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Studies on the Structure of Polysaccharide from *Trichophyton mentagrophytes*

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An alkali-soluble polysaccharide, designated as S-Iaw, has been isolated from the mycelia of *Trichophyton mentagrophytes*. It gave a single peak on high-speed liquid chromatography. The only component sugar was D-mannose, and its molecular weight was found to be about 24000.

Methylation, periodate oxidation, and acetolysis studies suggested that S-Iaw is composed of repeating units of *O*-(2-*O*-α-D-mannopyranosyl)-6-*O*-α-D-mannopyranosyl-α-D-mannopyranose.

Keywords——*Trichophyton mentagrophytes*; polysaccharide; high speed liquid chromatography; acetolysis; methanolysis; trisaccharide repeating unit

Trichophyton mentagrophytes is a dermatophyte that causes tinea pedis and other cutaneous lesions in humans.[2)] Barker reported that the D-mannose units in the galactomannan peptide from *T. mentagrophytes* which gave both immediate and delayed reactions in sensitized guinea pigs were joined predominantly by 1→2 and 1→4 linkages.[3)] Ito *et al.*[4)] have already reported that the main component of the immunologically active substance from the mycelia was a galactomannan with 1→2 and 1→6 linkages (no 1→4 linkages). However, the whole structure and molecular weight of this polysaccharide, designated as S-Ia, are unknown.

We re-examined the structure of this polysaccharide and also tried to isolate it from whole cells without the mechanical treatment described previously.[4)]

In the present investigation, whole cells of *T. mentagrophytes* were extracted with hot aqueous alkali to give mannosecontaining polysaccharides. The polysaccharides, which showed 4 peaks on high-speed liquid chromatography (HPLC), were purified through their insoluble copper complexes formed with Fehling's solution. Recovery of polysaccharide from the insoluble copper complex yielded an ash-free, nitrogen-free product (S-Iaw).

S-Iaw showed a single peak on HPLC in sodium chloride solution, and gave a single spot on glass-fiber paper electrophoresis in alkaline borate buffer. A hydrolysate of S-Iaw contained only mannose, which was identified by gas-liquid chromatography (GLC) as the trimethylsilyl (TMS) derivative of methyl mannoside. The high and positive value of the specific rotation ($[\alpha]_D^{22}$ +184°) indicates that the majority of glycosidic linkages in S-Iaw has the α-configuration. The basic physical and chemical properties are very similar to those of S-Ia ($[\alpha]_D^{24}$ +172°, sugar component; mannose, trace of galactose).

Gas liquid chromatography of the methanolysis products of S-Iaw methyl ether prepared by the Hakomori method[5)] and the Kuhn procedure revealed the liberation of approximately equal amounts of methyl 2,3,4,6-tetra-*O*-methylmannopyranoside, methyl 2,3,4-tri-*O*-methylmannopyranoside and methyl 3,4-di-*O*-methylmannopyranoside, which were identified by

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2) C.D. Wu-Yuan and T. Hashimoto, *J. Bacteriol.*, **129**, 1584 (1977).
3) S.A. Barker, O. Basarab, and C.N.D. Cruickshank, *Carbohyd. Res.*, **3**, 325 (1967).
4) Y. Kitazima, Y. Banno, T. Noguchi, Y. Nozawa, and Y. Ito, *Arch. Biochem. Biophys.*, **152**, 811 (1972).
5) S. Hakomori, *J. Biochem.* (Japan), **55**, 205 (1964).

comparison with authentic samples. The acetolysis of S-Iaw afforded a mannose pentaacetate and a mannobiose peracetate which were separated by silica gel column chromatography. The purified mannobiose peracetate was deacetylated with triethylamine in the usual way and converted into a permethylate by the Hakomori method. Methanolysis of the permethylate gave methyl 2,3,4,6-tetra-*O*-methyl and methyl 3,4,6-tri-*O*-methylmannopyranosides in a 1:1 ratio as identified by GLC with authentic samples. This is in fair agreement with the report[6] that α-(1→6)-linked D-mannobiose was cleaved faster than α-(1→2)-linked D-mannobiose. These results suggest that a single-unit side chain of D-mannopyranose is attached to every other unit of the basic chain, which is composed of 1→6 linkages of D-mannopyranose, through the C-2 position.

The relative susceptibilities of the sugars in fully acetylated S-Iaw, which was prepared by treatment with acetic anhydride and pyridine, to oxidation with chromium trioxide[7] were investigated to permit assignment of the anomeric configurations of the glycosidic bonds. After this procedure, only mannose was detected by GLC, so its glycosidic linkage was determined to be α. This result was supported by the infrared (IR) spectrum of S-Iaw, which shows an absorption at 810 cm^{-1} due to α-glycosidic linkages.[8]

When S-Iaw was subjected to periodate oxidation, 1.72 mol of periodate per mannose residue was consumed. This result is in good agreement with the theoretical value deduced from methylation analysis, which predicted a periodate consumption of 1.67 mol/mol. Periodate oxidation followed by reduction and acid hydrolysis gave glycerol and glycolaldehyde, but no erythritol.

Based on the above evidence, it is proposed that S-Iaw of *T. mentagrophytes* is composed of repeating units of a trisaccharide having the following structure (Chart 1).

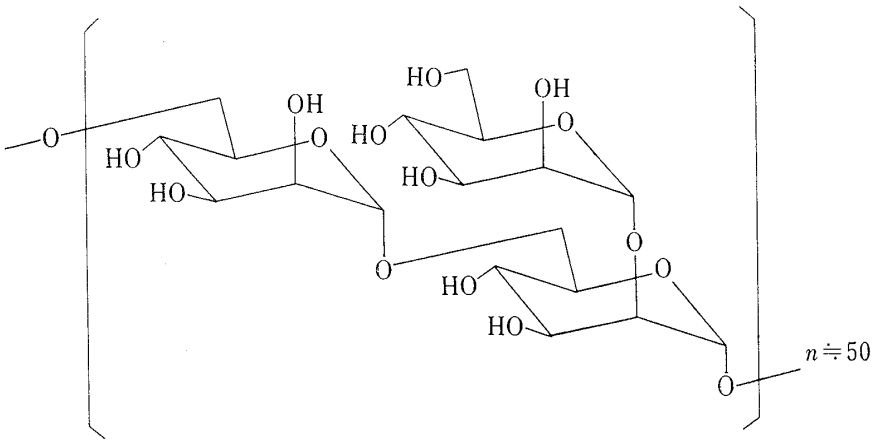

Chart 1

The average chain length of S-Iaw, based on estimation of reducing end groups by the Park-Johnson method[9] and quantitative analysis of the methanolysis products, was determined to be about 150 mannose units.

Studies on the structural determination of the other polysaccharides are under way. In addition, the serological activity of S-Iaw and the other polysaccharides will be reported elsewhere.

Experimental

Optical rotation was measured with a JASCO DIP-2 spectrometer. Infrared spectra were measured with a JASCO IRA-2 spectrometer. HPLC was carried out on a Toyo Soda HLC-802 UR unit equipped with

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an RI detector. The eluent (0.1 M NaCl) flow rate was 1.2 ml/min at 40° on a column of G2000 SW+G3000 SW.

Organism and Growth Conditions——A strain of *Trichophyton mentagrophytes* was grown at 28° for 4 days in 50 Erlenmayer flasks, each containing 200 ml of Sabouraud's medium (4% glucose, 1% polypeptone and 0.5% yeast extract). The mycelia were harvested by filtration and washed five times with distilled water.

Isolation of S-Iaw——Dried whole cells were treated with 1 N NaOH at 100° for 5 hr. After filtration, the filtrate was dialyzed and then poured into absolute ethanol. The precipitate (S-Iw) was collected by centrifugation. S-Iw was dissolved in water, and Fehling's solution was added. The precipitate was collected by centrifugation after 5 hr, washed with water, and decomposed by adding 5% HCl–MeOH. The solution was concentrated to a small volume under reduced pressure and poured into ethanol. The precipitate was collected and dissolved in water. The solution was dialyzed and lyophilized. The yield of S-Iaw based on the weight of dried mycelia was 2%. $[\alpha]_D^{22}$ +184° (c=0.25, H_2O), IR ν_{max}^{KBr} cm^{-1}: 810. The average molecular weight was *ca.* 2.4×10^4 as determined by the Park-Johnson method.

Sugar Components of S-Iaw——S-Iaw was methanolyzed with 5% HCl–MeOH in a sealed ampoule at 100° for 5 hrs. After concentration, the residue was converted into the trimethylsilyl ether and the glycosides were identified as methyl mannoside by GLC using a glass column packed with 2% OV-17 chromosorb WAW DMCS (80—100 mesh) at 150°, with a flow rate of 50 ml of N_2 per min.

Methylation Analysis——S-Iaw (40 mg) was dissolved in dimethylsulfoxide (5 ml) under a nitrogen atmosphere. The solution was treated with methylsulfinyl carbanion (2 ml) for 4 hrs at room temperature, and then with methyl iodide (2 ml) at 20° for 1 hr. The reaction mixture was extracted with $CHCl_3$. After concentration, the residue was methylated twice by Kuhn's method[10] using DMF (5 ml), Ag_2O (0.5 g) and CH_3I (2 ml) at 40° in the dark. The product showed no hydroxyl absorption in the infrared spectrum. The methylated product was methanolyzed with 5% HCl–MeOH (2 ml) in a sealed ampoule for 5 hrs. The resulting methyl *O*-methylmannosides were analyzed by GLC on a Shimadzu GC-6A gas chromatograph equipped with a hydrogen flame ionization detecter, using a glass column (0.3 cm × 2 m) packed with 10% DEGS-Chromosorb W (60—80 mesh) at 170°, with a flow rate of 50 ml of N_2 per min. Relative retention times (TM: relative to methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside): methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside (TM, 1.00); methyl 2,3,4-tri-*O*-methyl-α-D-mannopyranoside (TM, 2.36); methyl 3,4-di-*O*-methyl-α-D-mannopyranoside (TM, 6.15).

Acetolysis of S-Iaw——S-Iaw (25 mg) was suspended in the acetolysis reagent (25 ml of acetic anhydride, 25 ml of acetic acid and 2 ml of sulfuric acid), and acetolysis was carried out at room temperature for 3 days.

The reaction solution was extracted with $CHCl_3$ and the organic layer was washed with H_2O then dried over Na_2SO_4. After removal of the solvent, the resulting syrup was chromatographed on silica gel using benzene: acetone (20: 1) as a developing solvent. The resulting mannobiose peracetate was dissolved in 50% MeOH and a few drops of Et_3N were added. After standing for one day, the solution was evaporated down and methylated by the Hakomori method. The mannobiose permethylate was methanolyzed and analyzed by GLC. Relative retention times: methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside (TM, 1.00); methyl 3,4,6-tri-*O*-methyl-α-D-mannopyranoside (TM, 2.34).

Periodate Oxidation, Smith Degradation, and Analysis of Products——S-Iaw (46 mg) was added to a solution of 0.02 M $NaIO_4$ (25 ml). Oxidation was carried out in the dark at 7°. Aliquots (5 ml) were removed from the solution at intervals for estimation of their iodate.[11] The oxidation was completed after 48 hrs. On acid hydrolysis, the polyalcohol obtained on sodium borohydride reduction of the polyaldehyde gave glycerol and glycolaldehyde, which were identified as the TMS derivatives by GLC.

Oxidation of S-Iaw Acetate——S-Iaw (10 mg) was conventionally acetylated with pyridine (1 ml) and acetic anhydride (1 ml) and the resulting mixture was allowed to stand at room temperature for 24 hrs. The reaction mixture was then poured into ice-water and dialyzed. The resulting suspension of acetylated polysaccharide was purified by precipitation from acetone with light petroleum to yield 12 mg of polysaccharide acetate. The product gave a strong acetyl absorption band at 1735 cm^{-1}, but no OH-group absorption in its infrared spectrum. The S-Iaw acetate was then oxidized with CrO_3–AcOH. The products were hydrolyzed with 2 M HCl at 100° for 16 hrs. After this procedure, D-mannose was still detected by GLC. The oxidation product was also dissolved in methanol and deacetylated with 0.2 N sodium hydroxide at 5° overnight. The deacetylated compound was similar to that of S-Iaw in its HPLC pattern and IR absorption pattern.

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