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Plant Mucilages. IV.[1] Main Structural Features of the Mucous Polysaccharide isolated from *Digenea simplex*.*

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As the constituents of *Digenea simplex* Agardh, α-kainic acid,[2] succinic acid,[2] sodium α-D-mannosido-D-glycerate,[3a,b] α-allokainic acid,[4] fifteen amino acids and several volatile organic acids[5] have been reported until present time. Although it has been known that the seaweed contains a mucilage, the structure of the polysaccharide in the seaweed was still unknown. We can see only a few reports[5,6] on the component sugars of the mucilage, and they showed the presences of D-galactose, D-xylose and a Seliwanoff's reaction positive substance.

The structural study described in this paper gives the evidence that the mucilage isolated from *Digenea simplex* belongs to agar-type polysaccharides, and this seaweed should be added to the agar-producing group in Rhodophyceae, in addition to species of *Gelidium*, *Gracilaria*, *Acanthopeltis*, *Ahnfeltia*, *Ceramium*, *Campylaephora*, *Phyllophora*, and *Pterocladia* spp.[7]

After repeated washing with water to the dried seaweed, the residue was extracted with hot water. The extract was treated with freezing and thawing method to yield grayish white fibrous flakes. The yield of the mucilage was 5.5%.

The complete methanolysis of the mucilage was accomplished by heating with 3% methanolic hydrogen chloride for thirty hours. The methanolysate was subjected to saponification with barium hydroxide and then treated with ion-exchange resins in succession for the removal of sulfuric acid residue. The acidic substance adsorbed by the anion-exchange resin was eluted with excess of acid and isolated as a barium salt. The salt was proved to be barium methylsulfate derived from sulfate ester in the mucilage.

The neutral methanolysate was analyzed by thin-layer chromatography, and 3,6-anhydro-L-galactose dimethylacetal, methyl D-galactopyranoside and methyl D-xylopyranoside were detected. On the one hand, the methanolysate was converted into trimethyl-

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1) Part III: M. Tomoda and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **20**, 778 (1972).
2) S. Murakami, T. Takemoto, and Z. Shimizu, *Yakugaku Zasshi*, **73**, 1026 (1953).
3) a) K. Kawaguchi, S. Yamada, and S. Miyama, *Nippon Suisangaku Kaishi*, **19**, 481 (1953); b) S. Murakami, T. Takemoto, and Z. Shimizu, *Yakugaku Zasshi*, **73**, 1028 (1953).
4) S. Murakami, T. Takemoto, Z. Tei, K. Daigo, and N. Takagi, *Yakugaku Zasshi*, **75**, 766, 1252 (1955).
5) Y. Takao, *Yakugaku Zasshi*, **38**, 507 (1918).
6) H. Watanabe and T. Takano, *Yakugaku Zasshi*, **73**, 529 (1953).
7) E. Percival and R. H. McDowell, "*Chemistry and Enzymology of Marine Algal Polysaccharides*", p. 127 (Academic Press, London and New York, 1967).

silyl derivative and analyzed by gas-liquid chromatography. The result also showed the presences of the same three component sugar derivatives. Determination of components showed that the mucilage is consisted of 43.8% of 3,6-anhydro-L-galactose, 43.6% of D-galactose, 3.0% of D-xylose, and 3.3% of sulfuric acid residue. We observed small amounts of insoluble residue after methanolysis of the mucilage. This may be cause some lowering of the measured values of component sugars.

To clarify the linkage form in the main part of mucilage molecule, the partial methanolysis of the material was carried out by heating with 0.5% methanolic hydrogen chloride for two hours. The partial methanolysate was treated with barium hydroxide and ion-exchange resins as described above, and the neutral part was applied to an active charcoal column chromatography. In addition to 3,6-anhydro-L-galactose dimethylacetal, methyl D-galactoside and methyl D-xyloside, agarobiose dimethylacetal was obtained in a good yield (45.3%) from the starting material. Crystalline agarobiose dimethylacetal was identified by comparison with the authentic sample prepared from agar.[8] These results gave the evidence that agarobiose represents the chief repeating unit of the mucilage molecule like the structure of agarose.[9]

The mucilage was acetylated by heating with pyridine and acetic anhydride, then the acetate was extracted with chloroform. Chloroform-soluble product was precipitated

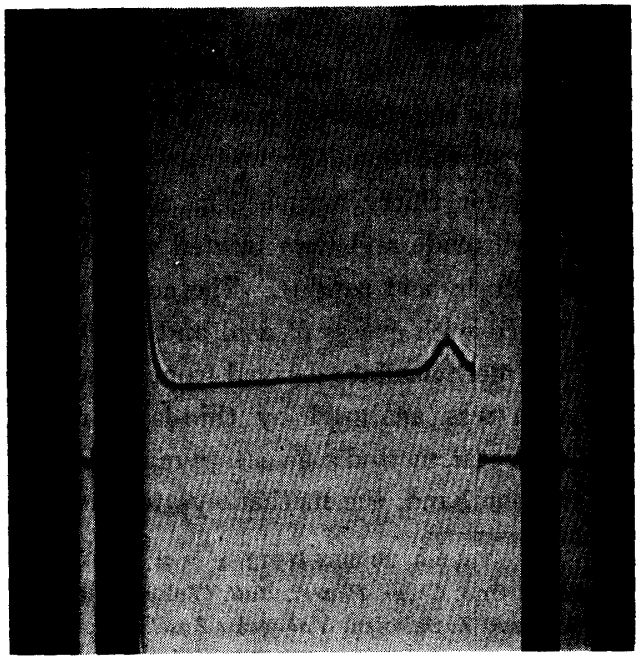

Fig. 1. Ultracentrifugal Pattern of Polysaccharide (Digenea-agarose)
0.5% dimethyl sulfoxide solution, 24°, 180 min, Hitachi model UCA-1A ultracentrifuge (60000 rpm)

8) C. Araki and S. Hirase, *Bull. Chem. Soc. Japan*, **27**, 109 (1954).
9) C. Araki, *Bull. Chem. Soc. Japan*, **29**, 543 (1956).

by addition of petroleum ether, and the dried precipitate was deacetylated by successive treatment with 1N ethanolic potassium hydroxide and 0.5N potassium hydroxide. After neutralization, the alkali-insoluble part was treated with freezing and thawing method to yield white fibrous mass. The product occupied the main part of the mucilage, and it was found to be a homogeneous polysaccharide by the ultracentrifugal analysis (Fig. 1).

Determination of components showed that the polysaccharide is consisted of 49.6% of D-galactose, 48.8% of 3,6-anhydro-L-galactose, 1.2% of D-xylose and 0.5% of sulfuric acid residue.

The polysaccharide yielded 86.9% of agarobiose dimethylacetal by partial methanolysis under the condition of heating at 70° with 0.5% methanolic hydrogen chloride.

After methylation with sodium hydride and methyl iodide in dimethyl sulfoxide,[10] methylated polysaccharide was methanolyzed and the methanolysate was analyzed by gas-liquid chromatography and thin-layer chromatography. Methyl glycosides of 2,3,4,6-tetra-O-methyl-D-galactose, 2,4,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose, and 2-methyl-3,6-anhydro-L-galactose were identified in addition to a few unknown minor products.

From the results of partial methanolysis and methylation studies, it is able to conclude that the main structure of the mucilage consists of alternately repeated units of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose.

Thus the main part of the mucilage has agarose-type structure like agar, but 6-methyl D-galactose, D-glucuronic acid and pyruvic acid, which have been reported as components of agar,[11] were not found. We also obtained the evidences of the presences of 1,4-linked D-xylose and sulfate ester in part of the polysaccharide. The name "Digenea-agarose" is proposed for the homogeneous polysaccharide isolated from the mucilgae. Methylation study showed that the non-reducing terminal of agarose type chain of this polysaccharide is D-galactose. This is a different structural conclusion from the other normal agarose.

10) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

11) a) S. Hirase and C. Araki, *Bull. Chem. Soc. Japan*, **34**, 1048 (1961); b) S. Hirase, *Bull. Chem. Soc. Japan*, **30**, 68 (1957).