Chemical Studies on Polyuronides. VI.[1] *On the Mucilage of Nori-utsugi Plant, Hydrangea Paniculata, Sieb. (1)*[2]

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Introduction

Nori-utsugi (*Hydrangea paniculata*, Sieb.), a species of Saxifragaceae, is a wild shrub growing widely in Japan. It contains a great amount of mucilage in the inner bark. The mucilagenous solution extracted from it with water, like that of the Tororo-aoi plant (*Abelmoschus Manihot*, Medic.), has been commonly used for the traditional paper-marking in Japan from olden times. In some cases the solution is said to have rather better properties than that of the Tororo-aoi plant, since the viscosity of the solution is not so easily decreased by stirring or heating. That is, the solution is suitable for paper-making in summer. These properties are due not only to the dispersing condition or molecular shape of the mucilage in the solution, but also to the chemical structure of the mucilage.

As the mucilage of the Tororo-aoi plant has been chemically studied in detail by the authors[1], the chemical structure of that of the Nori-utsugi plant was studied for purposes of comparison with it.

Formerly, Sawamura[3] found that the mucilage is a kind of polysaccharide, and Hara[4] showed that it is a galactoaraban. Komatsu[5] reported galactose, arabinose and rhamnose among the component sugars.

But the authors presumed that the mucilage is a polyuronide, seeing that the mucilagenous solution has very high viscosity and thread-forming ability even in low concentration. The chemical structure of the polyuronide was also studied.

Experimental Results and Discussion

1. Materials.—The Nori-utsugi plant grown in Funagata-cho, Yamagata Prefecture was employed in the present study.

2. Crude Mucilage.—The inner bark (100 g.) stripped from the plant was extracted twice with cold water (1 *l*) for a day; the liquid collected was then filtered through linen. Using a portion of the clear solution, the viscosity was measured by an Ostwald's viscosimeter at 20°C and the re-

1) Part V: S. Machida and N. Uchino, *J. Chem. Soc. Japan*, **74**, 615 (1953). Works on this subject are reviewed by S. Machida and N. Uchino in "A Summary of Chemical Studies on Polyuronides Part I.," *Bull. of the Faculty of Textile Fibers, Kyoto Univ. of Ind. Arts & Text. Fibers*, **1**, 116 (1954), written in English.

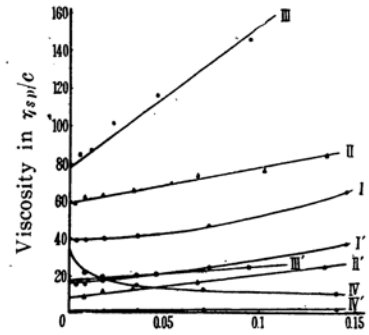
2) Read at the Ordinary Meeting of the Kinki Branch of the Chemical Society of Japan on May29, 1954.

3) M. Sawamura, *J. Chem. Soc. Japan*, **17**, 174 (1896); *Mem. Coll. Agri. Tokyo Imp. Univ.*, **5**, 259 (1902).

4) R. Hara, *Shigyo-zasshi*, **12**, No. 8, p. 1 (1918).

5) S. Komatsu, et al., *Mem. Coll. Sci. Kyoto Imp. Univ.*, **8**, 51 (1925).

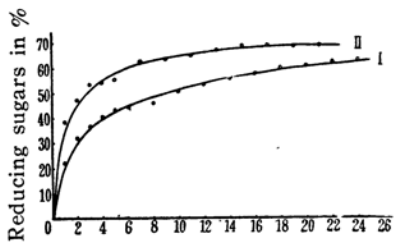
sults were shown in curve I in Fig. 1. The solution remaining was poured into acetone (ca. 2 *l*), and the mucilage, flocculated was filtered, washed by means of acetone, and then ether, and finally dried in vacuo over phosphoric oxide to give a white product of about 30% yield. The mucilage obtained did not reduce Fehling's solution, but gave the strong color reaction for uronic acid with naphthoresorcinol. It contained 12.13% moisture, 14.85% ash and 0.80% nitrogen. The sample freed from water and ash was shown to contain 49.20% uronic acid lactone by the carbon dioxide evolution method, and to give 25.66 cc./g. acid value titrated with 0.1N NaOH. The viscosity of the solution was shown in curve II in Fig. 1.



Concentration of mucilage in g./100 cc.

Fig. 1. Viscosity of mucilagenous solution.

3. Acid Hydrolysis.—With a view to determining the component sugars, the mucilage was heated and hydrolysed with 4% sulfuric acid in a bath of boiling water, and the reducing sugars produced were determined by Bertrand's method from time to time. It was found, as shown in curve I in Fig. 2, that the sugar production reached a maximum after twenty four hours, whose value calculated as glucose was 62.6%. In order to make it hydrolyse in more severe conditions, 8% sulfuric acid was used at the same temperature. The maximum value of reducing sugars in this condition was 68.6%, which was attained after fifteen hours, as shown in curve II in Fig. 2.



Reaction time in hr.

Fig. 2. Hydrolysis of mucilage.
I. 4% H_2SO_4 II. 8% H_2SO_4

Under these conditions, the uronic acid and pentose produced seemed to be further decomposed to some extent, since furfural was detected in the hydrolysate by the test with aniline-acetic acid paper. The fact that the maximum value of the sugar content was at most within about 69%, suggests the presence of linkage not being apt to hydrolyse in the polysaccharide molecule. The reaction was stopped, and the hydrolysate solution was filtered from the remaining substance. The filtrate was neutralised with barium hydroxide, filtered, evaporated to a small volume and poured into methanol. The precipitate was filtered, redissolved in water, filtered through an active carbon layer several times, evaporated again in reduced pressure to a small volume and added to methanol. The precipitate was again purified by reprecipitation with methanol and finally dried. The white powder obtained reduced Fehling's solution in the hot state and gave a beautiful color reaction of uronic acid with naphthoresorcinol. (Found: Ba 26.05%. $(C_6H_9O_7)_2Ba$ requires Ba 26.24%). With cinchonine, the free acid gave a white crystal, m.p. 178°C, alone or mixed with authentic cinchonine salt of D-galacturonic acid; and by oxidizing with nitric acid, it gave crystals of mucic acid, m.p. and mixed m.p. 213°C. These facts indicate that the uronic acid obtained is D-galacturonic acid.

The methanol solution of the hydrolysate which was removed from the precipitated barium uronate by filtration was concentrated in vacuum to a syrup. The syrup was dissolved in water, de-ionised by Amberlite resin IR-4B and IR-120, and again concentrated. The mixture of sugars was separated into its components on a sheet of Toyo-Roshi No. 50 filter-paper by partition chromatography and the use of a mixture of phenol (80%) and water (20%) as the solvent and aniline hydrogen-phthalate as the spraying reagent. As the constant value of R_f was not strictly expected due to some inconvenience of the experimental apparatus used, the value of R_{GI} of the unknown sample was estimated by comparison with that of the known sample tested at the same time in each experiment. The sugars detected were galactose (R_{GI} 1.07), arabinose (R_{GI} 1.36) and rhamnose (R_{GI} 1.48), where the known samples were galactose (R_{GI} 1.10), arabinose (R_{GI} 1.36) and rhamnose (R_{GI} 1.50).

From these results, it was found that the mucilage was a polyuronide composed of arabinose, galactose, rhamnose, and D-galacturonic acid.

4. The purified Mucilage.—From the mucilagenous solution appeared, however, on standing for several days a deposit, which might be composed of impurities. As in the case of the mucilage of the Tororo-aoi plant[1], the supernatant solution removed from the precipitates was expected to contain a polyuronide, which was the essential part of the mucilage of the Nori-utsugi plant. The purified polyuronide was obtained by adding the supernatant solution to a large volume of acetone. It contained 9.37% moisture, 7.42% ash; and the sample freed from water and ash was found to contain 52.32% uronic acid lactone.

The component sugars besides D-galacturonic acid were shown by paper chromatography of the hydrolysate to be galactose (R_{GI} 1.10) and rhamnose (R_{GI} 1.48), which procedure was the same as that mentioned above. Arabinose was not found;

perhaps the linkage between arabinose and the skeleton in the mucilage molecule is so weak that it is apt to auto-hydrolyse and liberate[6].

The viscosity of the solution of the purified mucilage was very high, as shown in curve III in Fig. 1. The data showed that the more the impurity is removed from the mucilage, the more viscous the solution becomes.

5. The degraded Mucilage.—As the solution of the purified mucilage was too viscous to handle, a conventient method was devised to purify the polyuronide, disregarding some degradation. The extracted mucilagenous solution (ca. 10 *l*) was heated at 120–125°C in an autoclave. As the viscosity of the solution decreased to a constant value within about two hours, the solution was filtered from the precipitate and added to a saturated solution of copper sulfate. The precipitate was filtered and soaked in a quantity of ethanol containing a few drops of hydrochloric acid. The ethanol solution was renewed until the copper ion was eliminated, and the mucilage was washed with absolute alcohol and ether to eliminate the chlorine ion, and dried.

The degraded mucilage thus obtained was a white amorphous powder (yield ca. 32 g.). Analysis showed that it contained 4.64% moisture and 0.25% ash; the sample freed from water and ash contained 58.92% uronic acid lactone, and gave 34.42 cc./g. of acid value titrated with 0.1N-NaOH. The component sugars besides galacturonic acid were shown by paper chromatography to be galactose (R_{GI} 1.07) and rhamnose (R_{GI} 1.48). A very faint red spot (R_{GI} 1.34?) was found with difficulty, which might be due to a trace of arabinose which still remained.

The degraded polyuronide was comparatively too hard to be dissolved in water, and owing to the degradation, the solution was found to have fairly low viscosity, as shown in curve IV in Fig. 1. The degraded polyuronide, however, is considered to represent the main molecular structure of the mucilage. It is further noticeable that curve IV indicates the characteristic feature of linear polyelectrolyte[7], which did not appear in the other curves. It may be explained on the basis of the presence of ionized carboxyl groups along the molecular chains. When sodium chloride was added to the mucilagenous solution, the viscosity decreased markedly and an almost linear curve was obtained. This is shown in the curves in Fig. 1, where I', II', III' and IV' indicate the addition of 1% sodium chloride to I, II, III and IV respectively. The essential part of the mucilage of the Nori-utsugi plant is, therefore, a polyuronide composed of galactose, rhamnose and D-galacturonic acid. In the crude solution, the properties of polyelectrolyte are perhaps interfered with some factors, for example metallic ion, and the viscosity curve of the crude solution gives a straight line.

6. Composition of Mucilage.—Then the quantitative relation between the component sugars in the polyuronide was determined. Boiling the purified mucilage with 12% hydrochloric acid solution gives rise to furfural and methyl furfural; the former is due to galacturonic acid and the latter to rhamnose, and both are precipitated as mixed phloroglucides. The amount of furfural-phloroglucide is calculated from the galacturonic acid content determined by the carbon dioxide evolution method. The quantity of furfural-phloroglucide is deducted from the total amounts of the mixed phloroglucides, and the amount of methyl furfural-phloroglucide is found, from which the rhamnose content of the mucilage is calculated. The glactose content is also directly found. The results thus obtained are shown in Table I.

TABLE I

COMPOSITION OF MUCILAGE

Sample (water, ash free)	Total phloroglucide (g)	Furfural-phloroglucide (g)	Methyl furfural-phloroglucide (g)	Rhamnose (g)	Rhamnose (%)	Galactose (%)	Galacturonic acid (%)
0.2123	0.0615	0.0465	0.0150	0.0274	12.92	34.76	52.32
0.2198	0.0639	0.0481	0.0158	0.0284			

It was found from these results that the essential part of the mucilage is a polyuronide composed of D-galacturonic acid, galactose and rhamnose in the molar ratio of 10:7:3; that is, the molecular formula is $[(C_6H_8O_6)_{10}\cdot(C_6H_{10}O_5)_7\cdot(C_6H_{10}O_4)_3]_x$. And the crude mucilage as it was extracted from the inner bark, is considered to have such a structure that the polyuronide skeleton is combined with some arabinose residues by some comparatively weaker linkages.

Summary

1. The mucilage of the inner bark of the Nori-utsugi plant (*Hydrangea paniculata*, Sieb.) was found to be composed of arabinose, galactose, rhamnose and D-galacturonic acid.

2. When the crude mucilagenous solution had stood for several days, a deposit appeared which might be composed of impurities. The purified mucilage was obtained from the supernatant clear solution. The purified mucilage was a polyuronide composed of galactose, rhamnose and galacturonic acid and it was considered to be the essential part of the mucilage.

6) E.L. Hirst, E.G.V. Percival and C.B. Wylam, *J. Chem. Soc.*, **1954**, 189.

7) H. Staudinger, "Die Hochmoleculare Organische Verbindungen", S. 1344 (1932); Wo. Panli. L. Sternbach, *Helv. Chim. Acta*, **24**, 317 (1941); S. Machida, N. Uchino, *J. Chem. Soc. Japan*, **74**, 183 (1953).

3. Degraded mucilage was obtained by heating the crude mucilagenous solution at 120–125°C in an autoclave. It had the same component sugars as the purified mucilage, and represented the main structure of the mucilage.

4. The essential part of the mucilage was a polyuronide composed of D-galacturonic acid, galactose and rhamnose in the molar ratio of 10 : 7 : 3; that is, the molecular formula is $[(C_6H_8O_6)_{10}\cdot(C_6H_{10}O_5)_7\cdot(C_6H_{10}O_4)_3]_x$

5. The solution of the degraded mucilage exhibited the typical electroviscous behavior of polyelectrolyte.

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