ing α-glycosides low coupling constants (1.5 – 3.0 Hz). The latter values are not very accurate, as, instead of a distinct doublet a single broadened peak was generally observed.

Also in agreement with earlier results, the equatorially oriented anomeric protons gave signals at lower fields than the axially oriented anomeric protons.

The chemical shifts are less predictable. For the substances studied, reduction of a disaccharide to the alditol produces an upfield shift for the (1→2) and (1→6) linked disaccharides but a downfield shift for the (1→4) linked disaccharides.

No anomeric pair of disaccharides containing D-mannopyranosidic linkages was available, and the methyl α- and β-D-mannopyranoside derivatives were therefore investigated. In agreement with previous results, no significant differences in chemical shifts or coupling constants between the isomers were observed.

Experimental. The disaccharides were either commercial samples or were available in this laboratory. Reduction of the disaccharides with sodium borohydride yielded the alditols. Trimethylsilylation was performed as described by Sweeley *et al.*[7] NMR spectra, in carbon tetrachloride, were recorded with a Varian A-60 A instrument, using TMS as internal standard.

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Structure of an Oligosaccharide Obtained on Degradation of the Lipopolysaccharide from *Salmonella typhimurium* LT2

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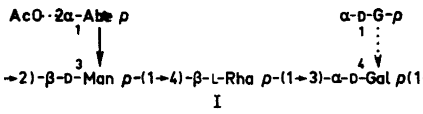
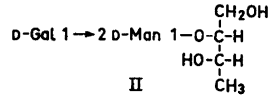
In the structure (I) proposed for the oligosaccharide repeating unit of the O-specific side-chains of the *Salmonella typhimurium* lipopolysaccharide (LPS),[1]



I

the anomeric configuration of the β-D-mannopyranose and α-D-galactopyranose residues was tentative and based upon earlier results.[2] Recently, however, Nikaido[3] has proposed, from enzymatic evidence, that the D-mannopyranose residue is α-linked. The anomeric natures of the sugar residues in the repeating units of LPS from different *Salmonella* serogroups have been determined by polarimetry and NMR studies on the oligosaccharides obtained after graded hydrolysis.[4–6] In the present paper, similar studies on an oligosaccharide obtained by Smith degradation of the *S. typhimurium* LT2 LPS are reported.

On periodate oxidation of the *S. typhimurium* LPS, the α-L-rhamnopyranose residues and the terminal D-glucopyranose residues but not other sugar residues in the O-specific side-chains should be oxidised. Subsequent borohydride reduction and mild acid hydrolysis (Smith degradation), which should also result in cleavage of the abequosidic linkage, would therefore yield the trisaccharide alditol II, containing the



II

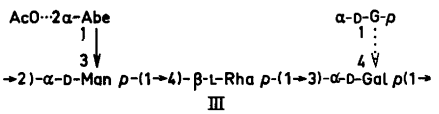
intact D-mannose and D-galactose residues and a 4-carbon fragment derived from the L-rhamnose residue.

This sequence of reactions was performed. Part of the reaction product was reduced with sodium borodeuteride, hydrolysed, reduced with sodium borohydride, acetylated and the mixture of alditol acetates investigated by GLC–MS. The presence of deuterium in the abequitol but not in the galactitol or D-mannitol established that whereas all the abequosidic linkages had been cleaved the galactosidic and the mannosidic linkages had remained intact.

The trisaccharide alditol (II) was isolated by chromatography on Sephadex G 15. Its optical rotation, $[\alpha]_{578}^{22}$ +111°, agrees well with that calculated for an entirely α-linked product, +115°, using Hudson's rules of isorotation, which give a value of $[\alpha]_{578}$ +50° for an α-D-galactopyranosidic and a β-D-mannopyranosidic linkage. Although these values are not very accurate, the results strongly support the assumption that both sugar residues are α-linked.

In the NMR spectrum of the trimethylsilylated trisaccharide alditol, a broad peak at $\tau = 5.05$ (2H) was present, indicating similar chemical shifts for the two anomeric protons. The absence of a signal with a large coupling constant (6 – 8 Hz) in this region, as expected for a β-D-galactopyranose residue, demonstrates that the D-galactose residue is α-linked.

The complete structure of the oligosaccharide repeating unit in the *S. typhimurium* LT2 may therefore be represented as follows (III).



III

Experimental. A solution of *S. typhimurium* LT2 LPS (100 mg) in 0.12 M sodium metaperiodate (20 ml) was kept in the dark at room temperature and the consumption of periodate was followed titrimetrically.[7] After 43 h, when the oxidation was complete, excess periodate was destroyed by addition of ethylene glycol. The solution was dialysed against distilled water, concentrated to 25 ml and sodium borohydride (500 mg) was added. After 6 h at room temperature excess of borohydride was destroyed with acetic acid and the solution dialysed as above and concentrated to dryness. The residue was dissolved in 0.05 M sulphuric acid (8 ml) and the solution was kept at 80°. The hydrolysis was followed in the polarimeter and after 4 h, when the decrease in optical rotation had ceased, the solution was cooled and neutralised with barium carbonate. The filtered and concentrated solution was fractionated on a Sephadex G-15, superfine, column (100 × 2.5 cm) which was irrigated with water (6.5 ml/h). The fractionation was followed polarimetrically and the major component was eluted in the trisaccharide region. A sugar analysis on the hydrolysate, using L-arabinose as internal standard, revealed that 2.4 mg of trisaccharide alditol had been obtained. The trisaccharide alditol was trimethylsilylated as described by Sweeley *et al.*[8] The NMR spectra were recorded at 60 MHz on a Varian A-60 A spectrometer fitted with a Varian C-1024 time-averaging computer device.

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