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LIV. ON CARRAGEEN (*CHONDRUS CRISPUS*). II<sup>1</sup>.  
ON THE OCCURRENCE OF ETHEREAL SULPHATES  
IN THE PLANT.

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INTRODUCTORY.

VARIOUS views have from time to time been put forward concerning the composition of carrageen or Irish moss mucilage; the discordant results may be attributed to the fact that some of the investigators have worked on the weed as such, while others have examined an aqueous extract, apparently assuming either material to contain only a single substance. In all cases previous workers have contented themselves with establishing the presence of certain carbohydrate residues by hydrolysing the material or by distilling it with mineral acids or oxidising with nitric acid. Working on these lines Tollens and his pupils have shown that the substance contains galactose and fructose residues, the latter in such quantity as to induce Tollens [1914] to place carrageen among the fructosans, without however assigning any definite formula to the substance. Sebor [1900] on the other hand goes a little further in expressing the opinion that carrageen mucilage is a complex carbohydrate produced by the union of galactose, dextrose and levulose residues together with a small quantity of pentosan or methylpentosan as an impurity.

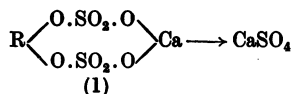
In the course of an investigation of the physical and chemical properties of an aqueous extract of Irish moss [Haas and Hill, 1921], it was however found that a hot water extract of the plant is not, as had been assumed by previous authors, a single substance but is composed of at least two constituents, which can be separated by making use of their different solubilities in cold water. A systematic investigation of these two substances, known for convenience as the cold and hot extracts, is now being undertaken, but although it is not as yet completed, the results so far obtained are thought to be of sufficient importance to justify publication at this stage.

It has long been known that *Chondrus crispus* yields on incineration a considerable amount of ash composed largely of calcium sulphate; practically all previous workers have drawn attention to this fact and have recorded

<sup>1</sup> For the first paper of this series see Haas and Hill [1921].

their inability to reduce the ash content by dialysis and, without offering any explanation for this anomaly, have confined their attention to the organic matter present, ignoring the possible significance of the high ash content.

Czapek [1920] commenting on the high percentage of sulphate found in the ash of many marine algae, states that it is not known what this is due to and a little later remarks: "Nicht unmöglich ist es, dass oft reichlich Gips vorhanden ist." That this remark does not apply in the present case is however now shown, by the fact that an aqueous extract of carrageen gives the reactions of the calcium ion but not of the sulphate ion; after hydrolysis, however, the addition of barium chloride produces a plentiful precipitate of barium sulphate showing that the calcium sulphate obtained by incineration is not present in the plant, as such, but is produced by the decomposition of an ethereal sulphate pre-existing in the undecomposed material. Owing to the bivalency of calcium such a compound must contain two sulphuric acid residues for each atom of calcium, as indicated by formula (1). The correctness of this assumption has been established by showing that the amount of sulphate in the solution after hydrolysis is double that found in the ash, since a compound of the type of formula (1) must on incineration lose half its sulphur while the remaining half is converted into calcium sulphate:



That this sulphur is really given off in the form of sulphuric acid on heating has been experimentally established and this decomposition accounts for the surprising readiness with which the extract of the weed tends to char when heated to constant weight in a steam oven.

While the occurrence of ethereal sulphates among products of animal origin is not uncommon, the same cannot be said of plant products and the present instance would appear to be the first recorded case of the existence of carbohydrate esters of sulphuric acid among plant products; apparently the only other known example of the occurrence of ethereal sulphates of vegetable origin is that of certain mustard oil glucosides which liberate potassium hydrogen sulphate on hydrolysis, but in any case the composition of these is very different from that of the substances here described.

Carrageen mucilage forms a very convenient material for demonstrating the ethereal sulphate grouping, since on boiling a little of the neutral solution with barium chloride no precipitate is formed; if a little concentrated hydrochloric acid is now added and the boiling continued a plentiful precipitate of barium sulphate is thrown down in the course of a minute or two.

The establishment of this ethereal sulphate grouping in carrageen necessitates a revision of many of the previously published statements concerning the composition of the water-soluble constituent of this material. Although it is not intended in the present instance to discuss the nature of the organic

complex with which the calcium sulphate residue is combined, it may be mentioned here that both the hot and cold extracts, as at present prepared, contain about 1 % of nitrogen, from which it has not as yet been found possible to free them.

#### EXPERIMENTAL.

At the outset of the present investigation an attempt was made to free the weed from adherent sea salt by a preliminary soaking in cold water; the first washings were distinctly saline but on removing these and allowing the weed to soak in distilled water all night it was found that the water had become quite syrupy and had extracted some material other than salts alone; the thick solution was therefore drawn off and replaced by fresh water and this process was repeated day after day until after about four weeks the amount of material extracted was practically negligible. The successive daily extracts were separately filtered and evaporated to dryness on tin lined copper pans and analysed.

The ash content of the thirtieth fraction was 21.5 % showing that this method could not be looked to for yielding an ash-free substance.

On digesting the residue from the cold water extraction with hot water over a boiling water-bath and evaporating the filtered solution the substance obtained was found to differ in some respects from that obtained from the cold water extraction, notably in the fact that strong hot water solutions gelatinise on cooling whereas the other material only produced thick gummy solutions, in equivalent strengths.

In view of the very tedious nature of the above described procedure and the fact that the hot extract was not entirely insoluble in cold water, the following improved method was adopted.

The handpicked weed was rapidly washed by squeezing it out a few times under a stream of running water and was then soaked for about an hour in distilled water. The aqueous extract was thereupon poured off and filtered, first through calico and then through a Chardin filter paper over a Buchner funnel. It was found advisable to replace the calico frequently owing to the clogging of the pores by the slimy particles of plant débris which were suspended in the liquid. The filtrate was evaporated to dryness over a water-bath; the residue so obtained is referred to for convenience as the Cold Extract or C.E.

The weed which remained after pouring off the water was then squeezed out as completely as possible and placed in a tall cylindrical vessel in which it was washed continuously for three days in running water by allowing the liquid to enter the vessel at the bottom and overflow at the top through muslin. At the end of this time the weed had lost its slimy feeling and was regarded as being practically free from any of the cold water extract; it was thereupon squeezed out, washed in several changes of distilled water and left to soak in the same over night; after one more squeezing out and washing it was spread out in thin layers and allowed to dry in the air. This exhaustively

extracted weed was then warmed with hot water over a water-bath and the resulting solution, after filtration and evaporation, yielded the so-called Hot Extract or H.E.

By collecting only the very first cold extract it was hoped to obtain a product as nearly as possible free from the H.E. since this dissolves but slowly in cold water, and by sacrificing most of the remaining C.E. together with some of the H.E. much time was saved and a fairly complete separation was effected. That the process is wasteful is shown by the fact that only about 37 % of the weight of the dry weed was obtained by this method, consisting of 14 % of C.E. and 23 % of H.E. respectively, whereas about 70 % of the weed is actually soluble in water.

#### *Comparison between the Hot and Cold Extracts.*

In external appearance there is no great difference between the two extracts; both form more or less transparent horny scales, although the H.E. is generally the clearer of the two; both are friable when dried in a steam oven but on exposure to moist air they absorb hygroscopic moisture and become soft and pliable; the amount of moisture taken up varies somewhat with the humidity of the atmosphere but the C.E. usually absorbs from 4 to 5 % more than the H.E.

When placed in cold water both swell up considerably, but the C.E. dissolves completely on stirring to form a gummy solution; the H.E., on the other hand, is only sparingly soluble in cold water and even if left to soak for a long time a large proportion of the material remains in the form of undissolved flakes; on warming, however, it dissolves fairly readily without however giving a perfectly clear solution; at a strength of about 1 %, such solutions, on cooling, show a tendency to gelatinise, although even a 2 % solution only forms a very thin jelly, and it is not until concentrations of from 3 to 5 % are reached, that a really stiff jelly is formed. The melting points of such jellies have been described elsewhere [Haas and Hill, 1921].

Towards salt solutions the behaviour of these two substances is different; thus while both the H.E. and the C.E. are precipitated from their solutions by half saturation with ammonium sulphate the H.E. only is precipitated by half saturation with sodium chloride or magnesium sulphate, the C.E. remaining unaffected thereby.

A further difference between the two is in their behaviour towards Rochelle salt; on adding five drops of a 20 % solution of this salt to 5 cc. of a 0.75 % solution of H.E. (which is too dilute to set to a jelly) the H.E. is thrown out in the form of a clear gelatinous precipitate which remains suspended in the aqueous medium, but on boiling and then setting aside to cool the whole liquid sets to a clear stiff jelly. Solutions of the C.E. are not affected in the same way. Other salts of the lyophilic series affect the H.E. to a less extent but observations on this subject are reserved for a future occasion.

Evidence of a chemical nature is also forthcoming to show that the C.E. and H.E. are different; this is based upon the colour reaction obtained from the two substances on warming them over a water-bath with alcoholic solutions of  $\alpha$ -naphthol and sulphuric acid; the deep violet colour given by the H.E. points to the presence of a ketonic sugar which would appear to be absent in the case of the C.E.

*The ash constituents of the Hot Extract.*

Mention has already been made of the fact that all previous workers had commented on the high ash content of carrageen; the figures given by different authors vary from 14.15 % [Church, 1876], for the air dried material, to 20.6 % quoted by Czapek [1920].

In the present investigation the high ash content of the material was once more confirmed, as the following figures will show:

Ash in the untreated weed ...	...	...	...	...	...	14.6 %
„ „ cold extract ...	...	...	...	...	...	21.8 %
„ „ hot „ ...	...	...	...	...	...	17.6 %
„ „ residue after extraction of all water-soluble material						5.19 %

These figures were in every case obtained by incineration of the oven-dried material. Some difficulty was experienced in these determinations owing to the tendency for the material to char quite suddenly while being heated in the steam oven to constant weight. That this should occur at the temperature of the steam oven was at first difficult to understand but was subsequently explained by showing that the material gives off sulphuric acid when it decomposes (see below, p. 475). This tendency to char adds considerably to the difficulty of accurate determinations of moisture content, more especially as the last traces of moisture are retained somewhat tenaciously.

Notwithstanding that earlier workers had recorded the fact that the ash content could not be reduced by dialysis and that one worker had actually observed an increase in the ash after dialysis [Moeller and Thoms, 1900], recourse was had to this method on several occasions. It was found that samples of the weed or of the C.E. which had a salt taste, lost it after dialysis with a corresponding falling off in the ash content; thus in one case the percentage of ash in a sample of C.E. was reduced from just under 40 to 21.79, but prolonged dialysis would not reduce it below the latter figure; the dialysing water was shown to contain appreciable quantities of chloride and sulphate and the experiment proved that while at least some of the ash constituents can be removed by dialysis, a considerable proportion of these resist such treatment. Similarly several days' dialysis did not reduce the percentage of ash in the H.E. below the average figure of 17.6.

With the object of establishing whether the reluctance to give up salts by dialysis was due to any inherent attraction of the colloidal material for inorganic salts in general, and sulphates in particular, samples of carrageen containing added sodium sulphate, carbonate or chloride were submitted to

dialysis and in every case these salts were completely removed after two or three days.

On the assumption that the reluctance to dialyse might be a peculiarity of the calcium sulphate a sample of H.E. was boiled with a solution of sodium carbonate, with the object of precipitating out the calcium, removing it by filtration and then dialysing away the sodium sulphate produced by double decomposition. On incinerating the dialysed material, however, it was found that the percentage of ash, instead of being reduced to zero, had been slightly raised, namely from 21.9 to 22.7<sup>1</sup>. The calcium had been successfully removed, since the ash now actually consisted of sodium sulphate, but, strange to say, this latter had not dialysed out. The slight increase, of course, is explained by the fact that the one atomic proportion of calcium (40) had been replaced by two of sodium (46).

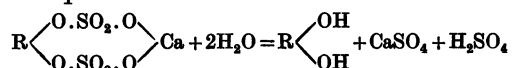
An attempt was then made by reversing the order of procedure of the previous experiment to remove the sulphate radicle first, with barium chloride, and then dialyse away the resulting calcium chloride. On adding the barium chloride, however, no precipitate of barium sulphate was formed even after prolonged boiling, whereas on the addition of a little concentrated hydrochloric acid and boiling again for a few minutes, a heavy precipitate of barium sulphate was thrown down.

From these experiments it was concluded that the H.E. contains ionised calcium but that the sulphate complex is united to an organic residue and therefore only becomes ionised after hydrolysis.

That the calcium is, in fact, ionised was proved by showing that it can be quantitatively precipitated from solution by ammonium oxalate and that the estimations so carried out agree with those made on the ash obtained after incineration. The results obtained were as follows:

1. Ca by direct precipitation ... 5.47 %
2. ,, incineration... .. 5.66 %

With regard to the sulphate residue it became necessary to assume the existence of a complex which would liberate ionised sulphate after hydrolysis, as indicated by the equation:



If this assumption is correct the amount of sulphate obtained by precipitation from the hydrolysed solution should be double that obtained by incineration, since the calcium present would only be sufficient to produce a single molecular proportion of calcium sulphate. That this assumption is borne out by experiment may be seen from the following figures:

	I	II
1. SO <sub>4</sub> by incineration...	12.15	11.8 %
2. ,, hydrolysis ...	24.55	23.81 %

<sup>1</sup> The material used in this case was a mixture of C.E. and H.E., the experiment having been carried out before the improved method of separation had been devised.

The discrepancy between the figures in the two columns is due, no doubt to a difference in moisture content, arising from the difficulty experienced in drying the material without charring (see below). Notwithstanding this the ratio of the two figures in each column is pretty closely 1 : 2, as required by theory.

Although the hydrolysis by boiling hydrochloric acid is sufficiently rapid to enable one to observe it qualitatively in the course of a few minutes, by the addition of barium chloride, it was found that in order to ensure complete hydrolysis, it was necessary to boil for some hours. In the above analyses about 0.5 g. was dissolved in 100 cc. of water and boiled with 5 cc. of concentrated hydrochloric acid for at least five hours.

Attention has already been drawn to the fact that the H.E. tends to char when heated to constant weight in a steam oven. This can be accounted for on the above assumption concerning the form of combination of the sulphate complex; on decomposition, a compound of the type indicated by formula (1) should give off sulphuric acid, whereas calcium sulphate, if present as such in the plant, would not do so. On dry distilling some of the H.E. in a small tube and passing the evolved vapours into water a strongly acid solution was obtained which gave an immediate precipitate with barium chloride, showing that sulphuric acid was actually evolved.

Without for the present expressing any opinion upon the nature of the organic complex present in carrageen, the experiments above described justify the conclusion that the hitherto accepted views concerning the composition of this material require some modification; the water-soluble constituent of Irish moss should in fact not be regarded as a more or less complex carbohydrate contaminated with a certain amount of inorganic salts, but as a mixture of salts of sulphuric acid united to a carbohydrate yielding complex, in other words as a mixture of ethereal sulphates.

#### SUMMARY.

1. The colloidal material extracted from *Chondrus crispus* by hot water consists of two substances.
2. These two substances may be separated from each other by taking advantage of their different solubilities in cold water.
3. The constituent which is more soluble in cold water produces thick viscous solutions; the other dissolves more readily in hot water and produces solutions which tend to gelatinise on cooling.
4. The Hot Extract is the calcium salt of an ethereal sulphate in which the calcium is freely ionised and can be quantitatively precipitated by the ordinary reagents for calcium, while the sulphate complex is not ionised until after hydrolysis of the compound. This accounts for the observation made by earlier workers that the high ash content of *Chondrus crispus* could not be reduced by dialysis.

It is hoped to continue the investigation of the constitution of both the hot and cold extracts and, if possible, also to extend the observations to other sea weeds.

In conclusion the author wishes to acknowledge his indebtedness to his friend and colleague Mr T. G. Hill for valuable criticism and advice during the course of this investigation and also to express his thanks to Miss Barbara Russell Wells for assistance with some of the analyses.

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