L. ON CARRAGEEN (*CHRONDRUS CRISPUS*).

IV. THE HYDROLYSIS OF CARRAGEEN MUCILAGE.

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THE chemical composition of carrageen has been the subject of investigation from time to time by various authors [*e.g.* Flückiger and Obermayer, 1868; Bente, 1875, 1876]. More detailed investigations were carried out by Haedicke, Bauer and Tollens [1887], who established the presence of fructose and estimated the galactose content at 28 %. Sebor [1900] confirmed the presence of galactose and fructose, found small quantities of pentosan and claimed to have found evidence of glucose, a question which will be referred to again later in the present paper.

All the above authors assumed the mucilage which is obtained by extracting the weed with water to consist of a single polysaccharide. Later, however, it was shown by Haas and Hill [1921] that the extract is in fact a mixture of two substances both of which are carbohydrate esters of sulphuric acid of the following type:

in which R stands for the carbohydrate radicle. These two ethereal sulphates, which were described as hot and cold extracts (H.E. and C.E. respectively), differed primarily in their solubilities and in the fact that the H.E. in 3 % solution forms a gel whereas the C.E. forms instead a viscous liquid. The present investigation is the outcome of an attempt to isolate the carbohydrate complex free from sulphate, but, as the result of a number of trials with various strengths of acid, it was found that the conditions which favour the separation from either H.E. or C.E. of the sulphate residue involve the complete breakdown of the carbohydrate complex.

It has been known that carrageen mucilage cannot be heated with dilute acids such as lemon juice or vinegar without sacrificing its gelatinising power. This observation seemed of some interest, and experiments were accordingly undertaken with the object of determining the strength of acid which would just destroy the gelatinising power of H.E. It was found that when a 2 % solution of H.E. was heated at 80° for 45 minutes with 0.15 N sulphuric acid the resulting liquid had acquired reducing properties, but, contrary to expectation, this was not due to the formation of free sugar but to the production of a non-dialysable product which proved to be still an ethereal sulphate.

In an actual experiment 250 cc. of 2 % H.E. were mixed with 250 cc. of 0.15 *N* sulphuric acid and heated on a water-bath for 45 minutes at 80°. The contents of the flask were then cooled and neutralised with calcium carbonate and, after filtration, evaporated to small bulk *in vacuo* and dialysed against distilled water for 2 days, then against running tap water for another 2 days, and finally once more against distilled water for a further 24 hours: the contents of the dialyser were then evaporated to dryness. The resulting product was designated the "residue."

The outer water from the first two days' dialysis was collected, evaporated to small bulk, filtered, heated to boiling, treated with a hot solution of baryta and left to stand overnight. After filtering, the excess baryta was precipitated with carbon dioxide, and the liquid heated to boiling, filtered and evaporated to dryness over a water-bath; this product was designated the "dialysate." As the residue was found to be slightly dialysable, the dialysate was further purified by being once again put into the dialyser, in this case only the first 12 hours' dialysate being collected and evaporated to dryness.

Examination of residue and dialysate.

Residue. This, consisting of light yellow horny scales and having an ash content of 19.75 %, was found to reduce Fehling's solution and to be optically active, specific rotation + 38.3°. It gave a positive Selivanoff reaction indicating the presence of fructose; the presence of pentose was also established by Bial's reagent and the production of furfural on heating with 12 % hydrochloric acid. On oxidation with nitric acid, crystals of mucic acid were obtained proving the presence of galactose. The solution gave no precipitate with barium chloride until after hydrolysis with hydrochloric acid, when a precipitate of barium sulphate was obtained which corresponded to a sulphate content of 26.72 %. An estimation of the sulphate contained in the ash obtained by incinerating a sample of the same material gave a value of 13.66 % calculated on the dry weight of the material. From these two figures the presence of an ethereal sulphate may be regarded as established [cf. Haas, 1921].

Dialysate. This was obtained as a brown, gummy material which it was not found possible to purify. It gave no precipitate with barium chloride until after hydrolysis with hydrochloric acid, from which it was inferred that it was also an ethereal sulphate. The substance gave the colour reactions for pentose and for fructose, but on oxidation with nitric acid yielded no mucic acid, showing the absence of galactose. It did not reduce Fehling's solution until after hydrolysis and was optically inactive.

The fact that the reducing properties were confined to the residue, which was unable to pass through a parchment dialyser, while the dialysable product had not reducing properties, showed that no free sugar had been produced as the result of the mild conditions of hydrolysis described above, since such sugars, if formed, would have passed out of the dialyser into the outer water.

From the description of the dialysate and residue given above it will be seen that they differ from each other and from the original H.E. The following table summarises the characters of the three substances:

	Dialysate	Residue	H.E.
Pentose (Bial's reagent)	+	+	+
Galactose (by mucic acid test)	-	+	+
Fructose (by Selivanoff)	+	+	+
Reduction of Fehling's solution	-	+	-
Optical activity	Inactive	Dextrorotatory	?*

* The optical activity of this material could not be satisfactorily determined owing to the fact that a sufficiently strong solution for the purpose is too opaque.

THE OCCURRENCE OF GLUCOSE IN CARRAGEEN MUCILAGE.

The presence of glucose in carrageen was originally suggested by Sebor [1900] on somewhat slender evidence; subsequently Muther and Tollens [1904] claimed to have established the presence of glucose on the basis of an analysis of the silver salt of a presumed saccharic acid. Since the silver content of saccharic acid is the same as that of mucic acid, a determination of the silver content furnishes no proof of the presence of glucose. It will be seen from the table given above that both dialysate and residue give the Selivanoff reaction for fructose before hydrolysis. When hydrolysed by heating on a water-bath for 3 hours with 5 % hydrochloric acid, however, the resulting solutions no longer gave the Selivanoff reaction, but on treatment with phenylhydrazine the residue alone gave a precipitate of glucosazone. Since the negative Selivanoff reaction showed the absence of fructose, the osazone could only have been formed from glucose.

In order to obtain further evidence in support of the presence of glucose, an attempt was made to prepare the acid potassium saccharate by oxidation with nitric acid; here a difficulty was encountered from the fact that, owing to the high calcium content of the material, the products of oxidation, namely mucic, oxalic and possibly saccharic acids, were obtained in the form of their sparingly soluble calcium salts. This circumstance makes it probable that saccharic acid, if present in small quantity, would escape detection, and in fact preliminary experiments failed to reveal any of this acid. To overcome this, recourse was had to the oxidation of a sample of the potassium salt of the H.E. For this purpose the mucilage was boiled on a water-bath with potassium carbonate, and subsequently filtered from the calcium carbonate formed. 2 g. of the resulting potassium salt were then oxidised with nitric acid, filtered from the crystals of mucic acid produced, and treated with anhydrous potassium carbonate and acetic acid in the usual manner. After the solution had stood for some days, perfectly typical crystals of acid potassium saccharate were obtained. This experiment, taken in conjunction with that in which glucosazone was obtained from the residue after the destruction of the fructose, establishes conclusively the presence of glucose in the mucilage of carrageen.

ALKALINE HYDROLYSIS.

A further attempt was made to isolate the carbohydrate residue by alkaline hydrolysis of C.E.¹ For this purpose various concentrations of aqueous sodium, potassium and barium hydroxides were successively tried both at the temperature of the water-bath and at higher temperatures in an autoclave; but, as in the case of acid hydrolysis, it was found that any attempt to split off the sulphate complex was accompanied by a breakdown of the carbohydrate with the formation in this instance of lactic acid and other products. Moreover, an examination of the products of alkaline hydrolysis revealed the fact that the quantitative liberation of the sulphate is a very slow process, as is shown by the following experiment. Portions of C.E. weighing 0.7 g. were heated with 40 cc. 3 % sodium hydroxide (free from sulphate) in an autoclave at 110° for varying lengths of time and the amount of free sulphate produced was estimated. The results obtained are presented below in tabular form. From the figures a curve was constructed which by extrapolation indicated that complete hydrolysis should have been effected in 54½ hours. Actual experiment showed, however, that out of a possible 30 % of sulphate only 20.5 % could be so obtained. This suggests that a portion of the sulphate is united more firmly than the rest, since it resists hydrolysis by 3 % sodium hydroxide at 110°.

Time heated hours	Sulphate obtained %	Time heated hours	Sulphate obtained %
1	10.42	9½	18.28
2	12.16	11½	18.18
3	14.78	16½	19.86
7	17.00		20.00

This conclusion is in agreement with the observations made upon acid hydrolysis where it was found that, while appreciable quantities of sulphate could be set free by a few minutes' boiling with dilute mineral acid, complete hydrolysis could only be effected by 6 hours' boiling with 4 *N* hydrochloric acid.

ACTION OF ALCOHOLIC POTASSIUM HYDROXIDE ON H.E.

12 g. H.E. were dissolved in 160 cc. water and 320 cc. 3 % alcoholic potassium hydroxide added. The H.E. was precipitated, but in spite of this the whole was heated on a water-bath under a reflux condenser for 15 hours. Examination of the residue showed that three different substances had been produced, a portion soluble in cold water, another soluble in hot water only and a third which was insoluble in either hot or cold water. That none of these three was the sulphate-free carbohydrate complex was shown by the fact that they all contained a higher percentage of ash than the original material. The hot water-soluble portion was found to have a gelatinising

¹ In this case the C.E. was selected for experiment since it is an ethereal sulphate of the same type as H.E. but takes considerably less time to prepare.

power five times as great as that of the original substance. It was shown to be an ethereal sulphate and is probably the potassium salt of the original H.E.; previous attempts to prepare this salt by double decomposition from the original mucilage, which is a calcium salt, had resulted in a compound which also had a greater gelatinising power than the original but contained, like the original, a certain percentage of nitrogen, whereas the product here obtained was nitrogen-free. It was not further investigated.

SUMMARY.

(1) The loss of gelatinising power resulting from mild hydrolysis of carrageen is not accompanied by production of free sugars although reducing properties are developed.

(2) The solution resulting from mild hydrolysis of carrageen contains two ethereal sulphates, which can be separated by dialysis. The product which is retained by the dialyser has reducing properties; the substance which passes through has no reducing power.

(3) The presence of glucose has been established in carrageen mucilage.

(4) Alkali hydrolysis of a cold water extract is shown to result in complete breakdown of the carbohydrate complex, and it was found that a quantitative separation of the sulphate could not be effected by this means.

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