ON THE MUCILAGINOUS SUBSTANCE OF FLORIDEAE

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The mucilaginous substance of Florideae is much used by Japanese as food stuff or for technical purposes. Agar-agar, manufactured from Gelidium is very familiar to us as food, as paste or as nutrient media in bacteriology. The mucilaginous substance of Chondrus, Gloiopeltis, Iridaea and others has also been applied in various industries as a valuable paste from an early time. Notwithstanding the large consumption of these products, both at home and abroad their chemical nature has not yet been fully investigated.

Payen[1] isolated a mucilaginous substance from *Gelidium corneum* and called it gelose and this substance was studied afterward by Morin[2] and Porumbau.[3] Euler[4] studied the constituents of carragheen moss (*Chondrus crispus*) and proved the presence of galactose, fructose and a methyl pentose among its hydrolysis products. From *Gloiopeltis sp.* Kawakami[5] lately identified galactose.

In the present paper are described the results of the investigation on some of the important species of Florideae, undertaken by the author to determine the chemical nature of their mucilaginous substance. The following species have been subjected to the research and the results will here be reported on: *Chondrus clatus* Holm. (*Tsunomata*), *Gloiopeltis furcata* var. *coliformis* J. Ag. (*Funori*) and *Iridaea laminarioides* var. *cornucopiae* J. Ag. (*Ginnanso*).

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1) Jahresb. ü. Fortschritte d. Chemie, 12, p. 5622 (1859).
2) ibid. 33, p. 1010 (1880).
3) „ 33, p. 1011 (1889).
4) Grundlagen und Ergebnisse der Pflanzenchemie, Brunschweig, p. 238 (1908).
5) Jour. Chem. Indus., Tokyo, 13, p. 343 (1910).

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I. Chondrus.

The sample of *Chondrus elatus* Holm taken for the investigation was obtained from the province of Boshu. It was air dried, yellowish brown in colour, and mixed with small shells and other impurities which were removed carefully previous to the research. General analysis gave the following results:

	In air-dry substance	In water-free substance
	%	%
Moisture	6.67	
Crude ash	17.81	19.58
Crude protein	8.94	9.58
Crude fat	0.20	0.21
Crude fiber	3.04	3.25
Nitrongen free extract	63.34	67.88
Total nitrogen	1.43	1.53
Non-protein nitrogen	0.34	0.36

Separation of mucilaginous substance.

Mucilaginous substance of the Chondrus was first separated from a small quantity of the sample and qualitative research as well as general analysis were made upon it.

Of the well purified and crushed sample 20 gms. were put into 1 liter of water and heated in a boiling water bath, then filtered through a large filter paper. The residue was treated again with 1 liter of water and filtered with the same filter. The operation was repeated three times and finally the filter was washed thoroughly with hot water. The filtrate and washings were put in together and made to a definite volume. With a portion of the solution, qualitative tests were first made upon the following:

a) Galactane—A part of the mucilage was oxidized with nitric acid of 1.15 sp. gr. in the usual manner. A large quantity of mucic acid was produced which indicates the presence of galactane.

b) Reducing sugar—10 cc. of the mucilage of the Chondrus was mixed with Fehling's solution and heated for sometime, but no reduction occurred.

c) Starch—By adding iodine solution to the pure colloid, no colour reaction for starch was observed.

d) Mannite—This was tested with the original substance. 10 gms of the crushed substance were heated with 50 c.c. of 85 per cent alcohol and put aside in a cool place until the next day, but no crystals of mannite were found in the liquid. The alcoholic solution was then filtered and the filtrate evaporated in a water bath ; when the concentrated solution was shaken with hydrochloric acid and benzaldehyde no precipitate was formed. No mannite is contained in the alga.

e) Pentosane and methyl pentosane—A small amount of the mucilage was heated in a test tube with dilute hydrochloric acid for about 10 minutes. The solution showed the characteristic absorption-spectrum of pentose, upon warming with phloroglucin and hydrochloric acid. On distilling the substance with hydrochloric acid of 1.06 sp. gr. a distillate was obtained which gave characteristic furfurol reaction with anilin acetate. The distillate was also tested for the presence of methylfurfurol by the spectral reaction of Oshima and Tollens as modified by Kondo and Oshima.[1] Of the distillate 300 c.c. was distilled again, and to 5 c.c. of the redistillate thus obtained a small amount of phloroglucin and an equal volume of concentrated hydrochloric acid were added and filtered after standing 5 minutes. The clear filtrate obtained showed no spectral reaction of methylfurfurol. Methylpentosane is not found in the mucilage.

f) Rotation—A filtered clear solution of the mucilaginous substance was examined in Schmidt and Haensch polariscope when a rotation toward the right was observed distinctly.

Another part of the mucilage was evaporated, dried and weighed, when the following results were obtained:

	In dry matter %
Substance soluble in warm water	77.56
Substance insoluble in warm water	22.44

1) Jour. of the Tokyo Chem. Soc., 39, pp. 185-198 (1918).

This figure for the water soluble substance can hardly be called a constant, as it differs according to manipulation. But, for comparison, the amounts were found in the way described above.

Further a general analysis was made with a part of the dried mucilage with the following results:

	In air-dry substance	In water-free substance
	%	%
Moisture	14.66	
Crude ash	21.26	24.89
Crude protein	4.47	5.24
Crude fat	Trace	Trace
Nitrogen free extract	59.61	69.87
Total nitrogen	0.72	0.84
Non-protein nitrogen	0.17	0.19
Galactane	22.27	26.09
Pentosane	10.20	11.69

The dry substance put in warm water swells gradually and finally dissolves into a viscid colloidal solution which is not precipitated by any acid, organic or inorganic. The mucilage does not form any precipitate with lead acetate, but is separated by basic lead acetate as a voluminous precipitate which can be changed again into colloidal form by digesting with dilute hydrochloric acid. But the mucilage obtained in such a way has a somewhat decreased viscosity. By strong alcohol the mucilage was separated as a voluminous precipitate which becomes again a mucous fluid in the original state when dissolved in water. So in the present investigation, alcohol was used for the purification of the substance as the manipulation was found to be quite simple.

Investigations upon the hydrolysis products.

(1) Preparation of the sample.

To the pure mucilage obtained by boiling the purified alga with water was added strong alcohol to the amount of about 80 per cent by weight,

when gelatinous precipitates were at once produced in large quantity. After standing 24 hours, it was filtered with suction, once more dissolved in water and again precipitated by alcohol. The precipitate obtained was evaporated in a water bath and subjected to hydrolysis.

(2) Optimum condition of hydrolysis.

Kawakami [1] found in hydrolysis of *Gloiopeltis sp.* that 26 hours' heating with 2 per cent sulphuric acid, which was added to eight times the weight of the sample, gives most favorable results and 17 hours' heating with 4.25 per cent sulphuric acid shows also good results. Prolonged heating makes the liquid dark coloured, separating insoluble humin substance and inverting the sugars once separated out. I intended first to determine the optimum condition of hydrolysis and then to treat the sample according to the result obtained.

The sample taken is the alcohol precipitate from the colloidal solution of the mucilaginous substance. Two gms. of this were put into 100 c.c. flasks with 20 c.c. of 2 per cent sulphuric acid. A similar set of flasks were made up with 3 per cent acid, and four with 5 per cent. The flasks were then heated for varying periods, from 6 to 30 hours in a water bath. The sample, while being mixed in sulphuric acid, swelled at first and dissolved by warming, insoluble humin substance separating. The colour of the liquid, too, changes from yellow to brown. At the end of the required period, it was filtered after being made up to a definite volume, neutralized with sodium carbonate and then the sugar determined in the usual way by means of Fehling's solution. The results were as follows:

1) Jour. Chem. Indus. Tokyo, 13, p. 352 (1910).

Weight of the sample (gm)	Percentage of sulphuric acid	Time of heating (hour)	Percent of sugar produced (as galactose)[1]
2	2	6	53.85
"	2	10	59.38
"	2	14	63.09
"	2	18	63.51
"	2	22	62.92
"	2	26	59.16
"	2	30	57.74
"	3	6	58.12
"	3	10	60.05
"	3	14	64.83
"	3	18	62.32
"	3	22	58.25
"	3	26	56.40
"	3	30	55.01
"	5	5	59.12
"	5	10	60.31
"	5	15	58.63
"	5	20	52.10

From the above table it was found that 14 hours' heating with 3 per cent sulphuric acid gives highest results in quantity of sugar. 18 hours' heating with 2 per cent and 10 hours' with 5 per cent also showed good results. Therefore I carried out hydrolysis thereafter with 3 per cent sulphuric acid, heating from 14 to 15 hours.

(3) Hydrolysis.

Two hundred grams of purified alcohol precipitate of the mucilage were mixed with 1 liter of 3 per cent sulphuric acid in a large flask and heated in a boiling water bath for 15 hours. During boiling the contents were stirred from time to time. At the end of the stated period the precipitates were completely disintegrated and the liquid was coloured dark brown, admixed

1) The percentage is calculated from the substance free from moisture and ash.

with a black fiber-like substance. When cooled it was filtered through muslin. The clear filtrate was then mixed with sulphuric acid and precipitated by a concentrated solution of phosphotungstic acid to eliminate the nitrogenous substances which were produced in hydrolysis. After standing overnight it was filtered. The filtrate obtained was neutralised by barium carbonate and allowed to stand overnight. On the following morning the barium salts were filtered off through a " Nutsch " filter with suction and the filtrate was then concentrated with the addition of a little calcium carbonate to about 200 c.c. in a partial vacuum. The solution thus obtained was put into a flask with 200 c.c. of 80 per cent alcohol and allowed to stand for about 10 hours when a blackish gummy substance and salts were precipitated to the bottom as well as to the side of the flask. The fluid was decanted and concentrated again in a partial vacuum to about 150 c.c. To the remaining syrup about 200 c.c. of 80 per cent alcohol were added and after standing overnight the clear solution was decanted and the alcohol evaporated. To the purified syrup about 100 c.c. of 95 per cent alcohol was added which produced a large amount of gummy substance. The gummy substance produced was separated by filtration, washed with 95 per cent alcohol, and dissolved in a small volume of water and reprecipitated by alcohol.

The purified gummy substance was once more dissolved in water. As the aqueous solution of the purified gummy substance thus obtained showed very sweet taste, it must contain much sugar which is hardly soluble in alcohol. So it was decolourised by stirring with animal charcoal, concentrated to a small volume and designated for investigation as syrup B.

The filtrate from 95 per cent alcohol was once more purified by shaking with absolute alcohol, decolourised with animal charcoal and preserved for investigation as syrup A. The yield of syrup A was 30 grams and of syrup B was 125 grams.

(4) Experiment with syrup A.

A) Qualitative tests.

Syrup A gave the following qualitative reactions:

a) It reduced Fehling's solution very strongly.

b) It rotated the plane of polarization toward the right.

c) It gave a red colour by heating with resorcin and strong hydrochloric acid according to Seliwanoff.

d) By heating with ammonium molybdate and acetic acid, it gave a pale blue colouration.

e) By heating the aqueous solution of the syrup with phloroglucin and hydrochloric acid, it gave a characteristic red colour and spectral reaction for pentose distinctly.

f) The distillate obtained by the redistillation of the syrup with hydrochloric acid of 1.06 sp. gr. gave no spectral reaction of methyl furfurol after the method of Kondo and Oshima.

g) It produced no characteristic crystal of cadmium bromoxylonate by the method of Bertrand.

h) Neither mucic nor saccharic acid was produced by the oxidation with nitric acid of 1.15 sp. gr. in the usual manner.

i) When a part of the syrup was mixed with a small quantity of phenylhydrazine the mixture, after a little while, became a crystalline mass. When examined under a microscope it showed large needle crystals, distinctly different from mannose phenylhydrazone. So there must be some other kind of sugar than mannose, forming a hydrazone with phenylhydrazine, which will be investigated later in detail.

j) Two drops of the syrup were placed on each of several object glasses and were seeded respectively with crystals of fucose, xylose, arabinose, fructose, galactose, mannose and glucose. After two days the drops which had been seeded with arabinose showed the formation of new arabinose crystals while in others the seeded crystals remained unchanged. On further standing, all drops, it was observed, were laden uniformly with some unknown fine crystals.

From the above qualitative reaction, it is safe to conclude that the syrup does not contain any dextrose, galactose, mannose nor xylose. On the other hand the presence of arabinose is highly probable. Besides this, it re-

vealed the existence, in no small quantity, of some unknown sugar which easily makes insoluble phenylhydrazone with phenylhydrazine like mannose.

B) Detection and isolation of a new sugar.

To prepare fully the hydrazone for further research, 30 gms of the syrup were mixed with 15 gms of water and 15 gms of pure phenylhydrazine and were put aside, sometimes stirring. The mixture soon became turbid and in course of 5 minutes became a yellow coloured mass. The crystalline mass was stirred with a mixture of ether and alcohol (3 : 1) filtered with suction, washed with ether-alcohol and finally recrystallized from 95 per cent alcohol. The product obtained in this manner was perfectly white and weighed 10.2 gms when dried over sulphuric acid in a vacuum.

The crystals of the hydrazone, examined under a microscope, showed a needle-like form. From the saturated solution of alcohol, however, crystals separated aggregated in bush-like formation. It was insoluble in cold water and ether but easily soluble in hot water, alcohol and acetone. From the hot water solution, it was crystallized in plate-like form. The melting point determined was 158°—160°C. For the separation of sugar from the hydrazone, benzaldehyde was used. The operation was carried out as follows: 10 gms of hydrazone, 25 gms of 95 per cent alcohol 10 gms of benzaldehyde and 8 gms of water were mixed. The mixture was heated one hour and a half in a water bath fitted up with a reflux condenser. The hydrazone melted on warming and upon cooling the long needle-like crystals of Benzaldehyde phenylhydrazone were formed. The melting point of the crystals was 155°—156°C which coincided with that of benzalphenylhydrazone and shows that the decomposition of the original phenylhydrazone had taken place. The filtrate from the crystals was shaken with ether in a separating funnel, repeated several times, and the watery solution obtained was evaporated after being decolourized with animal charcoal. On cooling the syrup became a white crystalline mass which was crushed with a glass rod, washed with absolute alcohol and ether and finally dried over sulphuric acid in a vacuum. The sugar thus obtained was perfectly white, as sweet as glucose and reduced

the copper solution strongly. On ignition it left no ash. The melting point was determined and found to be 152—153°C.

Of the carefully dried sugar 1 gm. was dissolved in water and increased to 25 c.c. and polarized in a 200 mm. tube in Schmidt and Haensch's polariscope. The newly prepared solution showed 9.69 on the scale toward the right and after twenty four hours it was observed 6.46° in the same direction. The specific rotatory power is

$$[\alpha]^{20}D=\frac{6.46\times 25}{1\times 2}=+80.75^{\circ}$$

The rotatory power obtained was pretty close to that of galactose.

Next the osazone was made as follows: 2 gms. of the sugar, 2 gms. of phenylhydrazine hydrochloride, 3 gms. of sodium acetate and 20 c.c. of water were mixed and heated in a boiling water bath. After half an hour yellow crystals were produced.

At the end of one hour the crystals were examined under a microscope. No other forms, besides the yellow, oblong, platelike crystals, looking like galactosazone, were observed. When cooled it was filtered with suction, washed with a little water and dried over sulphuric acid in a vacuum. The crystals were orange in colour and appeared to be extremely pure. The melting point was determined and found to be 195° C. The crystals were then recrystallized from 70 percent alcohol and their melting point was again determined and found to be 193°C.

The crystals were soluble in methyl and ethyl alcohol but not in water. The crystal forms as well as the melting point were found pretty close to galactosazone. But the difference was that the osazone of galactose has low solubility in methyl alcohol while the osazone obtained dissolved easily in the same medium.

To ascertain whether the isolated sugar is galactose or not, the test for mucic acid was carried out repeatedly with nitric acid of 1.15 sp. gr. in the usual manner, but no crystals were obtained by either method.

To determine the crystal form, a part of the sugar was dissolved in a little water, one drop of which was placed on an object glass and left untouched in a desiccator. After three days, fine crystals of oblong

plate, a little thick in the central part, were observed under a microscope. The crystal form of the sugar was quite unlike that of galactose which crystallizes generally, from its pure solution, in hexagonal plates.

From the above research it was found that the specific rotatory power and melting point of the phenylhydrazone and phenylosazone of the isolated sugar were fairly close to those of galactose while the crystal form and the melting point are different. Its high solubility in alcohol also shows a distinct difference from galactose. The failure to form mucic acid is a further proof that the sugar at hand is different from galactose.

An attempt was then made to detect the sugar by systematic research upon monoses.

Qualitative reactions were first tried with the sugar. It gave, by heating with picric acid and a few drops of caustic soda solution, a brownish yellow colour. By heating with resorcin and hydrochloric acid, it gave no characteristic ketose reaction and no Pinoff reaction with ammonium molybdate and acetic acid. Further it produced neither mucic acid nor saccharic acid by oxidation with nitric acid.

It gave no characteristic colouration and absorption spectrum of pentose by phloroglucin and hydrochloric acid. When a part of the substance was distilled according to the method of Ellet and Tollens[1] with hydrochloric acid 1.06 sp. gr., the distillate obtained indicated no colouration with anilin acetate up to about 60 c. c., after that pale pink colouration was observed until more than 400 c.c.. Further the absorption spectrum was observed at the beginning of blue in the spectrum, when examined by Oshima and Tollens method.[2]

When 0.2 gm. of the sample was distilled quantitatively with hydrochloric acid by the method above noted, alcohol-insoluble furfurolphloroglucid was 0.0031 gm. by weight, that is

Alcohol insoluble furfurolphloroglucid	1.20%
Alcohol soluble furfurolphloroglucid	1:65%

As many hexoses produce by distillation with hydrochloric acid oxymethyl-

1) Ber. D. Chem. Ges., 38, pp. 492—499 (1905).
2) ibid., 34, pp. 1425 (1901).

furfurol beside furfurol, the observed absorption band may probably be inferred to be that of oxymethylfurfurol.

To ascertain this, investigation was next made by the method of Oshima and Tollens modified by Kondo and Oshima. Of the sample 0.5 gm. was distilled with hydrochloric acid of 1.06 sp. gr. until 300 c.c. of the distillate were obtained in the way already noted above. Five c.c. of the distillate taken in a test tube was mixed with 5 c.c. of strong hydrochloric acid, and hydrochloric solution of phloroglucin was added. The absorption spectrum was then observed evidently nearer to the line E than the line F as observed by Tadokoro and Oshima[1] upon oxymethylfurfurol and the solution was found orange-yellowish coloured as well. Next the distillate was redistilled and the distillate therefrom was again examined with a spectroscope. No characteristic absorption spectrum of methylfurfurol was observed at all. From these results it may be decided that the sugar at hand is of different nature from pentose or methylpentose.

The purified sample was subjected to elementary analysis and to the determination of molecular weight. For this investigation the sugar was dried at 60°C in a vacuum.

0.3000 gm. of the substance gave 0.4353 gm. CO_2 and 0.1787 gm. H_2O

	C	H
Calculated for $(CH_2O)_6$:	39.97	6.77
Found :	39.58	6.62

Five grams of the sample was dissolved in 25 gms. of water and the depression of the freezing point was observed by Beckmann's apparatus.

Depression of 0.482°C was observed:

$$\frac{100 \times 5 \times 19}{25 \times 0.472} = 179.3$$

As the calculated molecular weight of hexose is 180.1, the analytical result and the molecular weight obtained agree closely with those of hexose.

To test whether the sugar is fermentable or not, 5 per cent aqueous

1) Jour. of the Tokyo Chem. Soc., 39, pp. 23—30 (1918).

solution of the sugar, mixed with beer-yeast, was exposed at 30°C accompanied by the parallel test with glucose.

The result was as follows:

	After 24 hours	After 48 hours	After 72 hours	After 96 hours	After 120 hours
1. Yeast extract+glucose+yeast	++				
2. Distilled water+glucose+yeast	−	+	+		
3. Yeast extract+isolated sugar+yeast	−	+	++		
4. Distilled water+isolated sugar+yeast	−	−	−	+	+

The isolated sugar is fermentable nicely by beer-yeast though not so well as glucose as may be observed in the above table.

Hexoses, in their general character, produce laevulic acid by heating in a boiling water bath with hydrochloric acid. So if the isolated sugar be a hexose, there must be detected the same acid when the sugar is treated in the above mentioned way. Therefore referring to the methods of Rischbieth[1] and others I carried out the process in the following manner:

Ten grams of the sugar were put in an Erlenmeyer's flask with 100 c.c. of hydrochloric acid of 1.1 sp. gr. (20 per cent as HCl) heated in a boiling water bath for twenty hours, provided with a reflux condenser, when the solution was highly coloured reddish brown and mixed with black humin substance. At the end of the stated period, it was filtered into a beaker. A part of the filtrate was taken in a separating funnel and extracted several times with ether. The ether layer separated was put in a porcelain basin, evaporated and dried for one hour in a steam drying apparatus. In testing the residue with iodine and caustic soda, the formation of chloroform was observed very distinctly, proving there is no doubt of the presence of laevulic acid. Therefore the other part of the filtrate was poured into a distillation bottle and sucked by an aspirator heating at the same time in a water bath. By this process water, formic acid and hydrochloric acid were distilled out. Then the residual syrup was put into an oil bath and distilled at 135—150°C under a vacuum. A yellow brownish liquid was obtained which after a

1) Ber. D. chem. Ges., 20, p. 1775 (1887).

short time became needle-like crystals. The melting point was 33—34°C which coincides exactly with that of laevulic acid.

The concentrated solution of the acid was saturated with silver oxide by which characteristic crystals of round form were obtained. In the analysis they were dried at 90°C in a vacuum.

0.2156 substance gave	0.1032 gr Ag.
Calculated as C_5 H_7 O_3 Ag:	48.04
Found:	47.88

The formation of laevulic acid was fully proved by these investigations.

We are now certain, from the results of above investigations, that the sugar in question is nothing but a hexose. Further it is probable that the sugar is an aldose, as it gave no characteristic colour reactions of ketose.

The sugar was next treated with methylphenylhydrazine with which ketose makes only its osazone. To 1 gm of the sugar dissolved in a small amount of water, 1 gm of methylphenylhydrazine and 4 c.c. of 50 per cent acetic acid were added, with which a small quantity of absolute alcohol was mixed. Then many fine colourless crystals were found already in the mixture. Now to change the hydrazone into osazone, it was heated in a water bath from five to thirty minutes. No osazone was at any time observed to have been formed. When examined under a microscope, the crystals were all in the form of colourless plates. Now the hydrazone was filtered, washed with ether and recrystallized from 70 per cent alcohol. The melting point was determined after the sample was dried over sulphuric acid in a vacuum and was found to be 191°C. The crystals were square plates hardly soluble in cold water, in methyl and ethyl alcohol and insoluble in ether. The melting points of methylphenylhydrazones are:—

arabinose	163°C[1]	galactose	180°C[2]
glucose	124°C[3]	mannose	178°C[4]
rhamnose	124°C[5]	fucose	180°C[6]

These figures are different from those of the prepared hydrazone.

1) Morrell and Crofts—Jour. chem. Soc., 75, p. 791 (1899)
2) Ekenstein and Robry de Bruyn—Recuil de travaux chimiques de Pays-Bas. 15, p. 226 (1896)
3) Neuberg—Ber. D. chem. Ges., 35, p. 965 (1902)
4) Morrell and Crofts—Jour. chem. Soc., 75, p. 790 (1899)
5) " " " ibid., 75, p. 790 (1899)
6) Determined by the author.

Fructose gives no characteristic hydrazone with the reagent.

Further we are aware that the sugar at hand is an aldose as no osazone was obtained by treating with methylphenylhydrazine.

To get more data for comparative investigation, parabromphenylhydrazone was prepared with the sugar, and the reduction product was also made and investigated.

Para-bromphenylhydrazone—A part of the sugar was dissolved in a little water to which was added the mixture prepared newly from 1 part of para-bromphenylhydrazine, 3.5 parts of 50 per cent acetic acid and 12 parts of water. A large quantity of hydrazone was formed after a little time which was filtered, washed with ether, crystallized once more from 90 per cent alcohol and dried over sulphuric acid in a vacuum. The melting point was 171—172°C.

The melting point of para-bromphenylhydrazone of arabinose is 165°C[1] and of fucose 181—183°C.[2] Xylose, galactose, fructose and glucose give no characteristic hydrazone with the reagent.

The recrystallized para-bromphenylhydrazone of the sugar forms a large needle easily soluble in alcohol but insoluble in ether and water.

Among hexoses heretofore described we can find none which has the characteristics shown by this sugar in the investigations above narrated. It may therefore be concluded that the isolated sugar is new to science and hence I propose here to name it Floridose. The is name derived from Florideae, the order to which the plant from which the sugar was first obtained, belongs.

Reduction of Floridose.

Many sugars may be reduced to corresponding alcohols. It would seem to be of interest to study whether floridose can also be reduced or not, and, if so, what is its reduction product. The experiment was carried out with favorable result as follows:

1) Ber. D. chem. Ges., 27, p. 2490 (1894)
2) ibid., 33, p. 140 (1900)

A solution of 3 gms. of the sugar in 30 c. c. of water was put in a beaker, in small amounts sodium amalgam (3 per cent) was then added and stirred violently with a motor. Throughout the operation the solution was kept as neutral as possible by adding dilute sulphuric acid drop by drop. After 15 hours the reaction was ended, consuming 75 gms of sodium amalgam when the solution showed no more reaction in heating in a test tube with Fehling's solution. Thereupon the fluid was separated from mercury by decantation, filtered and neutralized with dilute sulphuric acid. The neutral solution obtained in such a way was concentrated in a water bath until a crystal film was formed over the surface of the fluid. Absolute alcohol warmed to the boiling point was then added until no further precipitate resulted, and filtered hot. The residue was again warmed with alcohol and filtered. When the filtrate was cooled there appeared fine colourless crystals adhering to the wall of the vessel which, on examination under a microscope, were observed to consist uniformly of rhombic prisms. After a little while the crystals were separated by filtration, recrystallized from warm 95 per cent alcohol, washed with cold alcohol and dried over sulphuric acid in a vacuum. The yield was 1.6 gms, about 63 per cent of the sugar used.

The crystals thus obtained were easily soluble in water as well as in boiling alcohol but scarcely in cold alcohol. So, from a warm alcohol solution, crystals may be separated pure by cooling, and the crystal form was uniformly a rhombic prism both from water and alcohol. The crystals had a very sweet taste and their aqueous solution was neutral to litmus paper. They had no reducing power with Fehling's solution, and no rotatory power. The characters above mentioned suggested mannite or dulcite. The reduction product has much similarity in all points to these.

Elementary analysis was made with the substance dried carefully at 105°C for 3 hours with following results:

0.2803 gms sample gave,	0.4037 gm CO_2	0.1851 gm H_2O
	C	H
Calculated for $C_6H_{14}O_6$:	39.53	7.74
Found:	39.18	7.34

The melting point was determined and found to be 186—187°C. The melting point of dulcite is 186°C,[1] of mannite 166°C[2] and of sorbite 175°C.[3] We find much difference between the figures obtained and those of mannite or sorbite, while we see a close resemblance to dulcite. But the crystals at hand produced no mucic acid by oxidation with nitric acid of 1.15 sp. gr. as in the case of dulcite. So the sample investigated seems not to be dulcite.

Further trial was made to test the reaction of the crystals upon benzaldehyde with which mannite and sorbite make characteristic mono- or dibenzalyl compounds. The solution of the crystals was made acid with a little hydrochloric acid, a small amount of benzaldehyde mixed with it and carbonic acid gas was then passed through the mixture by which no precipitate was formed. The test for the dibenzalyl compound was not made, because the sample was insufficient.

As we see from the result of the investigation, the crystalline substance obtained by the reduction of floridose is also a new form of hexavalent alcohol which I shall name floridite.

The summary of the results of my investigation of the new sugar is as follows:

a. It is hexa-aldose.

b. The sugar crystallizes from aqueous solution in oblong plates, a little thick in central part. Sweet as glucose and reduces copper solution strongly. The crystals dissolve easily in strong alcohol and melt at 152—153°C.

c. By heating the aqueous solution with phloroglucin and hydrochloric acid, it gave a brown colour and no absorption spectrum of pentose. By heating with a picric acid and caustic soda solution, it showed yellow colour. It gave no Seliwanoff and Pinoff reaction for ketose.

d. It showed strong birotation. When observed after twenty four hours:

1) Hecht—Ann. d. Chem., 165, p. 148 (1873)
2) Favre—Annales de chemie et de physique, (3) 11, p. 76 (1845)
3) Stahl and Fischer—Ber. D. chem. Ges., 24, p. 144 (1911)

$[\alpha]D = +80.76°$ at 20°C.

e. It produces easily phenylhydrazone of silky lustre with phenylhydrazine in a cold place. The crystal form is needle-like, sometimes curved and aggregated bush-like. The hydrazone is soluble in methyl and ethyl alcohol and hot water, and crystallizes in square plates from the latter on cooling. Its melting point is 158—160°C.

f. It gives yellow osazone of oblong plates, soluble in methyl and ethyl alcohol, insoluble in hot water. The recrystallized osazone melts at 193°C.

g. It produces neither mucic nor saccharic acid, upon oxidation with nitric acid of 1.15 sp. gr.

h. It ferments by beer-yeast.

i. It easily produces methylphenylhydrazone with methylphenylhydrazine in a cold place. Its crystals are square plates melting at 191°C.

j. It combines with para-bromphenylhydrazine in a cold place and produces hydrazone of needle-like form, soluble in alcohol. Its melting point is 171—172°C.

k. By heating with hydrochloric acid it produces laevulic acid.

l. When it is reduced with sodium amalgam it changes to a hexavalent alcohol resembling dulcite. The melting point is 186—187°C.

Isolation of Floridose from *Ahnfeltia plicata*.

As described in preceding pages, a new kind of sugar was isolated from a Chondrus and named floridose. I attempted to detect the same sugar in other kinds of Florideae, and succeeded in separating it from *Ahnfeltia plicata* as well as from a species of Iridaea. The former is a kind of Florideae being now used as a material for agar-agar in Saghalien Island. The account on Iridaea will be found later on.

Five hundred grams of the Ahnfeltia was subjected to hydrolysis just as in the case of the Chondrus. The substance chosen, washed well with water, was heated in a boiling water bath with 3 per cent sulphuric acid. After 15 hours it was cooled, precipitated with phosphotungstic acid, the filtrate

therefrom was neutralized with barium carbonate. The filtrate from the barium salts was concentrated and treated first with 80 per cent alcohol of equal volume. A large quantity of black gummy substance was precipitated to the bottom. The fluid was decanted and concentrated to syrup. This syrup was stirred well in a basin with 95 per cent alcohol and decanted repeatedly. In this manner the part soluble in alcohol was extracted out. The alcoholic extract was evaporated, decolourized with animal charcoal and then concentrated in a partial vacuum at low temperature. Almost colourless, sweet but somewhat bitter syrup was obtained which after standing 12 hours became a crystalline mass. A small amount of 85 per cent alcohol was added, well stirred, filtered by suction and washed thoroughly with absolute alcohol and ether. The sugar thus obtained was 2.6 grams in weight, white in colour and as sweet as glucose. On ignition it left no ash. The melting point was found to be 152°C.

Half a gram of the sugar dried carefully at 60°C in a vacuum was dissolved in a little water and made up to 25 c.c. and polarized in a 200 mm. tube in the Franz Schmidt and Haensch's apparatus. Strong birotation was observed. After standing 24 hours, the rotation was 3.19 on the scale toward the right. The specific rotatory power is

$$[\alpha]^{20}D = \frac{3.19 \times 25}{0.5 \times 2} = +79.50^{\circ}$$

The result obtained coincides with that of floridose which is separated from the Chondrus.

To prepare phenylhydrazone with the sugar, 0.5 gm was dissolved in a little water to which a small amount of phenylhydrazine was added and put aside, sometimes stirring. After 10 minutes it showed the first crystal and after thirty minutes it became a yellow crystalline mass. The hydrazone was filtered by suction, washed with a mixture of ether and alcohol, then recrystallized from 95 per cent alcohol into fine needles. The crystals thus obtained were completely colourless and had a silky appearance. The melting point was determined in a capillary tube and found to be 158—160°C which coincides with that of floridose.

A small part of the sugar was dissolved in a little water to which a

mixture of 1 part of para-bromphenylhydrazine, 3.5 parts of 50 per cent acetic acid and 12 parts of water were added. After a short time the mixture was found filled with many crystals. It was filtered, washed with ether and recrystallized from 90 per cent alcohol. After the crystals were dried over sulphuric acid in a vacuum, the melting point was determined and found to be 171—172°C. The crystals were large needles, soluble in alcohol and acetone, but not in ether.

Further, methylphenylhydrazone was made and examined. A small part of the sugar dissolved in water was mixed with 4 c.c. of 50 per cent acetic acid and a little absolute alcohol. Then, after a little while, fine crystals appeared in great number which were, after a short time, filtered and washed by ether. When examined under a microscope, they were colourless square plates. After drying in a vacuum at 80°C, the melting point was determined and found to be exactly 191°C.

The crystals were scarcely soluble in alcohol, easily soluble in boiling water. The melting point as well as other characteristics were identical with those of floridose from *Chondrus elatus*.

C) Osazone test.

A part of the syrup A was subjected to the osazone test. 3 grams of the syrup, 4 grams of phenylhydrazine, 6 grams of sodium acetate and 40 c.c. of water were mixed and heated in a boiling water bath. At the end of one hour, fine yellowish crystals were produced. When cooled, it was filtered and washed with a little water. The osazone obtained was recrystallized from 70 per cent alcohol and dried over sulphuric acid in a vacuum. The melting point was determined and found to be 150—166°C. which showed the crystals were not from simple osazone. So separation was next tried according to solubility. The refined osazone was first treated with boiling water by which the crystals were separated into two parts: the crystals soluble and insoluble in water. The latter were treated with 50 per cent, 70 per cent and 95 per cent alcohol. The melting point of each osazone was determined, with the following results:

Osazone, soluble in boiling water	154—156°C.
Osazone, soluble in 50 per cent alcohol	not constant.
Osazone, soluble in 70 per cent alcohol	192—193°C.
Osazone, soluble in 95 per cent alcohol	192—193°C.

The melting point of the osazone which was soluble in boiling water, coincides with that of arabinose. Not only the melting point of the crystals coincides closely, but the fibrous crystal form, when examined under a microscope, was identical with arabinose phenylosazone. Crystals soluble in 70 per cent and 95 per cent alcohol showed the melting point to be 192—193°C. These fractions will probably be osazone of floridose above described.

D) Isolation of Arabinose.

A part of the syrup purified with absolute alcohol and animal charcoal was left untouched for two weeks when it was found thickly laden with fine crystals. A small amount of 95 per cent alcohol was added to the syrup, mixed, filtered by suction and washed with absolute alcohol and ether. The sugar thus obtained was slightly yellowish in colour, but upon recrystallization from alcohol it was obtained in pure state. The yield was 3.2 gms. The specific rotatory power was determined and found to be

$$[\alpha]^{20}D = \frac{6.19 \times 25}{1 \times 2} = +77.43^{\circ}$$

With a part of the sugar phenylhydrazone and methylphenylhydrazone were prepared. The melting points of these hydrazones were determined and found to be

Phenylhydrazone	158—159°C.
Methylphenylhydrazone	190°C.

From these figures the sugar isolated was floridose.

The mother liquor filtered off from the crystals formed a small quantity of new crystals after two days. At the end of a week the crystals were again separated by filtration with suction and washed with alcohol and ether.

From the specific rotatory power and crystal form, it was determined also to be floridose.

The mother liquor filtered off from the crystals of the second fraction was allowed to stand for more than two weeks but it did not show any sign of forming new crystals. The isolation of arabinose was then attempted by the use of para-bromphenylhydrazine. Para-bromphenylhydrazine easily forms with arabinose a hydrazone hardly soluble in warm 95 per cent alcohol, while the floridose hydrazone, as already shown, is easily soluble in the same medium. Taking this fact into consideration, I carried out the isolation in the following manner.

The syrup was dissolved in a little water to which a sufficient quantity of the mixture of para-bromphenylhydrazine, 50 per cent acetic acid and water was added. The solution soon became turbid and in the course of 30 minutes, abundant crystalline precipitates were formed. The crystals were separated by filtration, washed with ether-alcohol and pressed in filter paper. The mixture of hydrazones was put in a beaker with a small quantity of 95 per cent alcohol, heated in a water bath at about 70°C for a short time and then filtered. After the extraction was repeated once with 95 per cent alcohol in the same manner, the residue upon the filter paper was dissolved in a small quantity of 60 per cent alcohol and recrystallized from it. The crystals formed were separated by filtration, washed by ether and crystallized once more from 50 percent alcohol. The melting point was determined with a well dried sample and found to be 170°C which agrees well with that of arabinose. The yield was 2.2 gms. The separation of pure arabinose para-bromphenylhydrazone was readily attained in such a manner. Next the hydrazone was decomposed by benzaldehyde in the usual way. A mixture of 1.8 gm of pure hydrazone, 4.5 gms of 95 percent alcohol, 2 gms of benzaldehyde and 1.8 gm of water were heated in a water bath fitted up with a reflux condenser. After 3 hours it was cooled, when the contents solidified into a crystalline mass. The melting point of the crystals, when recrystallized from 95 per cent alcohol, was found to be 129—130°C which coincides with that of benzalbromphenylhydrazone and shows that the decomposition of the original hydrazone had taken place. The filtrate filtered off

from the hydrazone was shaken repeatedly with ether and the water solution was then evaporated. On cooling, crystals were formed in a few days. They were separated by filtration, washed with absolute alcohol and ether and finally dried over sulphuric acid in a vacuum. The sugar obtained in such a manner was almost colourless, very sweet in taste, gave distinctly all the pentose reactions. The yield was about 0.6 gm.

Half a gram of the sugar was dissolved in water, made up to 25 c.c. and polarized in a 200 m.m. tube. Bi-rotation was observed. After 24 hours 4.1 toward the right was read

$$[\alpha]^{20}D = \frac{4.1 \times 25}{0.5 \times 2} = +102.5$$

The rotatory power coincides closely with that of arabinose. The isolated sugar is therefore arabinose.

(5) Experiment with Syrup B.

A) Qualitative tests.

Syrup B gave the following qualitative reactions:

a) It reduced Fehling's solution very strongly.

b) It rotated the plane of polarization toward the right.

c) A characteristic red colouration was produced by heating with resorcin and hydrochloric acid by Seliwanoff reaction.

d) In heating the syrup in a boiling water bath with ammonium molybdate and a little acetic acid according to Pinoff, blue colour was observed.

e) By heating the aqueous solution of the syrup with phloroglucin and hydrochloric acid it gave characteristic colouration and spectral reaction of pentose.

f) The distillate obtained by the redistillation of the syrup with hydrochloric acid of 1.06 sp. gr. showed no spectral reaction of methylfurfurol.

g) It produced no characteristic crystals of cadmium bromoxylonate by the method of Bertrand.

h) Mucic acid was produced upon oxidation with nitric acid of 1.15 sp. gr. in the usual manner.

i) Saccharic acid was detected as acid potassium salt in the oxidized solution separated from the crystals of mucic acid by the usual method.

j) Two drops of the syrup were put on each of several object glasses and seeded respectively with a crystal of xylose, arabinose, glucose, fructose, galactose, mannose and floridose. After twenty four hours the drops which had been seeded with galactose and floridose showed a thick formation of new crystals while others remained unchanged.

From the above qualitative reactions it is clear that syrup B contains galactose and floridose. Besides that the presence of glucose and fructose is probable.

B) Osazone test.

Three grams of the syrup, 4 gms. of phenylhydrazine hydrochloride, 6 gms. of sodium acetate and 40 c.c. of water were mixed and heated in a boiling water bath. At the end of one hour, yellowish crystals were obtained. When cooled the mixture was filtered, washed with a little water and recrystallized from 70 per cent alcohol. As the osazone thus obtained was a mixture of various shapes of crystals, separation was attempted in the following manner:

The osazone was first treated with boiling water and separated into two parts. The part insoluble in boiling water was then treated with 50 per cent, 70 per cent, and 95 per cent alcohol and separated according to solubility. The melting point of each fraction was determined and found as follows:

Osazone, soluble in boiling water	154—156°C.
Osazone, soluble in 50 per cent alcohol	171—180°C.
Osazone, soluble in 70 per cent alcohol	193—195°C.
Osazone, soluble in 95 per cent alcohol	193—199°C.

The melting point of the osazone soluble in boiling water corresponds to that of arabinose. When examined under a microscope, the crystals, fibrous in shape, coincided well with those of arabinose mixed with no other crystals. I could not detect xylose osazone which dissolves also in warm water and crystallizes into characteristic needle form on cooling. The osazones soluble in 70 and 95 per cent alcohol will be those of galactose or floridose as the melting point of the osazones of these sugars is about 195°C. The osazone soluble in 50 per cent alcohol seems not to have a constant melting point.

From the above results it may be inferred that the amount of glucose in the syrup is comparatively small as the melting point of osazone soluble in alcohol was not higher than that of galactosazone.

C) Isolation of Floridose and Galactose.

The syrup B, which was pale yellow in colour, was left untouched in a cold place seeded with crystals of floridose. After one week it was found thickly laden with fine crystals. A small amount of 50 per cent alcohol was added, mixed thoroughly, filtered by suction and washed with 50 per cent alcohol and then ether. The sugar thus obtained was then recrystallized from a little water into pure crystals, which were dried over sulphuric acid in a vacuum. The yield was 12 grams in weight. The sugar was perfectly white in colour, very sweet in taste, and the melting point found was 151—152°C. One gram of the sugar was dissolved in a little water and made up to 25 c.c. and polarized in a 200 mm. tube. Bi-rotation was observed. After twenty-four hours the dextro-rotation 6.41 on the scale was noted

$$[\alpha]^{20}D = \frac{6.41 \times 25}{1 \times 2} = +80.11$$

No mucic acid was produced when a part of the sugar was treated with nitric acid of 1.15 sp. gr. by the usual method.

It may be inferred from these results, that the sugar isolated is no other than floridose.

The mother-liquor filtered off from the crystals of floridose was put aside for three weeks longer in a cold place, but it did not show any sign

of forming new crystals. The syrup was then mixed with a small amount of 75 percent alcohol, left standing as it was for a longer period after being seeded with crystals of galactose. After one month the syrup was found filled with plenty of new crystals. After being stirred with 50 per cent alcohol, the crystals were separated by filtration, washed by alcohol and ether and dried at 60°C. The yield was 28.7 gms in weight. The sugar thus obtained was slightly yellow in colour, so it was recrystallized from a small amount of dilute alcohol after being decolourized with animal charcoal. The crystals obtained were white, very sweet in taste and left no ash on ignition. For investigation it was dried over sulphuric acid in a vacuum. The melting point was determined and found to be 160—161°C. One gram of the sugar was dissolved in 25 c.c. of water and polarized in a 200 mm. tube in Schmidt and Haensch's polariscope. After 24 hours 6.37 toward the right was read. The specific rotatory power is

$$[\alpha]^{20}D = \frac{6.37 \times 25}{1 \times 2} = +79.66$$

A large quantity of mucic acid was obtained by oxidizing the sugar with nitric acid of 1.15 sp. gr. in the usual manner.

The melting point, rotatory power and other characteristics of the sugar were closely identical with those of galactose.

D) Detection of Mannose.

The mother-liquor filtered off from the crystals of galactose was allowed to evaporate of itself in a desiccator. Ten grams of the syrup thus obtained were mixed with 5 gms. of water and 5 gms. of pure phenylhydrazine. In the course of one hour it was found laden thickly with fine crystals. When examined under a microscope, the characteristic round grains of mannose phenylhydrazone mixed with fine needle-like crystals in small quantity were observed; the latter perhaps being the hydrazone of galactose or floridose.

After further standing, it was filtered with suction, washed with ether-alcohol and recrystallized from 95 per cent alcohol. The melting point was determined and found to be 167—172°C. As the melting point of mannose

phenylhydrazone is 195—200°C, there is a great difference between them. When crystallized three times from 95 per cent alcohol the melting point was found to be 191—195°C which is fairely close to that of mannose.

Further investigation was omitted due to the small amount of the hydrasone and its lack of purity, but judging from the crystalline form and the melting point, the presence of mannose in the syrup is highly probable.

Summary

a) From the hydrolysis products of the mucilaginous substance of *Chondrus elatus*, galactose, arabinose and an unknown sugar having much similarity to galactose were isolated.

b) The name floridose was given to the unknown sugar.

c) Floridose was separated also from another member of Florideae, *Ahnfeltia plicata*.

d) The presence of mannose, glucose and fructose is probable.

II. Gloiopeltis.

On the chemical nature of Gloiopeltis an investigation has been made lately by K. Kawakami[1] who, carrying out hydrolysis of the whole body of the plant, obtained galactose. Beside his statement there is no literature upon the constituents of the mucilaginous substance of the plant. The sample taken as the material of the present research was a product of Hokkaido, of *Gloiopeltis furcata* var. *coliformis* J. Ag. The general analysis showed the following results :

	In air-dry substance	In water-free substance
	%	%
Moisture	25.47	
Crude ash	28.96	38.65
Crude protein	9.05	12.13
Crude fat	0.34	0.41
Crude fiber	4.76	6.38
Nitrogen-free extract	31.42	42.43
Total nitrogen	1.45	1.94
Non-protein nitrogen	0.24	0.32

Separation of mucilaginous substance.

As in the case of the Chondrus the mucilaginous substance was first separated quantitatively and then the qualitative research as well as the general analysis were made.

20 gms of the well washed and dried material were put in 1 liter of water, heated in a boiling water bath for one hour and then filtered with suction. After the residue was treated three times in the same manner as above described, the filtrate and washings were put in together, and then the qualitative research was made as follows :

1) Jour. of Chem. Indst. Tokyo. 13, pp. 431-377 (1910).

a) Reducing sugars—When 10 c.c. of the mucilage of the Gloiopeltis were mixed with Fehling's solution and heated for a while, no reduction occurred. It shows that there is no reducing substance in it.

b) Starch—In adding iodine solution to the pure mucilage either in dilute or in concentrated condition, it gave no colour reaction. And even if we dip the section of the plant in the iodine solution no colouration was observed also. We know that no starch is present in the mucilage.

c) Mannite—This was investigated with the original substance, that is, 10 gms of the crushed material were heated with 50 c.c. of 85 per cent alcohol, and put aside in cool place, but no crystal of mannite was found in the fluid. The alcohol solution was then filtered and the filtrate, evaporated in a water bath. When the concentrated solution was shaken with hydrochloric acid and benzaldehyde, no precipitate was formed.

d) Pentosane and methylpentosane—When a small amount of the mucilage was heated with phloroglucin and hydrochloric acid, the fluid showed the characteristic red colour and absorption spectrum of pentose. On distilling the substance with hydrochloric acid of 1.06 sp. gr., a distillate was obtained which gave characteristic furfurol reaction with aniline acetate. The distillate also gave distinctly the characteristic absorption spectrum of methylfurfurol by the Kondo and Oshima method. Both pentosane and methylpentosane are present in the mucilage.

e) Galactane—Mucic acid was produced by oxidizing the sample with nitric acid. The presence of galactane is beyond question.

f) Rotation—A filtered clear solution of the mucilage was examined in a Schmidt and Haensch's polariscope, and rotation was observed.

A part of the mucilage was evaporated, dried and used for the general analysis. The results were as follows :

	In dry matter %
Substance soluble in warm water	80.01
Substance insoluble in warm water	19.99

	In air-dry substance	In water-free substance
	%	%
Moisture	14.10	
Crude ash	12.32	14.34
Crude protein	10.31	12.00
Crude fat	Trace	Trace
Nitrogen-free extract	63.27	73.66
Total nitrogen	1.64	1.90
Non-protein nitrogen	0.34	0.39
Galactane	20.70	24.09
Pentosane	1.64	1.90

The mucilaginous substance of the Gloiopeltis reacts just in the same way as that of the Chondrus. It is neither precipitated by any acid nor by lead acetate, while it separates as a voluminous precipitate with alcohol or with basic lead acetate. Alcohol was used, as in the case of the Chondrus, to purify the mucilage.

Investigations upon the hydrolysis products.

(1) Preparation of sample.

Into the pure mucilage obtained by boiling the purified seaweed, strong alcohol was poured, up to about 80 per cent by weight, and thus the gelatinous precipitates were produced at once in a large quantity. After 24 hours, they were filtered with suction. The filtrate therefrom was evaporated in a water bath to a small volume and precipitated again by strong alcohol. The two precipitates were mixed, dried in a water bath and used for the hydrolysis.

(2) Hydrolysis.

One hundred grams of the purified alcohol precipitate of the mucilage of

the Gloiopeltis were mixed with 1 liter of 3 per cent sulphuric acid in a large flask and heated in a boiling water bath for 15 hours. When cooled, it was filtered with suction. The dark coloured filtrate was then precipitated with phosphotungstic acid after the solution was mixed with sulphuric acid, until the content of the acid becomes about 5 per cent. Next day it was filtered, neutralized with pure barium carbonate on a water bath and left overnight. Thereupon the barium salts were filtered off, and the filtrate was concentrated in a partial vacuum to about 200 c.c. The warm solution thus obtained was put into a flask with 100 c.c. of 85 per cent alcohol and allowed to stand for about 12 hours, when a blackish gummy substance was precipitated on the bottom. The fluid part was decanted and concentrated in a partial vacuum to about 100 c.c. To the remaining syrup about 300 c.c. of 95 per cent alcohol was added, and much yellowish gummy substance was produced again. After a few hours, the clear solution was decanted. The gummy substance was dissolved in a small quantity of water and again precipitated by shaking with 95 per cent alcohol, then upper solution was decanted. The solutions decanted were brought together and concentrated to a small volume. The syrup was once more purified with 100 c.c. of absolute alcohol. The clear solution thus obtained was evaporated to about 50 c.c., decolourized with animal charcoal and preserved for investigation as syrup A. The total weight was 27 gms.

The gummy substances produced by shaking with 95 per cent as well as absolute alcohol were dissolved in a small amount of water, decolourized by animal charcoal, and again it was concentrated to a small volume. About 58 grams of yellowish syrup having a very sweet taste were obtained, and the ignition of a small quantity of it left no ash. The syrup thus obtained was preserved for further investigation as syrup B.

(3) Experiments with syrup A.

A) Qualitative tests.

a) It reduced Fehling's solution very strongly.

b) It rotated the plane of polarization slightly toward the right.

c) It gave the Seliwanoff reaction with resorcin and hydrochloric acid.

d) By heating with ammonium molybdate and acetic acid, it gave pale blue colourization.

e) By heating the syrup with phloroglucin and hydrochloric acid, it gave the characteristic red colour, and showed distinctly the spectral reaction of penstose.

f) The distillate, obtained by redistillation of the syrup with hydrochloric acid of 1.06 sp. gr., distinctly showed the spectral reaction of methylfurfurol.

g) It produced no characteristic crystal of cadmium bromoxylonate by the method of Bertrand.

h) Neither mucic acid nor saccharic acid was produced upon oxidation with nitric acid of 1.15 sp. gr. in the usual manner.

i) When a part of the syrup was mixed with a small amount of phenylhydrazine, the crystalline mass was made in a little while, and the crystals under a microscope showed the form of a long needle. The crystals were separated with suction, washed with alcohol and ether, and recrystallized from 80 per cent alcohol. The hydrazone, by heating in a capillary tube, melted at 172°C. which coincides closely with that of fucose phenylhydrazone. The presence of fucose therefore is highly probable.

j) Crystals of fucose, xylose, arabinose, galactose and glucose were respectively put into one drop of the syrup, placed on an object glass. After twenty-four hours, the drop which had been seeded with fucose showed the formation of new crystals while others remained unchanged.

From the above qualitative reactions, it is almost certain that the syrup under examination contains fucose, while for other sugars there remains a question.

B) Isolation of fucose.

To prepare much of the phenylhydrazone, another 100 gms of the pure mucilage of the Gloiopeltis were hydrolysed with 1 liter of 3 per cent sul-

phuric acid as above mentioned, and 28 gms. of purified alcohol-soluble syrup were obtained.

Twenty five grams of the syrup were mixed with 12 gms of phenylhydrazine and 12 gms of water. The mixture, after two hours, became a yellow crystalline mass. The hydrazone was stirred with ether-alcohol, soon filtered with suction and recrystallized from 95 per cent alcohol. The yield weighed 12 gms after being dried in a vacuum. The melting point was determined, and found to be 171—172°C.

The purified hydrazone was then decomposed by means of benzaldehyde. The hydrazone was mixed with 10 gms of water, 18 gms of 95 per cent alcohol and 12 gms of benzaldehyde, and the mixture was heated for one hour in a boiling water bath, provided with a reflux condenser. The hydrazone was dissolved soon by warming, and long needle-like crystals of benzalphenylhydrazone melting at 155°—156°C separated on cooling. The filtrate from the crystals was shaken repeatedly with ether, decolourized by animal charcoal and evaporated to a small volume. The syrup which resulted was almost colourless and reduced Fehling's solution strongly.

After standing three days the syrup became a fine crystalline mass which was filtered with suction after being mixed with 70 per cent alcohol. The crystals thus obtained were once more crystallized from warm alcohol, and quite pure needle-like crystals were prepared. The yield weighed about 1.7 gms. Having been dried at 60° in a vacuum, the rotatory power was determined by the use of a polariscope.

1 gm of the substance was dissolved in 25 c.c. of water and polarized in a 200 mm. tube. Strong birotation was observed, and after 24 hours, 5.86 toward the left was noticed.

$$[\alpha]^{15}D = \frac{5.86 \times 25}{1 \times 2} = -73.25^{\circ}$$

The specific rotatory power of fucose is -74° according to Widtsoe and Tollens.[1] The sugar under examination therefore is no other than fucose.

1) Ber. D. chem. Ges., 33, p. 141. (1900).

Phenylosazone and para-bromphenylhydrazone were further prepared with the sugar:

Phenylosazone—0.5 gm of the sugar, 1 gm of phenylhydrazine hydrochloride, 1.5 gm of sodium acetate were mixed with 10 c.c. of cold water. When, after a little while, the mixture was dissolved, there appeared crystals of hydrazone. By warming in a water bath, the hydrazone was again dissolved, and yellow osazone separated after long boiling. The crystals were separated by filtration, washed and recrystallized from 75 per cent alcohol. The melting point was 158—159°C which agrees with that of fucose.

Para-bromphenylhydrazone—1 gm. of the sample was dissolved in water to which a mixture of para-bromphenylhydrazine, 50 per cent acetic acid and water were added. After a little while a large amount of hydrazone was formed. The hydrazone was then filtered, washed with ether-alcohol and recrystallized from 70 per cent alcohol. The melting point was determined and found to be 181—182°C. The hydrazone had a silky appearance and was scarcely soluble in 95 per cent alcohol. The melting point of fucose para-bromphenylhydrazone, according to Widtsoe and Tollens[1] or Günther and Tollens,[2] is 181—183°C which coincides closely with that of the prepared hydrazone.

C) Osazone test.

Two grams of the syrup, 4 gms of phenylhydrazine, 6 gms of sodium acetate and 40 c.c. of water were mixed and heated in a boiling water bath for one hour. Fine yellow crystals were thereby produced. When examined under a microscope, the crystals did not consist of a single osazone. When cooled, they were filtered, washed with a little water, recrystallized from 70 per cent alcohol and dried over sulphuric acid in a vacuum. The melting point was determined and found to be 155—156°C. Then the osazone was stirred with boiling water in a beaker and filtered soon. The filtrate deposited many crystals on cooling, which were filtered off and recrystallized

1) Ber. D. chem. Ges., 33, p. 132 (1900).
2) Lieb. Ann., 271, p. 81 (1892).

from hot water. When examined under a microscope, no other forms but the yellow fibrous crystals, curved at their end, were observed. The crystals were washed with a little cold water and dried over sulphuric acid. The melting point was determined and found to be 155°C.

The melting point as well as its characteristic crystal form were quite identical with those of arabinose.

The residue, insoluble in warm water, was recrystallized from 85 per cent alcohol, and then the melting point was determined after drying over sulphuric acid. The figure obtained was 158—160°C. The fraction may probably be considered as fucosephenylosazone.

D) Isolation of arabinose.

From the osazone test above described, the presence of arabinose in the syrup is highly probable. So the isolation of arabinose was next attempted in the following manner:

To separate fucose out of the syrup first, a part of it was purified once more with animal charcoal to almost colourless and set untouched in a cold place, seeded with crystals of fucose. After several days it was found thickly laden with new crystals. Then the syrup was mixed with a small amount of 95 per cent alcohol, filtered and washed with absolute alcohol. The sugar thus obtained was almost colourless, and the melting point was found to be 138—139°C. The aqueous solution showed a remarkable laevo-rotation. From these characteristics the isolated sugar may well be considered as fucose.

The mother liquor filtered off from the crystals of fucose was again concentrated to a small volume in a partial vacuum, and allowed to stand in a cold place, and seeded this time with arabinose crystals. After two weeks many new crystals were formed. The syrup was stirred with a small amount of alcohol, filtered and washed with absolute alcohol and then ether. By recrystallization from water it was obtained in pure state. The crystals were perfectly white, very sweet in taste and gave all the pentose reactions. The

yield was about 0.8 gm. The melting point was determined and found to be 158—159°C.

Half a gram of the crystals was dissolved in 25 c.c. of water and polarized in a 200 mm. tube. Bi-rotation was observed, and after 24 hours a dextro-rotation of 4.09 was noticed. The specific rotatory power is:

$$[\alpha]^{20}D = \frac{4.09 \times 25}{15 \times 2} = +102.3^{\circ}$$

Half a gram of the pure sugar was mixed with 1 gm of phenylhydrazine hydrochloride, 1.5 gm of sodium acetate and 10 c.c. of water, and then the mixture was heated for one hour and a half in a water bath. Yellow crystals were thus produced. After recrystallization from boiling water their melting point was determined and found to be 159°C.

The specific rotatory power and the melting point of this osazone coincide closely with those of the osazone of arabinose.

(4) Experiments with syrup B.

A) Qualitative tests.

Syrup B gave the following qualitative reactions.

a) It reduced Fehling's solution strongly.

b) It rotated the plane of polarization toward the right.

c) It gave the Seliwanoff reaction with resorcin and hydrochloric acid.

d) By heating with ammonium molybdate and acetic acid it gave a blue colouration.

e) By heating the syrup with phloroglucin and hydrochloric acid it gave a characteristic red colour and the spectral reaction of pentose.

f) The spectral reaction of methylfurfurol was observed in the distillate which was obtained by distillation of the syrup with hydrochloric acid of 1.06 sp. gr.

g) It produced no characteristic crystal of cadmium bromoxylonate by the method of Bertrand.

h) Upon oxidation with nitric acid of 1.15 sp. gr., mucic acid was produced.

i) Saccharic acid was also produced from the filtrate of mucic acid.

j) Two drops of the syrup were placed on each of several object glasses and were seeded respectively with crystals of galactose, fructose and glucose. After twenty four hours, the drops which had been seeded with galactose showed the formation of new crystals while the others remained unchanged.

From the above qualitative reactions there can hardly be any doubt that the syrup under examination contains galactose. Moreover the formation of saccharic acid and the positive reaction for ketose suggest the presence of glucose and a ketose, probably fructose.

C) Isolation of galactose.

Though the syrup B was left untouched for one month it did not show any sign of forming new crystals of its own accord. The syrup was next dissolved in a small amount of water, decolourized with animal charcoal and again concentrated in a partial vacuum. The pale yellowish syrup thus obtained was mixed with a small amount of alcohol, and left as it was for three weeks after being seeded with pure crystals of galactose, when it was found laden with new crystals. A small amount of 85 per cent alcohol was added to the syrup, well mixed, filtered with suction and washed with absolute alcohol and ether. The sugar obtained was 6.5 grams in weight and slightly yellowish in colour, but upon recrystallization from alcohol with the use of animal charcoal it became perfectly white and left no ash on ignition. The melting point was 162°C.

One gram of the carefully dried sugar was dissolved in 25 c.c. of water and polarized in a 200 mm. tube. The rotation on the scale was 6.44 toward the right. The specific rotatory power is :

$$[\alpha]^{20}D = \frac{6.44 \times 25}{1 \times 2} = +\ 80.86°$$

A large amount of mucic acid was produced upon oxidation of the sugar with nitric acid of 1.15 sp. gr.

From the rotatory power and other characteristics, there is little doubt that the isolated sugar is galactose.

D) Separation of fucosephenylhydrazone.

Five grams of the syrup were dissolved in 3 gms. of water, to which 3 gms. of phenylhydrazine were added and the mixture was well stirred. After twenty minutes the mixture became a crystalline mass. No other forms but long needle-shaped crystals were obtained when examined under a microscope. The crystals were separated by filtration, washed with a small amount of 75 per cent alcohol and then recrystallized from 95 per cent alcohol. The crystals thus obtained were perfectly white with silky lustre and weighed 0.1 gm when dried over sulphuric acid in a vacuum. The melting point was 172—173°C.

From the crystal form and the melting point, the separated hydrazone was no other than that of fucose.

Summary.

a) Fucose, galactose and arabinose were isolated from the hydrolysis products of the mucilaginous substance of *Gloiopeltis furcata* var. *coliformis* J. Ag..

b) Fructose and glucose probably present in the hydrolysis products, while the existence of floridose is excluded.

III. Iridaea.

The alga is also a kind of *Florideae*, which is often used for technical purposes. By heating it with water, a viscid mucilaginous substance is easily produced. And it is for this cause, that this red seaweed is commonly used instead of the Chondrus, where the latter is hard to be found. The sample taken for the present investigation is the product of Hokkaido, its scientific name being *Iridaea laminarioides* var. *cornucopiae* J. Ag.

On the constituents of the alga, an investigation has lately been made by Hoagland and Lieb[1] and they described the presence of galactose in the hydrolysis products. Besides the description above mentioned, there seem to be no others upon the same subject up to date.

The material was first freed from the adhering salts or admixed sands by repeated washing with cool water, and then was subjected to a general analysis.

	In air-dry substance	In water-free substance
	%	%
Moisture	9.13	
Crude ash	8.63	9.49
Crude protein	12.56	13.82
Crude fat	0.89	0.98
Crude fiber	1.98	2.18
Nitrogen-free extract	66.81	73.53
Total nitrogen	2.01	2.21
Non-protein nitrogen	0.48	0.52

The pure mucilaginous substance was next separated from 10 gms of the substance by the same process as I have mentioned before in the case of Chondrus, and then the general analysis as well as qualitative research were made as follows:

a) Reducing sugar—A small portion of the mucilage was mixed with Fehling's solution and heated, by which no reduction was observed.

1) The Journ. of Biol. Chem., 33, pp. 287—297 (1915).

b) In adding iodine solution to the pure mucilage, it gave no colour reaction. When the section of the plant was dipped in the iodine solution, the characteristic colouration was observed at the interior tissue of it. But, as in the case of the Chondrus, starch did not come in the mucilage by boiling the sample with water in the usual way.

c) Mannite—The original substance was extracted with 85 per cent alcohol in the same way as I have mentioned before, and no crystal was obtained. The presence of mannite is excluded in the mucilage.

d) Pentosane and methylpentosane—When a small amount of the mucilage was heated with phloroglucin and hydrochloric acid, the solution showed the characteristic colouration and absorption spectrum of pentose. The distillate, obtained by distilling the substance with hydrochloric acid of 1.06 sp. gr., gave the characteristic furfurol reaction with aniline acetate, but no characteristic spectrum of methyl-furfurol was observed in it by the method of Oshima and Tollens modified by Kondo and Oshima. The presence of pentosane is highly probable.

e) Galactane—A large quantity of mucic acid was obtained by nitric acid in the usual manner.

f) Rotation—A slight dextro-rotation was observed when a filtered, clear solution of mucilaginous substance was examined by a Schmidt and Haensch's polariscope.

The percentage of mucilaginous substance was as follows:

	In dry matter %
The substance soluble in warm water	76.68
The substance insoluble in warm water	23.32

General analysis gave the following results.

	In air-dry substance	In water-free substance
	%	%
Moisture	6.55	
Crude ash	20.94	22.40
Crude protein	6.07	6.49
Crude fat	Trace	Trace
Nitrogen-free extract	66.44	71.11
Total nitrogen	0.97	1.04
Non-protein nitrogen	0.25	0.27
Galactane	23.33	25.07
Pentosane	1.55	1.66

Investigations upon the hydrolysis products.

(1) Preparation of sample.

The mucilaginous substance was extracted as much as possible from the purified alga with water, by heating in a water bath. Then it was filtered with suction, evaporated and dried. The dried substance was used directly as the material for the hydrolysis, without treating with alcohol.

(2) Hydrolysis.

Of the substance, obtained in the above way, 200 gms. were mixed with 1 liter of 3 per cent sulphuric acid and heated in a boiling water bath for 14 hours, then the fluid in the flask was found coloured dark brown. When cooled, it was filtered with suction, and the filtrate therefrom was then precipitated with phosphotungstic acid as in the case of the former research. Next day it was filtered, neutralized with barium carbonate and left over night. The solution filtered off from barium salts was then concentrated to about 300 c.c. in a partial vacuum. The solution thus obtained was put into a flask with 200 c.c. of 85 per cent alcohol and allowed to stand for twenty hours, when a blackish gummy substance was precipitated. The fluid part was decanted and concentrated again to about 200 c.c. To the remaining syrup, about 300 c.c. of 95 per cent alcohol were added, which produced

much yellowish gummy substance. After standing for a few hours, the clear solution was decanted. The gummy substance produced was dissolved in a small amount of water, and again precipitated by thorough shaking with 95 per cent alcohol. The clear solutions decanted were brought together and concentrated to a small volume. The syrup was then once more purified by shaking with 100 c.c. of absolute alcohol. The solution thus obtained was evaporated to about 40 c.c., decolourized with animal charcoal and preserved for investigation as syrup A. The total weight was 28 gms.

The gummy substance, produced by shaking with 95 per cent and absolute alcohol, was dissolved in a small amount of water, decolourized with animal charcoal and again concentrated to a small volume. About 73 gms. of the syrup were obtained which had very sweet taste and left no ash on ignition. The syrup was preserved for further investigation as syrup B.

(3) Experiments with Syrup A.

A) Qualitative tests.

Syrup A gave the following qualitative reaction.

a) It reduced Fehling's solution strongly.

b) It rotated the plane of polarization toward the right.

c) It gave no Seliwanoff reaction with resorcin and hydrochloric acid.

d) By heating with ammonium molybdate and acetic acid, it gave no colouration.

e) By heating the syrup with phloroglucin and hydrochloric acid, it gave a characteristic red colour and spectral reaction of pentose distinctly.

f) The distillate, obtained by the distillation of the syrup with hydrochloric acid of 1.06 sp. gr., showed no spectral reaction of methylfurfurol.

g) It produced no characteristic crystal of cadmium bromoxylonate by the method of Bertrand.

h) Neither mucic acid nor saccharic acid was produced by the oxidation of the sample with nitric acid of 1.15 sp. gr. in the usual manner.

i) When a part of the syrup was mixed with a small quantity of phenylhydrazine, hydrazone was formed after a while. The crystals, recrystallized from alcohol, showed their melting point to be 160°C.

j) Two drops of the syrup were placed on each of six different object glasses, and were seeded respectively with crystals of fucose, xylose, arabinose, galactose, fructose and floridose. After twenty four hours, the drops which had been seeded with floridose, showed the formation of new crystals, while the others remained unchanged. After three days, all the drops were found laden with fine plate-like crystals, which reminded me of the floridose crystals.

k) A part of the syrup was heated for one hour with phenylhydrazine and acetic acid. The resulting osazone was carefully washed and recrystallized from 70 per cent alcohol. When the purified osazone was treated with warm water, a part of it was dissolved and separated on cooling in fibrous crystals, which melted at 155°C. by heating in a capillary tube. The melting point and crystal form were identical with those of arabinosephenylosazone.

From the above qualitative reactions, it would seem that the syrup under examination probably contains floridose and arabinose.

B) Isolation of floridose.

As it has become very probable that the syrup containes floridose, the isolation of it was first attempted, depending upon the fact that the sugar very easily crystallizes from the purified solution. The syrup A was left untouched for two weeks seeded with crystals of floridose. Then it was found thickly laden with fine crystals. A little 95 per cent alcohol was added to the syrup, well stirred, filtered with suction and washed with absolute alcohol and then ether. The sugar thus obtained weighed 3.7 gms, and was almost colourless. Upon recrystallization from alcohol by the use of animal charcoal, it was obtained in perfectly pure state and left no ash on ignition.

One gram of the sugar was dissolved in 25 c.c. of water and polarized in a 200 mm. tube. The specific rotatory power was

$$[\alpha]^{20}D = \frac{6.38 \times 25}{1 \times 2} = +79.75^{\circ}$$

Melting point of the crystals was determined and found to be 152—153°C.

A small amount of the sugar was treated with nitric acid of 1.15 sp. gr., but no crystals of mucic acid resulted.

Phenylhydrazone was made with phenylhydrazine. The melting point was determined with its pure crystals, obtained by recrystallization from 95 per cent alcohol, and found to be 158—160°C.

A part of the sugar was dissolved in a little water to which a small amount of methylphenylhydrazine, 50 per cent acetic acid and alcohol were added. After a little while fine colourless plate-like crystals were formed. They were filtered, recrystallized from warm water and dried at 80°C in a vacuum. The melting point was 191°C.

Para-bromphenylhydrazone was also prepared with the mixture of para-bromphenylhydrazine and acetic acid. The crystals formed were separated by filtration, washed by ether and then recrystallized from a little water. After being carefully dried in a vacuum, they were subjected to the determination of the melting point, which was found to be 171—172°C.

The rotatory power and all other characteristics closely coincide with those of floridose. There is little doubt that the sugar in question is floridose.

C) Isolation of arabinose.

The mother-liquor filtered off from the crystals was once more decolourized with animal charcoal and concentrated again in a partial vacuum. The syrup obtained was seeded with crystals of floridose and put aside for one more week. The crystals formed were separated by filtration with suction, washed with alcohol and ether. The yield weighed 1.2 gms.

The mother-liquor, filtered off from the second crop of crystals, was concentrated again to syrup, allowed to stand in a cold place, after being seeded with the crystals of arabinose. After two weeks, a part of the syrup was examined under a microscope, when it was observed filled with fine boat-like crystals. Thereupon the syrup was mixed with a little 90 per cent alcohol,

filtered with suction and washed with absolute alcohol and ether. The sugar thus obtained was 0.8 gm in weight and almost colourless. It was purified by dissolving in a little water, decolourizing with animal charcoal and recrystallizing over sulphuric acid. The crystals were then perfectly white and showed all the pentose reactions.

Half a gram was dissolved in 25 c.c. of water, and after 24 hours, it was polarized in a 200 mm. tube.

$$[\alpha]^{20}D = \frac{4.07 \times 25}{0.5 \times 2} = +101.8^{\circ}$$

Osazone was made with phenylhydrazine. The resulting crystals were soluble in hot water and crystallized in fibrous shape. Their melting point was determined and found to be 156°C. The specific rotatory power and melting point of the osazone closely coincide with those of the osazone of arabinose.

(4) Experiments with syrup B.

A) Qualitative tests.

Syrup B gave the following qualitative reactions.

a) It reduced Fehling solution strongly.

b) It gave no Seliwanoff reaction with resorcin and hydrochloric acid.

c) It gave characteristic colour reaction and spectral reaction of pentose.

d) The distillate obtained by the distillation of the syrup with hydrochloric acid of 1.06 sp. gr., shows no spectral reaction of methylfurfurol.

e) A large quantity of mucic acid was produced upon oxidation with nitric acid of 1.15 sp. gr. in the usual manner.

f) A small quantity of saccharic acid was detected in the solution, separated from the crystals of mucic acid by the usual method.

g) It produced no crystals of cadmium-bromoxylonate by the method of Bertrand.

h) It produced phenylhydrazone with phenylhydrazine. The melting point was 158°C.

i) One drop of the syrup was placed on each of five object glasses, and seeded respectively with crystals of floridose, glucose, fructose, arabinose and galactose. After a few days, the drops which had been seeded with floridose and with galactose, showed the formation of new crystals, while the others remained unchanged.

From the above qualitative reactions it is almost certain, that the syrup under examination contains floridose and galactose.

B) Isolation of galactose.

The syrup B was left untouched nearly one month, but it did not show any crystal of its own accord. Thereupon the syrup was dissolved in a little water, decolourized with animal charcoal to pale yellow colour, and set aside in a cold place, after being seeded with crystals of floridose. Several days after, the crystals formed were separated by filtration. The filtrate therefrom was seeded this time with crystals of galactose, and set aside in a cold place for a long time. Then the syrup was found laden with many fine crystals. A little alcohol was added to the syrup, filtered and washed with absolute alcohol. The sugar thus obtained was recrystallized from a small amount of alcohol. The crystals obtained were perfectly white and very sweet in taste.

One gram of the sugar, carefully dried in a desiccator, was dissolved in 25 c.c. of water and polarized in a 200 mm. tube. The specific rotatory power was

$$[\alpha]^{15}D=\frac{6.4\times 25}{1\times 2}=+80.0^{\circ}$$

A large quantity of mucic acid was produced, when a part of the sugar was oxidized with nitric acid in the usual manner.

The isolation of galactose was fully demonstrated.

Summary

a) From the hydrolysis products of *Iridaea laminarioides* var. *cornucopiae* J. Ag., galactose, floridose, and arabinose were isolated.

b) The presence of glucose is probable, but the presence of fructose is not clear.

As we see in the preceding pages, many sugars were found in the hydrolysis products of the mucilaginous substance of Florideae. Galactose and arabinose were found in all the three samples investigated, and beside these, fucose, mannose and floridose were isolated or detected in the products.

Isolation of floridose is most interesting, and it was separated from the Chondrus, Iridaea and *Ahnfeltia plicata*, the first one yielding the largest amount. On the occurence of this sugar in other kinds of seaweeds, no investigation has yet been made.

To determine what quantity of sugars is produced when the original substance is split up by hydrolysis, 1 gram of the pure mucilaginous substance of each was subjected to hydrolysis by heating for fifteen hours in a water bath, and the amount of sugar produced was determined by Allihn's method, after the solution was neutralized by potassium carbonate. The result was as follows:

Chondrus	64.8 %
Gloiopeltis	61.3 %
Iridaea	60.6 %

The percentage was calculated as galactose from the substance free from ash and moisture.

From the results obtained it may be inferred that the greater part of the mucilaginous substance of these seaweeds consists of the anhydrides of the sugars above mentioned.

The mucilage of the Florideae does not contain any alginic acid nor "Tangsäure," the normal constituents of the mucilage of Laminariaceae. They are precipitated by acid from neutral or alkaline solution, while the solution of the mucilaginous substance of Florideae gave no such reaction by the reagent. Kawakami[1] states that, when the mucilaginous substance of *Gloiopeltis sp.* is heated to boiling with dilute acid, a part of it separates as a voluminous precipitate, while no change occurs in the cold. But this phenomenon, by my experiment, occurs only when the mucilage is mixed with impurities. The clear, filtered solution shows no change by boiling with dilute or concentrated acid of any kinds.

1) Journ. of Chem. Indust. Tokyo, 13. p. 370 (1910).

General characteristics of the mucilaginous substance of Florideae are much similar to those of fucoidin, which is a gummy substance obtained by Kylin[1] from *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Laminaria digitata*. He described as its characteristic the following reactions:

a) Fucoidin is prescipitated by alcohol from its aqueous solution.
b) Fucoidin is precipitated by basic lead acetate, while no reaction occurs with lead acetate.
c) It is precipitated by lime water, after being acidified with acetic acid, and the precipitate is soluble in saturated solution of sodium chloride.
d) It is not coloured by chlorzinciodine solution.
e) It shows pentose reaction.
f) The sugar produced by hydrolysis is not clear.
g) Fucoidin solution rotates the plane of polarization toward the left.

As the mucilaginous substance of Florideae shows, on the whole, the similar reactions described above, it may be considered as a kind of fucoidin. The rotatory power, however, deviates remarkably from that of fucoidin. The mucilage of the Chondrus and the Iridaea rotated toward the right, while that of the Gloiopeltis showed no rotation. The specific rotatory powers were determined in a 100 mm. tube by the use of Schmidt and Haensch's half shadow polariscope with following results:

Chondrus:

$$[\alpha]^{20}D=\frac{2.3\times 0.346\times 50}{0.388\times 2}=+102.55^{\circ}$$

Iridaea:

$$[\alpha]^{20}D=\frac{1.7\times 0.346\times 50}{0.377\times 1}=+7.80^{\circ}$$

The rotation power, it seems, varies much according to the constituents of the mucilage.

On the other hand, it may be said that these mucilaginous substances are a kind of gums, as we find their characteristics very similar to those of the gummy substances of land plants. When the latter substance is

1) Hoppe-Seyler's Zeits. f. physiol. Chem., 83, pp. 171-197 (1913).

put in water, it becomes a viscid colloid, and the greater part of it splits into sugars by hydrolysis. And further it gives with many reagents reactions somewhat similar to those in the case of the mucilaginous substance from Florideae.

		Mucilage of Chondrus	Mucilage of Gloiopeltis	Mucilage of Iridaea	A sample of gum Arabic	Fucoidin (By Kylin)
I	Acids	no ppt.	no ppt.	no ppt.	no ppt.	no ppt.
II	Alcohol	ppt.	ppt.	ppt.	ppt.	ppt.
III	Lead acetate	no ppt.	no ppt.	no ppt.	no ppt.	no ppt.
IV	Basic lead acetate	ppt.	ppt.	ppt.	ppt.	ppt.
V	Ferric chloride	no ppt.	no ppt.	ppt.	no ppt.	no ppt.
VI	Acetic acid in excess	no ppt.	no ppt.	no ppt.	ppt.	no ppt.
VII	Per cent of sugar produced by hydrolysis (as galactose)	64.83	61.3	60.6	88.0	—
VIII	Many metallic salts	Increase generally its viscosity				
IX	Chlorzinc iodine solution	no reaction	no reaction	no reaction	no reaction	no reaction
X	Rotation	+	o	+	—	—

Summary.

The results of the present investigation may be summarized as follows:

a) The mucilaginous substances of *Gloiopeltis furcata* var *coliformis* J. Ag., of *Chondrus elatus* Holm. and of *Iridaea laminarioides* var. *cornucopiae* J. Ag. were investigated.

b) The mucilaginous substances obtained from these plants do not contain any alginic acid or "Tangsäure."

c) The greater part of the mucilaginous substances of the marine algae changes into sugars by hydrolysis.

d) From the hydrolysis products of the Chondrus, galactose, arabinose and an unknown sugar, having much similarity to galactose, were isolated.

e) To the new sugar the name floridose was given.

f) Floridose is readily reduced by sodium amalgam into a hexavalent alcohol, to which the name floridite was given.

g) Glucose, fructose and mannose were also detected in the hydrolysis products, in small amounts.

h) From the hydrolysis products of the Gloiopeltis, galactose, arabinose, and fucose were isolated, while the presence of floridose is excluded. The presence of glucose and fructose is also probable.

i) From the hydrolysis products of the Iridaea, galactose, arabinose and floridose were isolated. The presence of glucose is probable while that of fructose is not clear.

j) Floridose was isolated also from *Ahnfeltia plicata*.

k) The mucilaginous substances of these algae must consist of mixtures of the anhydrides of the sugars detected. Galactane and arabane, it seems, play the important role, as both galactose and arabinose were found to be present in the hydrolysis products of all the samples investigated, and also, the anhydride of floridose must be an important component, forming the mucilaginous substance of Florideae.