CHEMICAL STUDIES ON THE GREEN ALGA,
MONOSTROMA NITIDUM WITTROCK—I.
COMPONENT SUGARS OF THE MUCILAGE (PART 1)

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Among the green algae, *Monostroma nitidum* is called as "HITOEAOA" and used for food in Japan. The main component of the alga is mucilage. However, there is no information of the chemical component of the mucilage of *Monostroma nitidum*. In this connection, it has been known that the mucilage of the other green algae is a sort of sulphated polysaccharide¹⁾⁻⁵⁾.

In the present work, component sugars of the mucilage of *Monostroma nitidum* were examined by paper chromatography and some specific colour reactions. It was thus confirmed that the mucilage contains xylose (1 part), galactose (4.2 parts), galacturonic acid (5 parts) and rhamnose (11.5 parts).

Experimental and Results

1. Material.

The thalli of *Monostroma nitidum* were collected at Hatsukaichi-chō, Saiki-gun, Hiroshima, in April 1960. The thalli were thoroughly washed with tap water and dried in the sun. The chemical composition was estimated by the usual method. The result is shown in Table 1.

2. Preparation of Mucilage.

The alga (600 g.) was extracted with 12 l. of cold water under occasionally stirring for 48 hours and then filtered with a three-fold gauze. The filtrate was concentrated under a reduced pressure at below 40°C. to about 400 ml.. Then, the mucilage was precipitated as barium salt with barium chloride and ethanol by the method used by MORI et al.⁶⁾ in their study of

Table 1. Chemical composition of
Monostroma nitidum.

Item	% on dry matter
Total nitrogen	2.47
Crude protein	15.44
Crude fat	0.57
Crude fiber	6.54
Crude ash	15.04
Nitrogen free ext.	62.23
Reducing sugar (as galactose)	37.45
Total S	5.55
Si	0.39
Ca	0.59
Mg	0.28

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carragheenin. The precipitate was redissolved in a small amount of hot water and precipitated by the same manner. The treatment was repeated three times and the precipitate was dissolved in hot water. The mucilage solution was treated twice with charcoal to remove any colouring matters. Finally, the mucilage was precipitated twice with ethanol. The mucilage obtained, in the form of barium salt was collected by filtration, washed thoroughly with ether and then dried over sulphuric acid in a vacuum desiccator. The yield was about 1.7% of the air-dried alga. The barium salt of mucilage obtained was a white-greyish powder. The chemical composition of the preparation is shown in Table 2.

Table 2. Composition of the mucilage (barium salt) of *Monostroma nitidum*.

Impurity %		% on water and crude protein free basis						
Water	Crude protein on water free basis	Ash	SO ₄ in ash	Ba	Total S	SO ₄ calcd.	Total sugar as rhamnose	Ba+SO ₄ +Total sugar
7.76	1.81	32.18	14.6	23.9	10.4	31.2	42.7	97.8

Hereon, the total sugar was determined, after hydrolysis with 3% hydrochloric acid for 5 hours, by the FEHLING-LEHMAN-SCHOORL method. As the same time, the total sulphur was determined as barium sulphate with an aliquot portion of the hydrolysate.

It is noteworthy that while any sulphate could not be detected until after hydrolysis, sulphate content, 31.2%, and that calculated from the ash, 14.6%, agrees with the ratio 2:1, as known to be characteristic of polysaccharide ethereal sulphates.⁷⁾ Therefore, sulphate in the mucilage is undoubtedly ethereally linked.

3. Detection of Component Sugars by Colour Reactions.

Component sugars of the mucilage were liberated on acid hydrolysis. They were detected by the following colour reactions. The results are as follows:

Pentose reaction (Resorcinol, Aniline acetate, and Phloroglucinol reactions)——Positive.

Methylpentose reaction (Acetone, and Phloroglucinol reactions)——Positive.

Ketose reaction (Selliwanoff's reaction)——Negative.

(Ammonium molybdate reaction)——Positive.

Hexuronic acid reaction (Naphthoresorcinol reaction)——Positive.

Hence, no anhydro- or ketose sugar residue in the hydrolysate and in the original mucilage was indicated by a negative Selliwanoff's reaction.

4. Fractionation of Component Sugars by Ion-exchange Resins.

According to the method of HASHIMOTO et al.⁸⁾ and NAKAMURA,⁹⁾ component sugars of the mucilage were fractionated by means of ion-exchange resins. The procedures are as follows:

The mucilage (5 g.) was hydrolysed by heating at 100°C. with N-sulphuric acid

(150 ml.) for 6 hours. The hydrolysate was neutralized to pH 7.2 with saturated barium hydroxide solution. Then, solid barium carbonate (0.8 g.) was suspended, and the mixture was warmed at 60°C. for 15 minutes to hydrolyse uronic acid lactones. After filtration, the filtrate was concentrated at below 40°C. *in vacuo* to about 50 ml.. The concentrated solution was passed through Amberlite CG-120 (H⁺ form) to remove Ba⁺⁺, and then the effluent was passed through Amberlite CG-400 (CO₃⁻ form) to remove uronate anions. The effluent was condensed to a syrup *in vacuo* (neutral sugar fraction).

The acid substance was eluted from CG-400 resin with 0.5 N-sulphuric acid, and the eluate was neutralized with barium hydroxide. After filtration, the filtrate was passed through CG-120 resin, and the effluent was condensed to a syrup *in vacuo* (acid sugar fraction).

The scheme of the aforementioned procedures is outlined in Fig. 1.

Fig. 1. Fractionation of hydrolysate of the mucilage (barium salt) by ion-exchange resins.

* The eluate was neutralized with barium hydroxide and then filtered. The filtrate was passed through Amberlite C G-120 to remove Ba⁺⁺.

5. Separation and Identification of Neutral Sugars by Paper Chromatography.

A small portion of the neutral sugar fraction was taken on a sheet of Toyo Roshi No. 50 filter paper (40×40 cm.). As control, some authentic sugars were corsspotted on the same paper. The sugars were developed by one-dimensional ascending method with a solvent system, n-butanol-acetic acid-water (4:1:2). The development was repeated twice with the same solvent. Spraying reagent used was 3% p-anisidine hydrochloride in n-butanol.

Paper chromatography revealed the presence of three components with Rf values of 0.56, 0.39 and 0.25, respectively, as tabulated in Table 3. Hence, the sugars in the neutral sugar fraction were identified as rhamnose, xylose and galactose by the comparison to Rf values of authentic sugars.

Table 3. Paper chromatographic data of the neutral sugar fraction of the mucilage and authentic specimens.

Sugars		Rf	Colour of Spot
Authentic specimens	Rhamnose	0.56	Yellow
	Xylose	0.40	Cherry red
	Arabinose	0.36	"
	Fructose	0.37	Yellowish brown
	Glucose	0.27	Brown
	Galactose	0.25	"
Neutral sugar fraction	Upper	0.56	Yellow
	Middle	0.39	Cherry red
	Lower	0.25	Brown

Method: Ascending (twice-development), at room temperature, for about 32 hours.

Filter paper: Toyo Roshi No. 50 (40×40 cm.).

Solvent: n-Butanol-acetic acid-water (4:1:2).

Spraying reagent: 3% p-anisidine hydrochloride in n-butanol.

6. Examination of Acid Sugar by Paper Chromatography.

The acid sugar fraction was examined by paper chromatography in the same manner as previously mentioned. Only one spot was made visible on the paper with the spraying reagent. The Rf value and colour of the spot were in good agreement with those of authentic galacturonic acid as tabulated in Table 4. Therefore, the

Table 4. Paper chromatographic data of the acid sugar fraction of the mucilage and authentic specimens.

Sugars		Rf	Colour of Spot
Authentic specimens	Glucurone	0.51	Cherry red
	Glucuronic acid	0.20	"
	Barium glucuronate	0.18	"
	Galacturonic acid	0.22	"
Acid sugar fraction		0.23	Cherry red

Chromatographic method is the same as in Table 3.

component in the acid sugar fraction is considered to be identical with galacturonic acid.

7. Examination of Acid Sugar by Specific Colour Reactions.

For examination of further components in the acid sugar fraction, the following specific colour reactions were employed; A) WILLIAMS' reaction¹⁰⁾ for hexuronic acid, B) DISCHE's thioglycolic acid reaction¹¹⁾ for glucuronic acid, and C) DISCHE's cysteine reaction¹²⁾ for galacturonic acid.

The position of absorption maximum and the shape of curve were measured with SHIMAZU QB-50 spectrophotometer.

NAKAMURA¹³⁾ reported that among the reactions he tested for the detection of glucuronic acid or its lactone, glucurone, the WILLIAMS reaction was most reliable as

Fig. 2. Absorption spectra of the WILLIAMS reaction of uronic acids.
I: Glucurone. II: Acid sugar fraction.

well as most sensitive and that the presence of this compound in the acid sugar fraction of *Euchuma muricatum* was clearly identified by the reaction.

Accordingly, the WILLIAMS reaction was carried out on the acid sugar fraction prepared in Experiment 4. The sample had two absorption peaks at 510 and 600 $m\mu$, which were in good agreement with these of authentic glucurone as illustrated in Fig. 2. Thus the presence of hexuronic acid was confirmed. However, it is difficult to draw from the above result a definite conclusion that acid sugar in this fraction corresponds to glucuronic acid, since the WILLIAMS reaction using naphthoresorcinol as colour developing reagent is considered to be common to all hexuronic acid.

Subsequently, in order to decide whether the hexuronic acid is identical with glucuronic acid or galacturonic acid, the DISCHE's reaction was carried out.

The thioglycolic acid reaction was not replied by a characteristic curve with the sample, while authentic glucurone showed an absorption peak at 510 $m\mu$. On the other hand, the sample and authentic galacturonic acid showed identical absorption curves having absorption maxima equally at 410 $m\mu$. The result is illustrated in Fig. 3.

Fig. 3. Absorption spectra of the thioglycolic acid reaction of uronic acids.
I: Glucurone. II: Galacturonic acid. III: Acid sugar fraction.

The cysteine reaction gave a characteristic curve having a maximum peak at 600 $m\mu$, which was identical with that given by authentic galacturonic acid as illustrated in Fig. 4.

Fig. 4. Absorption spectra of the cysteine reaction of uronic acids.

I: Galacturonic acid. II: Acid sugar fraction.

Taus the presence of galacturonic acid in the acid sugar fraction was confirmed by the DISCHE's thioglycolic acid and cysteine reactions.

8. Quantitative Paper Chromatography of Sugars of the Mucilage.

According to the method of FLOOD et al.,¹⁴⁾ sugars of the mucilage were quantitatively separated on a paper chromatogram.

The procedures are as follows:

The mucilage (123.7 mg.) was hydrolysed by boiling for 6 hours with N-sulphuric acid (6 ml.) in a sealed ampule. After cooling, the contents were washed into a solution of fructose (32.7 mg.) in water. A foreign sugar, fructose, served as reference sugar. The solution was neutralized with barium carbonate and filtered, and the filtrate was condensed to a syrup *in vacuo*. An aliquot of the syrup was placed on a sheet of filter paper. After development the sections of paper containing the sugar were cut off into pieces which were eluted, in turn, with 20 ml. of boiling water for 45 minutes. Each sugar eluted was colorimetrically determined by the phenol-sulphuric acid method¹⁵⁾⁻¹⁶⁾.

In the case of (A) rhamnose, xylose and galacturonic acid, 2 ml. of the solution (20-70 μ g. of sugar) was added with 0.1 ml. of 5% aqueous phenol and 5 ml. of 96% sulphuric acid, and was heated at 100°C. for 3.5 minutes. On the other hand, in the case of (B) galactose and fructose, 2 ml. of the solution (20-70 μ g. of sugar) was added with 0.7 ml. of 5% aqueous phenol and 5 ml. of 96% sulphuric acid, and was heated

at 100°C. for 6 minutes. The optical density of the yellow solution was measured at 480 m μ (A) and 490 m μ (B), respectively, against a blank prepared by extracting a sugar-free piece of paper from the same chromatogram.

The sections of paper containing the sugars were thus found to respectively contain rhamnose, 11.875, xylose, 1.050, galactose, 4.270, galacturonic acid, 5.100, and fructose, 13.625 mg.. The absolute amount of each sugar in the original mucilage was calculated from these figures, complete recovery of fructose being assumed. The results are summarized in Table 5. The results showed that the mucilage contains xylose (1 part), galactose (4.2 parts), galacturonic acid (5 parts), and rhamnose (11.5 parts).

Table 5. Composition of component sugars of the mucilage.

Sugars	Calcd. as	Amount, mg	Proportion	%	*% in the dried mucilage
Rhamnose	C ₆ H ₁₀ O ₄	25.371	11.5	52.95	22.67
Xylose	C ₅ H ₈ O ₄	2.218	1.0	4.63	1.98
Galactose	C ₆ H ₁₀ O ₅	9.223	4.2	19.25	8.24
Galacturonic acid	C ₆ H ₈ O ₆	11.104	5.0	23.17	9.92
Sum		47.916		100.00	42.81

* On water and crude protein free basis.

Summary

Mucilage of *Monostroma nitidum* was analyzed. Component sugars of the mucilage were divided by ion-exchange resist into neutral and acid sugar fractions. The component sugars in each fraction were examined by paper chromatography.

From the results, it was confirmed that the neutral sugar fraction consists of rhamnose, xylose, and galactose, and that the acid sugar fraction galacturonic acid.

In a further examination, a hexuronic acid was detected in the acid sugar fraction by the WILLIAMS reaction. The acid sugar was confirmed to be galacturonic acid by the DISCHE's thioglycolic acid and cysteine reactions.

Quantitative paper chromatographic analysis showed that the mucilage contains xylose (1 part), galactose (4.2 parts), galacturonic acid (5 parts), and rhamnose (11.5 parts).

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