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STRUCTURE OF A POLYSACCHARIDE OF *UMBILLICARIA MAMMULATA**

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Abstract—The polysaccharide isolated from *Umbillicaria mammulata* is a $\beta(1\rightarrow6)$ linked glucan (degree of polymerization: *ca* 150) with 9% of the glucose units acetylated at C-3. It is very similar to a polysaccharide recently isolated from the related lichen *Gyrophora esculenta*.

INTRODUCTION

LICHENS are, like other algae, rich sources of various polysaccharides. The starch-like substances in lichens have been extensively studied during the past centry. Among them, lichenin [a $\beta(1\rightarrow3$ and $1\rightarrow4)$ glucan], isolichenin [a $\alpha(1\rightarrow3$ and $1\rightarrow4)$ glucan] and pustulan [a $\beta(1\rightarrow6)$ glucan] are the best known examples.[1]

Shibata and his co-workers have recently isolated an acetylated glucan from *Gyrophora esculenta* and some other related species.[2] The glucan has been shown to be a $\beta(1\rightarrow6)$ homologous polymer of about 120 units similar to pustulan but acetylated in the C-3 hydroxyl groups in about 10% of the glucose units. The Japanese group further demonstrated that these glucans possess marked antitumor activity specifically against subcutaneously implanted ascites sarcoma-180 in mice.[2,3]

The purpose of this research was to examine a glucan in the locally abundant lichen, *Umbillicaria mammulata*, which is closely related to *G. esculenta*.

RESULTS AND DISCUSSION

Hot water extraction of the thalli of *U. mammulata* afforded a crude polysaccharide in a 21·8% yield. The crude polysaccharide was further purified by reprecipitation to a material which showed one main peak on Sephadex gel filtration. The purified polymer has a negative rotation, $[\alpha]_D$ −41·4°, which is suggestive of the presence of the β-linkage in the molecule. It is devoid of nitrogen, and the analysis of a thoroughly dried sample gave a value close to that for anhydroglucose. The IR spectrum showed absorptions at 1730 and 1252 cm^{-1} for an ester function in addition to the typical carbohydrate spectrum pattern.

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[1] CHANDA, N. B., HIRST, E. L. and MANNERS, D. J. (1957) *J. Chem. Soc.*, 1951.

[2] (a) SHIBATA, S., NISHIKAWA, Y., TAKEDA, T., TANAKA, M., FUKUOKA, F. and NAKANISHI, M. (1968) *Chem. Pharm. Bull.* **16**, 1639; (b) SHIBATA, S., NISHIKAWA, Y., TAKEDA, T. and TANAKA, M. (1968) *ibid.* **16**, 2362; (c) NISHIKAWA, Y., TAKEDA, T., SHIBATA, S. and FUKUOKA, F. (1969) *ibid.* **17**, 1910.

[3] (a) FUKUOKA, F., NAKANISHI, M., SHIBATA, S., NISHIKAWA, Y., TAKEDA, T. and TANAKA, M. (1968) *Gann* **59**, 421; (b) SHIBATA, S., NISHIKAWA, Y., TANAKA, M., FUKUOKA, F. and NAKANISHI, M. (1968) *Z. Krebsforsch.* **71**, 102.

Molecular weight estimation on Sephadex G-75 and G-100 gave an apparent value of 20000–40000, using Dextran T_{10}, T_{20} and T_{40} as standards.

The only sugar component of the polymer was glucose. The ester group was determined as an acetate by the PMR spectrum which showed a singlet at 2·10 due to a methyl signal of an acetate moiety. It was further confirmed by a paper chromatographic detection of acetic acid obtained after hydrolysis. The acetate content was evaluated by two methods. Calculation based upon the ratio of the acetyl signal to the sugar proton signals in the PMR spectrum resulted in a value of 0·07 mol of acetate/glucose unit. The second value, 0·092 mol/glucose units, was obtained by an alkaline hydrolysis-titration method. The discrepancy between the two values was probably due to difficulty in the PMR integration or titration of a small amount of acetate. Alkaline treatment afforded a polysaccharide, which showed no carbonyl absorption at 1730 cm^{-1}.

TABLE 1. PERIODATE CONSUMPTION OF LICHEN POLYSACCHARIDE BEFORE (**1**) AND AFTER DEACETYLATION (**2**)

Time (hr)	Moles periodate consumed* (1)	(2)	Moles formic acid formed* (1)	(2)
2	1·17	1·18	0·56	0·71
15	1·43	1·66	—	—
18	1·46	1·71	0·65	0·85
24	1·61	1·74	0·83	0·90
112	1·64	1·97		

* Data presented as moles per anhydroglucose unit.

Treatment with β-glucosidase and α-amylase gave no detectable amount of mono- or oligosaccharide, while hemicellulase gave glucose and maltose after long incubation. Periodate consumption of the original polysaccharide and the deacylated polymer was measured in triplicate (Table 1). The fact that *ca* 2 mol of 0·05 N periodate are consumed supports the 1,6-linkage. A slight difference in the periodate consumed by the acetylated and deacetylated polymers indicates that the acetyl moiety is partially blocking the vicinal glycol structure. The final proof for the 1,6-linkage came from exhaustive methylation and hydrolysis. Upon methylation by Hakomori's method followed by acid hydrolysis, the original polysaccharide (**1**) afforded 2,3,4-tri-*O*-methylglucose.

In order to determine the site of the acetate attachment, the method originally devised by Bouveng[4] and applied for the glucan from *G. esculenta* by Nishikawa *et al.*[3c] was followed. The polymer was treated with phenylisocyanate until there were no free hydroxyl groups. The resulting phenylcarbamate was methylated to replace the acetyls by methyl groups. The methylated product was then reduced with lithium aluminum hydride, and hydrolyzed. Examination of the hydrolysate by paper and gas chromatography revealed the presence of glucose and a lesser amount of 3-*O*-methylglucose. The result is consistent with a structure with 3-*O*-acetyl groups, which is the same as that of the polysaccharide isolated from *G. esculenta*.[2c]

Direct comparison with the polysaccharide from *Lasalia papulosa*, which had been considered identical with that from *G. esculenta*,[2a,2b] was done. The partial hydrolysis products and IR spectra of both polysaccharides were identical.

[4] BOUVENG, H. O. (1961) *Acta Chem. Scand.* **15**, 96.

EXPERIMENTAL

General. Optical rotation measurements were carried out with a Rudolph polarimeter using a 1 dm tube in the specified medium. IR spectra were recorded on KBr micropellets. PMR spectra were recorded using dimethyl-2-silapentane-5-sulfonate as the internal standard. Gas chromatography was done using a Varian 1200 equipped with a flame ionization detector.

Plant material. The lichen, *Umbillicaria mammulata* (Ach.) Llano, was collected in Aug. and Sept., 1971, at two locations, Arcadia Management Area, Rhode Island, and Purgatory Chasm, Uxbridge, Massachusetts. *U.* (*Lasalia*) *papulosa* was also collected at the same time. The identity of the specimen were confirmed by Dr. Jerome Jacobs of Clark University.

Isolation of the polysaccharide (**1**). Two hundred grams of ground *U. mammulata* was extracted with 800 ml of distilled water on a steam bath for 3 hr. The water was poured off through cheese cloth, and the marc was resuspended in 800 ml of water and heated for an additional 6 hr. The combined filtrate was centrifuged and the supernatant was added to 500 ml ethanol. The light brown precipitate formed was collected by filtration and dried to brown flakes (yield: 43·5 g). This material was dissolved in 300 ml of distilled water by heating. The insoluble portion was filtered off and then the filtrate was frozen. After slow thawing of the frozen material, separated ribbons of the polysaccharide were collected by centrifugation, washed with water twice, and then suspended in acetone and filtered (yield: 13·2 g). For elemental and spectroscopic analyses, the sample was passed through a Sephadex G-75 column and freeze dried. $[\alpha]_D$ −41·4° (1 N NaOH, C 0·65). Found: C, 41·74; H, 6·54%. IR: ν_{max} cm^{-1} 3400–3600 (OH), 1730, 1252 (acetate). NMR: δ (D_2O) ppm 2·10 (*s*, COMe), *ca* 3·5 and 3·7 (*m*, HC–OH).

Molecular weight estimation using sephadex G-100 and G-75. A mixture of 104 mg Dextran T_{10} (MW 9300)–105 mg Dextran T_{20} ($\bar{M}W$ 41·800) was dissolved in 1 ml of water and added to the column (22 × 400 mm). The 3·8 ml fractions were taken at a flow rate of 0·8 ml/min, and the polysaccharide content was determined by the anthrone–sulfuric acid color reaction. A solution of the polysaccharide (**1**), 30·1 mg in 1 ml of water was passed through the column under the same conditions. The average estimated molecular weight, $\bar{M}W$ 35000, was obtained. A similar experiment was done using a column of G-75 (18 × 410 mm). The results indicate a $\bar{M}W$ of 27000. Complete acid hydrolysis was carried out on 108·8 mg by refluxing in 1 N H_2SO_4 (10 ml) for 15 hr. After cooling, the solution was neturalized with barium hydroxide. The precipitated barium sulfate was filtered off and the filtrate was then lyophilized to dryness. The residue showed an $[\alpha]_D$ +53·4 (H_2O). A 2·2 mg portion of the acid hydrolysate was treated with a mixture of dry pyridine (0·5 ml) and Tri-Sil Z and analyzed by GLC (183 × 0·32 cm column 3% SE 52 on Chromosorb, oven temperature: 180°, 30 ml/min N_2 flow). Two peaks with retention times of 10·6 and 14·2 min were observed. Glucose gave the same retention times corresponding to the α and β anomers. Paper chromatography of the hydrolysate in two systems gave spots identical to those of glucose. An osazone of the hydrolysate, m.p. 201–201·5° was also identical to glucoosazone.

Partial hydrolysis. A solution of the polysaccharide (49·7 mg) in 1 N H_2SO_4 was heated on a steam bath for 1·6 hr. The solution was neturalized with a saturated solution of barium hydroxide, filtered and lyophilized. A portion (40·8 mg) of the acetylated polysaccharide of *Lasalia papulosa*, $[\alpha]_D$ −40·0° (C = 0·4, 2 N NaOH) extracted according to the method outlined by Shibata *et al.*[2a,2b] was also partially hydrolyzed and worked up in the same manner as above. Paper chromatography of the partial hydrolysates of the unidentified polysaccharide and *L. papulosa* polysaccharide was run in a *n*-BuOH–pyridine–water (6:4:3). Both samples showed identical chromatograms with spots at R_f 0·42 (glucose), 0·21, 0·09 and 0·04.

Enzymatic hydrolysis. To a solution of hemicellulase (12·5 mg) in 10 ml acetate buffer at pH 4·6 was added a 5 ml solution of the lichen polysaccharide 2 mg/ml. The mixture was kept at room temperature for 1 week. The contents were then lyophilized and analyzed by paper chromatography using EtOAc–HOAc–H_2O (3:1:3). The spots revealed by anisidine-hydrochloride spray had R_f's of 0·24 and 0·14. Those of glucose and maltose are respectively 0·22 and 0·13. Incubation with emulsin, and of the deacetylated polymer (see below) with α-amylase under the same condition did not give any hydrolysis products.

Deacetylpolysaccharide. A suspension of 36·1 mg of the polysaccharide in 5 ml of 0·2% NaOH was stirred for 30 min. The mixture was centrifuged and successively washed with water, ethanol and ether. The material was dried over $CaSO_4$ *in vacuo*. The IR spectrum showed no carbonyl absorption.

Methylation and hydrolysis. Methylation was carried out in the usual manner with NaH, dimethyl sulfoxide and MeI. The methylated polymer (30·2 mg) was dissolved in 10 ml of 72% H_2SO_4 at room temperature. Then water (80 ml) was added to bring the acid concentration to 6%. The solution was kept at room temperature for 24 hr and on a steam bath for 5 hr. The solution was neutralized with $BaCO_3$ and filtered through Celite. The filtrate was extracted with $CHCl_3$ (3 × 100 ml), and after evaporation, the residue was used for GLC analysis on the trimethyl silyl ether on a 3% SE-52, 183 × 0·32 cm stainless steel column and 5% neopentylglycol succinate on Gas Chrom Q column at 180° under N_2 flow rate of 30 ml/min gave the same retention times as the authentic 2,3,4-tri-*O*-methylglucose trimethyl silylate. TLC [isopropyl ether–MeOH (83:17) silica gel] also gave the same spot as 2,3,4-tri-*O*-methylglucose (R_f 0·49).

Determination of site of acetate attachment. The procedure of Nishikawa *et al.*[3] was used. TLC and PC of the resulting hydrolysate indicated the presence of glucose and 3-*O*-methylglucose.