CCLXXIV. ON CERTAIN SIMPLE PEPTIDES OCCURRING IN MARINE ALGAE

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Some time ago Haas & Hill [1931; 1933, 1] described the isolation of water-soluble peptides from the brown alga *Pelvetia canaliculata* forma *libera* S. M. Baker and the red alga *Corallina officinalis* Linn. It was then suggested that the presence of peptides in algae was due to a lack of metabolic balance determined either by desiccation or by low illumination. To obtain further evidence in support of this it was decided to examine as many algae as possible and preferably calcareous forms, as these, owing to their incrustation, would naturally be exposed to a much reduced illumination. Accordingly the following were selected: *Corallina squamata* Ellis, *Lithophyllum incrustans* Foslie, *Amphiora capensis* Aresch and *Galaxaura subverticillata* Kjell. A number of unencrusted algae were also examined, but, so far, the only two found to contain peptides were *Pelvetia canaliculata* forma *libera* and *Griffithsia flosculosa*.

The peptides contained in the above calcareous algae and that of *Pelvetia* are described in the present communication with special reference to the number and nature of their constituent amino-acids, but an account of the observations made on *Griffithsia flosculosa* will be the subject of a future paper.

Methods

The general procedure for the isolation of the peptides was as follows. The air-dried material was digested three times in water over a boiling water bath for about 30 min. The resulting extract was poured off and the residual plant material freed from adherent liquid in a tincture press. The combined extracts were next precipitated with basic lead acetate, and the filtrate, after freeing from Pb with H_2SO_4, was treated with $Ba(OH)_2$ to remove SO_4 and was concentrated under reduced pressure; the solution was then precipitated by mercuric acetate, and the Hg salt, after washing, decomposed with H_2S, filtered from HgS and evaporated to dryness in a vacuum.

Nature and properties of the peptides

The peptides prepared as above are all amorphous, more or less viscous substances, varying in colour from a light lemon-yellow to a dark brown. They all give a green colour with Bial's reagent for pentose, but the intensity of the reaction varies, being least for *Pelvetia* and highest for *Lithophyllum*. Although detectable by means of the above colour reaction, the actual amount of carbohydrate was too small to estimate in the material available.

The peptides all give the biuret reaction, the colour varying from a reddish mauve to a blue-violet colour, but as the tint varies somewhat with dilution the reaction is of no value for characterization.

With the object of obtaining some information on the number of amino-acid molecules forming the peptide chain, the ratio of amino-N before and after

hydrolysis was determined. In order to eliminate the disturbing influence of NH_3 in the determination of amino-N, the following procedure was adopted.

A given amount of peptide was made up to a known volume, and two equal aliquots were measured out. One was then placed in a flask with dilute aqueous Na_2CO_3 and heated at 60° while a rapid current of air was drawn through. After all ammonia had been removed, the contents of the flask were carefully washed out, neutralized with acetic acid, evaporated and finally washed into a graduated flask and made up to 25 ml. The amount of amino-N in this solution was then determined by the Van Slyke method.

The other aliquot was heated with twice its volume of conc. HCl in an autoclave at 105° for 4 hr. After evaporating the HCl the residue was dissolved in water, made alkaline with Na_2CO_3 and heated over a water bath until no more NH_3 was evolved; it was then acidified with acetic acid, made up to 25 ml. and the amino-N determined.

From these two determinations the number of amino-acid groups in the peptide chain was obtained.

Products of hydrolysis of the peptides

The hydrolyses were carried out as described above and the dark brown solutions obtained were evaporated to dryness over a water bath to remove excess of acid, taken up with water and filtered from humic matter. As the quantity of material available was relatively small, the ordinary large scale methods for isolating and identifying the various acids were impracticable, and for this reason the tests employed are given in detail.

Pelvetia canaliculata forma libera. This alga was collected at Blakeney Point, Norfolk, during the month of April.

The crude peptide obtained was a light lemon-yellow syrup; the yield, calculated on the air-dried alga, was 0·728 %.

After hydrolysis and removal of excess acid by evaporation the solution was tested for phenylalanine (Kapeller-Adler test) and tyrosine (Mörner's test), in both cases with negative result.

After removal of ammonia, a portion of the resulting solution was tested with phosphotungstic acid and, as no precipitate resulted, the remaining solution was concentrated to small bulk and saturated with HCl to remove glutamic acid, whose presence among the products of hydrolysis had already been established on an earlier occasion [1931].

In order to determine whether any other amino-acid remained after complete removal of the glutamic acid, the filtrate was concentrated, again saturated with HCl and once more filtered from a small quantity of precipitated glutamic acid hydrochloride; it was then boiled to drive off the acid, diluted and heated with excess of ZnO. After 40 hr. the solution was filtered and found still to give a strong ninhydrin reaction. A control experiment on authentic glutamic acid treated in the same way gave no ninhydrin reaction, showing that glutamic acid can be completely removed by this method. It was therefore concluded that a second amino-acid must be present. The solution was accordingly concentrated, freed from NH_3 and treated with a few crystals of phenol and an excess of NaOCl; an immediate deep blue colour resulted, suggesting glycine, alanine or leucine. To distinguish between these a little of the concentrated solution was treated with a few drops of saturated copper acetate; as no precipitate resulted, glycine and leucine were excluded, and it only remained to establish the presence of alanine. To this end the solution was deaminated with HNO_2 and tested for the presence of lactic acid, after treating with conc. H_2SO_4, by Hopkins' test with

copper sulphate and thiophene. A positive result was obtained, thereby establishing the presence of alanine.[1]

The ratio of amino-N before and after hydrolysis was found to be 11. In the earlier communication [1931] this ratio was given as 8 and the only acid detected was glutamic acid. Whether this discrepancy is due to seasonal variation or not is now under investigation.

The five following algae are all encrusted forms, but, as will be seen from the results described below, there is considerable variation in the composition of their constituent peptides.

Corallina squamata. The material used for extraction of the peptide was a composite sample, collected at different times of the year, from Dancing Ledge, Dorset.

The peptide was a dark amber-coloured substance of a resinous consistency, which however readily melted, on warming, to a viscous liquid. The yield of crude peptide calculated on the dry weight of the alga was 0·1 %.

After hydrolysis, the solution was treated with phosphotungstic acid; the heavy precipitate produced was washed and suspended in acetone-water and treated with excess of baryta solution; a rapid current of air was drawn through until all NH_3 had been removed; excess of baryta was removed with CO_2, and the resulting solution was shown to contain arginine by both the Sakaguchi test and Harden's diacetyl test.

The filtrate from the phosphotungstic acid precipitate was then treated with baryta to remove excess phosphotungstic acid and the precipitate, consisting of Ba phosphotungstate and $BaSO_4$, was filtered off. The filtrate gave only a feeble ninhydrin reaction, which suggested adsorption on the precipitate; the latter was therefore boiled with a strong solution of Na_2CO_3, and filtered; the filtrate now gave a strong positive reaction. After neutralization it was tested for aspartic and glutamic acids, but with negative results. On the other hand it gave a blue colour with phenol, followed by NaOCl, indicating glycine, leucine or alanine. Since it gave no precipitate with copper acetate the two former acids were excluded. The presence of alanine was, however, established by deamination with HNO_2, followed by Hopkins' thiophene test for lactic acid.

The test for phenylalanine (Kapeller-Adler) was negative.

The failure to find aspartic acid, after repeated trials, among the products of hydrolysis of this alga is significant in view of the fact that aspartic acid was definitely established in the other species, namely *Corallina officinalis*, as reported on an earlier occasion [Haas & Hill, 1933, 2].

The ratio of amino-N before and after hydrolysis of the peptide of *C. officinalis* was 4.

Lythophyllum incrustans. The material used for this investigation was collected at Studland Bay, Dorset, in May, by chipping off the mauve superficial layer covering the chalk which is exposed only at low spring tide.

The thickness of the actual algal covering was only a few mm. and the main bulk of the sample was composed of the rock substratum. The material was crushed into small fragments and extracted three times with hot water, the aqueous extract being worked up in the usual manner. The weight of crude peptide obtained from different samples varied considerably from 0·055 to 0·185 %. These figures are only approximate and depend entirely upon the amount of chalk or limestone adhering to the weed.

[1] A separate experiment showed that authentic phenylalanine when treated as above likewise gives a positive reaction. Since however the solution obtained from the hydrolysed peptide failed to give the Kapeller-Adler reaction for phenylalanine it was concluded that the presence of alanine was established.

The crude peptide was a dark brown substance of a gummy consistency. After hydrolysis it gave a precipitate with phosphotungstic acid, which after the usual treatment yielded a residue which gave good positive tests for arginine by the Sakaguchi test and by Harden's test with diacetyl. The filtrate after the usual treatment for the removal of Ba gave a positive reaction for phenylalanine (Kapeller-Adler).

The ratio of amino-N before and after hydrolysis was 4.

Amphiora capensis. This alga is a South African species allied to *Corallina*.

The air-dried material, extracted in the usual manner, yielded just under 0·3 % of crude peptide, in the form of a dark brown semi-solid residue with a peculiarly unpleasant smell.

The amount of material available was, unfortunately, not sufficient to allow of a detailed examination, and the only amino-acid which could be definitely established was arginine. The filtrate remaining after removal of the arginine gave a blue colour with phenol and NaOCl, suggesting glycine, alanine or leucine. The addition of saturated copper acetate to the concentrated solution gave a turbidity on standing, much as leucine does, but it was not found possible to establish the presence of this amino-acid conclusively. Lack of material rendered it impossible definitely to exclude glycine or alanine, or to make a determination of the ratio of amino groups before and after hydrolysis.

Galaxaura subverticillata. This is an encrusted green alga from Florida.

Extraction by the usual method gave a yield of about 0·2 % of a dark brown semi-solid peptide. The amount of material available was small, and only one amino-acid, namely phenylalanine, was definitely established. The hydrolysed product was precipitated with phosphotungstic acid without previous removal of ammonia; the resulting precipitate gave a negative test with ninhydrin, and as it gave no positive test for proline it was rejected.

The filtrate from the phosphotungstic acid precipitate was treated with baryta to remove excess of phosphotungstic acid and filtered. The filtrate was freed from Ba and precipitated with mercuric acetate. The resulting precipitate was decomposed with H_2S and filtered. This filtrate gave an amorphous precipitate with copper acetate, which was not further characterized owing to lack of material. The filtrate from the mercuric acetate precipitate, however, gave positive tests for phenylalanine. It may therefore be concluded that *Galaxaura* peptide contains at least two amino-acids of which one is phenylalanine.

The ratio of amino-N before and after hydrolysis was 2.

In addition, a further calcareous alga of South African origin, namely *Cheilosporium corymbosum*, was examined, but the amount of material available was too small for detailed examination. All that was established was that the weed yielded about 0·1 % of crude peptide in the form of a dark brown resinous material in which the ratio of amino groups before and after hydrolysis was 2.

For convenience of reference the results obtained are summarized in the following table:

	Alanine	Arginine	Aspartic acid	Glutamic acid	Phenyl-alanine
Amphiora capensis	+	+	.	.	.
Corallina squamata	+	+	.	.	.
Corallina officinalis	.	.	+	.	.
Galaxaura subverticillata	.	.	.	.	+
Lithophyllum incrustans	+	+	.	.	.
Pelvetia canaliculata forma *libera*	+	.	.	+	.

Although various other amino-acids were tested for, only those which gave positive results are recorded in the above table.

SUMMARY

1. The occurrence of peptides is reported in marine algae: *Pelvetia canaliculata* forma *libera* (Phaeophyceae), *Corallina officinalis*, *Corallina squamata*, *Amphiora capensis*, *Cheilosporum corymbosum*, the two latter from South Africa, *Lithophyllum incrustans* and *Galaxaura subverticillata* from Florida. Of these algae all except the first are encrusted forms.

2. The amount of crude peptide occurring in the algae varies from 0·05 to 0·29 % of the dry weight; in *Pelvetia*, however, the amount is as high as 0·728 %.

3. The peptides are all water-soluble and diffusible substances; they all contain a small proportion of pentose sugar.

4. The following amino-acids have been shown to occur in these compounds: alanine, arginine, aspartic acid, glutamic acid and phenylalanine.

5. In one genus, namely *Corallina*, a difference in composition of the peptide has been noted in different species, *C. officinalis* containing aspartic acid, while *C. squamata* does not, but contains instead alanine.

6. The number of peptide linkages as expressed by the ratio of amino groups before and after hydrolysis varies in the different peptides.

In conclusion we take this opportunity of acknowledging our indebtedness to Prof. T. A. Stephenson and Dr Janet Maclagan for supplying the South African algae, and to Dr F. C. Steward for the specimen of *Galaxaura* from Florida.

REFERENCES

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