CHEMICAL STUDIES ON THE GREEN ALGA, *MONOSTROMA NITIDUM* WITTROCK—II. LOW-MOLECULAR CARBOHYDRATES IN THE ALGA

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On the constituents of low-molecular carbohydrate fraction of green algae, a few reports have been presented¹⁻³⁾. However, there is no information of those in *Monostroma nitidum*.

In the present experiments, the low-molecular carbohydrate fraction which has been extracted with 80 per cent ethanol from the alga were analysed.

Experimental and Results

1. Material.

The alga used in this study was the same as that described in the previous paper⁴⁾.

2. Extraction of Sugars and Clarification of the Extract.

The procedures were carried out by the following methods⁵⁻⁶⁾ which are generally used.

Preparation of Sample for Paper Chromatography: Sugars were extracted in a SOXHLET apparatus with 80 per cent ethanol from the ground alga (50 g.). The extract was taken off occasionally to avoid prolonged heating of it during the extraction. Then all the extracts were condensed to a small volume *in vacuo*, treated with neutral lead acetate and filtered into a beaker containing solid sodium oxalate. After removal of lead oxalate, the filtrate was decolorized with active carbon, and then de-ionized by passage through a column of Amberlite IR-120 (H⁺), followed by passage through Amberlite IRA-400 (CO₃''). The de-ionized extract was condensed to a syrupy liquid *in vacuo*.

Preparation of Sample for Determination of the Sugar Contents: Sugars were extracted with 80 per cent ethanol (100 ml.) from the ground alga (10 g.) on a water bath (85°C.) for 2 hours. The extraction was repeated five times. After evaporation of the solvent, the extract was transferred quantitatively into a volumetric flask and cooled to room temperature. Then the solution was treated with neutral lead acetate and diluted to mark with distilled water. After standing for 10 minutes, the solution was filtered through a dry filter paper into a beaker containing solid sodium oxalate. To the filtrate a few drops of toluene were added and was allowed to stand overnight in a refrigerator. Then the precipitated lead oxalate was removed by filtration. The

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cleared sugar solution thus obtained was subjected to determination of the sugar contents.

3. Identification of Sugars by Paper Chromatography.

Method of paper chromatographic analysis was the same as that described in the previous paper⁴⁾ except the colour reagent used. In this experiment, the following colour reagents were employed; ammoniacal silver nitrate solution, phloroglucinol-HCl ethanol solution and resorcinol-HCl butanol solution.

Paper chromatography was carried out on an aliquot of the syrupy liquid prepared in Experiment 2. Then three spots with Rf values of 0.31, 0.25 and 0.15 could be visualized on the chromatogram by the colour reagents. They were identified as fructose, glucose and sucrose, respectively, by the comparison to Rf values and colour of spots of authentic sugars. On the other hand, for detection of the component sugars of oligosaccharide, an aliquot of the preparation was hydrolysed with hydrochloric acid (final concentration 0.1%) at 100°C. for 30 minutes. The hydrolysate obtained was chromatographed on a sheet of filter paper, and then only two spots corresponding to fructose and glucose were found. The results are summarized in Table 1.

Table 1. Paper chromatograms* of low-molecular carbohydrates in *Monostroma nitidum*.

Sugars	Spots	Rf	Colour of Spot developed by			Corresponding to
			A	B	C	
Low-molecular carbohydrates	Spot 1	0.31	Dark brown	Orange brown	Pale red	fructose
	Spot 2	0.25	Dark brown	Colourless	Colourless	glucose
	Spot 3	0.15	Colourless	Orange brown	Pale red	sucrose
Sugars in hydrolysate**	Spot 1	0.31	Dark brown	Orange brown	Pale red	fructose
	Spot 2	0.25	Dark brown	Colourless	Colourless	glucose

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

Solvent: n-butanol-acetic acid-water (4:1:2 by volume).

Colour reagents: (A) ammoniacal silver nitrate solution, (B) phloroglucinol-HCl ethanol solution, and (C) resorcinol-HCl butanol solution.

Development method: Multiple ascending method.

**Sugars were hydrolysed with 0.1 per cent hydrochloric acid at 100°C. for 30 minutes.

4. Determination of the Sugar Contents.

On the sugar solution prepared in Experiment 2, determination of the sugar contents was carried out by the following methods.

Total Sugar and Free Reducing Sugar: For determination of the total sugar, hydrolysis was carried out on 50 ml. of the preparation with 5.55 ml. of 1 per cent hydrochloric acid at 100°C. for 30 minutes. The total sugar and the free reducing sugar were determined as glucose by the FEHLING-LEHMAN-SCHOORL method.

Fructose: By the camphor-sulphuric acid reaction⁷⁾, fructose was colorimetrically

determined. Namely, a mixture of 2 ml. of the preparation and 3 ml. of 1 per cent solution of camphor in dilute sulphuric acid (3:1) was heated at 60°C. for 30 minutes. After cooling, the absorption of the reaction mixture (pink in colour) was measured at 510 m μ with SHIMAZU QB-50 spectrophotometer.

Glucose: The method of HIRST, HOUGH and JONES⁸⁾, a modification of WILLSTÄTTER-SCHUDEL method, was adopted for the direct determination of glucose. However, the results obtained was unsatisfactory as shown in Table 2. Therefore, the glucose value was calculated by deducting the fructose value from the free reducing sugar value.

Sucrose: For convenience sake, the difference between the free reducing sugar value and the total sugar value was regarded as the sucrose value. The results are shown in Table 2.

Table 2. Contents of low-molecular carbohydrates in *Monostroma nitidum*.
(% on water free basis)

Fructose*	Glucose	Sucrose	Free reducing sugar** as glucose	Total sugar** as glucose	Aldose*** as glucose
0.45	0.44	1.27	0.89	2.16	3.27

*Determined by the camphor-sulphuric acid reaction.

**Determined by the FEHLING-LEHMAN-SCHOORL method.

***Determined by the modified WILLSTÄTTER-SCHUDEL method.

Discussion

TSURUGA et al.²⁾ found fructose, glucose and sucrose as the constituents of low-molecular carbohydrate fraction in *Enteromorpha intestinalis*. ENDÔ³⁾ found only glucose in *Codium lactum*, and only fructose in *Cladophora Wrightiana*. Hence, it may be mentioned that the distribution of sugars in *M. nitidum* resembles to that of *E. intestinalis*. However, the former's reducing sugar value is about twice higher than the latter's. On the other hand, the author's preparation did not contain uncommon sugars such as mannitol and meso-inositol which were found in *E. compreseae*, and maltose and maltotriose found in *Chlorella*¹⁾. It is of interest biochemically and physiologically that each alga shows significant differences in kind or content of the low-molecular carbohydrates.

SUZUKI⁹⁾ determined aldose by the hypiodite method, and total sugar by the BERTRAND method in the hydrolysates of several algae, and also MORI et al.¹⁰⁾ in the hydrolysates of mucilage of *Chondrus ocellatus*. In these algae, the aldose value was higher than the total sugar value. From this fact they suggested that the unknown substance which strongly reduces iodine used as the reagent is contained in these hydrolysates. The similar fact was recognized in the present work, although determination of the aldose content was carried out on the extract of the alga. That is to say, the aldose value is about 50 per cent higher than the total sugar value as shown

in Table 2. Therefore, attempt to estimate the fructose content by the difference method failed.

Summary

Low-molecular carbohydrate fraction in *Monostroma nitidum* was analysed, and the following results were obtained:

1. Fructose, glucose and sucrose were found by means of paper chromatography; fructose and glucose were found also as the products of hydrolysis of sugars.
2. In the alga, 0.45 per cent of fructose, 0.44 per cent of glucose and 1.27 per cent of sucrose were included.

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