of the O-antigen polysaccharide of *Escherichia coli* O4. *Carbohydr. Res.*, **134**, 283–91.

Jeener, J., Meier, B. H., Bachmann, P. & Ernst, E. E. (1979). *J. Chem. Phys.*, **71**, 4546–53.

Kabat, E. A. & Mayer, M. M. (1971). *Experimental Immunochemistry*. C. C. Thomas, Publ., Springfield, Ill., USA, pp. 538–41.

Kocharova, N. A., Knirel, Y. A., Shashkov, A. S., Kochetkov,

N. K. & Pier, G. B. (1988). Structure of an extracellular cross-reactive polysaccharide from *Pseudomonas aeruginosa* Immunotype 4. *J. Biol. Chem.*, **263**, 11291–5.

Kusecek, B., Wloch, H., Mercer, A., Väisänen, V., Pluschke, G., Korhonen, T. & Achtman, M. (1984). Lipopolysaccharide, capsule, and fimbriae as virulence factors among O1, O7, O16, O18 or O75 and K1, K5, or K100 *Escherichia coli*. *Infect. Immun.*, **43**, 368–79.

Moll, A., Kusecevk, B., Pluschke, G., Morelli, G., Kamke, M., Jann, B., Jann, K. & Achtman, M. (1986). A re-examination of the O1 lipopolysaccharide group of *Escherichia coli*. *Infect. Immun.*, **53**, 257–63.

Myerowitz, R. L., Albers, A. C., Yee, R. B. & Ørskov, F. (1977). Relationships of K1 antigen to biotype in clinical isolates of *Escherichia coli*. *J. Clin. Microbiol.*, **6**, 124–27.

Niedermeier, W. & Tomana, M. (1974). Gas chromatographic analysis of hexosamines in glycoproteins. *Anal. Biochem.*, **57**, 363–68.

Nimmich, W. & Zingler, G. (1984). Biochemical characteristics, phage patterns, and O1 factor analysis of *Escherichia coli* O1:K1:H7:F11 and O1:K1:H⁻:F9 strains isolated from patients with urinary tract infections. *Med. Microbiol. Immunol.*, **173**, 75–85.

Phillips, L. R. & Frazer, B. A. (1981). Methylation of carbohydrates with dimsyl potassium in dimethyl sulfoxide. *Carbohydr. Res.*, **90**, 149–52.

Robbins, J. B., McCracken, G. H., Gotschlich, E. C., Ørskov, I., Ørskov, F. & Hanson, L. A. (1974). *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. *New Engl. J. Med.*, **290**, 1216–20.

Sarff, L. D., McCracken, G. H., Schiffer, M. S., Glode, M. P., Robbins, J. B., Ørskov, I. & Ørskov, F. (1975). Epidemiology of *Escherichia coli* K1 in healthy and diseased newborns. *Lancet* **i**, 1090–104.

Shashkov, A. S., Lipkind, G. M., Knirel, Y. A. & Kochetkov, N. K. (1988). Stereochemical factors determining the effects of glycosylation on the $^{13}C$ chemical shifts in carbohydrates. *Magn. Reson. Chem.*, **26**, 735–47.

Stellner, K., Saito, H. & Hakomori, S. (1973). Determination of amino sugar linkages in glycolipids by methylation. Amino sugar linkages of ceramide pentasaccharides of rabbit erythrocytes and of Forssman antigen. *Arch. Biochem. Biophys.*, **155**, 464–72.

Wagner, G. & Wüthrich, K. (1979). Truncated driven nuclear Overhauser effect (TOE). A new technique for studies of selective $^1H$-$^1H$ Overhauser effects in the presence of spin diffusion. *J. Magn. Reson.*, **33**, 675–80.

Westphal, O. & Jann, K. (1965). Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. *Meth. Carbohydr. Chem.*, **5**, 83–91.

Wilson, D. M. & Ajl, S. (1957). Metabolism. of L-rhamnose by *Escherichia coli*. I. L-Rhamnose isomerase. *J. Bacteriol.*, **73**, 410–14.