CLXIX. THE LIPOCHROMES OF SEA ANEMONES.

I. CAROTENOID PIGMENTS OF *ACTINIA EQUINA*, *ANEMONIA SULCATA*, *ACTINOLOBA DIANTHUS* AND *TEALIA FELINA*.

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ALTHOUGH during the past few years the structure of the plant lipochromes has been more or less completely elucidated, comparatively little is as yet known concerning the polyene pigments of marine animals. Indeed, up to the present, only one such pigment, astacene, which appears to be widely distributed in marine fauna, has been isolated and its structure determined [Kuhn and Lederer, 1933; Kuhn, Lederer and Deutsch, 1933; Karrer and Benz, 1934; Karrer and Loewe, 1934; Karrer *et al.*, 1935].

Euler *et al.* [1933] have described the occurrence of a pigment, salmenic acid, in the salmon whilst Euler and Hellström [1934] have isolated a further pigment, asterinic acid, from the starfish (*Asterias rubens*). Lönnberg [1931, 1, 2; 1932; 1933] has recorded spectroscopic data for the pigment extracts of numerous species of marine invertebrates. Various other pigments have been isolated by Lederer [1933; 1934, 1, 2] from different species of ascidia, mollusca and crustacea, the more important being pectenoxanthin, m.p. 182°, from the mollusc, *Pecten maximus*; glycymérine, m.p. 148–153°, from the scollop, *Pectunculus glycimeris*, and cynthiaxanthin, m.p. 188–190°, from the ascidian, *Halocynthia papillosa*. None of the above pigments has been examined in any great detail owing to the extreme difficulty of obtaining them in adequate quantity.

In the case of the pigments of various species of sea anemone, only one member has previously received attention. From *Actinia equina* Fabre and Lederer [1934] isolated a pigment, actinioerythrin, m.p. 85°, which they showed was a carotenoid ester. Prior to the appearance of these authors' paper we were ourselves engaged upon an examination of this pigment and, working on a larger scale, have obtained from it a hydrolysis product, m.p. 191–192°, which is, so far as we know, unique among carotenoids both as regards colour and absorption spectrum. Whereas actinioerythrin has absorption maxima in carbon disulphide at 574, 538 and 497m μ the new pigment, for which we propose the name "*violerythrin*", shows maxima in the same solvent at 625, 576 and 540m μ .

In addition to the above, three other species, *Actinoloba dianthus*, *Tealia felina* and *Anemonia sulcata*, have so far been examined. Like *Actinia equina*, both *Actinoloba dianthus* and *Tealia felina* contain, apart from carotene, only esterified carotenoids. The red pigment of *Actinoloba dianthus* is readily hydrolysed, yielding an acid, m.p. 195–196°, both acid and ester showing a single absorption maximum in carbon disulphide near 495m μ . From *Tealia felina* two pigments have been isolated; of these one is possibly identical with actinioerythrin whilst the second on hydrolysis yields an acid, m.p. 205–208°, with a

single absorption maximum near $500m\mu$ in carbon disulphide. *Anemonia sulcata* differs from the other three species in containing no esterified pigments. The main lipochrome, *sulcatoxanthin*, for which the formula $C_{40}H_{52}O_8$ is suggested by analytical data, is characterised by three absorption maxima in carbon disulphide at 516, 482 and $450m\mu$.

The present communication deals with the method of isolation of the pigments and certain preliminary observations as to their nature.

EXPERIMENTAL.

1. *Actinia equina*.

Isolation of actinioerythrin. The anemones were minced and repeatedly extracted with a mixture of ether and acetone (1:1) at room temperature. The combined extracts were concentrated to small bulk and diluted with water and the pigment was transferred to light petroleum. Phosphatides and sterols were removed by precipitation from the concentrated light petroleum solution with acetone, followed by further concentration and freezing to -10° . The pigment was again transferred to light petroleum and adsorbed on alumina (Merck standardised Brockmann diluted with an equal volume of inactive alumina); the chromatogram was developed with benzene; the actinioerythrin formed an intense violet-black zone on the upper half of the column, whilst a yellow pigment, spectroscopically identified as carotene, washed rapidly through. The ester was eluted with light petroleum-methyl alcohol and readsorbed from light petroleum on calcium carbonate, whereby remaining traces of sterol, terpene material and a further yellow pigment (absorption maxima at 502, 472 and $442m\mu$ in carbon disulphide and probably a taraxanthin ester) were removed. After elution and removal of solvent, the crude actinioerythrin was crystallised from absolute alcohol giving microscopic red-black needles, m.p. 83° (uncorr.) (Fabre and Lederer [1934] give m.p. 85°). In agreement with these authors we find that whereas in carbon disulphide the pigment is characterised by three absorption maxima at 574, 538 and $497m\mu$, in alcohol only one band with head at $511m\mu$ is present. On partition between light petroleum and 90 % methyl alcohol actinioerythrin is wholly epiphasic (the yield of crystalline pigment from 500 anemones is approximately 30 mg.).

Isolation of violerythrin. Fabre and Lederer [1934], working with a minute quantity of the ester, noted its ready hydrolysis but state that the resultant "acid" decomposed on liberation. We have found, however, that under carefully controlled conditions of saponification a crystalline product can be isolated. As a result of numerous experiments the following method has been found the most satisfactory. A solution of actinioerythrin (5 mg.) in light petroleum was saponified by shaking at room temperature with methyl alcoholic sodium hydroxide (20 ml. of 2.5 %) until the pigment was completely transferred to the alcohol phase (approximately 1 hour). The latter was diluted with water and rendered faintly acid with 5 % acetic acid, the end-point being readily observed by a colour change from cherry-red to orange. The pigment was extracted with ether and the washed solution dried over anhydrous sodium sulphate. After removal of solvent the crude solid was recrystallised from aqueous pyridine, when violerythrin (1 mg.) separated in deep violet micro-crystals, m.p. $191-192^\circ$ (absorption maxima at 625, 576 and $540m\mu$ in carbon disulphide). The pigment exhibits a remarkable range of colours in various solvents; in benzene it is deep blue, in pyridine and acetone bright blue, in ether and alcohol violet-red and in carbon disulphide purple.

From the above it would be anticipated that violerythrin is itself acidic in nature but this does not appear to be the case since dilute alkali is without action upon it. Probably the initially formed "acid" rearranges from an enolic to a stable ketonic form; more detailed study of the hydrolytic process is being carried out.

2. *Anemonia sulcata*.

500 animals were finely minced and extracted with ether-acetone (1:1). Whereas the initial extracts were of a deep reddish yellow colour, the later ones were typically chlorophyll-like in character. The combined extracts were concentrated, diluted with water and extracted with light petroleum and the resulting solution was partitioned with 65 % methyl alcohol. The residual petroleum phase was found to contain, in addition to carotene and chlorophyll, a small quantity of a xanthophyll pigment (absorption maxima at 478 and 450 $m\mu$ in carbon disulphide) which passed readily into 90 % methyl alcohol.

The sulcatoxanthin contained in the 65 % methyl alcohol was transferred to benzene and adsorbed on activated alumina. The chromatogram was developed by washing with benzene-ether (4:1), the pigment being held as a bright red zone near the head of the column. After elution and removal of solvent, the residue was dissolved in carbon disulphide and re-adsorbed on calcium carbonate. On addition of light petroleum to the concentrated ether eluate the sulcatoxanthin separated as a flocculent deep scarlet precipitate (50 mg.).

Properties of sulcatoxanthin. The pigment is insoluble in light petroleum, sparingly soluble in carbon disulphide and readily soluble in benzene and alcohol. It possesses no sharp melting-point but suddenly shrinks at 110°, softens at 125° and is completely melted at 130°. In carbon disulphide it shows three absorption bands with maxima at about 516, 482 and (450) $m\mu$. It is strongly adsorbed on sugar from carbon disulphide as a uniform rose-coloured zone (further evidence of its homogeneity) and this, together with its ready partition into 65 % methyl alcohol, is clearly indicative of a high state of oxidation. Treatment with alkali, even under mild conditions, causes complete destruction of the pigment, whilst with concentrated sulphuric acid a blue colour is produced. (Found: C, 72.4, 72.4; H, 8.1, 7.9 %. $C_{40}H_{52}O_8$ requires C, 72.7; H, 7.9 %.)

Chlorophyll-a from Anemonia sulcata. The presence of a chlorophyll-like substance in this species was first observed by MacMunn [1885] and confirmed by Elmhirst and Sharpe [1920], who attributed its occurrence to the presence of symbiotic algae in the tentacles. We have identified this pigment as chlorophyll-a in the following manner. The tentacles were removed from a dozen freshly killed animals and the total pigments transferred to light petroleum (B.P. 60–80°). The sulcatoxanthin was removed with 90 % methyl alcohol and the residual emerald-green solution examined spectroscopically and found to be identical with pure chlorophyll-a examined in the same solvent (absorption maxima at 657, 612, 571, 532, 504, 494, general abs. from 465 $m\mu$).

3. *Actinoloba dianthus*.

The pigment from 50 anemones was obtained in light petroleum solution in the usual manner. Partition with 90 % methyl alcohol indicated the presence of epiphasic carotenoids only. By adsorption of the light petroleum solution on calcium hydroxide one pigment was retained as a reddish brown band whilst a second passed through the column (absorption maxima at 501 and 471 $m\mu$ in carbon disulphide). The adsorbed pigment was transferred to light petroleum and shaken with calcium carbonate by which it was readily held. From this a

deep red, low-melting solid was obtained which could not be crystallised. On saponification with alcoholic sodium hydroxide (10 ml. of 5 %) at room temperature the ester was rapidly hydrolysed, giving on dilution an insoluble red sodium salt. The free acid crystallises from aqueous pyridine in deep violet-red prisms, M.P. 195–196° (2 mg.), showing a single absorption maximum in carbon disulphide at 495 $m\mu$.

4. *Tealia felina*.

The degree of pigmentation of this species of anemone varies considerably but on the whole is small. Two varieties were examined, a small shore form and a larger deep water animal. The pigments were obtained in light petroleum and a partition experiment with 90 % methyl alcohol showed the absence of hypophasic carotenoids. The chromatogram, developed with benzene on activated alumina, exhibited a violet-red zone, whilst a yellow pigment (carotene) washed through the column. Re-adsorption on calcium carbonate furnished three zones, coloured violet, red and yellow respectively, the last being present in only minute amount. The violet zone yielded, after crystallisation from absolute alcohol, a violet-black wax, M.P. 65–73°, with absorption maxima in carbon disulphide at 569, 530 and 497 $m\mu$. This pigment shows a close resemblance to actinioerythrin, but definite identification can only be established when larger quantities are available.

From the red zone an orange-red low-melting ester was obtained (absorption maximum at 500 $m\mu$ in carbon disulphide), hydrolysing to give on dilution an insoluble red sodium salt. The free pigment, obtained in the usual manner, separated from aqueous pyridine in black feathery needles, M.P. 205–208° (1 mg.), having the same absorption spectrum as the parent ester.

SUMMARY.

1. A preliminary investigation into the carotenoid pigments of four species of sea anemone has been made.

2. Actinioerythrin (M.P. 85°), the principal pigment of *Actinia equina*, has been isolated and hydrolysed to a non-acidic pigment, *violerythrin*, M.P. 191–192°.

3. The pigment of *Anemonia sulcata* consists mainly of a new lipochrome, *sulcatoxanthin*, probably $C_{40}H_{52}O_8$. The presence of chlorophyll-a in the tentacles has been demonstrated.

4. From the species *Actinoloba dianthus* a red acidic pigment, M.P. 195–197°, has been isolated.

5. Two lipochrome esters, one possibly identical with actinioerythrin, have been isolated from *Tealia felina*.

6. Spectroscopic data concerning the above and other polyenes present in these species have been obtained.

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