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Observations on the Chemical Composition of Laminaria digitata and Chondrus crispus

By Margaret R. Butler

The work on which the following report is based is closely related to previous work done partly in the graduate school of Dalhousie University and partly at the St. Andrews Experimental Station. It helps to correlate this work and furthermore it forms a basis for future work.

The report lends itself to division into the following parts:-

I. Iodine Content of Laminaria digitata.
II. Crystals of unknown substance extracted from L. digitata.
III. Preparation of carbohydrate samples.
IV. The Polysaccharide extracted from Chondrus crispus.

(a) Review of properties.
(b) Dialysis experiments.
(c) Calcium determinations.
(d) Sulphur determinations.
(e) Optical activity.

EXPERIMENTAL

I. Iodine Determinations

The iodine content of Laminaria digitata having been found to far exceed that of any other of the twelve species examined previously, it was thought advisable to repeat this determination on a fresh sample of the plant, in order to check the previous results.

The iodine content of a sample of Laminara digitata collected at Halifax, N.S. (Thrum Cap, MacNab Island) in September was found to average 0.368%. These results were obtained on air dry samples, and calculations made to a moisture-free basis.

The sample used for the present determinations was collected at Halifax (Herring Cove), in June, and was analysed in a moisture-free condition. The average in this case was .....0.340% dry weight.

The check thus shows the previous results to have been of the proper order of magnitude. It is quite probable that the variation observed is a seasonal one.

An attempt was made to determine the relative ease with which the iodine could be extracted by water and by alcohol. This was done in the hope that it might prove a clue to the form in which the iodine is combined. Aqueous and alcoholic extracts were made in approximately one per cent solutions. After five hours on the water bath these extracts were filtered and both filtrates and residues dried. The percentage of iodine in each was then determined.

Residue from aqueous extract contained		.03%	Iodine
" " alcoholic	"	.13%	"
The dried aqueous filtrate		.57%	"
" " alcoholic	"	1.17%	"

Since the alcoholic residue has greater bulk than the aqueous residue, on account of the solubility of carbohydrate material in water, the difference in the percentage of iodine remaining is intensified. Therefore, from the fact that more of the iodine present is capable of extraction with water than with alcohol one might surmise it to be, for the most part, combined inorganically. Since more iodine remains in the alcoholic residue than does in the aqueous residue, part at least of the iodine which is soluble in water must be insoluble in alcohol. Whether or not the iodine insoluble in water is soluble in alcohol cannot be shown from these results.

It was to be expected that the percentage of iodine in the dried filtrate of the aqueous extract would be low as compared with that of the alcoholic extract, because more of the plant substance is soluble in H_2O than in alcohol and the higher the total solid, the lower the proportion of iodine.

II. Unknown Crystals from L. digitata.

An extract of Laminaria digitata, made in hot alcohol, and left to cool over a period of twenty four hours, was found on examination to contain a large quantity of crystalline material. The crystals were white, and formed in rosettes. Under the microscope they were long and narrow. Some of these crystals were collected, washed with absolute alcohol, dried and kept.

They are very soluble in water, optically inactive, with an ash value of approximately 36.9%. No further observations have been made on this material.

III. Preparation of Carbohydrate Samples

Purified samples of carbohydrate were prepared from five different species of marine algae, viz., Chondrus crispus, Gigartina mamillosa, Rhodymenia palmata, Ascophyllum nodosum and Fucus vesiculosus.

The method of preparation used was the same in all cases. After boiling for 4-6 hours on a water bath, the 2% aqueous solution was filtered through sand on a Buchner funnel. The filtrate was then concentrated, and poured into twice its volume of 90-95% alcohol. The precipitate so formed was then dehydrated through 95% alcohol and two washings each of absolute alcohol and anhydrous ether. It was left at least twelve hours in each, and often much longer. After this treatment the material was dried in a vacuum desiccator, and ground to a fine powder in a mortar.

A finely ground portion of the whole plant was used in the preparation of these samples, except in the case of Fucus, when only the receptacles were used. The whole plant was used in the first attempt on Fucus, but the amount of carbohydrate precipitated was so small as to make it useless to continue. The same situation was encountered with Agarum Turneri. A carbohydrate sample was prepared from Fucus, using only the receptacles, which were cut from the dried plants. The final product was a pale fawn coloured powder. The sample from Ascophyllus closely resembled this, being perhaps slightly darker in colour, while that from Rhodymenia palmata was more of a grayish yellow colour. These three were very similar in the way in which they were precipitated by alcohol, and different from Chondrus and Gigartina in this respect. The two latter very closely resemble one another in being precipitated as a stringy, fibrous mass, while the former came down as an amorphous powder.

These samples are being preserved for future study and were prepared with a view to obtaining the highest possible degree of purity rather than with any idea of getting a maximum yield.

Samples prepared from different collections of the same plant have been kept separate; and the following is a key to the collection of the material used:

Chondrus crispus	(1)	Thrum Cap.	August 10th.
" "	(2)	Devil's Island	June 24th.
" "	(3)	Peggy's Cove	August 17th.
Gigartina mamillosa		Peggy's Cove	August 17th.
Rhodymenia palmata		St. Andrew's N.B.	July
Fucus vesiculosus		Bedford Basin	August 5th.
Ascophyllum nodosum		Bedford Basin	August 5th.

IV. Polysaccharide from Chondrus crispus.

The remainder of this report is concerned with a sample of carbhhydrate material prepared from Chondrus crispus, the known properities of which are as follows.-

(1) It is a grayish white crystalline powder.
(2) It is nitrogen free.
(3) It is soluble in water, forming in 2% solution a firm gel which is almost colourless.
(4) It is insoluble in cold alcohol.
(5) It will not reduce Fehlings solution.
(6) It is dextrorotatory, (specific rotation unknown).
(7) It has an ash content of 17.35%.
A sample prepared from a different collection gave ash value of 18.48%.
(8) With ammonium oxalate a precipitate forms, indicating the presence of Ca^{++}.
(9) The effect of the colloidal precipitants upon a solution of the substance is indefinite.

Dialysis Experiments

In order to determine how much of the ash value is attributable to inorganic salts, dialysis experiments were tried, using collodion membranes made in Erlenmeyer flasks. The distilled water against which the solution was dialysed was frequently changed.

The original solution contained Cl^- and Ca^{++}. At the end of 3 days all the Cl^- had dialysed away, but the Ca^{++} remained, even at the end of three weeks. Tests, with barium chloride, for SO_4^{--} were indefinite, throughout, giving a non-typical flocculation. The dialysate during the first six hours gave a positive test for Cl^- but not for Ca^{++} or SO_4^{--}. After five days it gave negative tests for all three. A sample of the combined dialysate which had been collected and evaporated was tested, with positive results for Cl^- and SO_4^{--}; Ca^{++}, however, if present at all was there only in very

minute quantity. The dialysate from an experiment which stood for three weeks was found to contain Ca^{xx} but no Cl or $SO_4^{=}$. In this case, however, there was evidence of bacterial action which might possibly have freed the calcium.

Some of the dialysed solution was evaporated to dryness and ashed.

Ash value of original material	17.38%
Ash value after dialysis	16.00%

Two facts are thus established, (1) that it is possible to remove by dialysis all the Cl present in the sample, and (2) that dialysis reduces the ash value very little. It seems reasonable to assume that the material removable by dialysis, being such a small quantity is not part of the compound, but is an impurity. It should be mentioned, in passing, however, that the material, dialysed for this length of time, appeared to have less gel forming power than the original.

Calcium Determinations

Calcium was determined quantitatively in a solution of the sample in two ways:

(1) Directly and (2) after oxidation with a potassium hydroxide and nitrate mixture (3:1). In both cases it was precipitated as the oxalate and weighed as calcium oxide. Results:

(1) Direct ppt.	2.67% Ca.	(Filter used here was not ash free
	2.15% Ca.	and therefore gave a high value for the CaO).
(2) Oxidized	3.48% Ca.	
	3.41% Ca.	

If as Haas says the calcium is freely ionized there should be no difference in the results obtained by the two methods unless there was Ca present in the reagents used. As no blank was run this possibility can not be overlooked but the difference seems too great to be accounted for by impurity of reagents. Therefore it would appear that some of the calcium is not freely ionizable.

Haas' Results: (1932)	Direct ppt ion.	5.47% Ca.
	After Incineration	5.66% Ca.

Russel-Wells (1928) Direct pption 4.03% Ca.
After incineration 5.98% Ca.

Sulphur Determinations

Sulphur determinations were made by precipitation as barium sulphate after oxidation with the potassium hydroxide, nitrate mixture. Results:

(a)	8.31% S
(b)	8.08% S
(c)	8.47% S
(d)	8.62% S
(e)	8.24% S
Average	8.34% S

In (e) sodium peroxide was used for the oxidation.

By calculation the average SO_3 of the whole sample 20.85%.
and " " SO_4 " " " " " 25.02%.

Haas: By incineration 12.15% $SO_4^=$
11.8 % $SO_4^=$
" hydrolysis 24.85% $SO_4^=$
23.81% $SO_4^=$

Russel-Wells. By incineration 15.95% $SO_4^=$
" hydrolysis 30.23% $SO_4^=$

Haas contends that one-half the $SO_4^=$ is lost in ashing which statement his figures bear out.

Haas has proposed the formula

to represent the carbohydrate present in Chondrus crispus. Assuming the R to be a hexose the atomic weight will be 395.

Worked on this basis the Calcium is 10.13%; and SO_4 20.26% theoretically. The results actually obtained were SO_3 20.85%. and Ca 3.4%. SO_3 therefore corresponds with the theoretical, while Ca is just one third of the theoretical.

Optical Activity

Attempts to determine the optical activity of the sample have proved unsatisfactory. That it is active, and dextrorotary seems certain; but it has been impossible to get the specific rotation. Readings were made with a Hilger polarimeter in a one decimeter tube, using a mercury arc lamp as light source. Readings were taken with both the D line and E line.

Summary of Results

Conc. of solution	Observed Rotation	Light	Calculated
.34%	.18°	D	5.27°
.34%	.23°	E	6.74°
.95%	.42°	D	4.41°
.95%	.45°	E	4.74°

The concentration of the solution was obtained by taking 10 cc. of the solution used for the rotation and evaporating it to dryness on a watch glass. This method gave the conc. of total solid in the solution and would therefore include any impurities e.g. NaCl which might be present, and hence lower the calculated specific rotation.

Rotation of Hydrolysate

An approximately 5% solution was boiled in 1% oxalic acid for 18 hours on a water bath. After neutralization with Calcium carbonate, the solution was filtered and the filtrate tested for optical activity, which was found to be as given.

Conc. of solution	Observed Rotation	Light	Calculated
0.58%	.12	D	2.05°
0.58%	.13	E	2.22°

Besides the error to be expected in handling such small quantities of dilute solutions a very considerable error is introduced into both these polarimetric experiments by the method in which the

concentration of the solution was obtained. In the case of the hydrolysate, after the readings recorded were made, the solution was evaporated, with the idea of making second readings on a more concentrated solution. With a very slight decrease of volume a large quantity of the calculated specific rotations were probably far from correct.

SUMMARY

I. The high iodine content of Laminaria digitata has been confirmed as 0.340% of dry weight.

II. It has been shown that a larger percentage of the iodine is extractable by water than by alcohol.

III. Crystals of an unknown compound were isolated from an alcoholic extract of Laminaria digitata.

IV. Carbohydrate samples were prepared from five different species of algae by extraction with water and precipitation with alcohol.

V. The polysaccharide from Chondrus crispus has been further purified by dialysis. A small amount of chloride was thus removed. No Ca^{++} is removable by this treatment. Free and combined calcium and total sulphur have been estimated, on the non-dialysable portion. It has been found impossible to obtain an accurate specific rotation for the polysaccharide.