Reports on General Survey and Investigation* on Agar.

By

Arao Itano, Ph. D.

[November 4, 1933.]

While the scientific and industrial uses of agar have increased markedly in a recent years, the detail information on agar is rather meager. Only very recently some scientific and general collected information became available. However the literature in English is very rare so far as the author is aware and it is the purpose of this paper to give a general survey of agar manufacture in Japan together with the results of investigation as to the iodine content in agar and hydrogen ion concentration of agar.

Part I. General Survey on Agar.

Historical :[1] The Japanese had been accustomed to use the seaweeds of various forms as their food since the ancient time, and one preparation called 'Tokoroten' which is the crude gel made from the seaweeds had been used quite popularly. But it was about two hundred and fifty years ago, a man near Kyôto accidentally discovered that exposing the crude gel to a severe cold, it freezes and on thawing it looses the water leaving a beautiful substance which had been known as 'Kanten' or agar. Ever since the preparation of agar had been practiced among the people round Kyôto and Ôsaka, and some on the commercial scale. Also in some parts in Shinano Prefecture, the industry started about eighty years ago.

Primarily the agar had been used in cooking as food but now it has become one of the big industries in Japan and it is exported to all over the world for various purposes which are briefly noted below :

I. Food uses :

In cerial foods ; pastries ; confectionary ; jellies and jams ; salada dressing ; thickener in soups etc.

---

* Assisted by Mr. Y. Tsuji of this laboratory.

II. Industrial uses :

Explosive ; hectograph ; soap ; water proof cloth and paper ; clarifier ; fly paper ; sizing in silk industry ; stencil patterns in art etc.

III. Pharmaceutical uses :

Adhesive dressings ; cold cream ; laxative ; suppositories ; stomach and intestinal remedies ; culture media for microbiological work etc.

Kind of Seaweeds used for Agar Manufacture :

Chiefly the seaweeds which belongs to Rhodophyceae is used for the agar manufacture. There are about fourteen families which include 30—40 species in the world. Around the Japanese islands, five families, namely Gelidium, Caulacathus, Pteroclodia, Acanthopeltis and Gatabella with 16—17 species are found among which Gelidium family is the most important one. Ten species in Gelidium are known in Japan and some of the more or less important ones are G. amansii Laux, G. Japonicum Okam, G. subcostantum Oham and G. pacificum Oham (see Plate IV, V and VI.). Among these G. amansii Laux is the best of all and used most extensively for the preparation of agar.

The seaweeds generally grow in the depth of 10—20 feet and their growth is influenced by light, temperature, concentration of salts and the depth. They are collected during the summer months from May to September by the women divers chiefly, by means of raking and also collected by draging with a specially constructed machine. Then, they are bleached first by the sun on sea shore, and upon reaching the agar manufacturers, the weeds are washed with fresh water and bleached repeatedly and crushed by pounding, and strained to get rid of the foreign matter such as the shells and sands etc. Next they are subjected to boiling for about ten hours in a boiler adding water about ten times of the volume of seaweeds ; strained through the cloth. To the strained material, a certain amount of acid such as acetic or sulfuric acid is added to precipitate the gel ; the coagulated gel is placed in a wooden frame and cut in any shape desired, usually square or threaded ; then subjected to freezing and thawing twice outdoors and dried by the sun completely on the bamboo screen so to eliminate the water as much as possible and at the sametime, the bleaching takes place satisfactorily while drying.

Chemical Composition of Seaweeds and Agar :

Some of the chemical analyses of seaweeds belong to Gelidium are given as follows :

(See Table I on next page.)

The chemical composition of gel prepared from Gelidium amansii is given by TAKAO as follows :

Table I.

Chemical Composition of Seaweeds (Gelidium,).[2]

Constituents.	Green.	Refined.
	%	%
H_2O.	7.36	6.82
Total nitrogenous compounds.	16.06	19.31
Soluble nitrogenous compounds...	7.87	8.37
Fat.	0.98	0.73
Non-nitrogenous extracts.	46.34	50.47
Pentosans.	2.85	3.41
Methyl-pentosan.	0.71	1.13
Fibre.	13.21	13.39
Ash.	12.49	5.74

Table II.

Chemical Composition of Gel prepared from Gelidium amansii Laux.[3]

H_2O.	N-compounds.	Non-N-compounds.	Fibre.	Fat.	Ash.
%	%	%	%	%	%
15.446	10.588	64.228	5.958	0.280	3.500

The general chemical analyses of some agar are given in Table III :

Table III.

Chemical Composition of some Agar.*

Sample No.		Moisture.	Ash.	Crude protein.	Crude fibre.	Soluble non-N-compounds.
		%	%	%	%	%
I.	Air dried.	23.897	2.904	1.428	16.135	55.636
	Dried in oven.	—	3.815	1.876	21.194	73.115
II.	Air dried.	23.120	2.465	1.504	19.267	53.644
	Dried in oven.	—	3.206	1.956	25.062	69.776

* Analyses made at Hokkaido University, informed thru the courtesy of Nozawa & Co.

The soluble non-nitrogenous compounds were analysed for galactose which is generally considered to be the substance which causes the agar coagulate and found as follows : (Table IV.)

Table IV.

Galactose found in soluble Non-N-compound in Agar.

Sample No.	Moisture.	Galactose.
I.	15.820	39.640
II.	23.120	33.400

As to the nature of agar gel, PAYEN[4] attributed it to the presence of 'Gelose' while BAUER, GREENISCH and MORIN claimed that the galactan is responsible. TOLLEN[5] considered d-Galactose as the coagulating agent. TAKAO[3] demonstrated the presence of pentose, fructose, galactan and glycogen in the agar.

Microbiological Consideration of Agar :

Since, in 1881, KOCH found the use of agar in the microbiological investigation in making the solid culture medium on account of its comparative inertness to the action of most microorganisms, it has been used quite extensively. In fact, so far as the author is aware, there are only several microorganisms which hydrolyse the agar, and some of them may be cited here for interest.

GRAN[6] isolated B. gelaticus from sea water and the organism was used by PRINGSHEIM[7] in connection with his experiment on the nitrogen fixation by Clostridium americanum and Azotobacter chroococcum in which he tried to use the hydrolysis of agar by B. gelaticus as a source of energy. In 1905, PANEK[8] described an organism, isolated from the sugar beet under the name of Bacterium beta viscosum. BIERNACHI[9] isolated Bacterium nenckii from raisins, which is a facultative anaerobe. GRAY and CHALMERS[10] isolated an organism in course of isolation of Spirochaeta cytophaga and named it as Microspira agar-liquefaciens. AOI[11] in 1925 isolated an organism from the stable manure as a new species of agar decomposer and named it Vibrio andoi. LATELY several reports appeared which deal with the agar decomposers: LUNDESTAD[12] isolated eight different species from sea water; WAKSMAN and BAVENDANUM[13] isolated an organism from marine sediment which attacks the hemicellulose complex of agar; GORESLINE[14] reported Achromobacter pastinator n. sp., Pseudomonas lacunogenes n. sp. and Pseud. segne n. sp.; NICHOLS[15] reported an agar liquefying bacterium which was isolated from the soil.

Part II. Investigation on Agar as to the Iodine Contents.

Since the agar is made from the seaweeds which contain a considerable amount of iodine, it is naturally suspected that some iodine is transfered to

the agar in the process of its manufacturing. However no definite information is available in this regard so far as the author is aware.

Again the physico-chemical as well as the physiological importance of iodine is well recognized and the agar is used extensively as food and also in the biological investigations so that it is important to ascertain as to the quantity of iodine may be found in the agar.

In the microbiological field, the information concerning the physiological influence of iodine are rarely found. Greaves[16], and Kooijmans[17] found that 1—100 to 1—1,000 of iodine stimulates the growth of yeasts; Scharrer and Schwartz[18] also investigated the influence on the yeasts and found that the ionic or organic iodine, in quantity of 0.000001 per cent stimulates the growth, and 0.25 per cent is harmful to most organism and noted that the molecular form of iodine is more poisonous than that of ionic form. Itano and Matsuura[19] investigated the influence on B. subtilis, Azotobacter and yeasts, and found that 0.007 per cent was the optimum for their growth and 0.2 per cent was harmful for them all. Gerschenfeld[20] tried a 3 per cent mixture of CaI_2, KI and NaI, and found it to be a powerful disinfectant.

In view of these facts, the following investigation was carried out.

Experimental:

With the method[33] previously described, the iodine content was quantitatively determined in:

1. seaweeds from different localities in Japan,
2. commercial agar of different grades and also that prepared in the laboratory,
3. elimination of iodine in agar by purification and by processing,
4. the residue after the agar is extracted.

(1) *Iodine in Seaweeds.*

From the plant physiological and iodine industrial standpoints, the iodine content of seaweeds have been investigated quite extensively and reported by various investigators, Dixit[21], Dangard[22], Kylin[23], and very recently by McClendon[24], and others. In general, those seaweeds which belong to Phaeophyseae contain a large amount and less in Rhodophyceae. Even the seaweeds of same family vary in their iodine content by the locality where they grow, the season, and also by the different part of the same plant. However very little is known about the iodine content of the seaweeds which belong to Gelidium which are used for the agar manufacturing chiefly. Endo[2] states in his book the absence of iodine in Gelidium amansii while Dangard and Kylin reported the presence of only a trace of iodine, and McClendon[24] reported as follows:

Gelidaceae :	Iodine per Kg. dry matter.
Gelidium amansii	130—140 mg.
G. subcostantum	300—340
G. japonicum	190—240
G. sp.	73—76

As to the form of iodine in the fresh seaweeds, Sargassum enerve Ag., Ecklonia cava KJELLM and EISNIA bicyclis (KJELLM) SETCH ; ESCHLE[25], OKUDA and ETO[26] reported that the iodine exists largely as water soluble organic compounds which is not decomposed by the action of weak sulfuric acid nor by sodium hydroxide. TSUKAMOTO and FURUKAWA[27] investigated Laminaria ochotensis MIYABE and found that the iodine existed in water soluble inorganic form. However no report on Gelidium was found concerning the form of iodine, although it bears relation with the iodine in agar ; and although this particular point is interesting, it was not investigated this time.

In this investigation, the seaweeds from different localities in Japan, which are chiefly used for agar manufacture, were purchased from an agar manufacturer in Hyôgo Prefecture and the total iodine was determined as in Table V :

Table V.

Iodine Contents of some Seaweeds.

No.	Prefecture.*	Classification of seaweeds.	Iodine in 1 g. dried seaweeds. (mg.)	Iodine p. p. m.
1.	Shizuoka.	Gelidium amansii Laux.	0.7262	726.2
2.	ibid.	Gelidium subcostantum Oham.	0.7962	796.2
3.	Chiba.	Gelidium linoides Kütz.	0.5742	574.2
4.	ibid.	Gelidium amansii Laux.	0.4302	430.2
5.	Kagoshima.	ibid.	0.4662	466.2
6.	Kochi.	Gelidium pacificum Oham.	0.4550	455.0

* Where the seaweeds were originally collected.

As Table V indicates, there are some variation, 430—796 p. p. m. in the amount of iodine content by the seaweeds of different origin.

(2) *Iodine Content in Commercial Agar of Different Grades and also Agar prepared in Laboratory.*

Judging from the nature of agar, it is possible that the agar adsorbs some of the soluble iodine in the process of its preparation. BUNGENBERG DE JONG[28] demonstrated that a piece of agar adsorbs iodine on shaking it in IKI solution, and a similar action may be taking place in agar manufacturing.

(a) *Commercial Agar of Different Grades :*

Three different grades of commercial agar was analysed for iodine and found as follows :

Table VI.

Iodine Content in some Commercial Agar.

Grade of Agar.	Iodine in 1 g. dried agar.	Iodine p. p. m.
	(mg.)	
I.	0.0118	11.8
II.	0.0265	26.5
III.	0.0428	42.8

It is noted in Table VI that the second and third grade agar contain much more iodine than the first grade by order. This difference may be due to the process of drying since the first grade agar is taken from the top layer of dried gel on the bamboo screen on which the gel is dried and the second, from the lower layer and the third, from the bottom, so that the drained water seems to carry down more soluble iodine toward the bottom in the course of drying.

(b) *Agar prepared in Laboratory :*

The agar was prepared in the laboratory from those seaweeds listed in Table I according to the following method which is very similar to the industrial process :

20 g. seaweeds were placed in 400 cc. boiling water in a 500 cc. beaker ; after a few minutes, 50 cc. weak sulfuric acid (12 cc. N/10 H_2SO_4 in 50 cc. H_2O) were added and heated slowly for an hour ; after that the solution was kept in an incubator at 80—85°C. for 12—14 hours ; the total volume was made up to 500 cc. and filtered into a large Petrie dish where the coagulation takes place within 2—4 hours ; the gel was cut into pieces of 0.5—1.0 cm. wide and placed on a bamboo screen and transfered into a galvanized iron box which imbeded in a freezing mixture, ice and salt ; after freezing, the content was taken out and placed in a warm room so that the frozen gel melts gradually and the water runs out ; when the dripping stops, it was taken outdoors and dried in the shade where the aeration is good ; then the gel dries up within 3—4 days. Thus the fairly good samples of agar was prepared. The air dried gel was dried further in a vacuum, $CaCl_2$ desiccator, and powdered after dried and subjected to the analysis of which results are given in Table VII :

(See Table VII on next page.)

Considering the results given in Table VII in the light of Table V, it is noted that larger the original content of iodine in the seaweeds, more iodine is transfered to the agar.

Table VII.

Iodine Content of Agar prepared in Laboratory.

No.*	Iodine in 1 g. dried agar.	Iodine p. p. m.
	(mg.)	
1.	0.0972	97.2
2.**	—	—
3.	0.1019	101.9
4.	0.0783	78.3
5.	0.0245	24.5
6.	0.0450	45.0

* Number corresponds to that in Table V.

** Failed to give agar gel.

From these results, it is noted that both the commercial and prepared agar contain iodine in amount of 10—101 p. p. m. Considering these amount in conjunction with the use of agar in the nutrient media for microorganisms and its subsequent influence, the following statement may be made:

Since 15 g. agar is usually taken for the preparation of 1 L. of the medium and the iodine content of 15 g. agar is 1.515 mg. utmost and 0.165 mg. lowest, the percentage of iodine in 1 L. of medium may be 0.00015—0.000016 per cent accordingly. So far as the KOOLMANS'[17] and other investigations are concerned, the presence of such a small amount of iodine is not harmful although it may stimulate the growth.

(3) *Elimination of Iodine from Agar by Purification and Processing.*

(a) *Influence of Washing:*

Besides the gelose, the ordinary agar contains various amount of crude protein, crude fibre, ash and iodine. For the cultivation of microorganisms, it is ideal to use the pure agar so that the food supply is strictly confined to just what are added experimentally. Consequently several methods of purification of agar have been reported by FELLER[29], MACDOUGAL[30] and others. In principle, the method consists of washing, filtration, dialysis or reprecipitation with alcohol and acetone. For example FAIRBROTHER and MASTIN[31] applied only filtration and dialysis for two weeks and succeeded in reducing the nitrogen content to 0.16 from 0.41 per cent, and also reduced ashes to 2.38 from 4.99 per cent. In this investigation, the effect of simple washing and dialysis was investigated in the following manner: 20 g. finely powdered agar was mixed with 1 L. distilled water and stood overnight, filtered and washed until the filtrate reached to 2—2.5 L. The filtrate was concentrated on the water bath and K_2CO_3 was added to make

the filtrate alkaline and evaporated to dryness ; the dried residue is dried further in an oven at 85°C. for a few hours, powdered and analysed for iodine. The results are shown in Table VIII.

Table VIII.

Elimination of Iodine from Agar by Washing.

Grade of Agar.	Iodine in 1 g. dried agar.		Ratio.
	Total iodine.	Iodine eliminated.	
	(mg.)	(mg.)	
I.	0.0118	0.00610	100 : 51.6
III.	0.0427	0.01704	100 : 39.8

Table VIII indicates that 40—50 per cent iodine can be eliminated by simply washing with water.

(b) *Elimination of Iodine by Dialysis and Reprecipitation :*

Next the agar was purified by the following method and the iodine content was determined : 20 g. powdered agar was placed in a collodion such in water, and changing the water twice a day and finally the agar was transfered on a filter paper, washed several times with water ; then thrown into the absolute alcohol to reprecipitate the agar ; separate the agar on the suction filter, washed with alcohol and ether and dried. The results are shown in Table IX.

Table IX.

Effect of Purification on Iodine Content.

Grade of Agar.	Iodine in 1 g. dried agar.	
	Before purification.	After purification.
	(mg.)	(mg.)
I.	0.0118	0.00609
II.	0.0265	0.00714

From these results, it is noted that even after such purification 6—7 p. p. m. iodine remains in the agar.

(c) *Influence of Sulfuric Acid used on the Iodine Content of Agar.*

In boiling the seaweeds for extraction of agar, a small amount of sulfuric acid is used, and the quantity of the acid added, influence the coagulation of agar owing to the hydrolysis of carbohydrates contained in the seaweeds. Consequently it was presupposed that the amount of iodine may be influenced by the amount of sulfuric acid used, and the following experiment was carried out :

To 20 g. seaweeds in 450 cc. water, various amount of N/10 H_2SO_4, as indicated in the table, was added and the iodine content of agar thus produced was determined. The results are shown in Table X.

Table X.

Iodine Content in Agar and Sulfuric Acid used.

cc. N/10 H_2SO_4 used.	Iodine in 1 g. dried agar.	Iodine p. p. m.
	(mg.)	
6	0.0198	19.8
12	0.0245	24.5
24	0.0649	64.9

Table X indicates that more the acid is used, more of the iodine is found in the agar.

(4) *Iodine Content in the Residue after Agar is extracted.*

The residue of seaweeds after the agar is extracted, has been used as fertilizer mainly but judging from the crude protein content, it may be used as the cattle feed. It was investigated here to ascertain what portion of iodine remains in the residue.

After the agar was extracted, the residue on the filter was washed with water and dried, and powdered to be analysed. The results are given in Table XI.

Table XI.

Iodine Content in the Residue.

No.*	Iodine in 1 g. dried matter.	Iodine p. p. m.
	(mg.)	
1.	1.3445	1344.5
2.	0.5515	551.5
3.	0.7470	747.0
4.	0.8605	860.5
5.	0.6910	691.0
6.	0.5585	558.5

* Same as in Table V.

The data indicate that with exception of No. 2, all others showed that the amount of iodine increased comparing with that of the original seaweeds. This may be due to the loss of the total weight of seaweeds in the process.

The relation of iodine content in the seaweeds, agar and the residue is shown in Table XII.

Table XII.

Relative Iodine Content in Seaweeds, Agar and Residue.

No.	1.	2.	3.	4.	5.	6.
Iodine (mg.) in 20 g. air-dried seaweeds.	12.180	13.320	8.900	7.200	7.800	7.570
Agar prepared. (g.)	6.15	—	6.12	7.02	4.80	5.55
Iodine (mg.) found in agar.	0.5301	—	0.5097	0.3310	0.0974	0.2145
Residue. (g.)	9.10	11.80	10.00	8.05	11.80	10.40
Iodine (mg.) found in residue.	11.3750	5.9590	6.9400	6.5964	7.6582	5.8084
Total iodine in agar + residue. (mg.)...	11.9051	—	7.4497	6.9274	7.7556	6.0229

Note: Since the amount of agar prepared from the same amount of seaweeds varies by such factors as boiling, freezing, drying etc., the values given in the table are the mean value of several experiments.

From the above results, it is noted that a large portion of iodine in seaweeds remains in the residue and very little was lost in the solution and a small portion remains in agar. These results are rather different from those which have been reported by other investigators. The difference may be due to such factors as the kinds, freshness of seaweeds which naturally may influence the amount of water-soluble iodine.

Part III. Hydrogen Ion Concentration of Agar.

The reaction of agar is generally considered as neutral and only a few reports on the subject are found which may be due to the difficulty of determining P_H value directly.

Burnes[32] reported that the P_H value of agar can be determined with antimony electrode. In this investigation, the commercial agar and the prepared agar in suspension of 1 : 20 and also 1 per cent solution at 35°C., were taken and the P_H values were determined by both the antimony and quinhydrone electrodes.

(1) *pH Value of Agar Suspension.*

One part of powdered commercial agar was suspended in 20 perts of water and P_H was determined at various intervals under different treatments as right after vigorous shaking, after standing for an hour and also for overnight. The results are given in Table XIII.

Table XIII.

pH of Commercial Agar Suspension.

Grade of agar.		Quinhydrone.	Antimony.
Right after shaking.	I.	6.82	6.54
	II.	6.98	6.67
	III.	6.81	6.56
After 1 hour shaking.	I.	6.84	6.71
	II.	8.02	6.81
	III.	6.95	6.63
After standing overnight.	I.	6.87	6.64
	II.	7.00	6.76
	III.	6.90	6.58

The results obtained by these two electrodes were very similar except those obtained right after shaking. As a whole, the reaction of agar is almost neutral.

Again the suspension of agar prepared in the laboratory was investigated and obtained the following results:

Table XIV.

pH of Laboratory Agar Suspension.

No.*	Quinhydrone.	Antimony.
1.	6.32	6.17
3.	6.85	6.68
4.	6.69	6.52
5.	6.73	6.55
6.	7.20	6.69

* Same as Table V.

Note: In this case the suspension stood for an hour before the determination.

Table XIV indicates that the antimony electrode gave lower P_H values than the quinhydrone electrode.

(2) *pH Value of Agar Solution.*

One per cent of agar was dissolved in water and the determination was carried out at 35°C. The results are shown in Table XV.

Table XV.

pH of 1% Agar Solution of Commercial and Laboratory Agar.

Kind of Agar.*	Quinhydrone.	Antimony.
I.	7.34	8.02
II.	7.27	7.85
III.	7.43	8.01
1.	6.89	6.92
3.	7.20	7.60
4.	7.15	7.55
5.	7.18	7.52
6.	7.16	7.59

* Same as noted in the previous tables.

The data indicate that the antimony electrode gave larger P_H values than the quinhydrone, and as a whole the larger P_H values were obtained for the agar. This may be due to the difference which is caused by the temperature although a proper temperature correction was applied.

Summary.

From the results obtained in the investigations reported in this paper as Part II and III, the following summary may be made:

1.) Six kinds of seaweeds which are chiefly used for agar manufacturing were tested for the iodine content and found that they contain from 0.045 to 0.079 per cent iodine.

2.) The commercial agar and the laboratory agar contain from 0.00118 to 0.01017 per cent iodine. Among the commercial agar, better grade of agar contain less iodine, and the iodine content of laboratory agar was in parallel with that of the seaweeds from which the agar is prepared.

3.) The iodine content in agar can be controlled to some extent by adjusting the amount of sulfuric acid used in the process, less the acid is used, less the iodine is found in agar.

4.) The iodine content of agar can be reduced by 40—50 per cent, by washing with water.

5.) A trace of iodine remains in agar even after dialyzation and reprecipitation.

6.) A large portion of iodine in the seaweeds remain in the residue after the agar is extracted.

7.) The hydrogen ion concentration of agar is somewhere near P_H 7.0 although some variations were found by the electrode used.

Literature.

1.) Report of Osaka Industrial Research Institute No. 6, 1929.
2.) ENDO, K., 'Kaisan Shokubutsu Gaku', p. 569. 1905.
3.) TAKAO, Y., 'Yakugaku Zasshi', p. 1061, 1921.
4.) Cited in GILTNER, Microbiology, p. 36, 1921.
5.) TOLLENS, B., Ber. deut. chem. Gesell., 34: 1434, 1901.
6.) GRAN, H. H., Bergenes Museum Aarbog, No. 2, 1902.
7.) PRINGSHEIM, E., Centbl. f. Bakt., II, 26: 227, 1910.
8.) PANECK, M. K., Bull. de l'Acad. Sci. de Crocovie, Nr. 1: 16, 1905.
9.) BIERNACKI, W., Centbl. f. Bakt., II, 29: 166, 1911.
10.) GRAY, P. H. H. and C. H. CHALMERS, Ann. Applied Biology, 11: 324, 1924.
11.) AOI, K., Centbl. f. Bakt., II, 63: 30, 1924; — and ORIKURA, J., ditto. 74: 321, 1928.
12.) LUNDESTAD, J., Centbl. f. Bakt., II, 75: 321, 1928.
13.) WAKSMAN, S. A. and W. BAVENDAMM, J. Bact., 22: 91, 1931.
14.) GORESLINE, H. E., Science, 76: 255, 1932.
15.) NICHOLS, A. A., Centbl. f. Bakt., II, 88: 177, 1933.
16.) GREAVES, J. E., ZOBELL, C. E. and GREAVES, J. D., J. Bact., 16: 409—30, 1928.
17.) KOOIJMANS, J., Centbl. f. Bakt., II, 82: 347, 1930.
18.) SCHARRER, K. and SCHWARTZ, W., Biochem. Z., 245: 218, 1932.
19.) ITANO, A. and MATSUURA, A., 'Nogyo and Engei', 9: 2062, 1933.
20.) GERSCHENFELD, L. and MILLER, R. E., J. Amer. Pharm. Assoc., 21: 894, 1932.
21.) DIXIT, S. C., J. Indian Chem. Soc., 7: 958, 1930.
22.) DANGARD, P., Compt. rend., 189: 862, 1919.
23.) KYLIN, H., Z. Physiol. Chem., 186: 50, 1929.
24.) MCCLENDON, J. F., J. Biol. Chem., 102: 91, 1933.
25.) ESCHLE, H., Z. Physiol. Chem., 23: 30, 1897.
26.) OKUDA, J. and ETO, T., 'Nogaku Kwaiho', 146: 1, 1914.
27.) TSUKAMOTO, M. and FURUKAWA, S., idid. 128: 1, 1913.
28.) BUNGENBERG DE JONG, H. G. and LANZIG, J. C., Kolloid-Beihefte, 35: 89, 1932.
29.) FELLERS, C., J. Ind. Eng. Chem., 8: 100, 1916.
30.) MACDOUGAL, A., Proc. Amer. Phil. Soc., 59, 150, 1920.
31.) FAIRBROTHER, F. and MASTIN, H., J. Chem. Soc., 123: 1412, 1923.
32.) BARNES, E. E. and SIMON, R. H., J. Amer. Soc. Agron., 24: 156, 1932.
33.) ITANO, A., Berichte d. Ōhara. Inst. etc., V, 53—58, 1933.

PLATE IV.

I. Gelidium amansii Laux.
(Shizuoka.)

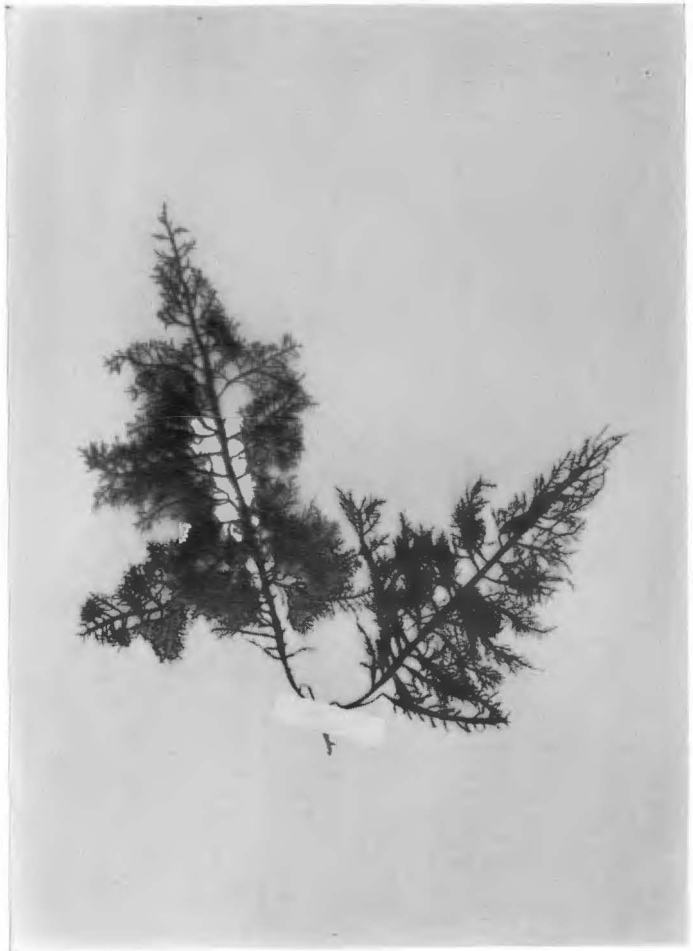
II. Gelidium subcostantum Oham.
(Shizuoka.)

PLATE V.

III. Gelidium linoides Kütz.
(Chiba.)

IV. Gelidium amansii Laux.
(Chiba.)

PLATE VI.



V. Gelidium amansii Laux.
(Kagoshima.)

VI. Gelidium pacificum Oham.
(Kochi.)