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By

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The presence of a polysaccharide in the ascidian test of which the composition is very similar to plant cellulose was first shown by Schmidt (1845) (1) and it was subsequently termed tunicin to distinguish it from the corresponding plant cellulose by Berthelot (1858) (2), because of its greater chemical resistance and peculiar tissue properties. Since then the plant and animal celluloses have been compared with one another in many investigations. The latter substance is as soluble in Schweitzer's solution, in an acid zinc chloride solution and in concentrated sulphuric acid as the former (1, 3, 4), but it shows a greater chemical resistance than the former (2, 3). Upon hydrolysis with mineral acid, both of them gave the same hydrolysates such as glucose (4), cellobiose (5, 6), cellotriose, cellotetraose, cellopentaose and cellohexaose (6). X-ray diffraction pattern served to identify animal and plant celluloses (7, 8). On the other hand, Endean (9) reported that the fibres of the test of *Pyura stolonifera* Heller are composed of a very insoluble polysaccharide which is similar to plant cellulose in some respects but differed therefrom by its insolubility in cellulose solvents or staining ability with cellulose dyes and emphasized that the chemical composition of the test of a majority of ascidian species has not been thoroughly investigated and that some other structural materials different from plant cellulose may arise in them.

In the present investigations, the authors have attempted to ascertain whether tunicin occurs in the test of *Cynthia roretzi* v. Drasche collected from the Tohoku District of Japan.

Experimental

1. *Preparation of tunicin*

The test of *Cynthia roretzi* v. Drasche defatted with acetone was ground, pulverized and treated with one percent of hydrochloric acid to remove the

minerals for 24 hours at room temperature. After washing with sufficient water, it was heated with an excess of one percent of sodium hydroxide on a boiling water bath for 20 hours. The alkali was renewed every five hours during the treatment. Then the alkali soluble part was discarded as supernatant liquid by centrifugation and its residues were washed with water and then by ethanol and ether. The white tunicin thus obtained was freed from any amounts of nitrogen and minerals.

2. *Hydrolysis of tunicin*

One gram of tunicin was dissolved in 100 ml of super-concentrated hydrochloric acid with gentle stirring and then a small amount of water was added, It was completely hydrolyzed by standing for 24 hours at 20°C. The partial hydrolysates were obtained by treating in the same manner for three hours. Both hydrolysates were concentrated to remove the hydrochloric acid under reduced pressure respectively and the remaining hydrochloric acid was neutralized with silver carbonate. The filtrate was examined by paper chromatography. Toyo filter paper No. 2 used as a standard cellulose was also hydrolyzed by the same procedures.

3. *Paper chromatography*

About one milligram of each hydrolysates was spotted on a sheet of Toyo filter paper No. 2, 40 cm in height, and developed with a mixture of buthanol-pyridine-water (3 : 2 : 1.5 v/v) by the ascending method at 25°C. A single spot was detected on the chromatogram of complete hydrolysates and identified with glucose by a chromatography mixed with authentic glucose. The five spots of the chromatogram of partial hydrolysates corresponded with each of five spots of the standard hydrolysates as indicated in Figure 1. Among them P_2 and P'_2 were identical with cellobiose by a chromatography mixed with authentic cellobiose.

It is well known that there exists a linear relationship between logarithm of a partition function α' as $\log(\frac{1}{Rf}-1)$ (10), $\log\frac{1}{Rf}$ (11) and $\log(\frac{Rf}{1-Rf})$ (12) and the polymerization degree of a saccharide series. Aso and Yamaguchi (13) presented the following equation to presume the correlation of Rf values of various glucose polymers with the chemical structure, using $\log(\frac{Rf}{1-Rf})$.

$$\log \alpha' = 0.477 - 0.373n_1 - 0.301n_2 - 0.210n_3 - 0.110n_4 - 0.512n_5$$

Where n_1 means the number of 1,1 α, α-, 1, 2 α- and 1,4 β-linkages and n_2, n_3, n_4, and n_5 mean the number of 1,2 β- and 1,4 α-, 1,3 α-, 1,3 β-, 1,6 α- and 1,6 β-linkages respectively.

The Rf values, $\frac{1}{Rf}-1$, $\frac{1}{Rf}$ and $\frac{Rf}{1-Rf}$ of tunicin series are seen inTable 1. The values plotted against the molecular sizes showed nearly a linear relation as indicated in Figure 2. The Rf values of cellobiose, cellotriose, cellotetraose

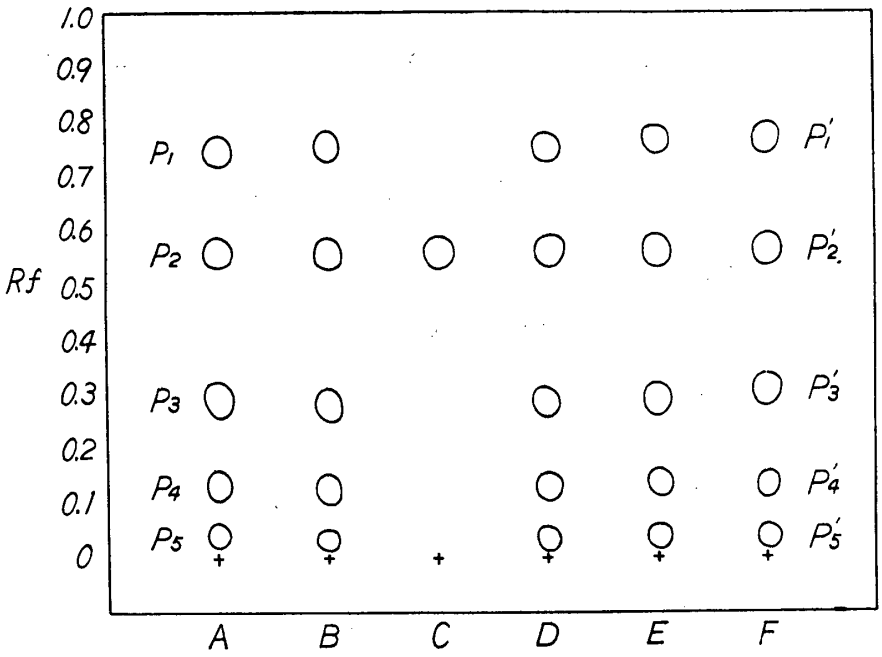

Fig. 1. Paper chromatograms of partial hydrolysates of tunicin and filter paper.

A: Tunicin
B: A mixture of tunicin and cellobiose
C: Cellobiose
D: A mixture of tunicin, filter paper and cellobiose
E: A mixture of filter paper and cellobiose
F: Filter paper

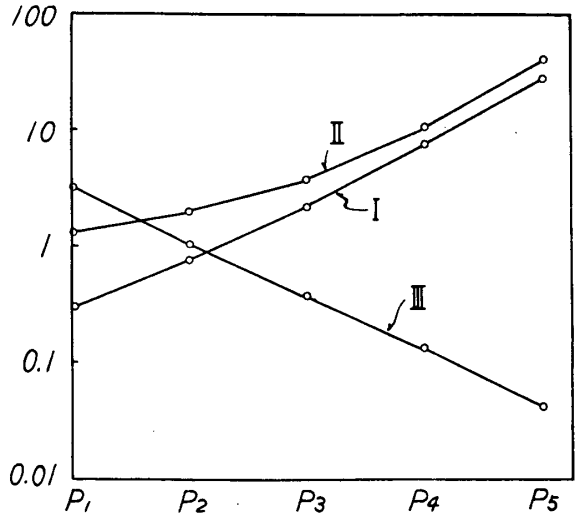

Fig. 2. log α' of partial hydrolysates of tunicin.

I. $1/Rf - 1$
II. $1/Rf$
III. $Rf/(1-Rf)$

and cellopentaose calculated from the above equation roughly corresponded with the *Rf* values of P_1, P_2, P_3, P_4 and P_5 respectively as seen in Table 2. From

Table 1. *Rf* and α' of partial hydrolysates of tunicin

	P_1	P_2	P_3	P_4	P_5
Rf	0.755	0.557	0.283	0.115	0.033
$\frac{1}{Rf}-1$	0.325	0.795	2.534	7.696	29.303
$\frac{1}{Rf}$	1.325	1.795	3.534	8.696	30.303
$\frac{Rf}{1-Rf}$	3.082	1.257	0.395	0.130	0.034

Tabel 2. *Rf* calculated from Aso's equation

	Glucose	Cellobiose	Cellotriose	Cellotetraose	Cellopentaose
Calculated	0.75	0.56	0.35	0.19	0.09
Found	0.76	0.56	0.28	0.12	0.03

the above two evidences, P_1, P_2, P_3, P_4 and P_5 in the partial hydrolysates of tunicin were identified to be glucose, cellobiose, cellotriose, cellotetraose and cellopentaose respectively.

Result

The tunicin of *Cynthia roretzi* v. Drasche gave only glucose by hydrolysis with hydrochloric acid and cellobiose, cellotriose, cellotetraose and cellopentaose by partial hydrolysis. Therefore it shows the same linkage of 1,4-β-glucoside as plant cellulose.

Concerning the little difference between tunicin and plant cellulose, Berthelot (2) and Schäffer (3) observed that the tunicin showed greater resistance than the plant cellulose to chemical acion. Rångby (8) reported that mercerized tunicin gave sharper X-ray diffraction pattern than that of plant and wood cellulose, and the micell strings of tunicin are somewhat thicker than those of the latter cellulose in electron microscopic studies.

The tunicin of *Cynthia roretzi* v. Drasche was soluble in Schweizer's solution, in an acid zinc chloride solution and in super-concentrated hydrochloric acid, but showed greater resistance to dissolve in the above reagents than cotton and wood cellulose.

Summary

The polysaccharide composing the test of *Cynthia roretzi* v. Drasche gave glucose, cellobiose, cellotriose, cellotetraose and cellopentaose on hydrolysis with hydrochloric acid. It therefore presents the 1,4 β-glucosidic linkage as in plant cellulose.

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