REFERENCES.

(1) Bordeianu, C. V., *Arch. Pharm.*, 272, 8 (1934), through *chem. Zentr.* (I) 2123 (1934).
(2) Kehrmann, Fr., *Ber.*, 22, 3266 (1889).
(3) Kamm, O., *Qualitative Organic Analysis*, 2nd Edition, 172 (1932).
(4) Kehrmann, Fr., *J. prak. Chem.*, (2) 39, 395 (1889).

A PHYTO-CHEMICAL STUDY OF IRIDÆA LAMINARIOIDES.*

BY JOHN ALAN ELLEGOOD.[1]

INTRODUCTION.

Seaweeds have been used for food since ancient times. In Japan to-day algæ of different varieties constitute an important factor in the dietary. Some varieties are cultivated as a crop and as such are harvested. However, the bulk of the supply is collected from natural sources. The demand is not attributed so much to the food value, which is extremely low, but to the flavor and consistency imparted to foods. Considerable work has been done on the vitamin content of seaweeds, some of which have been found to contain varying large amounts of Vitamins B and C. (1). Many of the Japanese soups are flavored with "Nori" a green alga Ulva lactuca. In American markets may be found "Dulse," (Rhodymenia palmata) one of the red algæ. "Dulse" is gathered from the coast of Newfoundland, dried and packaged for sale. It is used as a pudding thickener and as a source of iodine. A candy has been made from the stipe of the giant kelp, Nereocystis Lutkeana, also "seatron," a substitute for preservation citron, (2) from the same source.

Attempts have been made from time to time to utilize seaweed as a stock food but with little success. It has been used by coast farmers to fertilize their land (3). Besides the aforementioned uses, seaweeds have a more important place in our lives. In Pharmacy, products obtained from algæ are extensively used. Chondrus Crispus and the Mucilage of Chondrus appear in the National Formulary VI. Fucus was official in the fifth edition but has been dropped from the sixth. Agar is included in the Pharmacopœia. Agar has so many uses and is so well known that a description here would be superfluous.

Since the greater part of the agar on the market is imported, it seemed desirable to determine if a substance similar in nature could be prepared from some of the native seaweeds, of which there is a great abundance.

With this in view, Red, Green and Brown algæ were collected[2] at the University of Washington Oceanographic Laboratory at Friday Harbor, Washington. Due to the vast amount of material available, collection was limited to those varieties which occurred abundantly and those whose thalli were comparatively large and simple in form. Twenty-three were finally selected for the preliminary work.

---

* Submitted as a partial requirement for the Degree of Master of Science in Pharmacy, College of Pharmacy, University of Washington, Seattle.

[1] This work was supervised by H. A. Langenhan, Professor of Pharmacy.

[2] Under the supervision of Dr. George B. Rigg, Professor of Botany.

Table I lists the species collected, the last column indicating whether gel forming constituents were found.

TABLE I. (4)

Family Species.	Description.	Results.
Green		
Ulva lactuca	Thin sheets	Non-gelling
Ulva latissima	Thin sheets	Non-gelling
Brown		
Costaria costata	Thick broad fronds, heavy round stipe	Non-gelling
Cytosiphon	Small, tubular	Non-gelling
Nereocystis lutkeana	Very large kelp	Non-gelling
Hedophyllum	Broad, crinkled, short stipe	Non-gelling
Laminaria saccharina	Broad, smooth	Non-gelling
Laminaria bullata	Broad, crinkled in the center	Non-gelling
Red		
Iridæa laminarioides	Broad form with undulating edge	Gelling
Porphyra naiadum	Very thin, edges wavy, a broad form	Non-gelling
Turnerella pacifica	Variable size, margin much lobed	Gelling
Prionitis (three varieties)	Small, much branched	Gelling
Gigartina papillata	Medium size, covered with spines	Gelling
Rhodymenia pertusa	Very long, 4′–5′; many nodules and perforations	Non-gelling
Agardhiella coulteri	Small form, much branched	Gelling
Polyneura latissima	Small, pleated thallus, toothed margin, veined	Non-gelling
Fauchea fryeana	Bladelike, small	Non-gelling
Anatheca	Somewhat dichotomously branched	Gelling
Nitophyllum mirabile	Membraneous, deeply lobed	Non-gelling
Opuntiella californica	Flat, disk-shaped frond	Gelling
Dasyopsis plumosa	Finely branched, small type	Gelling
Callophyllis	Medium size, lobed	Non-gelling
Constantinea subulifera	Circular frond, leathery texture	Non-gelling

Iridæa laminarioides was selected for the first detailed study, as it is fairly abundant and the thalli are large and unbranched thus facilitating the cleaning and drying of the large amount required for this work.

The original method employed in the preparation of a gel was that used commercially in the preparation of agar (5). It consists briefly of boiling the bleached seaweed in a large excess of water for about eight hours. It is then expressed through muslin and the residue again boiled for another period of eight hours. In this second extraction, sulfuric acid is added to about 0.5%. Practically all of the plant is dissolved, leaving very little to strain out. Later this method was modified to the extent that the sulfuric acid was omitted as it was found that partial hydrolysis took place thus decreasing the yield of the gel.

After straining, the extract is reduced to a thick gel. This must be thick enough to retain its shape when cooled. The gel is then frozen solid and allowed to melt quite rapidly, allowing the water to run off and carrying with it the water soluble portion. The residue is then completely dried and is ready for use.

Each of the specimens listed in Table I were treated in the same manner. Many were found that would produce a good gel (see Table I), however, the thallus was either too finely branched to handle well or the whole plant was so small that it would have been quite impracticable to collect for commercial purposes.

OCCURRENCE.

Iridæa is reported as being found on the Pacific Coast from Santa Barbara northward (6). Agardh reports it off the coast of Peru, and later investigators place its habitat farther south in the cooler waters of Chile (7). Iridæa edulis, also known as Dilsea edulis and Schizymenia edulis is common around the British Isles (8). Still another variety is Iridæa mertensiana which occurs

in Japan (9). The species used in the present investigation was collected in Puget Sound in the vicinity of the Oceanographic Laboratory of the University at Friday Harbor, Washington. This alga appears to be fairly common in the cooler latitudes; however, it has not been reported growing on the Atlantic coast.

Iridæa laminarioides is a member of the red algæ (Rhodophyceæ). Family, Gigartinaceæ. According to Kylin it is identical with I. heterocarpa (10). It grows attached to rocks in the upper sublittoral zone, being exposed only at extremely low tides. All of the specimens found were growing in areas where swift tide currents prevailed. There are cystocarpic and tetrasporic plants, indicating the alternation of generation common to red algæ. It is not known whether Iridæa is annual or perennial.

DESCRIPTION.

The thallus of Iridæa is short and broad and usually has an undulating margin. The stipe is extremely short, rounding out to form a modified disk-shaped holdfast. The color ranges from a deep maroon through purplish red to cinnamon-brown. In older plants and those that have been subjected to extended desiccation bleached patches will be found. These patches are very fragile and fall to pieces with handling. When submerged Iridæa is very iridescent. This property is retained through desiccation, bleaching and subsequent immersion.

COLLECTION OF MATERIAL.

The material for this work was collected from a group of rocks lying off Brown's Island across the bay from the Laboratory. The plants were accessible only during minus tides making collection somewhat delayed. Approximately two gunny sacks of air-dried material was collected. The actual collecting was done from rowboats, the material being pulled from the rocks and placed in tubs of salt water. On returning to the station, the plants were stored in live-boxes until such time as they could be worked upon, which was never more than twenty-four hours after collection. Cleaning consisted of going over each thallus by hand to remove the larger adhering foreign material. They were then washed in running sea water followed by fresh water. Long strips of muslin were placed in the sun and the cleaned seaweed spread upon this. After it had dried, it was collected, macerated in fresh water and returned to the muslin strips. This was repeated until the thalli were bleached to a pale straw color. The bleached Iridæa was then dried and stored in tightly closed manila bags.

ANALYTICAL DATA.

Moisture.—The moisture of the fresh thallus was determined by first removing the surface water with dry cloths and then drying to constant weight at 100° C. Moisture content—89.47%.

Ash.—The methods used for determining ash and its constituents were those of the A. O. A. C. (11). Samples were run in duplicate and repeated for accuracy. All determinations were based on material dried to constant weight at 100° C.

Total ash	16.08%
Insoluble	3.27%
Soluble	12.81%
Ca	0.391
Na	0.53%
K	2.34%
SO_4	3.52%
Cl	1 54%
P_2O_5	0 7373%

Iodine.—Considerable difficulty was experienced in obtaining a suitable method for the determination of iodine. The colorimetric method of Tang and Whang (12) was attempted, but the results were not consistent. McKean (13) developed a method based on the extraction of the sample with water. This method would not apply to Iridæa as the plant swells and becomes very viscous in water and is impossible to filter. The U. S. P. method for Thyroid could not be used because of the very small amount of iodine present. The smallest trace of chlorine would give a very high iodine value. The following method was found to give 96.26% recovery of added iodine (as KI) to agar. It is essentially the method for the assay of Thymol Iodide U. S. P. XI.

The material is ignited to a dull red with anhydrous sodium carbonate. When it is thoroughly charred, it is washed onto a filter paper and the whole mass washed with about 150 cc. of hot distilled water. Enough solution of potassium permanganate is added to produce a pink color. Ethyl alcohol is added to reduce the excess permanganate and the solution made to 2,000 cc. with water. This is then filtered, the first 25 to 50 cc. being discarded and a 100-cc. aliquot taken for titration. KI and dilute H_2SO_4 are added to the aliquot which is then titrated with 0.001 normal sodium thiosulfate. In standardizing the method blanks were run on the reagents, on agar and on agar plus a measured amount of KI. To determine the recovery, exactly 1 Gm. of KI was diluted to 100 cc. and one cc. of this, representing 0.01 Gm. was added to two Gm. of agar Simultaneously the blanks were run. After deducting the titration values of the blanks, it was found that of the 0.00764 Gm. of iodine added (as KI), 0.007354 Gm. were recovered. Following the above procedure Iridæa was found to contain 24.32 parts per million of iodine.

Nitrogen.—The method used was the Kjeldahl-Gunning Arnold method (14). Duplicates were run along with a blank of the reagents used. The results: Iridæa 1. 3.23% nitrogen.

EXTRACTIVES.

Selective Solvents.—A series of extractions were made using a soxhlet extractor and extracting for a period of eighteen hours.

Table II gives the results of the extraction. In each case the residue left after the evaporation of the solvent was a very thin greasy film on the bottom of the soxhlet flask. The amounts were so small that it was not considered feasible to attempt an analysis.

TABLE II.—SEPARATE SAMPLES FOR EACH SOLVENT.

Solvent.	Per Cent of Extract.
Ethyl ether	0.24
Chloroform	0.21
Petroleum ether	0.19
Alcohol	0.70

Fractional Extraction.—Another set of extractions was made using petroleum ether, chloroform, ethyl ether, ethyl alcohol and water, in the above order.

TABLE III.—ONE SAMPLE EXTRACTED SUCCESSIVELY.

Solvent.	Per Cent of Extract.
Petroleum ether	0.1968
Chloroform	0.1937
Ethyl ether	0.2050
Ethyl alcohol	0.4530
Water	86.34

Qualitative Tests.—An alcoholic extraction was made of a comparatively large amount of the material. Following the method of Hassid (15), the alcoholic solution was filtered and reduced in volume until a syrup consistency was obtained. This was allowed to stand for several days when a few crystals were obtained. These were redissolved in a very small amount of alcohol and allowed to recrystallize. The melting point of the crystals was 183° C., agreeing with the melting point for dulcitol. The few remaining crystals were hydrolyzed with nitric acid to mucic acid. This was checked by the melting point. From this it was assumed that dulcitol was obtained from the alcoholic extract.

Both aqueous and alcoholic extracts responded to the following tests.

Carbohydrate	Molisch test	Positive
Reducing sugars	Benedict's	Negative
	Fehling's	Negative
Pentosan	Phloroglucinol-HCl	Negative
Galactan	Mucic acid	Positive

Crude Fiber.—Following the method as outlined in the A. O. A. C. (16). Crude fiber was determined on Iridæa laminarioides with the following results: Crude fiber 0.705%.

PHARMACEUTICAL.

An attempt was made to substitute the extract obtained from Iridæa laminarioides for some of the gels and gums used in official preparations.

As a preliminary step various dilutions of tragacanth were made and these compared with similar dilutions of the Iridæa extract by the falling ball method of Nicholls (17). It was found that an aqueous dilution of 1:40 of Iridæa compared with an aqueous dilution of 1:100 of tragacanth.

The following preparations were made substituting the extract for the tragacanth; glycerite of tragacanth; jelly of ephedrine sulfate; and an emulsion of olive oil (18).

In each case the product was not as attractive as that in which tragacanth was used. The color resembled agar products. The consistency was similar if about $2^1/_2$ times as much Iridæa extract was used in the place of tragacanth. Emulsions made with the extract tend to separate more readily than those made with tragacanth or acacia.

SUMMARY.

A survey was conducted on various Red, Brown and Green algæ to determine their gelling properties. Iridæa laminarioides was chosen as the first upon which to make a detailed study. The chemical constituents were determined, and a comparative pharmaceutical study of the aqueous extract was made. For one part of tragacanth $2^1/_2$ parts of Iridæa extract are required in order to obtain a product of comparable consistency.

BIBLIOGRAPHY.

(1) Norris, E. R., *J. of Nutrition*, 13, 4 (April 1937).

(2) Tilden, A., *Algæ and Their Life Relations*, Univ. of Minnesota (1925).

(3) Gray, S. O., *British Seaweeds*, L. Reeve & Co., London (1904).

(4) Kylin, H., *Marine Red Algæ in the Vicinity of Friday Harbor, Washington.* Harrowitz, Leipzig (1928).

(5) Tressler, D. K., *Marine Products of Commerce*, Chemical Catalog Co., New York City (1926).

(6) Hervey, A. B., *Sea Mosses*, Orange Judd Co., New York (1895).

(7) Howe, M. A., *Algæ of Peru*, New Era Press, Lancaster (1910).

(8) Newton, L., *Handbook of British Seaweeds*, Trustees of the British Museum, London (1933).

(9) Okamura, K., *Icones of Japanese Algæ*, 3, 83 (1913–1915).

(10) Kylin, H., *Marine Red Algæ in the Vicinity of Friday Harbor, Washington.* Harrowitz, Leipzig (1928).

(11) *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, 4th Ed., XII, 4, 10, 14, 15, 28, 36, 30 (1933).

(12) Tang, Pei-Sun, and Whang, Pao-chun, *Chinese J. Physiol*, 9, 285–289 (May 1935).

(13) McKean, J. B., *The Analyst*, 61, 11 (January 1936).

(14) *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, 4th Ed., II, 22 (1933).

(15) Hassid, W. Z., *Plant Physiology*, 8, 480 (July 1933).

(16) *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, 4th Ed., XII, 52 (1933).

(17) Nichols, A. B., Jour A. Ph. A., 26, 823 (1937).

(18) *Pharmacopœia of the United States*, 11th Revision, Mack Printing Co., Easton, Pa. (1936).