[Chem. Pharm. Bull. 24(8)1718—1723(1976)]

UDC 547.458.02.05 : 576.8.098

Studies on Fungal Polysaccharides. XIX.[1] Chemical Structure of a Water Soluble Polysaccharide from the Cell Wall of *Cladosporium trichoides*

Toshio Miyazaki[2a] and Yasuta Naoi[2b]

Tokyo College of Pharmacy[2a] and Tokyo Metropolitan Research Laboratory of Public Health[2b]

(Received September 23, 1975)

Major water-soluble polysaccharide obtained from the cell wall of *Cladosporium trichoides* by diethylaminoethyl-cellulose column fractionation is a heteroglycan, $[\alpha]_D^{20}$ +50° (c=1, H_2O), which is composed of D-galactose, D-glucose, D-mannose, and small amount of L-rhamnose (1.4: 1.4: 1.0: 1.0). The results of periodate oxidation, Smith-type degradation, methylation, and gas–liquid chromatography–mass spectrometry spectra show that the polysaccharide has branched structure and contains 1,2-, and 1,2,6-linked galactofuranosyl, 1,3,6-linked mannopyranosyl, and a small amount of 1,4-linked glucopyranosyl residues as the main units. The terminal residues are glucopyranosyl and mannopyranosyl residues.

In our previous papers,[3] secreted and cell wall polysaccharides from *Cladosporium herbarum* and the polysaccharide secreted from *C. tricholides* were described in detail. In this paper, chemical structure of a polysaccharide from the cell wall of *C. trichoides* is discussed. *C. trichoides* is known as a kind of pathogenic fungi which cause chromoblastmycosis.

The purified cell wall was prepared from the filamentous cells with a French press, followed by repeated washing and fractional centrifugation. The cell wall was microscopically free from cytoplasmic material. The crude polysaccharides were prepared by three consecutive extractions from cell wall as described in the experimental part. The crude polysaccharide extracted from the cell wall with hot water was treated with Pronase and then by the Sevag method. The pronase-treated crude polysaccharide was purified by diethylaminoethyl (DEAE)-cellulose column chromatography, using the water-0.01M, 0.05M, and 0.1M sodium hydrogen carbonate-0.1N sodium hydrooxide systems and the water-0.01M, 0.05M, and 0.1M sodium borate-0.1N sodium hydroxide systems for the elution. The major fraction eluted with distilled water was further purified by zone electrophoresis. The purified major polysaccharide (CTCW-1) thus obtained showed $[\alpha]_D^{20}$ +50° (c=1, H_2O), and was revealed homogeneous by paper electrophoresis (PE) with borate buffer and sedimentation analysis. CTCW-1 contained neither nitrogen (elemental analysis) nor phosphorus (the procedure of Fiske and Subbarow[4]), and the proportion of sugar in CTCW-1 was 95.2% (as glucose, by the procedure of Dubois, *et al.*[5]).

The component sugars of CTCW-1 were identified as galactose, glucose, mannose, and a small amount of rhamnose by paper chromatography (PC) of the acid hydrolysate, and their molar ratio was determined to be 1.4: 1.4: 1.0: 0.1, respectively, by the procedure of Dubois, *et al.*[5]

1) Part XVIII: T. Miyazaki and Y. Naoi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1752 (1975).
2) Location: *a) Kitashinjuku 3-chome, Shinjuku-ku, Tokyo 160, Japan; b) 24-1 Hyakunin-cho 3-chome, Shinjuku-ku, Tokyo 160, Japan.*
3) T. Miyazaki and Y. Naoi, *Chem. Pharm. Bull.* (Tokyo), **22**, 1360 (1974); *idem, ibid.*, **22**, 2058 (1974); *idem, ibid.*, **23**, 157 (1975).
4) C.H. Fiske and Y. Subbarow, *J. Biol. Chem.*, 66 375 (1925).
5) M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, **28B**, 350 (1956).

On periodate oxidation of CTCW-1, the amount of periodate consumption, and formic acid and formaldehyde liberated per sugar unit were 1.15, 0.04 and 0.11 mol, respectively. Smith-type degradation[6] of CTCW-1 gave arabinose, threitol, erythritol, glycerol, and unoxidized component sugars, and their molar ratio was approximately 5.1: 1.0: 2.0: 1.4: 1.0: 1.0: 7.5 (Gal–Glc–Man–Ara–Thr–Ery–Gly). These results suggested the presence of 1,2- or 1,3-linked galactofuranosyl (source of arabinose), 1,4-linked galrctopyranosyl or 1,5-or 1,6-linked galactofuranosyl (source of threitol), 1,4-linked hexopyranosyl (source of erythritol), 1,2- or 1,6-or terminal-linked hexopyranosyl (source of glycerol), and 1,3-linked or branching point of each component sugar residue.

Partial acid-hydrolysis of CTCW-1 using sulfuric acid was examined by successive treatment with (*a*) 0.01N at 100°, 4 hr; (*b*) 0.1N at 100°, 3 hr; (*c*) 0.5N at 100°, 2 hr; and (*d*) 2N at 100°, 6 hr, and the fragments released were separated by dialysis. The dialyzable fragments were submitted to PC, and an aliquot of the non-dialyzable materials was hydrolyzed for determination of sugar composition. Oligosaccharides liberated by treatment (*a*) and (*b*)

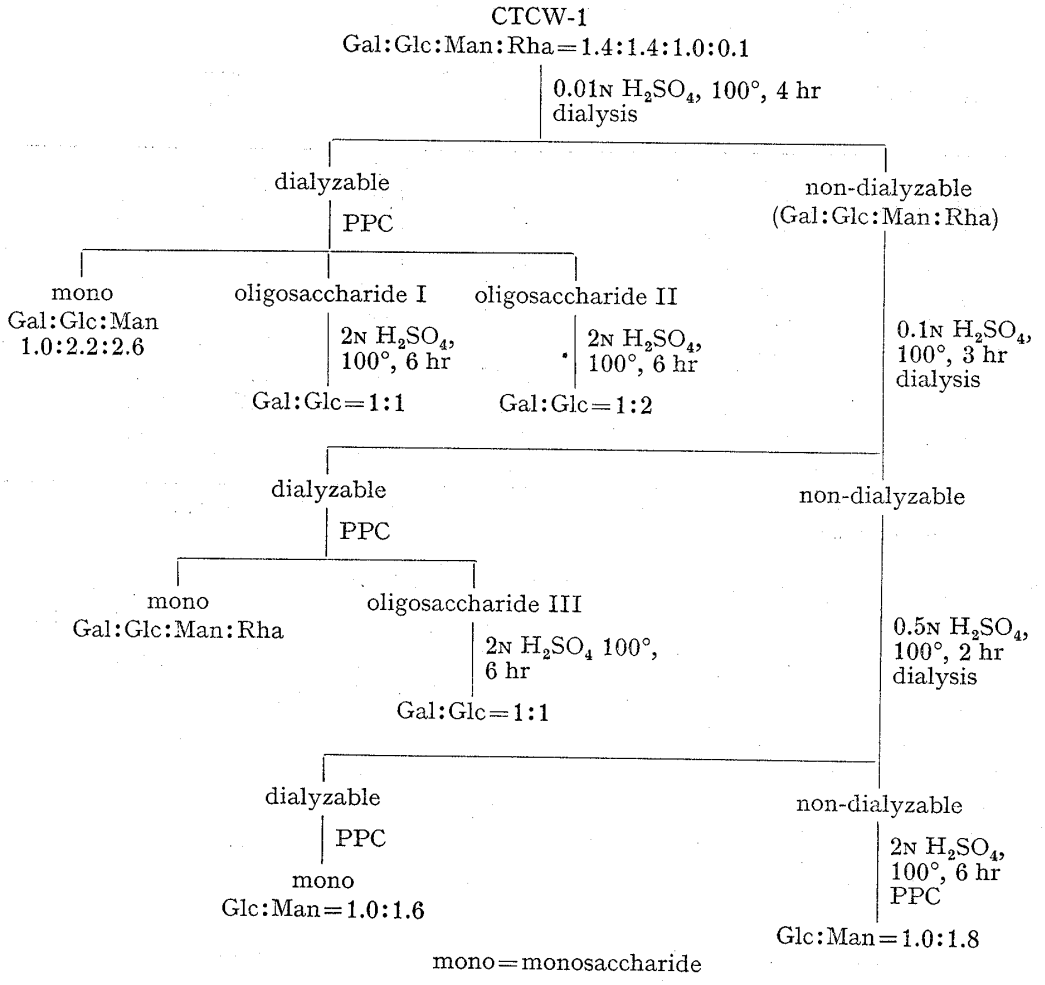


Chart 1. Partial Acid Hydrolysis of the Polysaccharide

were hydrolyzed by treatment (*d*). The component sugars of oligosaccharides I, II and III were galactose and glucose, and their molar ratio was determined to be 1: 1 (oligosaccharides I and III) and 1: 2 (oligosaccharide II). The results of partial acid-hydrolysis suggested that this galactoglucomannan has branching moieties including acid-labile galactosyl residues.

6) J.K. Hamilton and F. Smith, *J. Am. Chem. Soc.*, **78**, 5907 (1956).

After CTCW-1 was methylated by the methods of Hakomori[7] and then of Purdie,[8] methanolysis and hydrolysis were carried out, and resulting *O*-methylated monosaccharides were analyzed by PC, PE, and gas–liquid chromatography (GLC). PC revealed the presence of di-*O*-methyl-, tri-*O*-methyl-, and tetra-*O*-methyl-monosaccharides in the ratio of 1.7 : 1.0 : 1.9. Di-*O*-methyl-monosaccharide fraction seemed to be 2,4-di-*O*-methylmannose and 3,5-di-*O*-methylgalactose (M*G* value, 0.01 and 0.71) from PE using 1% sodium borate. Tri-*O*-methyl-monosaccharide fraction was found to be 3,5,6-tri-*O*-methyl-, (M*G* value, 0.67), 3,4,6-tri-*O*-methyl- (M*G* value, 0.32), and 2,3,4-, 2,3,6-, or 2,4,6-tri-O-methyl-monosaccharides (M*G* value, 0.00) from PE. The methyl *O*-methyl glycosides fraction obtained by mathanolysis of methylated CTCW-1 were analyzed by GLC, and this fraction was identified to be methyl 2,3,4,6-tetra-*O*-methyl-glucopyranoside and -mannopyranoside. The *O*-methylated alditol acetates from CTCW-1 were prepared by the procedure described in our previous paper[3] and then the products were analyzed by GLC. The values of relative retention time of the *O*-methylated alditol acetates from CTCW-1 were consistent with the values in literature[9] as shown in Table I.

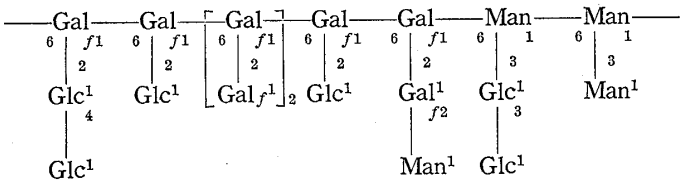
Table I. Identification of Permethylated Alditol Acetates

O-Acetyl-*O*-methyl alditol	ECNSS-M column		OV-225 column		Molar ratio
	observed t_R[a]	literature t_R[a]	observed t_R[a]	literature t_R[a]	
1,5-Di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol	0.46	0.46	0.34	0.35	0.1
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol	1.00	1.00	1.00	1.00	9.8
1,4-Di-*O*-acetyl-2,3,5,6-tetra-*O*-methylgalactitol	1.15	1.15	1.10	1.10	0.1
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol	1.00	1.00	1.00	0.99	4.3
1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-methylglucitol	1.94	1.98	1.83	1.83	1.1
1,2,4-Tri-*O*-acetyl-3,5,6-tri-*O*-methylgalactitol	2.25	—[b]	1.95	—[b]	1.4
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol	2.47	2.50	2.30	2.32	3.2
1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-methylmannitol	2.47	2.48	2.19	2.19	1.0
1,4,6-Tri-*O*-acetyl-2,3,5-tri-*O*-methylgalactitol	3.28	3.28	2.75	2.76	1.1
1,3,5,6-Tetra-*O*-acetyl-2,4-di-*O*-methylmannitol	5.40	5.44	4.46	4.51	3.8
1,2,4,6-Tetra-*O*-acetyl-3,5-di-*O*-methylgalactitol	6.23	6.35	5.12	5.10	11.5

a) *T* is retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol.
b) —=unknown

The *O*-methylated alditol acetates from CTCW-1 were analyzed by GLC-mass spectrometry (GLC–MS), and those results were consistent with the values reported in literature.[10] 1,2,4-Tri-*O*-acetyl-3,5,6-tri-*O*-methyl-D-galactitol was identified by GLC-MS (*m/e*: 43, 45, 87, 89, 101, 129, 145, 161, and 189). Therefore, the source of arabinose in the Smith degradation[6] product should be 1,2-linked galactofuranosyl residue. 1,3,5,6-Tetra-*O*-acetyl-2,4-di-*O*-methylgalactitol and 1,2,4,6-tetra-*O*-acetyl-3,5-di-*O*-methyl-galactitol were distinguished by the change of *m/e* in deuterium labeling experiment as described in our previous paper.[1]

From these results, it is concluded that CTCW-1 has a branched structure and the probable structure of basic portion in the polysaccharide would be formulated as follows:
CTCW-1:



7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
8) T. Purdie and T.C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).
9) B. Lindberg, *Methods Enzymol.*, **28B**, 178 (1972).
10) H. Björndal, B. Lindberg, and S. Svensson, *Carbohyd. Res.*, **4**, 433 (1967).

As discussed in our previous paper,[3] extracellular polysaccharide (CTP-1) from *C. trichoides* has a highly branched structure consisting of 1,2,6- and 1,2,3-linked mannan core and contains 1,6-linked mannopyranosyl, 1,4-linked galactopyranosyl, and 1,2-linked galactofuranosyl residues as the main units, while CTCW-1 has a branched structure consisting of 1,2,6-linked galactofuranosyl and 1,3,6-linked mannopyranosyl core, and contains terminal glucopyranosyl and mannopyranosyl residues as the main units. Therefore, these polysaccharides from *C. trichoides* have a different structure from each other.

Experimental

Preparation of Cell Wall——*Cladosporium trichoides OUT 4294*[11] was grown in a Sabouraud medium (dialyzable polypeptone 1%, glucose 4%) in an incubator at 25° for 20 days. The culture flasks were shaken once a day. The mycelium was separated from the medium by filtration through a Nylon cloth, washed thoroughly with distilled water, and the cell wall was separated with a French press. This procedure was repeated 3 times under the pressure of 400 kg/cm^2. This was usually sufficient to achieve total cell rupture. The cell wall was separated immediately from cytoplasmic debris and intracellular soluble materials by repeated centrifugation at 3000 rpm for 10 min to prevent enzymic degradation of the cell wall. The supernatant was discarded, the residue was resuspended in an aqueous solution of 0.1% sodium dodecylsulfate and the suspension was stirred vigorously for 24 hr. This treatment was repeated 8 times. The sedimented cell wall preparation was washed free of sodium dodecylsulfate with distilled water and dried over P_2O_5 *in vacuo*. A grayish fine powder was obtained in yield of 6.3 g per 130 g of cell. Microscopic examination of this material showed that it was free from cytoplasmic contamination (tested with 0.02% Methylene Blue staining).

Extraction of Crude Polysaccharide from Cell Wall——Extraction of crude polysaccharide from cell wall was performed successively under three different conditions.

1) Extraction with Water at 100°: The cell wall (20 g) was extracted with H_2O at 100° for 2 hr. After centrifugation, this procedure was repeated 9 times. The combined supernatant was dialyzed against running water for 3 days, and the internal solution was concentrated *in vacuo*. To the concentrate, 10 volumes of ethanol was added, and the precipitate that appeared was collected by centrifugation, washed successively with ethanol, acetone, and ether, and dried *in vacuo*. Yield of the water-soluble substance was 3.4 g (corresponding to 16.0% of the wall).

2) Extraction with 1N KOH at Room Temperature: The water-extracted cell wall material (15.6 g) was extracted with 1 N KOH at a room temperature for 2 hr. After centrifugation, this procedure was repeated 7 times. The combined supernatant was dialyzed against running water for 3 days, and the internal solution was concentrated to a small volume *in vacuo*, 10 volumes of ethanol was added to the concentrated, and the precipitate that formed was collected by centrifugation and treated as described above. Yield of the alkali- and water-soluble material was 0.8 g (corresponding to 5.1% of the wall).

3) Extraction with 1 N KOH at 100°: The treated cell wall was further extracted with 1 N KOH at 100° for 2 hr. After centrifugation, this procedure was repeated 9 times. The supernatant and sediment were treated as described above. Yield of the alkali- and water-soluble material was 0.22 g (corresponding to 1.1% of the wall), and that of the sediment was 0.65 g.

Protease Digestion and Separation of Hot Water Extracted Polysaccharide——The crude polysaccharide (3.4 g) obtained by hot water extraction was dissolved in 100 ml of H_2O and adjusted to pH 7.8 with Na_2CO_3. The solution was treated with protease (Pronase, Kaken Kagaku Co., Tokyo), and then fractionated by DE-AE-cellulose column chromatography as described in our previous paper.[3] Yields were as follows: H_2O eluate (W-1), 0.59 g (21.1%); 0.01 M $Na_2B_4O_7$ eluate (CTCW-2), 0.168 g (6.0%); 0.05 M $Na_2B_4O_7$ eluate, 0.448 g (16.3%); 0.1 M $Na_2B_4O_7$ eluate (CTCW-3), 0.392 g (11.6%), 0.1 N NaOH eluate, trace. Purification of W-1 (100 mg) was carried out by zone electrophoresis using Pevicon C-870 (polyvinyl resin, M and S Instruments Inc., *Japan*) as the supporting medium, which was equilibrated with 0.026 M borate buffer (pH 9.2).

Homogeneity of CTCW-1——(1) Electrophoresis of the CTCW-1 on Whatman No. 1 filter paper in 0.026 M borate buffer at pH 9.2 (50 volt/cm, 90 min) gave a single spot by periodate-Schiff reagent[12] which moved to the anode. (2) CTCW-1 was dissolved in 0.1 M aqueous NaCl to a concentration of 1.0% and the sedimentation pattern was observed at 60000 rpm using Hitachi Model 282 analytical ultracentrifuge equipped with schlieren optical system. CTCW-1 showed a single symmetrical peak on analytical centrifugation.

Component Sugar of Main Polysaccharide CTCW-1——CTCW-1 (20 mg) in 5 ml of 1N H_2SO_4 in a sealed tube was heated in a boiling water bath for 6 hr, and the mixture was treated as described in ous previous paper.[3] Galactose, glucose, mannose, and rhamnose were identified in a molar ratio of 1.4: 1.4: 1.0: 0.1 (by the procedure of Dubois, *et al.*[5]).

11) This strain was supplied from the Faculty of Engineering, Osaka University.
12) E. Köiw and A. Gronwall, *Scand. J. Clin. Invest.*, **4**, 244 (1952).

Properties of CTCW-1——Sugar content of CTCW-1 was 95.2% (as glucose, by the procedure of Dubois, *et al.*[5]) and CTCW-1 was free from phosphorus (by procedure of Fiske-Subbarow[4]) and nitrogen (elemental analysis). CTCW-1 showed $[\alpha]_D^{20}$ +50° ($c=1$, H_2O), and was slightly soluble in water.

Periodate Oxidation of CTCW-1——CTCW-1 (20 mg) was oxidized with 0.018 mol $NaIO_4$ in 50 ml of water at room temperature in the dark. A blank solution without glycan was processed similarly. A 3-ml aliquot was taken at different periods for determination of $NaIO_4$ consumption,[13] and the formation of formic acid[14] and formaldehyde.[15] The moles of $NaIO_4$ consumed per anhydro component sugar unit are given in Table II.

Table II. Results of Periodate Oxidation of CTCW-1

Mole/unit	Time (hr)							
	1	3	6	12	24	48	72	96
IO_4	0.61	0.91	1.01	1.05	1.11	1.13	1.15	1.15
HCOOH	0.04	0.08	0.12	0.24	0.32	0.46	0.45	0.45
HCHO	0.08	0.10	—	0.11	0.10	0.10	0.11	0.10

Smith-type Degradation of Periodate-oxidized CTCW-1——After oxidation of CTCW-1 by $NaIO_4$, the reaction mixture was submitted to the Smith-type degradation as described in our previous paper,[3] and seven spots corresponding to standard galactose, glucose, mannose, arabinose, threitol, erythritol, and glycerol were detected as the main products on the paper chromatogram by spraying a solution of alkaline–$AgNO_3$[16] and *p*-anisidine–HCl.[17] The relative molar ratios of hexoses and arabinose were determined by the method of Dubois, *et al.*,[5] and those of threitol, erythritol, and glycerol by the method of Lambert-Neish.[18] The molar ratio was approximately 5.1: 1.0: 2.0: 1.4: 1.0: 1.0: 7.5 (Gal-Glc-Man-Ara-Thr-Ery-Gly).

Partial Acid Hydrolysis of CTCW-1——CTCW-1 (100 mg) was hydrolyzed with 0.01 N H_2SO_4 (3 ml) at 100° for 4 hr and the hydrolysate was dialyzed against distilled water (300 ml) for 24 hr. This dialysis procedure was performed 4 times. The external solution (1200 ml) of the hydrolysate was evaporated to about 20 ml *in vacuo*. After neutralization with barium carbonate and filtration, it was concentrated to a syrup. Paper chromatographic examination of the syrup using AcOEt–pyridine–H_2O (10: 4: 3) (solvent system A) showed the liberation of galactose, glucose, and mannose (molar ratio, 1.0: 2.2; 2.6) and two oligosaccharides (oligosaccharide I and II). The internal solution was concentrated to dryness and weighed. A part of the non-dialyzable material was hydrolyzed with 2 N H_2SO_4 at 100° for 6 hr and treated as described above, galactose, glucose, mannose, and rhamnose were detected. Other part of the non-dialyzable material was hydrolyzed with 0.1 N H_2SO_4 (3 ml) at 100° for 3 hr. The dialyzable fragments from the hydrolysate, which was afforded by the same procedure as above, gave four components, galactose, glucose, mannose, and rhamnose (molar ratio, 6.0: 8.0: 2.1: 1.0), and an oligosaccharide (oligosaccharide III). The non-dialyzable material was hydrolyzed with 0.5 N H_2SO_4 (3 ml) at 100° for 2 hr. The external solution of the hydrolysate was concentrated to a small volume, neutralized with barium carbonate, and filtered. The filtrate (free from barium ions) was evaporated to a syrup and treated as described above, glucose, and mannose (molar ratio, 1.0: 1.6) were detected. The non-dialyzable material, containing glucose, and mannose in approximate molar ratio of 1.0: 1.8, was hydrolyzed with 2 N H_2SO_4 at 100° for 6 hr. These oligosaccharides were hydrolyzed with 2 N H_2SO_4 at 100° for 6 hr, respectively, and the hydrolysates neutralized with barium carbonate, and filtered, and the filtrates were concentrated to a small volumes. The hydrolysates were reduced with sodium borohydride (100 mg), and the resulting alditols were treated with a mixed solution of acetic anhydride and pyridine (1: 1) at 100° for 1 hr and the products were analyzed by GLC on a column (200×0.3 cm) containing 5% ECNSS-M (60—80 mesh) at 180°. The results are given in Chart 1.

Methylation Analysis——Dry sample (50 mg) of the CTCW-1 was dissolved under a nitrogen atmosphere in dimethyl sulfoxide (2.5 ml) and treated with methylsulfinyl carbanion (0.5 ml) at room temperature for 5 hr. The resulting polysaccharide alcoxide was then permethylated at room temperature with methyl iodide (0.2 ml) by the method of Hakomori[7]. The same procedure was repeated (three times). The methylated CTCW-1 thus obtained was dissolved in methyl iodide (5 ml) and then stirred in a water bath of 50° for 8 hr with occasional addition of silver oxide (50 mg). When methylation was incomplete, as judged from the

13) L. Malaprade, *Bull. Soc. Chim. France*, **51**, 833 (1934).
14) R.L. Whistler and J.L. Hickson, *J. Am. Chem. Soc.*, **76**, 1671 (1954).
15) J.F. O'Dea and R.A. Gibbons, *Biochem. J.*, **55**, 580 (1953).
16) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature* (London), **166**, 444 (1950).
17) L. Hough, J.K.N. Jones, and W.H. Wadman, *J. Chem. Soc.*, **1950**, 1702.
18) M. Lambert and A.C. Neish, *Can. J. Res.*, **28B**, 83 (1950).

infrared spectrum, the methylation procedure was repeated. A portion (15 mg) of the methylated CTCW-1 was methanolyzed with 0.7 N methanolic hydrogen chloride at 100° for 8 hr. After neutralization with silver carbonate the mixture of methyl glycosides was analyzed by GLC. GLC analysis of the methyl *O*-methyl glycosides was effected in a Schimadzu GC-5A unit, equipped with a flame ionization detector, using a 200×0.3 cm glass column packed with 15% polybutane-1,4-diol succinate on celite 545 (60—80 mesh); column temperature, 175°; nitrogen flow rate, 50 ml/min. Methyl 2,3,4,6-tetra-*O*-methyl glucopyranoside and -mannopyranoside were detected. Another portion (30 mg) of the methylated CTCW-1 was heated with 90% formic acid in a boiling water bath for 5 hr. Formic acid was distilled off and the residue was further hydrolyzed with 1 N H_2SO_4 for 5 hr in a boiling water bath. The hydrolysate was neutralized with barium carbonate, filtered, and the filtrate was evaporated to a syrup. The methylated sugars in the hydrolysate were reduced with sodium borohydride or borodeuteride (100 mg). After the decomposition of excess reagent by addition of Amberlite IR-120 (H^+) resin, boric acid in the residue was removed as methyl borate by repeated evaporations with methanol at 30° in a water bath. The mixture of alditols was acetylated by heating with pyridine and acetic anhydride (1: 1) at 100° for 1 hr. GLC analyses of *O*-acetyl-*O*-methyl alditols derived from the methylated CTCW-1 were carried out under the conditions of gas flow rate of 40 ml N_2/min on a glass column (200×0.3 cm) containing 5% (w/w) of ECNSS-M on Chromosorb W (aw-dmcs, 60—80 mesh), at 180°, and of gas flow rate of 20 ml N_2/min on a glass column (200×0.3 cm) containing 3% (w/w) of Silicone OV-225 on Chromosorb W (aw-dmcs, 60—80 mesh), at 170°. Retention values were quoted relative to the mobility of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. For GLC-MS spectrometry, alditol acetates derived from methylated CTCW-1 were dissolved in acetone and injected into a Schimadzu Model LKB-9000 gas-mass spectrometer. The mass spectra were recorded at 70 eV, trup current 60 μA, temperature of the ion source, 310°. 1,3,5,6-Tetra-*O*-acetyl-2,4-di-*O*-methylmannitol deuterium: *m/e* 43, 87, 118, 129, and 1,2,4,6-tetra-*O*-acetyl-3,5-di-*O*-methylgalactitol deuterium: *m/e* 43, 88, 117, 130, and 190.

PC and PE of the Hydrolysate of Methylated CTCW-1——The methylated CTCW-1 was hydrolyzed by formic acid and 1 N H_2SO_4.[19] Paper chromatogram of the hydrolysate using the solvent system AcOEt–AcOH–H_2O (9: 2: 2), showed three spots corresponding to tetra-*O*-methyl- (*Rf* value, 0.84), tri-*O*-methyl- (*Rf* value, 0.65), di-*O*-methyl-monosaccharide (*Rf* value, 0.40). Di-*O*-methyl-monosaccharide fraction seemed to be 2,4-di-*O*-methyl-D-mannose (*MG* value, 0.01) and 3,5-di-*O*-methyl-D-galactose (*MG* value, 0.71) from PE using 0.026 M borate buffer (pH 9.2). Reported values[20] for di-*O*-methyl-mannose are 0.49 (3,4-), 0.43 (4,6-), 0.09 (2,6-), 0.14 (2,3-), and 0.39 (3,6-).

Component Sugar of Minor Polysaccharides, CTCW-2 and CTCW-3——The minor polysaccharides were hydrolyzed with 1 N H_2SO_4 at 100° for 6 hr, and the resulting sugars were detected by PC as described above. Galactose, glucose, mannose, rhamnose, and glucuronic acid were detected.

19) H.O. Souveng, H. Kiessling, B. Lindberg, and J. Mckay, *Acta Chem. Scand.*, **16**, 615 (1962).
20) T. Miyazaki, *Chem. Pharm. Bull.* (Tokyo), **9**, 831 (1961).