

Note

Identification of D-galacturonic acid in the specific capsular polysaccharide of pneumococcal type XXV*

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It has long been known that the capsular polysaccharide of pneumococcal type XXV (S XXV) contains an otherwise unidentified uronic acid and amino sugar¹ S XXV, the principal type-specific antigen of pneumococcal type XXV, has become of interest because of the cross-reactivity of polysaccharides of widely different origin in antipneumococcal type XXV antiserum^{2,3} More details of its constitution were therefore sought

Hydrolysis of S XXV (8.8 mg, preparation 3, not listed in ref. 1) with M H₂SO₄ (5 ml) for 20 h at 100° was accompanied by considerable charring. Removal of H₂SO₄ with BaCO₃ and chromatography in both 5:5:1:3 (v/v) ethyl acetate–pyridine–acetic acid–water (Solvent A) and 18:3:1:4 (v/v) ethyl acetate–acetic acid–formic acid–water (Solvent B) gave spots corresponding to galactose, galacturonic acid, galactosamine, and glucosamine. The hydrolyzate was separated on Whatman No. 3MM paper by means of solvent A and the portion containing the uronic acid was eluted with water, de-ionized with Amberlite IR-120 (H⁺) ion-exchange resin, and evaporated to dryness *in vacuo*. The residue was converted into the methyl ester methyl glycoside by heating under reflux with 1% methanolic HCl for 8 h. The solution was evaporated to dryness *in vacuo*, and again after 5 additions of dry methanol (2 ml) to remove HCl. Reduction of the residue with potassium borohydride⁴ and hydrolysis with 0.5M sulfuric acid gave the corresponding hexose, chromatographically identified, in solvent A, as galactose⁵. A portion of this galactose was treated with galactostat (Worthington Biochemical Corp., Freehold, N.J.). It

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§A somewhat faster spot on the chromatogram, possibly due to residual methyl galactoside, disappeared on prolonged hydrolysis.

gave rise to a yellow color, as did D-galactose, and with the same absorption maximum at 425 nm. This identifies much of the galacturonic acid in S XXV as the D isomer.

Since the sample of S XXV used contained C-polysaccharide (which does not contain a uronic acid)⁵, no attempt was made to characterize the other sugars more definitely.

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