CHARACTERIZATION OF 3-DEOXY-D-MANNO-OCTULOSONIC ACID AS A COMPONENT OF THE CAPSULAR POLYSACCHARIDE ANTIGEN FROM NEISSERIA MENINGITIDIS SEROGROUP 29-e*

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SUMMARY. 3-Deoxy-D-manno-octulosonic acid (KDO) has been characterised as the major component (53%) of the capsular polysaccharide antigen of N. meningitidis serogroup 29-e. This is the first reported occurrence of KDO in any biological polymer other than its well established occurrence in the lipopolysaccharides of gram-negative bacteria.

INTRODUCTION

Since it was first identified as a product of enzymatic synthesis (1), 3-deoxy-octulosonic acid has been shown to be a component of the cell wall lipopolysaccharides of E. coli (2) and other species of gram-negative bacteria (3-5) including N. meningitidis (6). The characterisation of this component as 3-deoxy-D-manno-octulosonic acid (KDO) has also been reported (7, 8). KDO is not a common constituent of biological polymers, and

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to date reports of its occurrence in these polymers have been exclusively restricted to the lipopolysaccharides of gram-negative bacteria, where it functions as a linkage point between the lipid and polysaccharide moieties (3, 9). We now report the first occurrence of KDO in a biological polymer other than a lipopolysaccharide. The capsular polysaccharide antigen of N. meningitidis serogroup 29-e is shown to contain equimolar proportions of KDO and 2-acetamido-2-deoxy-galactosamine as its sole constituents.

MATERIALS AND METHODS

N. meningitidis serogroup 29-e (strain 550, Laboratory Centre for Disease Control) was grown on a chemically defined medium and the group specific polysaccharide was isolated and purified by identical procedures described previously for the serogroup X polysaccharide (10, 11). The polysaccharide was de-O-acetylated by its incubation at 37° for 4 h in 0.1M NaOH solution.

Paper chromatographic analysis was performed by the descending method on Whatman No. 1 and 3MM papers using the following solvent systems (v/v): (A) butan-1-ol - pyridine - 0.1N HCl (5:3:2), (B) propan-2-ol - acetic acid - water (54:8:18), and compounds were detected by alkaline silver nitrate (12) and periodate-thiobarbituric acid (13) spray reagents. Their rates of movement are quoted relative to that of D-galactose (R_{Gal}).

Gas-liquid chromatography (g.l.c.), at 190°, was performed on an F and M model 402 chromatograph using a hydrogen flame detector. The glass column was packed with (i) 3% SE-30 on gas chrom Q, 80-100 mesh, and retention volumes are quoted relative to that of penta-O-acetyl-L-arabinitol (T_A).

Melting points were recorded on a Fisher-Johns apparatus and are uncorrected, and optical rotations were determined at 21 ± 3° on a Perkin Elmer 141 polarimeter.

RESULTS AND DISCUSSION

The capsular polysaccharide from N. meningitidis serogroup 29-e was homogeneous by gel filtration (Sephadex G-200) and was shown to contain O-acetyl substituents by ^{13}C n.m.r. spectroscopy (14). Following its de-O-acetylation the polysaccharide was hydrolysed (0.5 M HCl at 80° for 2 h), and the presence of a 2-keto-3-deoxy-acid was confirmed by the method of Weissbach and Hurwitz (15). Paper chromatographic analysis of the hydrolysate indicated the presence of two major components at R_{Gal} 0.3 and 1.3 in solvent A, and R_{Gal} 1.0 and 1.3 in solvent B. In both solvents the slower component had an identical mobility to that of authentic KDO and the faster component to that of authentic 2-acetamido-2-deoxy-galactose. Quantitative estimation of KDO (15) in the de-O-acetylated polysaccharide (after hydrolysis with 0.02N H_2SO_4 at 100° for 15 min) indicated that the polysaccharide contained 53% by weight of KDO.

Periodate oxidation of the de-O-acetylated polysaccharide resulted in the consumption of 1 mole of sodium metaperiodate per mole of KDO with no release of formaldehyde. Smith degradation (16) of the polysaccharide followed by paper chromatography in solvent B showed the presence of unoxidized 2-acetamido-2-deoxy-galactosamine and the presence of another faster moving component. No KDO could be detected. The faster moving component had an identical mobility to that of erythritol in solvents A and B and was isolated by preparative paper chromatography in solvent B. Crystallization from water-ethanol gave crystals of D-erythritol of m.p. and

mixed m.p. 118-120° and $[\alpha]_D$ +7.5° (c, 1.6 in water). This evidence indicates that the KDO is in the D form and is linked at C-7 or C-8 in the polysaccharide.

The hydrolysate of a large-scale hydrolysis of the de-O-acetylated polysaccharide (100 mg) was passed through a column of Rexyn 201 (carbonate form), and the column was washed with water to remove the neutral component. The acidic component was then eluted with 0.5M NH_4HCO_3 solution and the inorganic salts were removed by repeated lyophylisation (X5) from water. Crystallization from water-ethanol gave crystals of ammonium 3-deoxy-D-manno-octulosonate (14 mg) of melting point 120-122° undepressed on admixture with an authentic sample.

The manno-configuration of the KDO obtained from the polysaccharide was also confirmed by g.l.c. analysis and paper chromatographic analysis (17). The ammonium salt of the isolated octulosonic acid after trimethylsilylation (18) and g.l.c. analysis using column i gave three peaks at T_A 3.1, 3.7 and 4.1 identical to those obtained from authentic trimethylsilylated ammonium 3-deoxy-D-manno-octulosonate. In contrast, using identical conditions, authentic trimethylsilylated ammonium 3-deoxy-D-gluco-octulosonate gave four peaks at T_A 1.7, 2.9, 3.4, and 4.4. In addition the isolated ammonium salt of the octulosonic acid and authentic ammonium 3-deoxy-D-manno-octulosonate gave the same two identical components, as shown by paper chromatographic analysis in solvent A (R_{Gal} 1.4 and 1.6), when reduced with sodium borohydride (17).

Thus all the evidence above supports the positive identification of 3-deoxy-D-manno-octulosonic acid (KDO) as a component of the capsular polysaccharide antigen of N. meningitidis serogroup 29-e. A detailed structural

investigation of this polysaccharide is now in progress and will be reported at a later date.

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