ACTA CHEMICA SCANDINAVICA 8 (1954) 985—988

Studies on the Chemistry of Lichens

VI *. The Structure of Pustulan

BENGT LINDBERG and JOHN McPHERSON

Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden

The occurrence of a galactofuranoside, umbilicin (Part V) in the lichen *Umbilicaria pustulata* suggested the presence of polysaccharides with galactofuranoside units. Total hydrolysis of the methanol-extracted lichen revealed the presence of galactose, glucose and mannose. When the hydrolysis was performed with 0.01 *N* hydrochloric acid overnight, only galactose was liberated, although in small amounts indicating that part of the galactose in the polysaccharides is furanosidic. Attempts to isolate a polysaccharide containing these units, however, were not successful, but during these experiments another polysaccharide, pustulan, was isolated in quantity. This polysaccharide was isolated from the same source by Drake [1], who proved that it was a glucan and assumed that the units were connected with β-1,6-linkages. (Drake called the polysaccharide pustulin, but following the suggestion of Whistler and Smart [2], the name pustulan is adopted.) Pustulan, therefore, should be related to the glucan lutean (original name, luteose), the malonic ester of which is produced by a *Penicillum* mould [3].

In order to study the structure of pustulan a sample was partially hydrolysed and fractionated on a charcoal column, using the gradient elution technique. Glucose, a di-, a tri- and a tetrasaccharide were isolated in this manner. The disaccharide was identified as gentiobiose. The β-acetates of the tri- and tetrasaccharide had the same values for m.p. and rotation as those of 6-β-gentiobiosido-glucose [4] and 6-β-gentiobiosido-gentiobiose [4]. For these saccharides we suggest the names gentiotriose and gentiotetraose. Not even traces of other di-, tri- or tetra-saccharides were observed, indicating that the pustulan molecule is linear, containing exclusively β-1,6-linkages.

The sugars were obtained as amorphous powders, chromatographically pure but containing small amounts of water. The purity of the samples and the concentration of solutions were determined by hypoiodite titrations. The data for the sugars and their acetates are given in Table 1.

* Part V, *Acta Chem. Scand.* 8 (1954) 821.

Table 1. Properties of the sugars obtained by hydrolysis of pustulan (19.7 g).

Sugar	Yield, g	$[\alpha]_D$ water ($c=2$)	β-acetate, m.p. Observed (uncorr.)	Lit.	β-acetate $[\alpha]_D$ chloroform ($c=2$) Observed	Lit.
Glucose	4.5	52.7				
Gentiobiose	3.25	9.5	191—192	196	— 5.3	—5.4
Gentiotriose	2.08	—6.5	211—211.5	219—220	—7.0	—8.0
Gentiotetraose	1.0	—14.5	134—135	135, 207—209	—10.9	—11.1

When, for the sugars or their acetates $[M]_D/n$ is plotted against $n—1/n$, where $[M]_D$ is the molecular rotation and n the number of glucose residues per molecule, a straight line is obtained (Fig. 1) in agreement with the Freudenberg-Blomquist relationship [5]. Glucose and β-glucose pentaacetate fall on the curve, although this has no significance. The other extreme, pustulan itself, is close to the curve. The observed specific rotation for pustulan is —46°, and from the values for the dextrins a value of —43° can be calculated. For the pustulan acetate the calculated rotation —17.4°, does not correspond at all with that observed, + 9.1°. The yield of chloroform soluble acetate by triple acetylation of pustulan with acetic anhydride in pyridine, after swelling in formamide, was only about 25 %, and the obvious conclusion is not that the Freudenberg-Blomquist relationship does not hold, but that the pustulan acetate does not go into true solution but is dissolved as large aggregates, the rotation of which should not be compared with that of the low-molecular acetates.

The R_F-values of glucose, gentiobiose, -triose and -tetraose in the solvent ethyl acetate-acetic acid-water, 12 : 5 : 6, were 0.32, 0.167, 0.091 and 0.047

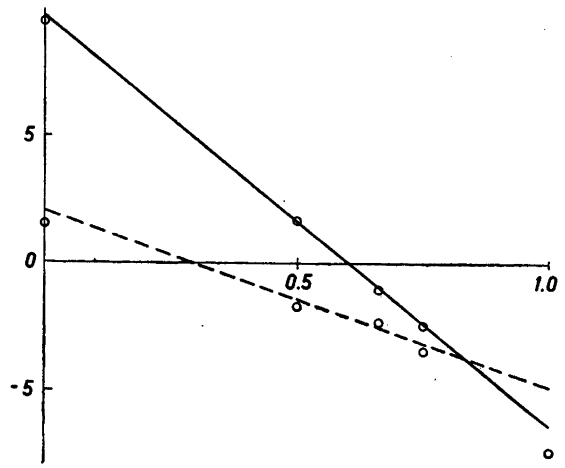

Fig. 1. Application of the Freudenberg-Blomquist relationship to the oligosaccharides (————) and their acetates (— — — — —). Ordinate: $10^{-3}[\alpha]_D M/n$. Abscissa: $n-1/n$.

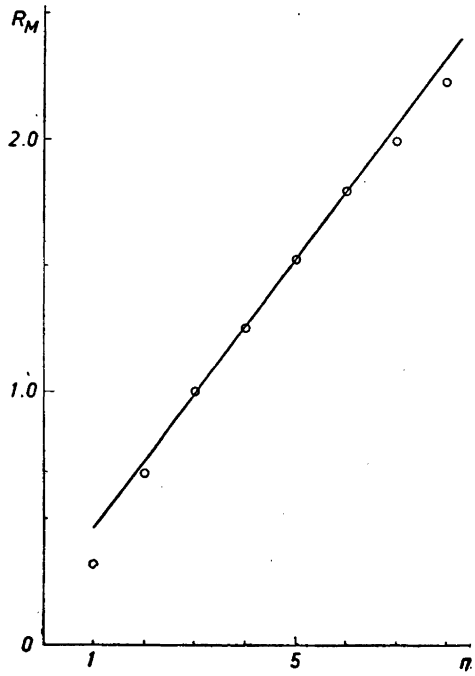

Fig. 2. Relation between R_M-values and chain length.

respectively. The fractions, which should contain the pentaose, hexaose, heptaose and octaose were not characterised, but their R_F-values were determined to be 0.029, 0.016, 0.011 and 0.006. When the R_M-values [6,7] are plotted against n, the number of glucose units in the oligosaccharide, a straight line is obtained (Fig. 2), strongly indicating that all the oligosaccharides belong to the same series.

These results indicate that pustulan is a linear glucan with β-1,6-linkages between the units, in agreement with Drake's original suggestion[1].

EXPERIMENTAL

Extraction of pustulan. The lichen *Umbilicaria pustulata* (200 g), after continuous extraction with ether for three days and with methanol for fourteen days, was given a pre-extraction with sodium carbonate solution (2 %, 2 × 2 l) for fourteen days, filtered and washed with cold water. The lichen was then extracted four times with boiling water (2 l) for a period of four hours. The hot solutions were filtered through linen and frozen solid overnight. Next morning the product was allowed to thaw. The crude polysaccharide was collected by filtration, drained well and the process of dissolution in hot water, freezing and thawing repeated a further five times. Before the last freezing small amounts of undissolved material were removed by centrifugation. The pustulan finally was washed with ethanol and ether and dried in a desicator over sulphuric acid to yield light buff flakes (14 g). Ash content: 0.52 %. Moisture 11.6 %. (Determined by keeping a sample at 110° overnight). The presence of glucose only in the hydrolysate of the purified pustulan was shown by paper chromatography.

Partial hydrolysis. After some preliminary experiments a larger amount of pustulan (19.7 g) was refluxed with *N* sulphuric acid (1 l) for 135 minutes, cooled and neutralised with barium carbonate. The mixture was filtered and concentrated under reduced pressure to yield a clear syrup. In order to remove higher dextrins, the syrup was dissolved in 10 % ethanol (200 ml) and filtered through a short column of carbon-Celite, which was then washed with 50 % ethanol (2 l) and the solutions concentrated. The syrup obtained, dissolved in 1 % ethanol (200 ml), was added to the top of a carbon-Celite column (50 × 6.5 cm) and subjected to gradient elution with 1 % to 40 % ethanol (8 l). The eluate was divided into fractions, which were investigated by paper chromatography. (Solvents: ethyl acetate-acetic acid-water, 3:1:1, and for the higher members 12:5:6). Similar fractions were combined and concentrated under reduced pressure. The separation was satisfactory, with empty fractions between all the sugars.

Each oligosaccharide was dried thoroughly and the chromatographic purity established. The percentage of oligosaccharide in the amorphous powder was determined to be 95, 93, and 94 for the biose, triose and tetraose by hypoiodite oxidation [8]. In addition to the sugars listed in Table 1, the pentaose to the octaose were isolated in yields of 0.78, 0.48, 0.33, and 0.25 g. They were investigated only by paper chromatography and found to be fairly pure.

Acetylation of oligosaccharides. Each oligosaccharide was acetylated with acetic anhydride in the presence of anhydrous sodium acetate by heating in a glycerol bath at 130° until the solid went into solution. The mixture crystallised from ice water and was recrystallised from ethanol to constant m.p.

Acetylation of pustulan. Pustulan (2 g) was given two treatments with sodium chlorite-acetic acid according to the method of Wise *et al.*[9] (Yield 1.6 g) $[\alpha]_D = -46°$ (water $c = 0.5$ or NaOH, $c = 0.5$). The bleached pustulan (1.1 g) was then acetylated three times following the method of Carson and Maclay [10]. The grey amorphous residue (1.6 g) was dried throughly and extracted with chloroform in a continuous extractor for 2 hours. The chloroform was evaporated under reduced pressure, affording a strong, clear film (0.41 g). The value for the specific rotation of this material, +9.1° (chloroform, $c = 0.3$), agreed well with that obtained by Drake [1], +9°. Acetyl content, 43.4 %, required for the completely acetylated polysaccharide, 44.78 %.

SUMMARY

The structure of pustulan as a linear glucan with β-1,6-linkages has been established by the method of partial hydrolysis and isolation and characterisation of oligosaccharides formed.

REFERENCES

1. Drake, B. *Biochem. Z.* **313** (1943) 388.
2. Whistler, R. L. and Smart, C. L. *Polysaccharide Chemistry*, New York 1953, p. 17.
3. Raistrick, H. and Rintoul, M. L. *Trans. Roy. Soc. (London)* **B 220** (1931) 255.
4. Helferich, B. and Gootz, R. *Ber.* **64** (1931) 109.
5. Freudenberg, K. and Blomqvist, G. *Ber.* **68** (1935) 2070.
6. Bate-Smith, E. C. and Westall, R. G. *Biochim. et Biophys. Acta* **4** (1950) 428.
7. French, D. and Wild, G. M. *J. Am. Chem. Soc.* **75** (1953) 2612.
8. Jeanloz, R. *Helv. Chim. Acta* **29** (1946) 57.
9. Wise, E. L., Murphy, M. and D'Addieco, A. A. *Paper Trade J.* **122** (1946) 35.
10. Carson, J. F. and Maclay, W. D. *J. Am. Chem. Soc.* **68** (1946) 1015.

Received March 22, 1954.