Studies on Fungal Polysaccharides. X.[1] Extracellular Heteroglycans of Absidia cylindrospora and Mucor mucedo[2]

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The main polysaccharide of A. cylindrospora, $[\alpha]_D + 15.0°$, in a highly complicated heteroglycan composed of fucose, mannose, glucose, galactose, N-acetylglucosamine, and N-acetylgalactosamine in a relative ratio of 10: 25: 4: 2: 2: 1 and that of M. mucedo, $[\alpha]_D + 30.4°$, contains the same componental sugars in a molar ratio of 10: 25: 2.1: 3.6: 4: 2. The results of periodate oxidation and partial acid hydrolysis of both polysaccharides indicate a similarity in their structural feature.

The minor polysaccharide of A. cylindrospora, $[\alpha]_D + 43.6°$, has the same componental sugars as the main polysaccharide but that of M. mucedo, $[\alpha]_D - 65.0°$, is an acidic heteroglycan consisting of fucose, galactose, and glucuronic acid.

Since a survey of fungal polysaccharides was reported by Martin, et al.[4] in 1956, chemistry of polysaccharides prepared from fungi has been serologically studied by several groups of workers in recent years as an aid to taxonomy. The object of this continuing program on extracellular polysaccharides of the fungi belonging to Mucorales, especially of Rhizopus nigricans, Absidia cylindrospora, and Mucor mucedo, is to relate polysaccharide structure to species difference and immunological properties.

The previous paper[5] reported that the main extracellular polysaccharide of R. nigricans, $[\alpha]_D + 68.1°$, is a complicated heteroglycan composed of fucose, mannose, glucose, galactose, N-acetylglucosamine, and N-acetylgalactosamine in approximate molar ratio of 1:22:4:5:4:4, and the minor polysaccharide is an acidic heteroglycan consisting of fucose, galactose, and glucuronic acid. The present paper describes gross structural features of extracellular polysaccharides from A. cylindrospora and M. mucedo.

The crude polysaccharides isolated from the culture liquids were treated with pronase and the Sevag method, followed by DEAE-cellulose column chromatography using sodium hydrogen carbonate for the elution and then rechromatography over DEAE-cellulose column in a borate-form. The main fraction (AI) from the culture liquid of A. cylindrospora, $[\alpha]_D + 15.0°$ ($c=0.66$, H_2O), which gave a single spot in paper electrophorogram using a borate buffer, contained 0.8% nitrogen, 88.7% hexose (by the procedure of Dubois, et al.[6]), 5.8% hexosamine (by the Blix method[7]), and no phosphorus (by the method of Fiske-Subbarow[8]). Reducing power of AI was 1:0.007 (as fucose, by the Park-Johnson method[9]). The main fraction (MI) from the culture liquid of M. mucedo, $[\alpha]_D + 30.4°$ ($c=0.95$, H_2O), which showed one spot in paper electrophorogram, had 1.6% nitrogen, 81.2% hexose, 13.7% hexosamine,

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and no phosphorus.   Reducing power of MI was 1:0.01.   The infrared spectra of AI and MI showed absorption bands due to acetamido group at 1640 and 1550 cm^{-1}, respectively.

The component sugars of AI and MI were identified as L-fucose, D-mannose, D-glucose, D-galactose, D-glucosamine, and D-galactosamine by paper chromatography of the acid hydrolysates, and the molar ratio of AI was estimated approximately as 10:25:4:2:2:1 and that of MI as 10:25:2.1:3.6:4:2 by the methods of Dubois, et al.[6] (for hexoses), Dische[10] (for methylpentose), Blix[7] (for total hexosamine), and Ludowieg[11] (for galactosamine).

On periodate oxidation of AI, comsumption of periodate from anhydro component sugar unit was 0.92 mole, the values of formic acid and acetaldehyde liberated from the unit were 0.32 and 0.005 mole.   Formaldehyde was not produced.   In the case of MI, periodate consumed was 0.87 mole, and formic acid and formaldehyde liberated were 0.19 and 0.022 mole. Acetaldehyde was not produced.   The periodate-oxidized AI and MI were treated by the Smith procedure.[12]   Paper chromatographic examination of the hydrolysates showed the presence of the componental sugars in addition to a large amount of glycerol and tetrahydric alcohol.   The molar ratio of AI treated by this procedure was estimated approximately as 2:22:1.1:1:32:5.5 (fucose, mannose, glucose, galactose, glycerol, and tetrahydric alcohol) and the case of MI, it was nearly 3:11:1:2:50:9 by the procedures of Dubois, Dische, and O'dea-Gibbons[13] (for polyhydric alcohol).   After oxidation, hexosamine contents of AI and MI changed to 3.0% and 6.1% (as glucosamine, by the Blix method[7]), respectively.

Partial acid-hydrolysis of AI and MI using sulfuric acid as mineral acid was examined by three successive steps (treatment I: 0.01N, 90°, 1 hr; treatment II: 0.01N, 100°, 2 hr (3 hr in the case of MI); and treatment III: 0.1N, 100°, 3 hr), and the fragments released were
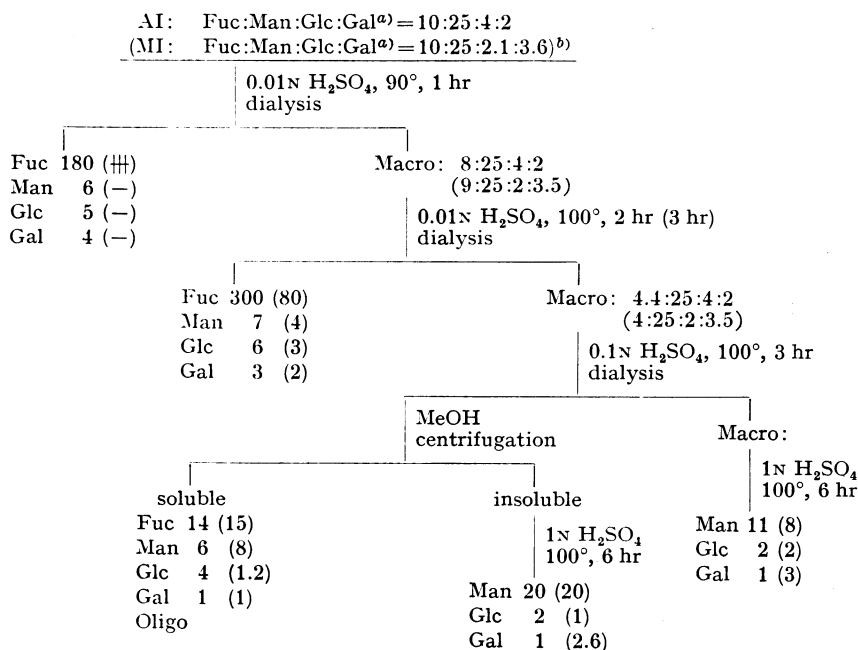
AI: Fuc:Man:Glc:Gal$^{a)}$ = 10:25:4:2
(MI: Fuc:Man:Glc:Gal$^{a)}$ = 10:25:2.1:3.6)$^{b)}$

| 0.01N H$_2$SO$_4$, 90°, 1 hr
| dialysis

Fuc 180 (卌)   Macro: 8:25:4:2
Man   6 (—)   (9:25:2:3.5)
Glc   5 (—)
Gal   4 (—) | 0.01N H$_2$SO$_4$, 100°, 2 hr (3 hr)
                          | dialysis

Fuc 300 (80)   Macro: 4.4:25:4:2
Man   7 (4)   (4:25:2:3.5)
Glc   6 (3)
Gal   3 (2) | 0.1N H$_2$SO$_4$, 100°, 3 hr
                          | dialysis

MeOH   Macro:
centrifugation

soluble   insoluble | 1N H$_2$SO$_4$
Fuc 14 (15) | 100°, 6 hr
Man 6 (8) | 1N H$_2$SO$_4$
Glc 4 (1.2) | 100°, 6 hr   Man 11 (8)
Gal 1 (1)   Glc 2 (2)
Oligo   Man 20 (20)   Gal 1 (3)
                        Glc 2 (1)
                        Gal 1 (2.6)

Chart 1.   Gradual Hydrolysis of AI and MI

a) except hexosamines
b) ( ): indicate molar ratios in the case of MI

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separated by dialysis.   Results of partial hydrolysis are summarised in Chart 1.   The dialyzable fragments were submitted to paper chromatography and an aliquot of the non-dialyzable material was hydrolyzed to determine its molar ratio, and the residue was submitted to the next step.   Alterations of the component sugars were particularly focused on the neutral component.

Majority of the dialyzable fragments liberated by treatment I was fucose (both in AI and MI) and after treatment I, the component sugars of the non-dialyzable material were fucose, mannose, glucose, and galactose in approximate molar ratios of 8:25:4:2 (AI) and of 9:25:2:3.5 (MI).   A large amount of the dialyzable fragments released by treatment II was also fucose in addition to traces of mannose, glucose, and galactose (both in AI and MI) and the non-dialyzable material after treatment II was composed of fucose, mannose, glucose, and galactose (molar ratio of 4.4:25:4:2 in AI and of 4:25:2:3.5 in MI).   The fucose contents liberated by treatment I and II corresponded to 56% (AI) and 60% (MI) of the total fucose. The dialyzable fragments obtained by treatment III was added to 8 volumes of methanol and the methanol-soluble and -insoluble fractions were separated.   The methanol-soluble fraction consisted of fucose, mannose, glucose, and galactose in approximate molar ratio of 14:6:4:1 (AI) and of 15:8:1.2:1 (MI), and the component sugars of the methanol-insoluble material were mannose, glucose, and galactose (molar ratio of 20:2:1 in AI and of 20.2:1:2.6 in MI).   The final non-dialyzable material was composed of mannose, glucose, and galactose (molar ratio; 11:2:1, (AI), and 8:2:3, (MI)).

The minor polysaccharides, AII and AIII from *A. cylindrospora* and MII and MIII from *M. mucedo*, were also isolated by DEAE-cellulose chromatography.   AII, $[\alpha]_D$ +43.6° (c=1.1, H_2O), corresponding to 8.8% of the crude polysaccharides, had the same componental sugars as AI (molar ratio, fucose: mannose: glucose: galactose: N-acetylglucosamine: N-acetylgalactosamine=10:24:12:2:1:0.5), and AIII, corresponding to 3.4% of the crude polysaccharides, was composed of mannose, glucose, galactose, and glucuronic acid (molar ratio, 20:19:10:4) in addition to small amounts of fucose and xylose.   MII, corresponding to 9.1% of the crude polysaccharides, consisted of fucose, galactose, and glucuronic acid (molar ratio, 3:2:1), and MIII, $[\alpha]_D$ −65.0° (c=1.23, H_2O), corresponding to 12.4% of the crude polysaccharides, contained the same componental sugars as MII (molar ratio, fucose: galactose: glucuronic acid=6:3:2).

On the bais of these data, it seems reasonable to assume the discussion described below; (1) the main extracellular polysaccharides, AI and MI, are both highly complicated glycans composed of fucose, mannose, glucose, galactose, N-acetylglucosamine, and N-acetylgalactosamine, and their fucose contents are extremely higher than that in *R. nigricans*; (2) 56% and 60% of the total fucose and a small amount of other componental sugars of AI and MI are liberated by mild acid hydrolysis (0.01N sulfuric acid, 100°, 2 hr); (3) results of gradual acid hydrolysis suggest that combinations of the component sugars in AI and MI are quite complicated, but the final non dialyzable fragments and the methanol-insoluble materials, formed by 0.1N sulfuric acid treatment, are free from fucose; (4) on periodate oxidation, large portions of fucose and mannose are oxidized and about one half of the total hexosamine is also oxidized; (5) as the main linkages in AI and MI, (1→2) and (1→4)-linked hexopyranose residues would exist because large quantities of glycerol and tetrahydric alcohol were formed by the Smith degradation; (6) part of the componental sugars would exist in (1→3)-linkage or branching point because they are not completely oxidized by periodate; (7) main polysaccharides, AI and MI, not only contain the same compon ental sugars, but their behaviour on periodate oxidation and partial acid hydrolysis is similar: and (8) amounts of acidic glycans elaborated from *R. nigricans* (6.8%) and *M. mucedo* (21.5%) are larger than that in *A. cylindrospora* (3.4%).

It may be concluded that these polysaccharides have genus or species specifity.   At present, our investigation is limited to a comparison of gross structural features of the polysac-

charides in each of the above three groups as revealed by periodate oxidation and partial hydrolysis. At a later stage, more detailed structual examination will be made through their serological investigations and methylations.

Experimental

Isolation and Protease Digestion of Extracellular Polysaccharides——Large batches of *Absidia cylindrospora* were grown in Sabouraud medium (dialyzable polypeptone 1%, glucose 4%) at $25 \pm 2°$ in an incubator for 14 days, and 21 days in the case of *Mucor mucedo*. In order to obtain a filamentous form and less aerial spore-formation, the culture flasks were shaken once a day. The medium was separated from the mycelium by filtration through a Nylon cloth. The filtrate was dialyzed in a Visking Cellophane tubing against running water for 1 day. The internal solution was concentrated to a small volume and 3 volumes of EtOH was added to the concentrate. The precipitate that appeared was collected by centrifugation, washed with EtOH, acetone, and ether, and dried *in vacuo*. Yield, *ca.* 1.7 g/liter (*A. cylindrospora*) and 0.6 g/liter (*M. mucedo*).

Protease digestion was carried out as described previously[5] and followed by the Sevag method. Yield, *ca.* 66 mg/liter (*A. cylindrospora*) and 85 mg/liter (*M. mucedo*).

Separation and Purification of Crude Polysaccharides by DEAE-Cellulose Column Chromatography——1) *A. cylindrospora*: Crude polysaccharides were separated on a DEAE-cellulose column using H_2O, $NaHCO_3$, and NaOH. A solution of the material (1.0 g) in H_2O (15 ml) was applied to a column (3.2 × 45 cm) and stepwise elution was effected with H_2O, and 0.02M, 0.05M, 0.1M, 0.2M, and 0.5M $NaHCO_3$, and then with 0.1N NaOH. Each fraction was obtained according to the procedure described in an earlier paper.[5] Yields were as follows: H_2O eluate (Fraction 1), 528 mg (52.8%); 0.02M $NaHCO_3$ eluate (Fraction 2, AIII), 34.2 mg (3.4%); 0.05M, 0.1M, and 0.2M $NaHCO_3$ eluate, trace; 0.5M $NaHCO_3$ eluate (Fraction 3), 98 mg (9.8%); and 0.1N NaOH eluate (Fraction 4), 52.3 mg (5.2%).

The water-eluted polysaccharide (Fract. 1, 400 mg) in H_2O (10 ml) was submitted to a column (3.2 × 33 cm) of DEAE-cellulose (borate form). Stepwise elution was effected with H_2O, and 0.01M, 0.02M, 0.05M, and 0.1M $Na_2B_4O_7$, and with 0.1N NaOH. Each fraction was treated as described above (except the borate-eluted fractions which were acidified with AcOH to decompose borate complex and dialyzed against distilled water until free of borate ion). Yields were as follows: H_2O eluate (Fract. 1-1), 254.3 mg (64%); 0.01M $Na_2B_4O_7$ eluate (Fract. 1-2, AII), 67.0 mg (17%); and other fractions, trace.

The water-eluted polysaccharide (Fract. 1-1, 254.3 mg), corresponding to 33.4% of the crude polysaccharides, was dissolved in H_2O (2 ml) and centrifuged at 4000 rpm for 10 min. The supernatant was mixed with 4 volumes of EtOH. The white precipitate (AI) thereby formed was centrifuged, washed with acetone and ether, and dried *in vacuo*. Yield, 213.0 mg.

The 0.5M $NaHCO_3$-eluted polysaccharide (Fract. 3, 98 mg) n H_2O (5 ml) was chromatographed on a column (2 × 25 cm) of DEAE-cellulose (borate form) and treated as above. Yields were as follows: H_2O eluate (Fract. 3-1), 17.2 mg (17.5%); 0.1M $Na_2B_4O_7$ eluate (Fract. 3-2), 16.6 mg (17.1%); 0.1N NaOH eluate (Fract. 3-3), 17.8 mg (18.1%); and other fractions were nil. Uronic acid contents of these fractions were below 1.1%.

2) *M. mucedo*: A solution of the polysaccharide (850 mg) in H_2O (15 ml) was applied to a column (3.2 × 40 cm) of DEAE-cellulose and treated as described above. Yields were as follows: H_2O eluate (Fraction 1), 137.2 mg (16.1%); 0.02M $NaHCO_3$ eluate (Fraction 2), 46.9 mg (5.5%); 0.05M, 0.1M, and 0.2M $NaHCO_3$ eluate, trace; 0.5M $NaHCO_3$ eluate (Fraction 3), 374.4 mg (44.0%); and 0.1N NaOH eluate (Fraction 4), 56.0 mg (6.5%). The water-eluted polysaccharide (Fract. 1, 137.2 mg) in H_2O (5 ml) was submitted to a column (2 × 33 cm) of DEAE-cellulose (borate form). Each fraction was collected as above. Yields were as follows: H_2O eluate (Fract. 1-1), 115 mg (84%); 0.1N NaOH eluate (Fract. 1-2), 19.7 mg (14%); and other fractions, trace.

The water-eluted polysaccharide (Fract. 1-1, 115 mg), corresponding to 13.5% of the crude polysaccharides, was dissolved in H_2O (1 ml) and centrifuged at 4000 rpm for 10 min. The supernatant was added to 4 volumes of EtOH. The white precipitate (MI) thereby formed was centrifuged, washed with acetone and ether, and dried *in vacuo*. Yield, 101.1 mg.

The 0.5M $NaHCO_3$-eluted polysaccharide (Fract. 3, 370 mg) in H_2O (15 ml) was chromatographed on a column (3.2 × 30 cm) of DEAE-cellulose (borate form) and treated as above. Yields were as follows: H_2O eluate, trace; 0.02M $Na_2B_4O_7$ eluate (Fract. 3-1, MII), 77.9 mg (21%); 0.05M $Na_2B_4O_7$ eluate, trace; 0.1M $Na_2B_4O_7$ eluate (Fract. 3-2), 20.5 mg (5.5%); and 0.1N NaOH eluate (Fract. 3-3, MIII), 106.0 mg (28.6%).

Properties of AI and MI——Paper electrophoresis of AI and MI using 0.026M borate buffer (pH 10.0) showed a single spot (detected with the periodate-Schiff reagent[14]). Both AI and MI had max. IR absorptions 1640 and 1550 cm⁻¹ (acetamido group), respectively. AI, $[\alpha]_D$ +15.0° (c=0.66, H_2O), contained 0 8%

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nitrogen, no phosphorus, 88.7 % hexose (calculated from standard curve of the solution containing component sugars, by the $C_6H_5OH-H_2SO_4$[6]), and 5.8% hexosamine (as glucosamine, by the Blix method[7]). Reducing power of AI was 1: 0.007 (as fucose, by the Park-Johnson method[9]).

MI, $[\alpha]_D + 30.4°$ ($c = 0.95$, H_2O), contained 1.6% nitrogen, no phosphorus, 81.2% hexose, and 13.7% hexosamine. Reducing power of MI was 1: 0.01.

Quantitative Estimation of the Componental Sugars of AI, MI, and Other Fractions—— Each sample (ca. 5 mg/ml 1N H_2SO_4) was hydrolyzed in a sealed tube at 100° for 8 hr and treated as described in the previous experiment.[5] A portion of the hydrolysate was examined by the ascending paper chromatography on Toyo Roshi No. 50 filter paper, using the following solvent systems (v/v); (a) EtOAc–pyridine–H_2O (10: 4: 3), (b) BuOH–AcOH–H_2O (5: 1: 2), (c) AcOH–pyridine–AcOH–H_2O (5: 5: 1: 3). Sugars were detected by the spray reagents of $AgNO_3$–NaOH,[15] p-anisidine-HCl,[16] and ninhydrin.[17]

1) AI; fucose, mannose, glucose, galactose, glucosamine, and galactosamine: AII, $[\alpha]_D + 43.6°$ ($c = 1.1$, H_2O), with 5.5% hexosamine; fucose, mannose, glucose, galactose, glucosamine, and galactosamine: AIII; mannose, glucose, and galactose in addition to glucoronic acid and xylose.

2) MI; fucose, mannose, glucose, galactose, glucosamine, and galactosamine: MII; fucose, glucuronic acid, and galactose: MIII, $[\alpha]_D - 65.0°$ ($c = 1.23$, H_2O); fucose, galactose, and glucuronic acid.

The componental sugars of these fractions were estimated by the methods of Dubois, et al.[6] (for hexoses), Dische[10] (for methylpentose), Blix[7] (for hexosamines), Ludowieg[11] (for galactosamine), and Bitter-Muir[18] (for uronic acid), and their molar ratios were calculated.

Periodate Oxidation of AI and MI——1) AI (16.2 mg) was oxidized in 25 ml of 0.018M $NaIO_4$ in the dark at room temperature. A blank solution without the sample was prepared similarly. The consumption of $NaIO_4$ and the formation of HCOOH, HCHO, and CH_3CHO were determined with an aliquot of this solution by the procedures of Maraprade,[19] Whilstler,[20] O'dea-Gibbons, [13] and Annison.[21] The number of moles of $NaIO_4$ consumed per anhydro Sugar unit of AI was as follows: 0.61 (1 hr), 0.72 (3 hr), 0.80 (6 hr), 0.83 (12 hr), 0.92 (24 hr), 1.01 (48 hr), and 1.04 (72 hr). The value of HCOOH was 0.13 (1 hr), 0.16 (3 hr), 0.18 (6 hr), 0.21 (12 hr), 0.31 (24 hr), 0.38 (48 hr), and 0.40 (72 hr), and the value of CH_3CHO was 0.005 (2 hr), 0.009 (48 hr), and 0.006 (92 hr). HCHO was not produced.

2) MI (12.3 mg) was oxidized as described above. Consumption of $NaIO_4$: 0.64 (1 hr), 0.71 (3 hr), 0.82 (6 hr), 0.85 (12 hr), 0.91 (24 hr), 0.96 (48 hr), and 1.00 (72 hr). Formation of HCOOH: 0.06 (1 hr), 0.12 (3 hr), 0.16 (6 hr), 0.20 (12 hr), 0.28 (24 hr), 0.29 (48 hr), and 0.41 (72 hr). Formation of HCHO: 0.017 (1 hr), 0.022 (24 hr), and 0.022 (72 hr). CH_3CHO was not produced.

Smith-type Degradation of AI and MI——1) AI (10 mg) was oxidized with $NaIO_4$ as described above. To destroy the excess periodate, ethylene glycol (0.2 ml) was added after 48 hr and the solution was dialyzed against running water for 1 day. To the internal solution concentrated to a small volume was added $NaBH_4$ (ca. 15 mg) with continuous stirring overnight and then the excess $NaBH_4$ was decomposed by acidification with AcOH. The reaction mixture was dialyzed against distilled water for 2 days, evaporated to a syrup, and hydrolyzed with 0.2N H_2SO_4 (1 ml) at 100° for 6 hr. The syrup (SO_4^{2-} free) was obtained by the usual manner and paper chromatographic analysis of the hydrolysate using the solvent system (a) showed six spots corresponding to glycerol, erythritol, fucose (and threitol), mannose, glucose, and galactose. In order to estimate relative molar ratio of these main products, the syrup was spotted on a filter paper and multiple development was carried out 5 times with the solvent system (a), as described above. Hexoses, methylpentose, and polyhydric alcohol were determined by the methods of Dubois,[6] Dische,[10] and O'deaGibbons.[13] After oxidation of AI, hexosamine content was 3.02% (as glucosamine, by the Blix method[7]). Contents of the Smith degradation products were as follows (in μg): fucose (38), mannose (450), glucose (23.5), galactose (20), glycerol (344), and tetrahydric alcohol (78).

2) MI (10m g) was treated with $NaIO_4$ for 48 hr. Subsequent procedure was the same as described above. Six spots corresponding to glycerol, erythritol, fucose (and threitol), mannose, glucose, and galactose were detected as the main products on the paper chromatogram. After oxidation of MI, hexosamine content changed to 6.1%. Contents of the Smith degradation products were as follows (in μg): fucose (54), mannose (217), glucose (20), galactose (40), glycerol (506), and tetrahydric alcohol (122).

Gradual Acid Hydrolysis of AI and MI——1) AI (54.1 mg) was hydrolyzed with 0.01N H_2SO_4 (15 ml) at 90° for 1 hr and the hydrolysate was dialyzed against distilled water (400 ml) for 24 hr. This dialysis procedure was performed 3 times. The external solution (1200 ml) of the hydrolysate was evaporated to about 20 ml in vacuo. After neutralization with $BaCO_3$ and filtration, it was concentrated to a syrup. Paper

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21) E.F. Annison and W.T.J. Morgan, *Biochem. J.*, 50, 460 (1952).

chromatographic examination of the syrup using the solvent system (a) showed fucose, mannose, glucose, and galactose (molar ratio, 180: 6: 5: 4). The internal solution was concentrated to dryness and weighed.

A part of the non-dialyzable material was heated with 1N H$_2$SO$_4$ at 100° for 7 hr, and treated as described above (molar ratio of fucose: mannose: glucose: galactose=8: 25: 4: 2). Other part of the non-dialyzable material (38.8 mg) was hydrolyzed with 0.01N H$_2$SO$_4$ (15 ml) at 100° for 2 hr. The dialyzable fragments from the hydrolysate, which was afforded by the same procedure as above, gave four components, fucose, mannose, glucose, and galactose (molar ratio, 300: 7: 6: 3).

The non-dialyzable material (29.7 mg), containing fucose, mannose, glucose, and galactose in approximate molar ratio of 4.4: 25: 4: 2, was heated with 0.1N H$_2$SO$_4$ (12 ml) at 100° for 3 hr. The external solution of the hydrolysate was concentrated to a small volume, neutralized with BaCO$_3$, and filtered. The filtrate (free from barium ions) was evaporated to a syrup and 8 volumes of EtOH was added to the syrup. The precipitate that appeared was separated by centrifugation and washed 7 times with MeOH (8 ml). The sugar content of MeOH-soluble fragments was 7.0 mg (as mannose, by the C$_6$H$_5$OH–H$_2$SO$_4$ method) and that of the MeOH-insoluble material was 11.5 mg. The MeOH-soluble fragments were examined by paper chromatography using the solvent system (a). Presence of fucose, mannose, glucose, and galactose was revealed in a molar ratio of 14: 6: 4: 1 in addition to small amounts of oligosaccharides. After hydrolysis of the MeOH-insoluble material (ca. 1.5 mg) with 1N H$_2$SO$_4$ (1 ml) at 100° for 6 hr, mannose, glucose, and galactose (molar ratio, 20: 2: 1) were detected in addition to small amounts of glucosamine and galactosamine. The material (11.2 mg) in the internal solution consisted of mannose, glucose, and galactose (molar ratio, 11: 2: 1) in addition to small amounts of glucosamine and galactosamine.

2) MI (20.4 mg) was heated with 0.01N H$_2$SO$_4$ (15 ml) at 90° for 1 hr and the fragments released were separated by dialysis as described above. The dialyzable fragments were composed of fucose and a small amount of hexosamine. A part of non-dialyzable material was hydrolyzed with 1N H$_2$SO$_4$ at 100° for 7 hr (molar ratio of the released sugars, fucose: mannose: glucose: galactose=8.9: 25: 2: 3.5) and the remain (12.9 mg) was treated with 0.01N H$_2$SO$_4$ (12 ml) at 100° for 2 hr. The dialyzable fragments from the hydrolysate gave four main components, fucose, mannose, glucose, and galactose (molar ratio, 80: 4: 3: 2). The non-dialyzable material (10.5 mg), containing fucose, mannose, glucose, and galactose in approximate molar ratio of 4: 25: 2: 3.5, was hydrolyzed with 0.1N H$_2$SO$_4$ (10 ml) at 100° for 3 hr. The external solution of the hydrolysate was evaporated to a small volume, neutralized with BaCO$_3$, and filtered. The filtrate freed from Ba^{2+} was concentrated to a syrup and the syrup was mixed with 8 volumes of EtOH. The precipitate thereby formed was washed 7 times with MeOH, and the supernatant, the MeOH-soluble fragments, was examined as above. Four main spots corresponding to fucose, mannose, glucose, and galactose were revealed in a molar ratio of 15: 8: 1.2: 1. After hydrolysis of the MeOH-insoluble material (3.3 mg) with 1N H$_2$SO$_4$ (1 ml) at 100° for 6 hr, mannose, glucose, and galactose were detected in a molar ratio of 20: 2.1: 2.6 in addition to small amounts of glucosamine and galactosamine. The non-dialyzable material (4.0 mg) consisted of mannose, glucose, and galactose (molar ratio, 8: 2: 3) in addition to small amounts of glucosamine and galactosamine.