CLXVIII. THE ALGAE.

III. THE ISOLATION OF LACTOFLAVIN.

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WHILST lactoflavin is widely distributed in both the animal [Kuhn *et al.*, 1934] and vegetable kingdoms [György *et al.*, 1934], it is still uncertain whether this flavin is the sole member of the group or whether there are several such compounds, *i.e.* hepatoflavin from horse liver [Stern, 1933], hepaflavin from malt [Karrer *et al.*, 1934], uroflavin from urine [Koschara, 1934] and renoflavin from ox kidney [Guha and Biswas, 1934].

In order to investigate the possible presence of lyochromes in the algae and to ascertain whether the flavin, if present, is identical with lactoflavin, we have examined the marine brown alga *Fucus vesiculosus* and the fresh water green alga *Cladophora sauteri* in detail.

During our investigations a communication appeared by Euler and Adler [1934] in which they claim to have detected the presence of flavins in several marine Phaeophyceae. As, however, these authors relied solely upon spectroscopic evidence, the question as to the identity still remained open.

EXPERIMENTAL.

Fucus vesiculosus.

The pale brown aqueous acetone washings from the fresh alga (50 kg. wet weight) [see Heilbron and Phipers, 1935] were very glutinous owing to large amounts of mucilage, thus rendering the usual procedure for the isolation of flavins by adsorption on fuller's earth impracticable. To overcome this difficulty we employed the methods described by Warburg and Christian [1933] whereby the photo-derivative lumilactoflavin is obtained. A portion of the extract (10 l.) was freed from acetone and made $N/2$ with respect to alkali by the addition of aqueous sodium hydroxide (380 ml. of 13*N*). After irradiation, the lumiflavin was extracted from the acidified aqueous solution with chloroform. The bright yellow pigment, after purification by conversion into the sodium salt and finally by crystallisation from water, melted at 310° decomp. (uncorr.) and exhibited an absorption maximum in chloroform at 447*mμ*. Warburg and Christian give for lumilactoflavin M.P. 320° (corr.) and an absorption maximum in chloroform at 445–455*mμ*. Kuhn, Rudy and Wagner-Jauregg [1933] give M.P. 328° (corr.) and report an absorption maximum in chloroform at 445*mμ*.

Cladophora sauteri.

The methods described by Kuhn and Kaltschmitt [1935] for extraction of lactoflavin from hay were employed. The alga (50 kg. wet weight) was covered with hot dilute hydrochloric acid (50 l. at p_H 1.2) and allowed to stand with

intermittent agitation for 12 hours. The flavin was removed from this aqueous extract by adsorption on fuller's earth and further purified by adsorption on lead sulphide and frankonite KL. The pigment was acetylated and chromatographically separated from impurities on a column of alumina. After elution, crystallisation of the pigment from aqueous methyl alcohol yielded red-brown crystals decomposing at 230° (uncorr.) with slight sintering at 180° (absorption maxima in water at 445 and $360m\mu$). Kuhn and Wagner-Jauregg [1933] give for tetra-acetyl-lactoflavin M.P. 235° decomp. (corr.) and absorption maxima in water at 445 and $374-376m\mu$.

SUMMARY.

Marine and fresh water algae as typified by *Fucus vesiculosus* and *Cladophora sauteri* both contain flavins. Lactoflavin has been obtained from the latter alga by direct adsorption from the aqueous extract, a process inapplicable to the former from which the photo-derivative lumilactoflavin was isolated.

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