CCXLIII. THE METABOLISM OF CALCAREOUS ALGAE. I.

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(Received October 10th, 1933.)

The rather specialised conditions of growth to which the calcareous algae are subject as a result of a thick incrustation of calcium carbonate [Haas and Hill, 1933] provided the motive for the present investigation. The results here communicated are concerned only with the isolation of the galactoside floridoside and of a new pentapeptide of aspartic acid.

The material selected for examination was the red alga, Corallina officinalis, collected in the early summer of 1932 at Lyme Regis, and the results here described were all obtained from the one sample of about 9 kg. of wet material.

Preliminary experiments had shown that a concentrated hot water extract of the weed gave a biuret reaction and a positive Molisch reaction. At the outset a known weight of the dried material was dissolved in an excess of standard hydrochloric acid and by back-titration it was found that the amount of carbonate, calculated as CaCO₃ was of the order of 80 %. This figure was sufficient to indicate that considerable quantities of the weed would be required, and accordingly quantities of 1 kg. at a time of dried material were roughly broken up and extracted three times with hot water; the combined extracts were evaporated somewhat and then precipitated with basic lead acetate. The filtrate after removal of the lead with sulphuric acid was treated with sufficient baryta to remove all sulphuric acid and the solution, still acid with acetic acid, was evaporated under reduced pressure before precipitating with mercuric acetate. The bulky white precipitate (A) thus formed was filtered and washed and further treated as described below (p. 1803).

The filtrate from A was found to reduce Fehling’s solution only after hydrolysis; the absence of pentoses and fructose was established by the usual colour tests, but on the other hand oxidation of the crude syrup obtained on evaporation yielded mucic acid, indicating the presence of galactose. The filtrate was accordingly evaporated under reduced pressure to a syrup (B). Prepared by this method the syrup contained considerable quantities of acetates, and it was not to be expected that a sugar would crystallise from this mixture. Extraction of the syrup with alcohol also yielded no crystallisable extract.

A different line of attack was therefore indicated, and this was provided by the work of Colin and Guépin [1930, 1, 2] who, extending the work of Kylin [1915], succeeded in isolating from Rhodymenia palmata a considerable quantity of a crystalline carbohydrate which, as in the case of the syrup B, did not reduce until after hydrolysis and also yielded mucic acid on oxidation. In a later paper these authors were able to show that this substance, which had been described by Kylin as trehalose, was in fact a galactoside of glycerol to which they gave the name of floridoside.
In view of the possibility that Corallina might likewise contain this substance it was decided to work up some fresh material following the procedure adopted by the above authors for isolating floridoside from Rhodymenia. Accordingly 250 g. of air-dried Corallina were extracted three times with hot water; the combined extracts were evaporated to about 200 cc. and treated with four times their volume of absolute alcohol; after some time the supernatant alcohol was poured off from a brown gummy deposit consisting of the peptide to be described later together with salts; the alcoholic solution was then evaporated to 200 cc. and the residue treated with eight times its volume of alcohol; after standing, the solution was filtered and once more evaporated under reduced pressure to a syrupy consistency; this was extracted with alcohol and the extract evaporated to dryness and kept in a vacuum desiccator. Repeated attempts to induce it to yield any crystals of floridoside were unsuccessful.

Having thus failed to isolate the carbohydrate by crystallisation from the syrupy extracts obtained by either of the two methods described, it was decided to try to isolate the substance by conversion into an acetyl derivative. With this end in view 2 g. of syrup were heated over a gauze with 4 g. of acetic anhydride and 2 g. of anhydrous sodium acetate, the mixture being continuously stirred until it just boiled, whereupon heating was at once stopped; the resulting viscous yellow-brown mass was allowed to cool and poured into water and extracted three times with ether. The ethereal extract, after washing free from acid with water and sodium carbonate, was dried and evaporated. A clear light yellow syrup, weighing about 0·6 g. resulted, which deposited crystals overnight; the latter were separated from the syrupy mother-liquors by stirring with a little cold alcohol and, after pressing on a tile, weighed about 0·2 g. This substance was soluble in hot alcohol, ethyl acetate, acetone, benzene or ether, but insoluble in light petroleum; it was recrystallised from a mixture of alcohol and light petroleum, from which it separated in aggregates of radiating prisms melting at 100–101°. (Found: C, 49·82; H, 6·04 %. Calc. for C_{21}H_{30}O_{14}: C, 49·80; H, 5·92 %.) These figures agree for the hexa-acetyl derivative of floridoside, a compound which had not been previously described; its specific rotation was \([\alpha]_D +108·5°\), in acetone solution.

Isolation of floridoside.

In order to establish the fact that this substance was actually the acetyl derivative of floridoside, a larger quantity was prepared and de-acetylated by the method of Helferich and Bredereck [1928]. For this purpose 1 g. of the hexa-acetate was dissolved in 7·5 cc. of dry chloroform and cooled to \(-15°\); to this were added, with constant shaking, 5 cc. of sodium methoxide whose titre had been previously determined with \(N\) sulphuric acid; the mixture, which set to a gel, was kept cold for 1½ hours and then treated with the amount of \(N\) sulphuric acid which was equivalent to the sodium methoxide; after thoroughly shaking, the chloroform was run off from below and the aqueous alcoholic layer was evaporated to dryness in a vacuum desiccator. The residual mixture was extracted repeatedly with absolute alcohol, and the combined extracts on evaporation yielded a crystalline solid which separated from alcohol in wart-shaped aggregates and melted at 126–127°. (Found: C, 42·38; H, 7·12 %. Calc. for C_{42}H_{18}O_{4}: C, 42·52; H, 7·12 %.) A molecular weight determination by depression of freezing-point in aqueous solution gave 241. C_{42}H_{18}O_{4} requires 254.

\(^1\) On subsequently determining the amount of reducing sugar produced after hydrolysis of the syrup the floridoside content of the dry weed was estimated to be 0·09 %.
Colin and Guégin obtained for their sample a value of 245, by the method of plasmolysis.

The specific rotation was [α]D +158·4° as compared with 160° given by Colin and Guégin.

A portion of the pure substance when oxidised with nitric acid yielded crystals of mucic acid m.p. 213–214°. Another portion was hydrolysed by heating for 2 hours at 70° with 5% hydrochloric acid; after neutralisation and evaporation the residual product was oxidised with bromine water, when on testing with α-naphthol and sulphuric acid it gave a characteristic green colour, indicating the presence of glycerol.

Taking all these facts into consideration it may be regarded as established that Corallina officinalis contains floridoside; the amount contained in this weed is, however, small and as it defied isolation by the method of Colin and Guégin, it is thought that the method of acetylation here described may be useful in other cases for establishing the presence of floridoside when only small quantities are present.

Examination of the precipitate A.

This material was suspended in water and decomposed with hydrogen sulphide: after removing the lead sulphide the filtrate was evaporated under reduced pressure at 40°; a yellowish-brown syrup resulted which, on keeping for some weeks in a vacuum, dried to a friable resinous material D. The weight of the crude material obtained from 1 kg. of dried weed was about 8 g.

This substance was readily soluble in water but insoluble in organic solvents; its aqueous solution was neutral to litmus and gave a marked biuret reaction of a reddish-pink tint.

The material was hydrolysed by boiling in aqueous solution over a sand-bath with twice its volume of concentrated hydrochloric acid for 3½ hours, after which the solution no longer gave a biuret reaction.

An estimation of amino-nitrogen gave a five-fold increase after hydrolysis, showing the substance to be a pentapeptide.

The nature of the product obtained on hydrolysis was determined as follows. 6 g. of the resinous substance D were dissolved in 10 cc. of water and boiled over a sand-bath with 10 cc. of concentrated hydrochloric acid for 3½ hours. The dark brown liquid resulting was evaporated to dryness over a water-bath and left in a desiccator over caustic potash. The residue was then taken up in water, decolorised with charcoal and filtered; the filtrate, still strongly acid with hydrochloric acid, was treated with caustic soda until the reaction was brought to about pH 3·6, when a precipitate appeared which was soluble both in acid and in alkali; it was filtered and crystallised from water from which it separated in prisms; heated in a sealed capillary tube the substance showed signs of melting with decomposition at about 270°.

Analysis showed the substance to be aspartic acid. (Found: C, 35·84; H, 5·26; N, 10·39 %. Calc. for C4H7NO4: C, 36·08; H, 5·27; N, 10·53 %.) Aspartic acid was further characterised by the fact that a cold aqueous solution of this substance gave with a solution of copper acetate a deposit of fine blue needles of the sparingly soluble copper salt.

It is proposed to examine the properties of the peptide more closely when more material is available and also to investigate its physiological significance.
Summary.

1. Extraction of the red sea weed, *Corallina officinalis*, with hot water yielded a solution containing floridoside and a new pentapeptide of aspartic acid.

2. The floridoside was isolated by conversion into its hexa-acetyl derivative m.p. 100–101°, a compound which has not been described before; this was then de-acetylated and the liberated floridoside was identified by analysis and by its physical constants.

References.

Helferich and Bredereck (1928). *Liebig's Ann.* 465, 166.