CVI. SOME PROPERTIES OF THE POLYSACCHARIDE COMPLEX EXTRACTED FROM A MARINE ALGA, *CHONDRUS CRISPUS*.

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THE chemical nature of the water-soluble polysaccharide constituent of *Chondrus crispus* has previously received considerable attention. The interest manifested in it is attributable to its possession of marked gelatinising power. On account of this property it lends itself to a variety of uses. Unfortunately, the results to be found in the literature are based on variable methods of extraction, many of which are not very rational. The primary aim of the present work was, therefore, the preparation of a pure standard extract, so that the data obtained might be significant and of general application. The establishment of a definite criterion of purity is no simple matter, as the material is non-crystallisable and decomposes at very low temperatures. However, despite this, a reasonably pure standard extract has been obtained and from it have been prepared salts which give evidence of an even higher degree of purity.

HISTORICAL.

Investigation of this particular species, *Chondrus crispus*, and the material extracted from it in aqueous solution, dates from the work of Flückiger and Obermayer [1868]. In Abderhalden's *Biochemisches Handlexikon* [1911] there appeared the statement that "the pure mucilage of carrageen¹ corresponds to the formula $C_6H_{10}O_5$." The evidence on which this formula is based was not given. Though it is conceivable that it might represent the carbohydrate moiety, this formula is quite inadequate for the complete substance, since it takes no account of the 20 % ash content. There is also present some nitrogen which, until proven to be an impurity, cannot be disregarded. Apart from the fact that it is largely carbohydrate, very little is definitely known of the nature of the material in *Chondrus crispus* which is responsible for gelation. Haas [1921] advanced the theory that it is a colloidal electrolyte—an ethereal sulphate of calcium, the general structure of which might be represented by formula I.

Harwood [1923], making use of physico-chemical methods, finds the evidence contradictory and points out that without further experimental work it is impossible to choose between the alternative formulae, I and II. He appears, however, inclined to agree with Haas that I is more acceptable.

¹ The aqueous extract of *Chondrus* is commonly referred to in this way; the plant itself is also frequently called carrageen or Irish Moss.

On the other hand Haas's assumption of this disulphate ester has been criticised by Nelson and Cretcher [1931], on the grounds that the analytical data are insufficient to indicate this composition of the esters and to preclude the presence of polymericides of monosulphate esters.

Even should one of these ultimately be found correct, for the present one serves as well as the other as a working hypothesis. It is not impossible that both types may be present in the extract.

According to Haas, the calcium is ionised, but the sulphate ion is masked by its combination with the carbohydrate part of the molecule. On heating, this type of compound breaks down thus:

Later Haas altered his procedure and instead of using the dried extract with which he had previously worked [Haas and Hill, 1921] he prepared two fractions. One was a cold water extract (C.E.) and the other a hot water extract (H.E.) of the plants from which, presumably, all the C.E. had been removed.

The occurrence of a similar calcium sulphate-carbohydrate compound in agar-agar was reported in the same year [Neuberg and Ohle, 1921]. Haas and Russell-Wells [1923] made a fairly extensive study of a number of seaweeds. They found that in nearly all species having high ash contents the presence of an ethereal sulphate in the plants was indicated.

The evidence on the strength of which the ethereal sulphate formula was assigned is as follows:

- (1) The calcium is freely ionised and can be quantitatively precipitated by ammonium oxalate even before hydrolysis.
- (2) The sulphate content of the ash is high.
- (3) The sulphate cannot be precipitated from the solution until after hydrolysis.
- (4) The amount of the sulphate in the ash is only half of that in the hydrolysed solution. This loss of half the sulphate on ashing is attributed to its escape as sulphuric acid, according to equation (i).

Experiments with algae other than *Chondrus crispus* showed variations in the 2:1 ratio of "sulphate by hydrolysis" to "sulphate in ash." Nevertheless, Haas believes it permissible to assume, when the amount in the hydrolysed solution is greatly in excess of that in the ash, that the sulphate occurred in the plant in an ethereal linkage of the type described. The discrepancies from the 2:1 ratio are explained, in the case of higher ash sulphate, as due to the retention by an alkaline ash of some of the sulphuric acid which should normally have escaped. In cases where the sulphate in the ash is less than the theoretical, the loss is attributed to the presence of an ammonium salt, which would leave no ash.

EXPERIMENTAL.

Preparation and analysis of samples.

Preparation. From a number of methods of extraction and precipitation investigated, the following was finally adopted as being the most satisfactory method of obtaining a standard preparation.

20 g. of *Chondrus crispus* plants are washed with cold distilled water until free from chlorides. (Three or four washings are usually sufficient.) The washed *Chondrus* is then transferred to a flask with a litre of distilled water, and heated on a boiling water-bath for 5 or 6 hours. The resultant viscous mixture is centrifuged and the clear liquid filtered through a Chardin filter-paper on a Büchner funnel encased with a hot water jacket. It is necessary to change the filter-paper

frequently. The filtrate, kept in constant motion by stirring, is evaporated on a water-bath to approximately 300 ml. It is then poured, slowly and with stirring, into one litre of 95 % alcohol. A stringy, fibrous material is precipitated, which is removed as soon as formed, allowed to drain through cheese-cloth and finally transferred to 250 ml. of 95 % alcohol, in which it remains until the next day. It is then transferred daily from one to the next of the following washes: (1) and (2) 200 ml. absolute alcohol; (3) and (4) 150 ml. anhydrous ether. The precipitate is then dried in a vacuum-desiccator for a day and finally ground to a fine powder. It is dried in air for at least 6 hours at approximately 80° before being used. If the temperature is allowed to rise above this for any length of time charring occurs. The product is a creamy-white, non-crystalline powder, soluble in both cold and hot water and forming in 2 % solution a firm gel.

Ash. The ash content of the extract does not represent simply salt content but combined inorganic material. It does not, as will be seen later, represent all of this, but it may be expected to represent approximately the same percentage of the combined inorganic material in all cases, since the percentage lost depends on the original structure. Ash determinations of a number of extracts prepared from different collections are given in Table II.

It will be observed that the differences in ash content are slight, in spite of the fact that these extracts are made from plants collected at different places and in different seasons (Table I).

Table I. *Identification of samples.*

Sample	Place of collection	Month of collection
S	South Shore, P.E.I.	August
N	Souris, P.E.I.	"
H	Halifax, N.S. (Pt. Pleasant)	February
T	" (McNab's Island)	August
D	" (Devil's Island)	June
P	Peggy's Cove, N.S.	August
C	Cavendish, P.E.I.	"
F	Details of collection missing. Extract made without any washes of absolute alcohol	

The standard extract used in the present investigations was prepared from sample C.

Table II. *Percentage of ash in different extracts.*

Sample	% ash (dry weight)	Sample	% ash (dry weight)
F	17.30	S	19.11
D	19.32	N	18.43
T	19.46	H	16.36
P	19.74	C	19.44

As previously noted, many investigators regard the ash content of marine algae as practically pure calcium sulphate. The fact that the SO_4 content of the ash (see Table VI) averages only 58 % negatives this assumption. (SO_4 calculated for calcium sulphate is 70.34 %.)

The ash was found to contain varying quantities of potassium up to 12 % (Table VI). Russell-Wells [1922] reports that the ash of her extract contained sulphate, calcium, magnesium and small quantities of sodium and potassium, with traces of iron. The extract, therefore, probably contains a calcium ethereal sulphate, with which there are associated analogous salts of other metals. There is found also some phosphate which was not originally precipitable as the phosphate ion, and which may be assumed to have been present in an organic combination.

Sulphate. During the present investigation, sulphate (SO_4) was determined in three different preparations: (1) in hydrolysed samples; (2) in oxidised

samples; (3) in the ash. In each case the sulphate was precipitated by means of barium chloride, ignited and weighed.

Hydrolysis. In order to ascertain when hydrolysis was complete, a number of methods have been tried. The results (Table III) show that the sulphate is much more resistant to hydrolysis by alkali than by acid. It is evident also that all the sulphate is freed by boiling directly, for 5 hours, in 5 % hydrochloric acid, whereas even after 13 hours on the water-bath nearly 2 % still remains combined. This latter fact might be taken to indicate that the sulphur occurs in more than one type of combination.

Table III. *Sulphate by hydrolysis of extract.*

Sample C:	Method of hydrolysis	Time (hrs.)	% sulphate (SO ₄)
	Boiled directly with 5 % hydrochloric acid	5	27.66
	" "	6	27.53
	" "	13	27.44
	On water-bath with 5 % hydrochloric acid	6	21.62
	" "	13	25.62
	Boiled directly with 5 % sodium hydroxide	6	13.32
Sample H:			
	Boiled directly with 5 % hydrochloric acid	6	25.18
	Boiled on sand-bath with 40 % hydrochloric acid*	6	25.38

* [Haas and Russell-Wells, 1923.]

Oxidation. The oxidation was accomplished by fusion with a hydroxide-nitrate (8:1) mixture. The melt was dissolved and acidified with hydrochloric acid, after which the sulphate was precipitated with barium chloride (Table IV).

Table IV. *Sulphur in extract after oxidation.*

Sample*	% sulphate (SO ₄)	Sample*	% sulphate (SO ₄)
I	29.04	W	26.70
	28.08		27.72
II	27.60	A	28.20
	29.07		29.28
III (a)	28.56	3X	30.24
	28.95		30.09
III (b)	28.02	5X	28.53
	27.96		
H	28.95		
	29.31		

* Differing symbols here indicate slight variations in the standard method of extraction, made with another purpose in view, but all are from the same collection of plants, namely sample C.

The sulphate after oxidation was found to average 28.38 %. The difference between this figure and that obtained by the hydrolysis, being very little outside the range of experimental error, seemed insufficient to warrant the assumption of any appreciable amount of unoxidised sulphur.

Ash. From the ash of the standard sample C, the sulphate was found to be 11.54 %, which figure does not satisfy equation (i), since in the present case only 35 % of the sulphate has been retained, rather than 50 %. Russell-Wells [1922] reports more than 50 % of the sulphate to be lost on ashing and attributes the excess loss to the presence in the original of some ammonium ethereal

sulphate, the sulphur of which would of course be completely lost on ashing. In the present case, however, the excess loss is too great to be explained in this manner, because the amount of nitrogen present would be sufficient to account for only 1 % of sulphate at the most (unpublished data).

As noted above, the ratio of "sulphate in hydrolysate" to "sulphate in ash" was found to be more nearly 3:1 than the 2:1 theoretically required by Haas's formula. This suggests the possibility that there might occur in the molecule three sulphate groups, only one of which is retained in the ash. An acid salt of the structure shown in the following equation would explain the observed results:

The sulphate retained in the ash of a compound such as III is one-third of that in the original.

Nelson and Cretcher [1931] found, in their analysis of the carbohydrate acid sulphate of *Macrocystis pyrifera*, that the percentage of ash obtained without oxidation was somewhat lower than when the sample was ashed in the presence of sulphuric acid. This they believe to be due, not to the retention of unoxidised sulphur in the latter case, but to the reduction of some sulphate in the former.

In order to determine whether or not the sulphate being lost here, in excess of that normally escaping as sulphuric acid, was due to this cause, approximately 0.2 g. of the sample was ashed in an electric muffle furnace, after having been moistened with nitric acid. A sample was ashed in the same manner with sodium carbonate (Table V). These figures show plainly that the sulphate loss

Table V. *Percentage of ash and sulphate in extract of sample C after different methods of ashing.*

Method	% ash (dry weight)	% SO ₄ calc. to original extract
Simple incineration	19.44	11.54
Ashed with nitric acid	19.33	11.67
Ashed with sodium carbonate	—	28.49

experienced on ashing cannot be attributed to reduction of the sulphate. That the sulphate obtained after hydrolysis is the true sulphate value is shown by the agreement between it and that obtained after ashing with sodium carbonate, as well as that from the fusion with hydroxide-nitrate mixture (see Table III).

Preparation of salts.

Potassium salts. A 1 % solution of the extract was dialysed in collodion membranes¹ against frequently replaced potassium chloride until the dialysate gave a negative test for calcium (5 % potassium chloride was used at first, then 2.5 %). The dialysed material was then tested to make sure that no calcium had been retained. Dialysis was then continued against distilled water, until the excess of potassium chloride was removed. It was noticed that shortly after dialysis against the potassium chloride began, the extract became opaque and soon set to a gel. During continued dialysis against distilled water the material gradually liquefied.

¹ These membranes were made with the following collodion solution: 5 g. pyroxylin, 25 ml. absolute alcohol, 65 ml. anhydrous ether, 10 ml. ethylene glycol. Membranes were allowed to dry 25 to 30 minutes before being filled with water [Pierce, 1927].

The material, now free from calcium, is concentrated on the water-bath, precipitated and dehydrated according to the standard method. The product, a fine, pure white powder, is the potassium salt or salts resulting from replacement of the calcium and other cations of the water-soluble salts which are present *in situ* in the plant.

This material was found to have an actual ash content of 25.56 %. In appearance, the ash obtained from the original extract is frequently a dirty fawn colour and fused, while that of the potassium salts is snowy white and powdery. This ash contained K, 45.18; SO_4 , 55.14 %. K_2SO_4 requires K, 44.89; SO_4 , 55.11 %.

Now the plant *Chondrus* itself contains¹ 4.58 %, and the standard extract 2.22 % of potassium. On the other hand, the material obtained by dialysis against the potassium chloride solution contains only 0.069 % of calcium. Further evidence of the purity of this product is found in the absence from it of any trace of phosphorus. Positive tests for phosphorus were given by all other extracts, except that in the preparation of which the plants had been washed for 3 days in running water before the extract was made. When the period of dialysis was prolonged the resultant preparations showed that most of the cations of the extract were replaced by potassium during the first few hours, and that subsequent dialysis affected but slightly the total amount of potassium introduced.

Ammonium salts. Ammonium salts were prepared in a similar manner to the potassium salts, except that the final solution of the ammonium salts was not precipitated but was evaporated to dryness. Complete dialysis in this case required from 2 to 3 weeks, sometimes more. This ammonium salt gives less than 1 % of ash, whereas that which Russell-Wells [1922] prepared by another method yielded 5 % ash. The original sulphate in this material was found by hydrolysis to be 28.49 %.

Calcium salts. The potassium salt prepared as described above is obviously a more nearly pure material than the original extract. It was determined therefore to regenerate the calcium salt from it by dialysing the potassium derivative against calcium chloride (a 2.5 % solution was used) until no potassium could be detected in the dialysate. This point was reached in about 48 hours, after which the excess of calcium chloride was removed by dialysis against distilled water. The solution of the "regenerated" calcium salt was then evaporated, precipitated and dehydrated as in the standard preparation.

The material obtained differed in appearance from both the original and the potassium salt and decomposed much more readily even than the original.

The ash values of the original and the "regenerated" calcium salt did not differ greatly. The sulphate and calcium in the ash, however, were found to be very different. Figures are given in Table VI, which includes the potassium salt, for the sake of comparison, as well as the potassium figures for all three.

Table VI clearly shows to be erroneous the assumption that the original extract is a calcium salt. For one thing its ash contains more potassium than calcium. The difference between the calcium contents of the original extract and of the "regenerated" calcium salt is very great. The simultaneous decrease in potassium is not sufficient to account for this, nor is the increase which would occur by substitution of calcium in place of the hydrogen in formula III sufficient to account for the difference. The presence of still other cations is thus

¹ This figure is for a different collection of plants from that on which the results presented here were obtained.

Table VI. *Comparison of ash and sulphate in prepared salts with standard extract.*

	% ash	% SO ₄ in ash	% SO ₄ in original calc. from ash	% SO ₄ in original by hydro- lysis	% calcium in ash	% calcium in original salt calc. from ash	% potas- sium in ash	% potas- sium in original salt calc. from ash
Original	18.67	58.44	10.91	26.82	2.88	0.52	11.99	2.22
extract	19.14	58.74	11.54	27.51	3.68	0.68	—	—
Regenerated	20.96	68.72	14.40	29.06	32.22	6.75*	0.76	0.16
calcium salt								
Potassium	25.73	54.47	14.05	27.32	0.28	0.07	45.42	11.70
salt	25.26	55.14	13.94	28.00	—	—	—	—
Ammonium	0.60	—	—	28.49	—	—	—	—

* Calcium by hydrolysis in this sample was 6.56 %.

indicated. Further, the percentages of calcium and sulphate in the ash of the "regenerated" calcium salt approximate to those of pure calcium sulphate (Ca, 29.66; SO₄, 70.34 %), while they differ markedly from those of the original extract.

It will be noted from Table VI that in the potassium salt the theoretical 50 % of sulphate is lost on ashing. If the cations of a substance such as that represented in formula III were replaced by dialysis against potassium chloride, a neutral potassium salt (IV) would be obtained. This, on ashing, would undergo the following changes:

It will be seen that here one-half the sulphate is converted into sulphuric acid and lost, as was actually found to be the case with the prepared potassium salt. This fact, together with the already noted absence of calcium and phosphate and the purity of the ash, leads to the conclusion that this preparation is a true ethereal sulphate. In the "regenerated" calcium salt also a 2:1 ratio of "sulphate from hydrolysis" to "sulphate from ash" is established. The original extract, being a mixture of sulphates, the ratio of "hydrolysis sulphate" to "ash sulphate" in it has not the same significance.

Further evidence that the extract of *Chondrus crispus* cannot rightly be regarded as a pure chemical compound was obtained from an examination of

Table VII. *Comparison of fractions with standard extract.*

Tests	C.E.	H.E.	Standard
50 % alcohol	No ppt.	Good ppt.	Good ppt.
Molisch	+	+	+
Biuret	—	—	—
Reduction	—	—	—
Reduction after hydrolysis	+	+	+
Potassium	+	— ?	+
Phosphate	+	—	+
Chloride	None visible	— ?	— ?
Calcium	?	++ +	++ +
Sulphate	— ?	—	—
Sulphate after hydrolysis	+	+	+
Ash	27.43 %	17.24 %	19.47 %
	26.43	17.35	19.42
	27.49	16.80	.
Nitrogen	0.32	0.16	0.24
	0.30	0.15	0.24

the fractions described by Haas [1921]. Extracts were made according to his method of separation and compared with one another and with the standard extract, as previously described. Instead of being simply evaporated to dryness, however, the fractions were precipitated and dehydrated in the same manner as the standard preparation.

Solutions of the two fractions differ greatly in appearance; the H.E. is almost water clear, while the C.E. has a yellowish colour and is very opaque, making it difficult to distinguish precipitates in it. In appearance, as in other properties, the standard is intermediate between the C.E. and H.E. which might be expected, as it is in effect a mixture of the two, containing little C.E. and much H.E. The marked difference in ash content of C.E. and H.E. might be attributed to salts adherent to the plant and taken into solution along with the polysaccharide complex. To obviate this possibility the method of fractionation was modified in the following way. A suspension of the finely ground plants was made, introduced into collodion membranes and dialysed against frequent changes of distilled water. The first dialysates were tested and found to contain chloride, sulphate, calcium and potassium ions. The absence of the chloride ion—because it is the most abundant in sea-water and is detectable longer in the dialysate—was taken to signify that all the salts had been removed. The dialysed suspension was filtered through Chardin filter-paper on a Büchner funnel. The filtrate, which from this point was treated according to the standard method of preparation, yielded the “dialysed cold extract” (D.C.E.) which was free from adherent salts. The residue was then extracted with water on a boiling water-bath for 5 hours. From the filtrate of this extract was obtained, by precipitation and dehydration as before, the “dialysed hot extract” (D.H.E.). It was now possible to make a comparison of the ash material actually combined in the two fractions with that combined in the standard extract.

Table VIII. *Comparison of ash and sulphate content of fractions with standard extract.*

Sample	% ash (dry weight)	% SO ₄ in ash	% SO ₄ in original salt calculated from ash	% SO ₄ in original salt by hydrolysis
Standard	19.42	58.74	11.54	27.51
D.C.E.	21.47	59.36	12.75	28.79
D.H.E.	17.63	60.90	10.74	27.83
H.E.	17.13	63.34	10.85	27.03
C.E.	27.12	—	—	—

Assuming that the difference between the ash of the C.E. and D.C.E. is due to free salts, there still remains a 4 % difference between the ash contents of the dialysed fractions (D.C.E. and D.H.E.). The ash content of the standard is intermediate between the fractions, as a mixture of the two would be. The ash of the D.C.E. contains more phosphorus than that of the D.H.E., which, of course, would help to increase the percentage of ash in the former.

A consideration of the sulphate content of the fractions may mean little or nothing, as the differences recorded are very small.

The original sulphate obtained both by hydrolysis and by calculation from the ash sulphate is higher in the D.C.E. The percentage of sulphate in the ash of the D.C.E. might be depressed somewhat by the presence there of phosphate. The difference in the “hydrolysis sulphate” between the fractions becomes more pronounced when the D.C.E. is contrasted with the H.E. instead of the D.H.E., because the H.E. contains none of the C.E., while the D.H.E. contains some

of it. In respect to sulphate content, therefore, as well as ash, the standard preparation would appear to fill an intermediate position.

It should be noticed, from Table VIII, that the proportion of "hydrolysis SO_4 " to "ash SO_4 " coincides no better with the 2:1 ratio in the case of the fractions, than it does in the standard as here prepared.

Comparison with agar-agar.

Carrageen is frequently compared with agar-agar. Both are products of marine algae, and possess a common physical characteristic of importance, namely the power to gel in aqueous solution. As the method of preparation of most commercial agars does not eliminate nearly all the impurities, a solution of agar was subjected to the precipitation and dehydration procedure described, before being compared with the standard extract of *Chondrus*. Table IX affords a comparison of the purified and unpurified agar with the standard extract of *Chondrus*.

Table IX. *Comparison of agar with standard extract of Chondrus.*

Tests	Unpurified agar	Purified agar	Standard <i>Chondrus</i> extract
Appearance of aqueous solution	Yellowish opaque	Bluish-white opaque	Colourless clear
Molisch	+	+	+
Reduction	?	?	-
Reduction after hydrolysis	+	+	+
Biuret	-	-	-
Calcium	+	+	++
Potassium	-	-	+
Chloride	+	+	-
Phosphate	-	-	+
Sulphate	+	?	-
Sulphate after hydrolysis	+	-	+
Ash	4.44 %	2.17 %	19.11 %
Nitrogen	2.51	0.07	0.24

Though it appears from this table that there is no sulphate in the purified agar, this is contrary to conclusions of the majority of investigators [Samec and Ssajevic, 1921; Fairbrother and Mastin, 1923], and as it is the result of a single observation no great importance is attached to it. Even the unpurified agar gives a sulphate test only after hydrolysis of a concentrated solution, or in the ash. However, the basic structure of agar cannot include an ethereal sulphate group, as it does in the case of the *Chondrus* extract. Even supposing the whole ash were composed of sulphates, it would be sufficient to account for the existence of only a small fraction of the original material as an ethereal sulphate similar to that present in the *Chondrus* extract, unless indeed this were an ethereal ammonium sulphate. That it could have been such is precluded by the small amount of nitrogen present in agar. We seem, therefore, to be led to the conclusion that though agar contains a small quantity of ethereal sulphates, they are not such essential constituents as in *Chondrus*. If, on the other hand, it is assumed that the structure of agar is based on the ethereal sulphate linkage, it follows from the difference in ash content that the carbohydrate group is ten times as complex as that in *Chondrus* extract.

DISCUSSION.

The data here presented afford evidence that the material extracted by water from *Chondrus crispus* is not a single substance, but a mixture of several, neither the whole nature nor the number of which is known.

The fractionation suggested by Haas [1921] appears artificial, as neither of the fractions is any nearer being a pure chemical compound than is the standard extract. Russell-Wells [1922] says, "the similarity of oxidation products of both (fractions), points to both consisting of substances belonging to the same group of compounds, but there is not evidence enough to show whether the differences are due to different bodies, or mixtures in different proportions of the same substances. On the whole the former of these alternatives appears the most likely." From the findings here presented, however, it seems more rational to regard the fractions as mixtures of the same or very closely allied substances, in different proportions and to suppose that in addition to the common components, each fraction may contain some substance or substances which do not occur in the other. Thus the C.E. contains inorganic and simple organic materials, *e.g.* phosphates, from which the H.E. is free. It is quite probable that some of the more complex constituents of the H.E. are entirely lacking from the C.E.

The evidence for the purity of salts prepared from the standard extract, on the other hand, is clear cut. In the case of the potassium salt, for instance, there is: (1) an agreement between the percentages of potassium and sulphate in the ash with those of pure potassium sulphate; and (2) an agreement of the sulphate content of the salt and its ash with the theoretical 2:1 ratio (Table VI). Furthermore (3) the removal of the phosphate ion which was present in the standard extract suggests that other impurities also have been removed.

If the potassium preparation is not a single salt, it can only be a mixture of ethereal sulphates of potassium, which are very similar in structure and differ only in complexity. The variations, being such as not to interfere with the agreements noted, must be either in the nature or the complexity of the carbohydrate radical with which the potassium sulphate is united, or in the number of potassium sulphate groups united with the (same) carbohydrate radical.

It has been determined that the sulphate forms 28 % of the whole potassium salt. On the basis of formula V, $R(OSO_2OK)_2$, the molecular weight would be roughly 685, or that of the carbohydrate radical 415. Based on formula IV, $R(OSO_2OK)_3$, the molecular weight would be approximately 1030, or that of the carbohydrate fraction 625. The properties of the material, however, are such as to suggest that the molecular weight is even greater than this. Therefore, one of the following formulae probably represents its constitution more correctly:

Since the total ash of this preparation can be accounted for it should prove a very satisfactory material for use in investigating the carbohydrate fraction of the complex.

SUMMARY.

A method which yields a standard and relatively pure substance is described for the preparation of the gelatinising constituent of *Chondrus crispus*. This material is largely carbohydrate in nature but has an ash content equivalent to approximately 20 % of its dry weight.

The extract previously described as an ethereal sulphate of calcium is now shown to be a mixture of sulphates, together with other substances, for example, phosphates.

The potassium salts of the ethereal sulphates which occur *in situ* have been prepared, and the preparation found to be superior in purity to the original extract.

The potassium of this preparation was then completely replaced by calcium and the differences between these "regenerated" calcium salts and the original extract determined. The results invalidate the assumption that the original is solely, or even chiefly, an ethereal sulphate of calcium.

The ammonium salts also were prepared and are compared with those of potassium and calcium and with the original extract.

It was found that more sulphate was lost on ashing the standard extract than corresponded to the theoretical loss, on the basis of the formula proposed by Haas [1921]. This leads to the suggestion of a modified formula—that of an acid salt—which more closely fits the facts. Such an acid salt would, by the method used in the preparation of the potassium, calcium and ammonium salts, be changed into a neutral salt. The sulphate loss on the ashing of these prepared salts corresponds with the theoretical.

The *Chondrus* extract is compared with a purified sample of agar-agar, to which it is similar in physical properties and origin.

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