Methylation Analysis of Pustulan

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The glucan pustulan was first isolated by Drake [1] from the lichen *Umbilicaria pustulata*. Its low optical rotation indicated that it was a β-glucan and periodate oxidation studies suggested that it was essentially (1→6)-linked. These findings were confirmed by Lindberg and McPherson,[2] who subjected it to partial acid hydrolysis and isolated a series of oligosaccharides, built up of (1→6)-linked β-D-glucopyranose residues. They were, however, unable to detect any other oligosaccharides. (1→6)-Linkages are the most resistant to acid hydrolysis and a low percentage of other linkages may therefore have been overlooked in these studies. As pustulan is the most readily accessible of the rather unusual (1→6)-β-glucans and has been used as a substrate in studies on β-glucanases, a more detailed investigation of its structure was desirable. In the present paper, a methylation analysis of pustulan is reported.

Pustulan was methylated in methylsulphoxide by treatment first with methylsulphinyl sodium and then with methyl iodide, following the procedure of Sandford and Conrad.[3] The fully methylated polysaccharide was hydrolysed, the methylated sugars converted into their alditol acetates and the mixture analysed by GLC [4]—MS.[5] One major peak was observed on GLC and the retention time was the same as that for the alditol acetate from 2,3,4-tri-*O*-methyl-D-glucose. MS of the component corresponding to this peak was indistinguishable from that given by an authentic sample of the alditol acetate [5] prepared from this sugar. This both proved its identity and established that it did not contain other components. Some minor peaks were also observed on the chromatogram. Two of these were tentatively identified from their retention times as the alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose (1 %) and 2,4,6-tri-*O*-methyl-D-glucose (0.4 %), respectively. Others, detectable only when the chromatograph was run at high sensitivity, amounted to less than 0.5 % of the main component and were not identified. A hydrolysate of pustulan contained a small amount of galactose (<0.5 %) and some peaks may consequently be derived from contaminating polysaccharide material. The results of the methylation analysis thus confirm that pustulan is essentially a (1→6)-linked β-glucan. If the polysaccharide contains other linkages, the proportion of these must be less than 1 %.

Experimental. The pustulan was prepared as previously described.[2] A hydrolysate contained glucose and less than 0.5 % of galactose. Pustulan (8 mg), in a 5 ml serum bottle sealed with a rubber cap, was dissolved in dry methylsulphoxide (1 ml). Nitrogen was flushed through the bottle and a solution of 2 M methylsulphinyl sodium in methylsulphoxide (0.8 ml) was added dropwise, using a syringe. The resulting, gelatinous solution was agitated in an ultrasonic bath (40 kc/sec) for 30 min, and kept for 8 h at room temperature. Methyl iodide (0.8 ml) was then added dropwise, under external cooling with ice-water, and the resulting, turbid solution was agitated for 30 min in the ultrasonic bath, whereby a clear solution was obtained. This solution was poured into water (50 ml), dialysed, and concentrated to dryness. A solution of the methylated polymer in 90 % formic acid (3 ml) was kept for 2 h at 100°, concentrated to dryness and the residue was dissolved in 0.13 M sulphuric acid (3 ml) and kept for 12 h at 100°. The sugars were converted into alditol acetates and analysed by GLC [4] on an ECNSS column. The MS of the main peak (T=2.50, relative to 1.5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol) was determined in an LKB 9000 combined gas chromatograph-mass spectrometer. The T-values of the minor peaks were 1.00, 1.96, 5.27, 7.25, 9.69, and 11.06. The corresponding values for the alditol acetates derived from 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose were 1.00 and 1.95, respectively.

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1. Drake, B. *Biochem. Z.* **313** (1943) 388.
2. Lindberg, B. and McPherson, J. *Acta Chem. Scand.* **8** (1954) 985.
3. Sandford, P. A. and Conrad, H. E. *Biochemistry* **5** (1966) 1508.

4. Björndal, H., Lindberg, B. and Svensson, S. *Acta Chem. Scand.* **21** (1967) 1801.
5. Björndal, H., Lindberg, B. and Svensson, S. *Carbohyd. Res.* **5** (1967) 433.

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Synthesis of Phenyl α-Tyvelopyranoside

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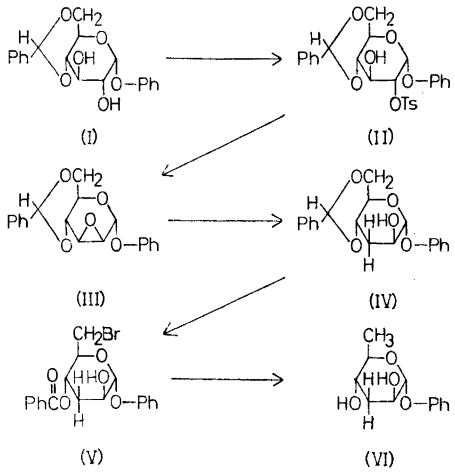
Phenyl α-tyvelopyranoside (phenyl 3,6-dideoxy-α-D-*arabino*-hexopyranoside, VI) was needed in connection with other studies and the present paper reports the synthesis of this substance. In a recent paper Stirm *et al.*[1] describe the synthesis of *p*-nitrophenyl α-tyvelopyranoside from tyvelose triacetate, *p*-nitrophenol, and mercuric chloride. The resulting mixture of α- and β-anomers was separated by chromatography but the yield of the α-glycoside was low and another route was therefore attempted.

Phenyl α-D-glucopyranoside was transformed into the 4,6-*O*-benzylidene derivative (I) and reacted with one equivalent of tosyl chloride in pyridine. A good yield of a monotosylate was obtained and shown by methylation analysis to be the 2-*O*-tosylate (II). On treatment with sodium methoxide in methanol it yielded phenyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannoside (III), which was reduced with lithium aluminium hydride into phenyl 4,6-benzylidene-3-deoxy-α-D-*arabino*-hexoside (IV). This intermediate, which gave a single spot on TLC, was obviously not pure as it showed a broad m.p. interval and gave a poor elemental analysis. Attempted purification by crystallisation was not successful. It was reacted with *N*-bromo-succinimide in carbon tetrachloride to yield amorphous phenyl 4-*O*-benzoyl-6-bromo-3,6-dideoxy-α-D-*arabino*-hexoside (V), which was purified by chromatography. The latter reaction generally gave good yields of V, but in some experiments the oxidation was slow and a complex mixture of products, containing only a low percentage of V, was obtained. The reason for this is not known, but as IV was not pure, it may be due to a contaminant, present in some samples but not in others. V was finally reduced with lithium aluminium hydride yielding phenyl α-tyvelopyranoside (VI). The overall yield of VI from phenyl α-D-glucopyranoside was 34 %.

The structure of VI is evident from its mode of synthesis and was further corroborated by converting it into tyvelitol tetraacetate; the product was indistinguishable by GLC and mass spectrometry from an authentic sample.



Experimental. Melting points are corrected. Concentrations were performed under reduced pressure, at a bath temperature not exceeding 40°. TLC was performed on Silica Gel (E. Merck AG, Darmstadt) using as irrigant ethyl ether-toluene (2:1, v/v) (A) or ethyl acetate (B). Compounds were detected with 8% sulphuric acid at 100°. GLC was carried out on a column (200 × 0.3 cm), containing 3 % (w/w) of ECNSS-M on Chromosorb G (80–100 mesh), at 200°. A Perkin-Elmer 881 Gas Chromatograph with flame ionisation detector was used.

For mass spectrometry the alditol acetates, dissolved in chloroform, were injected into an ECNSS-M column, mounted in an LKB 9000 combined gas chromatograph-mass spectrometer. The mass spectra were recorded at an inlet temperature of 250°, ionisation potential of 70 eV, ionisation current of 60 μA and a temperature of the ion source of 290°.

Phenyl 4,6-O-benzylidene-α-D-glucoside (I) was prepared from phenyl α-D-glucopyranoside,