Chemical Studies on the Proteins and the Carbohydrates of *Iridaea laminarioides*

By

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Introduction

This report is an account of the experimental work which was done between March 1932 and December 1934 in our laboratory, by the author and the following collaborators, viz., M. ABE, K. YOSHIMURA, Y. SASAKI and M. YANASE. Chemical studies on the constituents of different algae have been reported by many authors so here are summarized only the important results of those authors' experiments. Among the mineral constituents of

algae have been reported the following rare elements (1) besides those elements common in land plants, viz., Mn, Br, Ag, As, Co, Cu, Ni, Pb, Zn, Sb, Ta, Ga, Mo, Ge, Cr, Ti, W and V. The coloring matters of algae (2) (3) (4) (5) have been stated to be chlorophyll, carotine, xanthophyll, phycoerythrin and phycocyan. Among these coloring matters, the latter two belong to the proteids. Only the chromoproteids of algae have been investigated; no other kinds of proteins have been reported. The carbohydrate of algae is a most complicated organic matter and numerous investigators (6) (7) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (23) (24) (25) (26) have reported about different constituents. To make a brief summary of these reports, the majority of the methylpentosans are composed of fucose and a majority of pentosans are composed of xylose and arabinose. The important hexosans are composed of glucose, mannose, galactose and of the polymer of hexuronic acids with which are contaminated Ca and SO_3. The fat of algae are composed of rich quantities of unsaturated fatty acids as olein, and $C_{10}H_{28-30}O_2$ but reports dealing with the fats are not numerous (20) (21) (22). These organic constituents of algae differ with the kinds as seen in the variation of coloring matter between green and red. Gaidukovs stated that when *Florideae* were exposed to the sun light, the red color changed to green. The seasonal change of iodine content of *Laminaria* has also been described as being more rich in the spring time than in the autumn.

Iridaea laminarioides grows in a fresh zone of sea tide and it contains red color like the kinds of algae which grow in the deep sea. This alga has much interest for its chemical constituents not only of coloring matters but also of proteins and carbohydrates. Further, economical interest is found in its mucilage, which belongs to the carbohydrates but has not yet been determined exactly, because this mucilage is used commonly as a cementing material in the covering of Japanese house walls. Anyhow, at first it is needed to examine the general chemical constituents of this alga and their seasonal variation, so the following experiments were undertaken. The majority of samples were collected at the sea side of Muroran in the province of Hokkaido, Japan, during 1931–1933. The materials were preserved in the air dry state.

I. General constituents of the alga

The general constituents of *Iridaea laminarioides* were determined from two samples collected from different localities of Hokkaido with the following results.

TABLE 1

Const. / Locality	Water	Ash	Protein	Proteid
Muroran	17.86	24.43	15.25	12.13
Mashike	8.98	12.55	19.52	18.95
Const. / Locality	Fat	Fiber	N-free exts	Pentosan
Muroran	0.22	1.17	42.04	1.01
Mashike	0.46	1.72	65.71	1.72
Const. / Locailty	Ratio = Proteid / Protein			
Muroran	79.89			
Mashike	97.08			

Next the distribution of amino nitrogen was determined by Van Slyke's method and the following values were obtained in the bases of the dry matter and of the total nitrogen percent.

TABLE 2

Nitrogen	Total-N	Amid-N	Insol-N	Humin-N
%	3.1247	0.2520	0.1327	0.0948
%	100.00	8.0648	4.2468	3.0339
Nitrogen	Basic-N	Cystin-N	Monoamino-N	
%	0.6105	0.0404	2.0347	
%	19.5379	1.2929	65.1166	

The qualitative test for sugars in the hydrolysed products of this alga was undertaken and the following results obtained.

TABLE 3

Test for Xylose.	Test for Galactose.	Test for Pentose.	Test for Methyl pentose
+	+	+	+
Molisch's Reaction	Seliwanoff's R.	Pinoff's R.	
+	+	+	

For the above data, the xylose test was carried out by Tollens and Widoe's Cd-xylonate method, the galactose was determined by the forma-

tion of mucic acid by oxidation, the pentose by phloroglucin reaction, while the methylpentose test was made by Oshima and Kondo's method.

Molisch's reaction was tested with α-Naphthol, Seliwanoff's reaction with resorcin and HCl, Pinoff's first reaction with ammonium molybdate and the second reaction with resorcin. From this syrup of sugars, methylpentose phenylhydrazone was forme dwhich was identified as fucose phenylhydrazon by nitrogen contents, viz. 11.02 and by the specific rotatory power, viz. $-74°.32$. Pentose osazone was also formed and identified as arabinose osazone by the melting point, viz. 163°, by the specific rotary power $+103°.4$ and by brom-phenylhydrazone which showed 167°C at the melting point.

II. Seasonal variation in the constituents of the alga

The seasonal chemical variations of algae have been observed by some authors who stated that the mineral constituents are increased in the autumn while the iodine content is rather rich in the spring time. The author investigated the seasonal variation of the general chemical constituents of *Iridaea laminarioides* and obtained the following results. In this table, the young are those collected in March and the old ones in July.

TABLE 4

Constit. Dry matter %	Protein	Proteid	Fiber	Fat	Ash	N-free extr.
Young	31.35	21.00	1.54	2.10	17.17	47.83
Old	18.57	14.75	1.42	0.27	28.52	51.18

Constit. Dry matter %	Reducing sugar	Galactose	Pentosan
Young	34.47	32.81	1.39
Old	43.61	39.49	1.22

Among the above results, the galactose was determined by mucic acid method and the pentosan by phloroglucin method. Thus the table shows that the young samples are rich in protein and fat while the old are rich in N-free extracts, reducing sugar, ash and galactose contents, compared with the former.

III. The chemical properties of proteids

Iridaea belongs to the *Florideae* which contain chromoproteids according to Kylin(27).

These chromoproteids were distinguished as phycoerythrin and phycocyan, the content of the former being stated to be less than that of the latter.

Nakano and Azuma(3) isolated both in their crystal form and Kitasato(4) has given the following elemental compositions to samples of them which were isolated from *Porphyra tenera*. He also determined the distribution of amino acid nitrogen of phycoerythrin.

TABLE 5

Elements	C	H	N	S	O
Phycoerythrin	50.87	7.04	15.34	1.76	24.99
Phycocyan	50.60	6.90	15.76	1.69	24.97
Amid-N	Humin-N	Cystin-N		Arginin-N	Histidin-N
7.45	4.04	4.70		10.12	4.00
Lysin-N	Monoamino-N		Nonamino-N		
10.01	48.98		11.65		

Svedberg determined the colecular weight of phycocyan as 10600, of phycoerythin as 20,8000 in the case of *Ceramium rubrum*; these values correspond to two or three times those of egg albumin. Also the isoelectric point of phycocyan was measured as pH 4.5 and of phycoerythrin as pH 4.3. Recently, Lemberg(28) examined the chromogens of both coloring matters by oxidation and obtained phycocyanobilin which resembles bilicyanin and phycoerythrobilin which resembles urobilin. He stated the relation of phycoerythin to phycocyan and the further chemical structure. There are very scanty reports of proteid radicals combined with coloring matters. There is also no report about the proteids other than chromoproteid.

A. Preparation of different kinds of proteids.

The air dried samples were extracted with 35% alcohol at 40°C and the coloring matters etc. without mucilage were obtained by filtering. The filtrate was evaporated and the syrup was extracted with aceton, leaving the choromoproteid in the residual precipitate.

The precipitate was extracted again with 35% warm alcohol and the evaporated residue was extracted with a mixture of dilute alcohol and ether; thus chromoproteid was obtained taking away the solvent.

The sample which did not dissolve in 35% warm alcohol was treated

with boiling water for two hours and the soluble mucilage was taken away completely, then the residue was extracted with 2% NaOH which showed no protein reaction. This residue was extracted again with 4% NaOH and the precipitate which is obtained from the extracts by means of HCl and alcohol showed intense protein reactions. The protein materials were dialysed, treated with alcohol and ether and dried in a H_2SO_4 desiccator. The filtrate still showed protein reaction so it was treated with alcohol; this substance was precipitated and dried also.

B. Alkali soluble protein A.

The alkali soluble protein A was precipitated with HCl and alcohol and found to show intensive Millon's and xanthoprotein reactions. This protein was hydrolysed for 24 hours with 20% HCl by direct flame and the nitrogen distribution in amino acids was determined by Van Slyke's method. The following results were obtained in total nitrogen percent. These results are compared with those of Kitasato's phycoerythrin and with prolamin and glutelin of grain plant seeds.

TABLE 6

Proteins / N-distr.	Alkali sol. protein	Phycoeryth.	Wheat glutelin	Barley glutelin
Amid-N	27.47	7.45	11.06–17.8	7.46–14.13
Humin-N	1.91	4.04	1.05– 1.32	2.49– 3.73
Diamino-N	19.26	11.65	—	15.88–22.24
Monoamino-N	46.34	48.78	—	59.27–72.79
Arginin-N	9.48	10.12	6.10–10.95	4.32– 8.85
Histidin-N	2.07	4.00	5.50– 6.17	0.08–11.83
Cystin-N	0.37	4.72	1.77– 5.43	1.16– 4.39
Lysin-N	7.33	10.01	3.04– 6.85	3.42– 9.05
	Ibid.	Prolamin		
		Rye.	Millet.	Barley.
Amid-N	27.47	23.32	20.76	18.91–24.38
Humin-N	1.91	1.81	1.35	1.56– 3.05
Diamino-N	19.26	17.56	9.34	14.06–20.59
Monoamino-N	47.34	57.31	69.85	57.01–65.51
Arginin-N	9.48	—	3.9	4.46– 6.28
Histidin N	2.07	—	1.71	3.58–13.79
Cystin-N	0.37	—	1.23	1.63– 6.35
Lysin-N	7.33	—	2.46	0.00

The alkali soluble protein A of *Iridaea* shows great difference in amino acid nitrogen distribution, especially in amid-N, and in humin-N, in comparison with phycoerythrin. When compared with glutelin of wheat and of barley, the amid-N and lysin-N also show great differences. When compared with prolamin of rye, barely and millet, the amid-N and humin-N contents are very similar, but the richness of lysin-N is the characteristic of the alkali soluble protein of this alga.

C. Chromoproteid.

The chromoproteid of *Iridaea* was extracted with 35% warm alcohol, precipitated with aceton and found to give intensive Millon's and xanthoprotein reactions. It resembles phycoerythrin and phycocyan in the soluble properties in salt solution, but not in water and it gives a bluish violet color and carmin fluorescence. In the reaction of yellow color with HNO_3, it is similar to phycoerythrin but it is different in solubility in a mixture of alcohol and ether. The following differences between phycoerythrin and phycocyan appeared in the absorption bands of spectra.

TABLE 7

Phycoerythrin	565–569	541–537	498–492
Phycocyan	610–624	573–577	—
Cromoproteid of *Iridaea*	613.6	?	495.7

In the above table, the absorption band of this chromoproteid at 613.6 coincides with that of phycocyan and at 495.7 to that of phycoerythin but the two bands corresponding to 565–569 and 541–537 were not found.

Next, the nitrogen distribution in amino acid was determined by Van Slyke's method as already stated above. The obtained results are compared to the corresponding values of phycoerythrin, haemoglobin and soja-

TABLE 8

Proteins / N-distr.	Chromoproteid of alga	Phyco-erythrin	Globin of haemoglobin	Glycinin of soy-bean
Amid-N	12.87	7.45	5.13– 6.31	8.61–10.14
Humin-N	5.28	4.04	2.30– 2.32	1.91– 2 41
Diamino-N	13.45	11.65	25.96–31.51	—
Monoamino-N	59.73	48.78	63.68–59.35	53.40–58.84
Arginin-N	8.02	10.12	6.64–10.63	13.81–16.13
Histidin-N	1.93	4.00	8.82–16.01	7.99– 9.72
Cystin-N	0.44	4.70	0.40– 0.72	0.36– 0.56
Lysin-N	3.05	10.01	3.94–10.66	7.72– 8.27

glycinin as in table 8.

In the above results, the contents of amid-N and cystin-N are far different from those of phycoerythin and they are like those of glycinin; the content of cystin-N is very near to that of globin of haemoglobin.

D. Alkali soluble proteins B and C.

The alkali soluble protein A was precipitated with HCl and alcohol from 2% NaOH extracts: the filtrate still contained protein B. The filtrate was evaporated to syrup, then the protein B was precipitated with alcohol and hydrolysed. The distribution of amino acid nitrogen was determined by Van Slyke's method.

The alkali soluble protein C was prepared from the residual alga of the 2% NaOH extraction, extracted with 4% NaOH solution. From the extracts of 4% NaOH solution, the protein containing precipitate was separated with HCl and alcohol, then its amino-nitrogen distribution was also determined. These values were compared on the basis of total nitrogen percent, with those of A and B proteins, in the following table.

TABLE 9

	Protein A	Protein B	Protein C
Insoluble-N	—	1.66	4.31
Amid-N	27.47	17.13	9.37
Humin-N	1.91	1.00	1.15
Monoamino-N	46.34	58.23	63.61
Diamino-N	19.26	21.98	21.56
Arginin-N	9.48	16.38	15.24
Histidin-N	2.07	0.87	2.06
Lysin-N	7.33	4.73	4.26
Cystin-N	0.37	0.00	0.00
Free amino-N	—	9.80	10.04

In the table, the alkali soluble proteins of *Iridaea* do not show the same chemical constitution. The protein which is easily soluble and precipitable, is rich in amid-N, lysin-N and cystin-N contents and poor in monoamino-N and arginin-N. On the contrary, the protein which is difficult to extract, is rich in monoamino-N and arginin-N content but poor in lysin and cystin-N.

IV. Chemical properties of polysaccharides

Chemical investigations on the mucilagenous carbohydrate of algae are very numerous. Hoagland and Loeb(6) found galactose while Taka-

hashi(7) proved the presence of galactose, floridose, and arabinose. Neuberg(8), Haas(9), Samec and Isajeric(10), Russel and Wells(11) and Bird and Haas(12) have stated that the mucilage is calcium salt of sulphuric acid ester of carbohydrate: Nelson and Cretcher(29) assumed that it is monosulphate of methylpentose which is condensed as $(R\text{–}O\text{–}SO_2OH)_4$ and formed principally from fucose. Kylin(14) applied the name froidin to the hot water soluble fraction matter; Stanford called the alkali fraction soluble matter algin and alginic acid. Bird and Haas (l. c.) stated that the hot water soluble fraction matter is Ca-sulphate ester of a condensed uronic acid with methyl-pentosan and proved fucose and sulphate ion in its hydrolytic products but their usage of the name uronic acid is not clear. Hoagland and Loeb (l. c.) found xylose and arabinose in the hydrolytic products of alginic acid. Schmidt and Vocke(15) assume that alginic acid is a condensed product of polyglycuronic acid while Cretcher and Nelson(16) stated that it is a condensed product of mannuronic acid and fucose derived from fucoidin and from a part of the cell walls. Recently, Takahashi and Shirahama(17) gave the following formula

$$R\begin{matrix}\diagup O\text{–}SO_2\text{–}O\diagdown \\ \diagdown O\text{–}SO_2\text{–}O\diagup\end{matrix}Ca$$

for Japanese agar-agar from the stand point of partial hydrolysis and it is proved to resemble pectin as $R\text{–}R'\text{–}O\text{–}SO_2\text{–}O(Me)(Me)$ in respect to containing galactose and pentose and being contaminated with a small quantity of uronic acid.

Kylin(l.c.) stated that alginic acid of *Laminaria* and of *Ascophyllum* gave pentose reaction but no mucic acid. Hoagland and Loeb (l. c.) isolated xylosazon from *Macrocystis*. Tomoda and Atsuki(18) stated that alginic acid of *Laminaria* is an anhydrous polymer of galacturonic acid and glycuronic acid. Schmidt and Vocke(l. c.) assumed that the alginic acid of *Fucus* is a polymer of glucuronic acid and Nelson and Cretcher(l.c.) that that of *Laminaria* and of *Macrocystis* is a polymer of mannuronic acid. Miwa(19) stated that the alginic acid obtained from *Desmarestia, Laminaria, Ecklonia, Crystophyllum, Fucus, Podina* and *Ulopteryx* is the same substance, i.e. a polymer of mannuronic acid.

From the hydrolytic products of *Iridaea*-mucilage, Takahashi obtained galactose, floridose, arabinose and glucose but no fructose and he could not find alginic acid in the hot water soluble fraction matter. Recently Lüdtke (23) found a reducing substance not ketose, also laevulinic acid and an organic acid not uronic acid. Fairbrother and Mastin(25), Hoffmann and

Gortner(26) stated that it is Ca-salt of agar acid. Takahashi and Shirahama(l.c.) named it "Kantensäure" which contains galactose, pentose, SO_3, and gave ketose reaction and hexose but no uronic acid nor methylpentose. The author separated the different polysaccharides and examined each one in order to identify the contents of the hydrolytic products, viz., 35% alcohol soluble, hot water soluble, 2% NaOH soluble and the insoluble polysaccharides.

A. Separation of polysaccharides.

The air-dried samples upon extraction with 25% alcohol at 40°C yielded no viscous liquid containing coloring matters. The evaporated extract was mixed with aceton and the precipitate was freed from aceton and extracted again with 35% alcohol to free from chromoproteid. The residue was hydrolysed with 5%H_2SO_4, neutralized with $Ba(OH)_2$, then filtered. The filtrate was evaporated and treated with alcohol to obtain the syrup of sugars. This syrup is made up of the hydrolytic products of the dilute alcohol soluble polysaccharides.

The residual algae after the alcohol extraction, was treated with fifteen times its volume of water, boiled for two hours in a water bath, until no more extract was obtained: the procedure was repeated three or four times and the preparate filtered. The clear light yellow filtrate was evaporated, made to a syrup, ground with quartz sand and extracted with 70% alcohol for three days.

The 70% alcohol extracts were reserved as samples for examination for araban-like matters: the residue was made paste and treated with 0.0625% HCl alcohol and the soluble part was preserved as sample for the same purpose. The precipitate in HCl-alcohol was dried and hydrolysed for eight hours with 2% HCl and the syrup obtained by the ordinary method was retained as the sample for tests for the hot water soluble polysaccharides, i.e. the principal constituents of mucilage of algae. Further the hot water insoluble residue was extracted with 2% NaOH and this residue was extracted with 4% NaOH sol. Both the alkali extracts were treated with HCl and alcohol and the precipitates were separated. The former was used as the sample for alkali soluble polysaccharide investigaand the latter for protein investigation. At last the 4% NaOH insoluble residue was kept as sample of fiber.

B. Dilute alcohol soluble polysaccharides.

The syrup, obtained from the hydrolytic products of the dilute alcohol

soluble polysaccharides showed the following qualitative reactions, viz., the strong reducing power of Fehling's solution, cherry red color with HCl and phloroglucin, strawberry red upon heating in water bath with aceton and HCl and the absorption band of methylpentose in the yellow part of the spectrum. The benzene soluble color heating with HCl and naphthoresorcin yielded very faint results and the ketose reaction with HCl and resorcin was strong but Pierart's fructose reaction which is detected by the reduction of Cu-hydroxide and glycocoll mixture in the presence of K-carbonate was negative.

After treating with 5n-HCl by the Sieben-Lucius(30) method, the ketose reaction of syrup could not be found and it still showed the strong reducing reaction, the pentose reaction and the dextro-rotation. From this solution, the phyenylosazon was formed, purified with 60% alcohol and found to be soluble in dilute aceton and to melt at 166°C in which it resembles pentose. This solution showed no more methylpentose reaction with aceton and HCl and it did show that during and at the same time as the treatment of the ketose decomposition, methylpentose was lost. Accordingly the operation of phyenylhydrazone formation was undertaken, treating the material with acetic acid and phyenylhydrazin at room temperature for 24 hours. The crystals were obtained and after recrystallization, they reacted positively laevo-rotation. They showed the melting point at 170–173°C which coincides to that of fucose-phenylhydrazone.

Next, for the detection of arabinose, brom-phenylhydrazone and β-naphthophenylhydrazone were formed. Both crystals were purified from dilute alcohol; the former showed the melting point at 162–168°C and the latter at 174–176°C. The former coincides to that of arabinose-brom-phyenylhydrazone and the latter to that of arabinose-β-naphthophyenyl-hydrazone. The following examination was made for the substance showing ketose resorcin reaction and methylpentose reaction in the syrup. This syrup shows very strong ketose resorcin but no Ihl's reaction(31) for fructose and (2) the precipitated substance which was obtained from the syrup by the addition of CaO, showed no further ketose resorcin reaction. So there is no positive reaction for fructose. The filtrate of the CaO-treated liquid still showed resorcin reaction but no positive Pierart's reaction of fructose with glycocoll and $Cu(OH)_2$, therefore there seems to be no fructose in the syrup.

For the separation of the substance which showed the ketose resorcin reaction, the syrup was extracted with a 3:1 mixture of ether and alcohol. In the syrup, freed from ether and alcohol, the ketose resorcin reaction

was observed markedly. The ether and alcohol insoluble part was then decolorised with animal charcoal. It showed methyl pentose reaction with aceton and HCl. From this syrup, phenylhydrazone was formed and after purification, it showed the melting point of 173°C and the specific rotatory power $[\alpha]_D^{20} = -5.3°C$ which coincide with the corresponding values of fucose phyenylhydrazone.

a. Separation of ether soluble syrup.

The syrup obtained from the dilute alcohol soluble and the HCl alcohol soluble polysaccharides, contains a substance which gives a strong ketose reaction with resorcin and HCl, in addition to arabinose and fucose. As above mentioned, the syrup which is soluble in the mixture of alcohol and ether, contains the substance, which gives the ketose resorcin reaction but it is not fructose and contains only a trace of fucose. When this syrup was extracted with ether only, the extract was freed from fucose in the following way. The syrup was extracted many times with ether and the residual syrup no longer showed the ketose resorcin reaction but gave a strong pentose and methyl pentose reaction. The ether soluble syrup gave a strong ketose reaction, the reduction of Fehling's solution and of iodine alcohol solution. The syrup is far different from the sugars in respect to this ether soluble property and also in the property of the iodine reaction, so it seems to be some cleavage fraction matter of sugars.

The phenylosazone of this substance was obtained in a small quantity and its melting point found to be at 248–250°C. Further investigation will be carried on in the future.

The residual syrup of the ether extracts which were obtained from the 70% alcohol and HCl alcohol soluble polysaccharides was proven to contain the same sugars by the following results. The syrup obtained from 70% alcohol soluble polysaccharides showed the pentose reaction with phloroglucin and HCl, but no mucic acid reaction of galactose. The bromphenylhydrazone melted at 168°C which corresponds to the melting point of arabinose and the phenylosazone at 166°C which corresponds to that of arabinose. The syrup obtained from HCl alcohol soluble polysaccharides showed the pentose reaction with phloroglucin and HCl, the methylpentose reaction with aceton and HCl but no galactose reaction. From this syrup, bromphenylhydrazone was obtained which melts at 168°C and a small quantity of phenylhydrazone which melts at 173°C.

From the above results, it is clear that the araban fraction i.e. the dilute alcohol soluble portion is composed of a large quantity of arabinose

and less fucose. Beside these two components, the syrup obtained from this fraction contains an ether soluble substance which gave the ketose resorcin reaction, the reducing reaction of Fehling's and iodine solutions.

C. Hot water soluble polysaccharides.

The residual samples obtained in the 35% alcohol extraction, were treated with water for two hours in a water bath; this treatment was repeated three times and the 70% alcohol and HCl alcohol soluble parts separated (the so-called araban fraction). The parts insoluble in 70% alcohol and HCl alcohol were hydrolysed with 2% HCl and the syrup obtained which was next treated with alcohol by the ordinary method. The syrup showed the strong reducing action of Fehling's solution and yielded cherry red color with phloroglucin upon boiling with 20% HCl and very faint methylpentose reaction with aceton and HCl. This syrup is different from the araban fraction in respect to showing no resorcin and no naphthoresorcin reaction, but it shows a strong mucic acid reaction upon treatment with HNO_3. So the crystals, obtained by oxidation with HNO_3 of sp. gr. 1.15 were treated with $(NH_4)_2CO_3$ and NH_4OH, filtered, then evaporated to purify.

The melting point of the purified crystals showed 212–214°C which coincides to that of music acid, gave the characteristic color reaction with $FeCl_3$ and the pyrrol reaction with pine wood and HCl. The syrup was diluted with H_2O, added KH_2PO_4 and fermented with beer yeast very easily.

For osazone test, the syrup was treated by the ordinary method with phenylhydrazin and acetic acid and the obtained phenylosazone was purified with 60% alcohol. The seperation of the osazone by different solvents was carried out and the water insoluble, the 32% aceton soluble and the insoluble in aceton, osazons were obtained. The water insoluble osazon showed the melting point at 166–168°C which coincides with that of arabinose. The 32% aceton soluble osazone showed 177–178°C which coincides with that of fucose and the aceton insoluble one showed 183–184°C which coincides to that of galactose. Further the osazone insoluble in hot water but soluble in 60% alcohol showed the melting point at 205°C, coinciding with that of glucose. Therefore, this syrup may contain fucose, arabinose, galactose and glucose. Among these sugars, the quantity of galactose was most abundant followed by fucose and least, glucose. The arabinose content was also less and if the precipitation of the hot water soluble polysaccharide was repeated, then the arabinose content became only avg.

1.3% as pentose (max. 1.34 and min. 1.14%).

In the hydrolysis of these polysaccharides with 1% H_2SO_4, a small quantity of the insoluble and the precipitated substances was found in the neutralization and large quantities of the substances insoluble and soluble in alcohol. Then this polysaccharide was hydrolysed with 1% HCl and the sugar contents of this liquid estimated at various times as shown in the following table.

TABLE 10

Hours	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Sugar%	25.98	33.70	37.04	40.38	45.19	45.79	47.07	49.30	48.30

As shown in the table the maximum quantities of the sugar were found at 4 hours after their hydrolysis and they correspond to ca. 50% of the total quantities.

a. Separation of galactan from fucosan.

This polysaccharide was hydrolysed for 0.5 hours with 1% H_2SO_4, then a small quantity of insoluble matter (I) was found, ca. 0.5%, and after the neutralization with $Ba(OH)_2$, a small quantity of the insoluble matter was found again. The first insoluble matter contained 2.61% of mineral salts. It was soluble in alkali solution, partially soluble in hot water but not soluble in alcohol nor in ether. Before and after hydrolysis (1), it did not form any reducing substance of Fehling's solution at all. The second insoluble matter was soluble in hot water and it has the power of reducing iodine and (2) Fehling's solution. It showed negetive orcin-HCl reaction and formed the insoluble barium salt; thus it was proved to be of an acidic nature. For the separation of fucose from galactan, to the filtrate obtained by the neutralization was added many times its volume of alcohol. The soluble part was found to be composed of fucose and the insoluble part of polysaccharide which is composed of fucose molecules. The syrup was prepared from the alcohol soluble portion and it showed the following color reactions, i.e. Molisch's α-naphthol, picric acid, phloroglucin and HCl and aceton and HCl reaction. Since the ketose resorcin reaction showed only dark brown color and the naphtho-resorcin and mucic acid reactions were negative, the syrup seems to contain methylpentose but no galactose. Two g. of syrup were mixed with four of α-methylphenylhydrazin and 1.25 g. alcohol and a hydrazone formed in the ordinary way. The hydrazone was recrystallized many times from alcohol

and white needle crystals obtained which are soluble in dilute aceton and pyridin but not soluble in ether nor in benzol; they melt at 179–180°C, that is to say, the material of the crystals corresponds to fucose-methylphenyl-hydrazone. The specific rotary power was estimated in pyridin solution and the following value obtained which concides with that of fucose-methyl-phenyldrazone reported by VAN DEN HAAR(32).

$$[\alpha]_D^{16} = \frac{0.03 \times 100}{2 \times 0.404} = +3°.17$$

The alcohol insoluble matter was hydrolysed with 5% H_2SO_4 and the syrup obtained in the ordinary way. This syrup showed Molisch's and picric acid reaction but neither Seliwanoff's, nor Pinoff's ketose reaction nor naphthoresorcin reaction. It reduced Fehling's solution strongly and large quantities of mucic acid were found upon oxidation. Then 0.2 g. of syrup, 1.3 g. of H_2O and 0.5 g. of phenylhydrazin were mixed, kept for three hours. Radial needle crystals were formed which melted at 155°C and the crystals showed the following specific rotatory power which coincides to the value for α-galactose-phenylhydrazone reported by FISCHER (33).

$$[\alpha]_D^{16} = \frac{0.29 \times 100}{2 \times 0.699} = -20°.8$$

Fischer's value:—The specific rotatory power $= -21°.6$. The melting pt. $= 158°$C.

The hot water soluble polysaccharides were hydrolysed with 1% H_2SO_4 in an autoclave at 4–4.5 At. press. for 15 minutes, neutralised with Ba $(OH)_2$, and then filtered. The filtrate was evaporated and extracted with alcohol. The syrup showed no uronic acid reaction of naphthoresorcin, no galactouronic acid reaction with Pb-acetate nor brucin nor cinchonin salts.

D. Separation of 2% NaOH sol-soluble polysaccharides.

The residual samples freed from hot water soluble matters were continuously extracted over night with 2% NaOH, the extracts were filtered and from the filtrate, the precipitates were formed with HCl and alcohol. The precipitates were hydrolysed with 5% H_2SO_4 for twelve hours then neutralised with $Ba(OH)_2$ and the syrup was formed from alcohol solution in the ordinary way. The syrup obtained from 95% alcohol extracts, was kept a long time in a desiccator and formed a crystal mass; then it

was kept a long time in a discator and formed a crystal mass; then it ether whereupon ca. two g. of yellow powder were obtained.

These crystals were very hygroscopic, reduced Fehling's solution at room temperature also reduced iodine solution when hot but did not decolorize methylen blue. They showed no naphthoresorcin-HCl reaction nor mucic acid reaction of galactose while the pentose reaction of phloroglucin-HCl and the methylpentose reaction of aceton-HCl were not clear.

The ketose reaction of resorcin-HCl and the phenylosazone reaction were very strong and when the phenylosazon was recrystallized from 60% alcohol it showed the melting pt. at 168–169°C. The methyl phenylosazone was also obtained, recrystallized from 60% alcohol and found to melt at 170–173°C. Its *p*-nitrophenylhydrazone showed the melting point at 128–129°C, but after being resorved in disccator for three weeks it melted at 118–122°C.

Its specific rotatory power was estimated from methylalcohol solution and the following value obtained:

$$[\alpha]_D^{16} = \frac{100 \times 0.815}{1 \times 2.5} = +32°.6$$

a. Separation of crystals into two fractions.

The crystals were ground in absolute alcohol, filtered and after a repetition of this treatment the residual crystals were dissolved in methyl alcohol. The absolute alcohol soluble part was freed from alcohol and treated with methanol; then the methylalcohol soluble part was freed from it and treated with absolute alcohol. Thus the separation of two fractions was completed and the absolute alcohol soluble fraction found to be very scanty corresponding to ca. 1/10 of the methanol soluble fraction. The absolute alcohol soluble fraction formed a phenylosazone melting at 166–168°C which resembled arabinose-phenylosazone but it did not coincide in following stated specific rotatory power, with pure sugar in 10% solution:

$$[\alpha]_D^{20} = \frac{100 \times 0.84}{1 \times 2.675} = +31°.4$$

The methanol soluble fraction formed phenylosazon melting at 125–130°C and showed the following specific rotatory power of pure sugar in 10% solution:

$$[\alpha]_D^{20} = \frac{100 \times 0.195}{0.5 \times 3.205} = +12°.17$$

There is needed further investigation of these two fractions in the near future.

E. Four% NaOH-sol. insoluble polysaccharides.

The algae belonging to *Floridea* have thick cellulose membrane and mucous substance (34) which are used as agar-agar. Until the present, the investigations on cellulose have been limited only to microscopical examination. Recently Naylor, Russel and Wells(35) proved the presence of cellulose microscopically while Dillon and O'Tuama(36) isolated cellulose and formed phenylosazone but not mannose-phenylhydrazone from it. But there are no published experimental results on the polymer structure, comparing it with the land plant cellulose.

Boedeker(37) estimated the glucose value of cotton cellulose, treated fifteen hours at 20°C in 41.4% HCl and obtained 90.65%. Fischer and Shroeder(38) estimated ⅓ residue, treating filter paper for three hours at 200°C in 4n-KOH. Klassen(39) distinguished cellulose into insoluble and soluble parts in treatment with n-HCl and Na-bisulphite. The author took the insoluble residue of *Iridaea laminarioides* in 4% NaOH extraction and boiled it in 1.25% H_2SO_4, next in water, in 1.25% KOH and again in water for 30 minutes each; then washed it with water, alcohol and ether. This material was compared in respect to the following properties with that of defatted cotton fiber and of filter paper. In the following table the values for reducing power were obtained after treatment with 35% HCl, the 4:1n-KOH soluble matter was treated at 150°C for 5 hours, the n-HCl and Na-bisulphite soluble matter was treated at 98° for 5 hours.

TABLE 11

Samples	The reducing power		n-KOH soluble mat.	n-HCl, Na-bisulphite soluble mat.
	8 hrs.	15 hrs.		
Cotton fiber	27.24	29.29	8.50	4.71
Filter paper	29.64	37.90	16.91	1.40
Fiber of algae.	19.55	24.74	15.75	10.19

In the above table, it may be observed that the reducing substance formation of *Iridaea* cellulose is a little less than that of cotton and of filter paper and in the treatment of cellulose with HCl and Na-bisulphite, larger quantities of soluble matter were contained in *Iridaea* than in cotton and filter paper. When the pentosan content of *Iridaea* was compared with that of cotton and of filter paper, treated by Tollens' method, it was

observed that they showed no great differences as indicated in the following results.

Cotton 2.18% Filter paper 1.29% Iridaea 2.82%

Next the α-cellulose content of *Iridaea* was determined by Hess's method and 91.10% obtained which is very slightly different from the 95.17% of cotton cellulose, determined by Hagglund and Klingstedt. Further oxycellulose was determined by Cross and Bevan's method in the following way: boiled the sample of α-cellulose for 1 hr. with 1% NaOH, then passed chlorine gas for 1 hr. and after freed from HCl, treated with 2% Na_2SO_4, NaOH and 0.1% $KMnO_4$, washed, dried and weighed. It showed the loss of only 0.71%. Therefore, the cellulose of *Iridaea* is different from that of land plant in the richness of the amorphous cellulose of Herzog.

V. Fermentation of arabinose and fucose by *Aspergillus oryzae.*

Reports of chemical investigations on the fermentation of pentose and methylpentose by organisms are not numerous, especially there is very little on methylpentose. Among the yeasts, it was reported by Lintner that *Schizosaccharomyces thermantitonum* ferments arabinose but other kinds of yeast do not. Many yeasts contain large quantities of methylpentosan, which are called yeast gummy substances (40) and the nucleic acid of yeast contains ribose, a kind of pentose (41). As to bacteria, it was reported by Schmidt and Peterson(42) that the aceton fermentation bacteria formed butylalcohol, aceton, volatile and non volatile acid with CO_2 and by Bertrand(43) that in sorbose fermentation, *Bacterium xylinum* formed arabonic acid. Lafer(44) stated that in oxalic acid fermentation was caused by *B. aceti, B. actigenum, B. acetosum, B. ascendens, B. Kützengianum, B. xylinum,* and by *Thermobacter aceti.* Grimbert(45) reported that in lactic acid fermantation, *Pheumococcus* formed lactic acid and acetic acid from arabinose and Tollens(46) found alcohol in the pentose culture media of *Bac. ethaceticus.* Seiler(47) stated that anhydropentose was changed to galactan, $C_{12}H_{20}O_{10}$, $C_6H_{10}O_5$ by *Bact. metarabinum* and by *Bact. persicae.* As above mentioned, in the bacterial fermentation of arabinose, the various products were formed but they were very different from those of fungus fermentation. There is only one report (48) that methylpentose, i. e. isorhodeose, was fermented by *Aspergillus niger* to form tartaric acid. Previously, it was stated by Behren(49) that *Botrytis cinerea, Rhizopus nigricans* and *Penicillium glaucum* utilized arabinose.

Niktinsky(50) reported that *Aspergillus niger* easily utilized arabinose as a carbon source and that there is no difference from *d*-glucose. Peterson, Fred and Schmidt(51) stated that when pentose, i.e. arabinose, was fermented by *Aspergillus, Penicillium* and by *Mucor,* they could not find any alcohol nor volatile acid excepting CO_2 only. While White and Willamen (52) reported that *Fusarium* formed alcohol from arabinose, Amelung isolated citric acid from *Aspergillus* culture media, containing arabinose. Butkewitsch(53) stated that citric acid is formed from arabinose by *Citromyces* while only a trace was formed by *Aspergillus.* Tamiya(54) found kojinic acid in arabinose fermentation by *Aspergillus flavus.* Schreyver (55) reported that *Aspergillus* formed fumaric acid from arabinose.

However there has been no report concerning the differences of the fermentation products between pentose and methylpentose when cultured by the same fungus under different conditions of the culture. The author had the opportunity of getting plentiful amounts of substances composed principally from arabinose and from fucose in the above experiments connected with the chemical studies of *Iridaea laminarioides.* The precipitate of aceton from 35% hot alcohol extracts of the alga is composed mainly of methylpentosan i.e. fucosan, contaminated by a small quantity of araban while the araban fraction which is obtained from dilute alcohol and HCl alcohol soluble part of the hot water extracts of residual alga is composed of araban. In this experiment, the both were hydrolysed with HCl or H_2SO_4 and, after neutralization, were mixed with the following nutritional salts. *Aspergillus oryzae* was then cultured at 30°C for a few days until the time of spore formation. The nutritional salts were prepared as follows:—$NaNO_3=4$, $KH_2PO_4=2$, $MgSO_4.7H_2O=1$, $FaCl_3=1$ drop in 100 cc H_2O.

A. Separation and detection of the fermentation products.

The fungus membrane on the surface of the fermentated liquid was taken away, the liquid was filtered and treated by steam distillation to free from volatile matters. The distillate was used as samples for the detection of acetaldehyde, aceton, alcohol, acetic acid and for formic acid.

The residual liquid was neutralised with NaOH and Cu-acetate added to precipitate kojinic acid, then filtered and the filtrate was acidified with acetic acid then precipitated with Pb-acetate. The Pb-precipitate may contain oxalic, citric, fumaric, tartaric and allied acids and when the filtrate is made alkaline with ammonia, the precipitate which is formed by

Pb-acetate may contain glycolic acid and its filtrate does contain lactic acid. The Pb-precipitate, containing oxalic, citric and allied acid was treated with H_2S and reprecipitated with Pb-acetate in 50% alcohol solution in the presence of ammonia. The oxalic acid fraction was insoluble and the citric acid fraction became soluble. The Pb-precipitate, containing citric and tartaric acid fraction was treated with H_2S then the filtrate was concentrated and mixed with two volumes of 95% alcohol; some K-acetate was added. Then the precipitate was found to be composed of tartaric and the filtrate was citric acid.

For the detection of acetaldehyde, the silver mirror reaction, the phenylhydrazin reaction and the *p*-brom-phenylhydrazin reaction were employed.

For the detection of aceton, Gablielsen's Na-nitroprussid reaction was used with ammonia.

For the detection of alcohol, the iodine and K-iodine reaction were carried out with KOH.

For the detection of acetic acid, the formation of the hexa-acetate of ferrisalts was used also the distillation test of Ca-acetate and the silver acetate crystals with $AgCO_3$.

For the detection of formic acid, the precipitate by Pb-acetate in ammonical solution, the color reaction with $FeCl_3$ and with resorcin and H_2SO_4 and the decoloration of methylblue with K-bisulphite were employed.

For the detection of kojinic acid use was made of the color reaction with $FeCl_3$, the reducing test with Fehling's solution, the diazo-reaction with diazobensosulphonic acid and determination of the melting point.

For the detection of lactic acid, the guajacol reaction with H_2SO_4, the acetaldehyde formation, treating with K-permanganate and the crystal-isolation of Zn-lactate were used.

For the detection of glycolic acid, the *p*-cresol reaction with acetic acid and H_2SO_4, Bülow's hydrazin reaction with $FeCl_3$ and HCO_4 and the crystals and the melting point of the phenylhydrazid were examined.

For the detection of citric acid, the formation of Hg–$HgSO_4$ acetondicarboxylic acid salt by Deniges' reagent, Stahres' reaction with K-permanganate and the Na-nitroprussid reaction were tested.

For the detection of tartaric acid, Fenton's dioxymaleic acid reaction, the color reactions with resorcin, with α-naphthol and with ammonium molybdate were examined.

For the detection of fumaric acid, the melting point of free acid and Ca-fumarate were determined.

For the detection of oxalic acid, the color reactions with $FeCl_3$, with resorcin and H_2SO_4 and the Ca-Ag-oxalate were examined.

For the detection of glycerinaldehyde, the orcin and the phloroglucin reaction, the reduction of Fehling's solution and the phenylosazone were examined.

For the detection of oxocarboxylic acid, the color reaction of *α*-naphthol, of phloroglucin, of orcin, of resorcin an dof naphthoresorcin were examined and the osazon test was made.

B. Fermentation products of arabinose.

The liquid of arabinose fermented by *Aspergillus oryzae* was filtered and treated by steam distillation. The distillate showed no silver mirror reaction, (56) no phenylhydrazin nor *p*-brom-phenylhydrazin reaction at all, therefore it showed no acetaldehyde. The same filtrate was negative to Gablielsen's (57) aceton reaction with Na-nitroprussid, acetic acid and with ammonia. The filtrate showed also no alcohol reaction, while a Pb-salt with $Pb\text{-}CO_3$ was formed in the ammoniacal solution. This salt was distinguished from acetic acid and showed strongly the color reaction of formic acid i.e. red color by $FeCl_3$ or orange by resorcin-H_2SO_4 and it faintly decolorized methylblue solution.

The residue from the steam distillation was neutralized with NaOH and formed Cu-salt by the addition of Cu-acetate solution. After the Cu-precipitate was treated with H_2S, its concentrated filtrate showed the intensive color reaction of $FeCl_3$, intensive color of diazo-reaction and reduced Fehling's solution. These reactions indicated the presence of kojinic acid in the fermented liquid. But by the extraction with ether, it was not possible to obtain crystals of kojinic acid, then its quantity may be very small.

The filtrate of Cu-salt was freed from Cu, treated with H_2S and acidified with acetic acid, Pb-acetate was added. The Pb-precipitate was next obtained which may contain oxalic and citric acid and the filtrate also contained Pb-salt. This filtrate was alkaline with ammonia in the presence of Pb-acetate. Then the Pb-precipitate was also obtained which may contain glycolic acid and a less quantity of Pb-salt which is assumed to be lactic acid was observed in its filtrate. The Pb-salt of glycolic acid was decomposed with H_2S and the free acid solution obtained. This solution gave green color with p-cresol, acetic acid and H_2SO_4, reddish violet with Bulow's hydrazin and $FeCl_3$-reagent and also formed phenylhydrazon crystals. From these results, the presence or glycolic acid in the liquid

is proven. The small quantity of Pb-salts in the filtrate of the glycolic acid fraction was observed to correspond to that in the lactic acid fraction. It gave the following reactions freed from Pb. The lactic acid reaction with K-permanganate and with $FeCl_3$ were negative and showed only rose red color and dark precipitate with guajac tincture and H_2SO_4. The color reactions were observed to be red with orcin-acetic acid and HCl, with phloroglucin-H_2SO_4 and also with resorcin and HCl. These reactions are common to glycerinaldehyde and dioxyaceton, but an absolutely certain conclusion could not be attained. The Pb-salt of the oxalic and citric acid fraction ws treated with H_2S, concentrated to a small volume; then it was treated by Fleischer' method (58) to separate oxalic acid from citric acid in the following way. The concentrated liquid was mixed with Pb-acetate, ammonia and made 50% alcohol solution, then the insoluble part contains the oxalic acid fraction and the soluble part contains the citric acid and tartaric acid fractions. For the separation of the latter two acids after the soluble part was treated with H_2S, the tartaric acid was precipitated, treated with 90% alcohol and K-acetate. But there was no precipitate obtained and citric acid enters the filtrate which had the following intensive reactions, viz., Deniges' reaction (59) with his reagent and $KMnO_4$ showed positive, Stahre's (60) reaction with $KMnO_4$, NH_4-oxalate and with H_2SO_4 also showed positive and Na-nitroprussid reaction showed rubin red color. Further the liquid formed precipitate in ammoniacal solution with $CaCl_2$ upon the addition of alcohol. The oxalic acid fraction gave the color reactions of green with $FeCl_3$ and of green to blue with resorcin and H_2SO_4 and it yielded Ca-oxalate precipitate which is insoluble in acetic acid when treated with Na-acetate, $NaCO_3$ and $CaSO_4$ solution. But the quantity of this precipitate was very small.

For the detection of glycerinaldehyde, the filtrate from which kojinic acid was separated, was freed from Cu and acetic acid and extracted with alcohol then with the mixture of alcohol and ether. The syrup of the alcohol-ether gave the reduction of Fehling's solution reaction intensively at room temperature. The orcin and the phloroglucin reactions were observed and the latter was distinguished from the reaction of dioxyaceton by the insoluble white precipitate in boiling water. Further, the phenylosazon was formed and showed the melting point at 129–130°C which coincides to that of Wohl and Neuberg's result (61).

In the above results, it was observed that in the arabinose fermentation by *Aspergillus oryzae*, there were formed formic acid, a trace of kojinic acid, glyconic acid, citric acid, a trace of oxalic acid, glycerinaldehyde but

a question as to the presence of lactic acid and there was no tartaric acid.

C. Fermentation products of fucose.

The liquid resulting from fermentation of fucose by *Aspergillus oryzae* was filtered and treated by steam distillation. The distillate showed neither of the acetaldehyde reactions, i.e. the phenylhydrazin nor the silver mirror-reaction and it showed no aceton reaction, i.e. with Na-nitroprussid, acetic acid and ammonia. The filtrate showed also no alcohol reaction, i.e. with KI, iodine and KOH and no acetic acid reaction, i.e. with $FeCl_3$. These results coincide with the results of the distillate of arabinose.

For the detection of formic acid with Pb-acetate, $FeCl_3$ and with resorcin and H_2SO_4, the reactions were very faint, therefore fucose seems not be adapted to formic acid formation, in comparison with arabinose.

The distilled residual liquid was neutalized with NaOH and the precipitate was formed, added Cu-acetate, filtered and the filtrate was freed from Cu-salts and the Pb-precipitate was formed by Pb-acetate after acidification with acetic acid.

For the detection of kojinic acid obtained from the H_2S decomposition of Cu-precipitates, the $FeCl_3$ reaction, the reduction of Fehling's solution, and the diazo-reaction were positive but when it was extracted with ether no crystals could be obtained. The Pb-precipitate with corresponds to the oxalic and citric acid fraction, was obtained as stated above by the treatment with Pb-acetate and acetic acid and from the filtrate the Pb-precipitate was obtained, after making it alkaline with ammonia, which corresponds to the glycolic acid fraction. After the separation of this precipitate, the filtrate contained also Pb-salts which correspond to the lactic acid fraction.

The Pb-precipitate of the oxalic acid and citric acid fraction was decomposed with H_2S and after being concentrated, Pb-acetate and ammonia were added in the presence of 50% alcohol. Therefore the oxalic acid fraction comes out as precipitate while the citric acid fraction remains in the filtrate. All these Pb-salts were decomposed with H_2S, filtered; the filtrate was concentrated and the various reactions were examined.

For the detection of glycolic acid, the concentrated solution was treated with ether and after the ether extract was freed from ether, the following eactions were tested. The cresol reaction with glacial acetic acid and H_2SO_4 showed green-greenish blue and the guajacol-reaction showed violet color. Bülow's reaction (62) with $FeCl_3$ and H_2SO_4 in the presence of phenylhydrazin showed reddish violet color. The phenylhydrazon was

formed, treated with phenylhydrazin on a water bath and freed from the excess of phenylhydrazin, extracted with ether and white crystals of the phenylhydrazid were obtained which melted at 117°C by the purification with acetic ester.

For the detection of lactic acid, after the concentrated solution was treated with ether, examination was made of the following reaction.

As to the formation of acetaldehyde, upon heating the solution with K-permanganate it was detected and the rose red color was also observed after treating with H_2SO_4, and guajac tincture and the bright yellow was seen with $FeCl_3$. Large quantities of the liquid were boiled with $ZnCO_3$ and crystals of Zn-lactate were obtained. When the ZnO content was determined by the ordinary method, it was found to correspond to 28.02%.

For the detection of oxalic acid, the precipitate in ammoniacal solution with $CaCl_2$ which is insoluble in acetic acid was observed; the resorcin-H_2SO_4 reaction showed green-blue, the $FeCl_3$ reaction showed greenish yellow. But the Cu-oxalate with Cu-acetate and the Ag-oxalate with $AgNO_3$ in the presence of HNO_4 were formed in small quantities so the liquid seems to contain only a small quantity of oxalic acid.

In the detection of citric acid, the white precipitate by Deniges' reagent and the characteristic color with Na-nitroprussid were not observed and the presence of citric acid was questionable. For the detection of tartaric acid, Fenton's color reaction (63) with $FeSO_4$ and H_2O_2 and the ammonium molybdate reaction with H_2O_2 were negative. Therefore the presence of tartaric acid is also uncertain. The resorcin-H_2SO_4 and the α-naphthol-H_2SO_4 reactions which are common to this acid and succinic acid were observed. But the pyrrol reaction of succinic acid was very faint and in the $FeCl_3$ reaction, no precipitate was formed and the material showed only a clear red color. From these results it may be stated that the presence of succinic acid is a question while that of oxocarboxylic acid is more probable. Further, in the phloroglucin-HCl and in the orcin-HCl reactions, the red color indicated amylalcohol and the reddish violet color indicated ether in the naphtho-resorcin-HCl reaction. The oxocarboxylic acid not precipitated by Cu-acetate, did not give the diazo reaction and no reduction of Fehling's solution; those points are distinguished from kojinic acid. This acid formed osazon with phenylhydrazin and acetic acid and it is soluble in ether with the melting point at 198–207°, so it is near to the melting point of glucuronic acid.

Summary

From the above reported chemical studies of the proteins and the carbohydrates of *Iridaea laminarioides,* the following results may be summarized.

I. In the general constituents, the proteid ratio of the total nitrogen and ash are different according to be localities of production of the alga.

II. As to the seasonal variation of the constituents, the young alga is rich in protein and in fat contents while the old is rich in nitrogen free extracts, reducing sugars, ash and galactan contents.

III. In the proteids of this alga, the chromoproteid and the alkali soluble proteins were distinguished and the chemical properties of the chromoproteid found to be near to phycoerythrin and to phycocyan but not the same. It is different from the latter two in the following characteristics, viz., in the absorption band of spectrum and the amino nitrogen distribution, especially in the rechness of amid-N and the less cystin N-contents.

The alkali soluble proteins have not the same properties, so the author distinguished A, B and C by the difference of solubility. The more soluble protein is rich in amid N, lysin-N and cystin-N content and poor in monoamino-N and arginin-N content. On the contrary, the protein which is difficult to solve is rich in monoamino-N and arginin-N content but poor in lysin and cystin-N.

IV. Regarding the polysaccharides of this alga, the author distinguished different kinds by the variation of their solubility.

A. The dilute alcohol soluble polysaccharide is not so viscous as the non-water-soluble one and it is composed mainly from araban and fucosan.

B. The hot water soluble polysaccharide is differentiated into three fractions, viz., the polysaccharide soluble in 70% alcohol or HCl-alcohol, easily soluble in water and not very strongly viscous is one of them. It is separated as araban fraction of pectin investigation and found to be composed principally from araban, contaminated with fucosan.

C. Among the hot water soluble polysaccharides, the HCl-alcohol insoluble fraction was separated and found to swell in water and to show strong viscosity.

This is a principal substance of the *Iridaea* mucilage which is used commonly as the cementing material of Japanese house walls. This is a mixture of two kinds of polysaccharides viz., fucosan and galactan, of course the latter occupies the larger part of it. When the mixed polysaccharides were hydrolysed with 1% H_2SO_4 for half an hour, the fucosan became

fucose and was separated from galactan by the alcohol precipitation. When this precipitate was hydrolysed with 5% H_2SO, there were formed large quantities of galactose.

D. The alkali soluble polysaccharide was precipitated by HCl-alcohol and after it was hydrolysed with 5% H_2SO_4, the crystal mass was obtained from the alcohol soluble syrup. Among the crystals, there are distinguished two substances, the one is soluble by absolute alcohol and the other is soluble in methanol, the former is not soluble in methanol and the latter not in absolute alcohol. The phenylosazon of the alcohol soluble part melts at 166–168°C, it resembles arabinose but it is different in that

$$[\alpha]_D^{20} = \frac{100 \times 0.84}{1 \times 2.575} = +31°.4$$

The phenylosazon of the methanol soluble parts melts at 125–130°C and showed

$$[\alpha]_D^{20} = \frac{100 \times 0.195}{0.5 \times 3.205} = +12°.17$$

E. The alkali insoluble polysaccharides are composed principally from fiber which contains cellulose and hemicellulose. The fiber was as far as possible freed from hemicellulose, treated with acid, alkali and by chlorinatio. This purified fiber was compared to cotton fiber and to filter paper. It was observed that the fiber of this alga is not very different from the composed substances from the formation of reducing substance and of KOH soluble matter from cotton and filter paper. But it is far different in the richness of HCl-, Na-bisulphite soluble matters from the other fibers. The pentosan, α-cellulose and oxycellulose content of the alga-cellulose are not very different from the others.

V. The fermentation products of arabinose and fucose in this alga by *Aspergillus oryzae* were examined and various organic acids, aldehyde and other substances, were found. Certain differences between the case of arabinose and of fucose were also observed. In both the fermented liquids formic, glycolic, a trace of ovalic, lactic, citric and kojinic acids were detected, further some oxobarboxylic acid was found. But the writer could not detect alcohol, acetaldehyde, aceton nor acetic acid. The case of fucose fermentation, was different from arabinose in the less content of formic, citric and kojinic acids while there were large contents of oxocarboxylic and lactic acid.

— End —

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