CCXXVI. PIGMENTED MARINE ANIMAL OILS.

I. PIGMENTS FROM THE ANGLER FISH (*LOPHIUS PISCATORIUS*), THE PRAWN (*NEPHROPS NORVEGICUS*) AND THE WHALE.

BY GEORGE NORMAN BURKHARDT, ISIDOR MORRIS HEILBRON,
HAROLD JACKSON AND EDRYD GWYLFA PARRY,

The Department of Chemistry, University of Manchester,

AND JOHN ARNOLD LOVERN,

Torry Research Station, Aberdeen.

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IN continuation of a comprehensive investigation into the constituents of marine animal oils [Morton *et al.*, 1931; Heilbron *et al.*, 1931; Castle *et al.*, 1934, *etc.*] we have commenced a study of pigmented specimens, and during the past year have made a preliminary examination of three such oils, *viz.*: angler-liver oil (*Lophius piscatorius*), red whale oil, and the oil of a particular species of prawn (*Nephrops norvegicus*).

The unusual pigmentation of the liver of the angler or monk fish and of the oil extracted from it, was first noted by one of us (J. A. L.). Lovern and Morton [1931] discussed the carotenoid nature of the pigment and its location and development in the liver and examined the absorption spectrum of the oil. Lovern [1931] reported the presence of two pigments, one a neutral substance with carotenoid properties and the other acidic in character. Subsequently he amplified this [1932] and gave an account of the adsorption of the unsaponifiable matter on alumina. We have continued this investigation with a view to the further separation, identification and isolation of the pigments. While our work was in progress Sørensen [1934] published the results of an investigation on the development of the colour in the liver of *Lophius piscatorius* by various treatments and concluded that it was present as a colourless protein-carotenoid complex from which it was liberated on extraction. From the behaviour of the oil and the unsaponifiable material on adsorption on calcium carbonate from carbon disulphide solution and from an examination of the absorption spectra of the carotenoid bands he concluded that the colour was due to the presence of a taraxanthin ester and epi- and hypo-phasic astacene esters [Kuhn and Lederer, 1931, 1]. Our results amplify and confirm those of Sørensen.

Angler fish-liver oil. The pigmented oil was obtained by intimately mixing the minced liver with anhydrous sodium sulphate and extracting with ether. A most striking difference in the pigmentation was observed between the oil obtained by rapidly working up single livers and that yielded by the slower operations involved in attempting to work up large amounts. The first process gives rise to a bright red, and the latter to a dull brown oil in which we have found that extensive destruction of the pigments has occurred. Livers maintained in cold storage still give bright oils, and all such oils retain their colour when kept under nitrogen. In the present communication only bright fresh oils are considered. A solution in chloroform (4.5 %) gave maxima at 518, 483 and 455 $m\mu$, $E_{1\text{ cm.}}^{1\%} = 0.135$ [*cf.* Lovern and Morton, 1931]. With antimony trichloride the oil gave a violet colour with a single clear band with maximum at 572 $m\mu$, $E_{1\text{ cm.}}^{1\%} = 0.135$, which was not affected by either benzoyl peroxide [Heilbron *et al.*, 1931] or methyldindole [Emmerie *et al.*, 1931; Morton, 1932].

Chromatographic analysis of the oil using a 10 % solution in light petroleum

(B.P. 40–60°) and alumina as adsorbent (Merck standardised Brockmann) gave three bands: a rather weak brownish red band (A) near the top of the column, an intense cerise band (B) less strongly adsorbed and a yellow band (C) which passed through the column giving a bright yellow filtrate. Elution by methyl alcohol removed a small portion of (A) and the bulk of (B), the remainder in each case being disengaged by glacial acetic acid. The pigment from the main fraction (B) gave a single broad absorption band with maximum at 497 $m\mu$ in carbon disulphide; the yellow filtrate showed maxima at 468 and 443 $m\mu$ in light petroleum, displaced to 496 and 465 $m\mu$ in carbon disulphide. The intensities of absorption expressed in terms of the original oil were:

$$E_{1\text{ cm. (light petroleum)}}^{1\%}, \text{ cerise band (astacene pigment)} 0.016;$$

$$E_{1\text{ cm. (light petroleum)}}^{1\%}, \text{ yellow filtrate (xanthophyll pigment), } 0.040.$$

The latter corresponds to approximately 2 mg. xanthophyll per 100 g. oil, the ratio of xanthophyll pigment to astacene pigment being 5:2, assuming the same extinction coefficient for the two pigments. The methyl alcohol eluate of band A gives no selective absorption (probably astacene oxidation products), while the pigment removed by glacial acetic acid gave the same absorption spectrum as (B) in carbon disulphide solution.

The pigment from band B re-adsorbed twice on alumina exhibited the typical astacene ester behaviour, being retained at the head of the column [cf. Kuhn and Lederer, 1933]. Re-adsorbed on calcium hydroxide from light petroleum, four bands were obtained: (i) at the top, an orange-red band, (ii) a brick-red band, (iii) a violet band and closely associated with it (iv) a very weak brown band. All these exhibited a broad absorption maximum closely resembling that of astacene, no clear difference between the fractions being detectable.

Saponification of (B) with 2*N* methyl alcoholic NaOH yielded a red sodium salt which separated at the interphase on addition of light petroleum. This behaviour is identical with that of the red sodium salt of astacene [Karrer and Benz, 1934]. The free pigment was obtained as a violet-black solid on acidification but the amount was too minute to allow of its further purification. For confirmatory identification we have compared the absorption spectra of the above pigment (465 $m\mu$ in light petroleum) with that of a sample of partially purified crystalline astacene from lobster (463 $m\mu$ in light petroleum, 499 $m\mu$ in carbon disulphide) which is in fair agreement with the value recorded by Sørensen [1934] (503 $m\mu$ in carbon disulphide).

In agreement with this author's statement, we have observed that the spectroscopic values for the yellow band (fraction C) closely resemble those of authentic taraxanthin, giving maxima at 468 and 440 $m\mu$ in light petroleum (taraxanthin, 469 and 443 $m\mu$ in light petroleum). In this connection it is interesting to note that Karrer and Loewe [1934] have recently established the constitution of astacene as 4:5:4':5'- or 5:6:5':6'-tetraketo- β -carotene, $C_{40}H_{48}O_4$, while Kuhn and Lederer [1931, 2] have found that taraxanthin is a four-oxygen xanthophyll. The co-existence of these two pigments in the angler oil suggests the possibility that taraxanthin may be the biochemical precursor of astacene in this case, a question which we are at present investigating.

Red whale oil. This material, received by the courtesy of Messrs Lever Bros., Ltd., was obtained from a whale caught in the Antarctic in 1931. Whales giving red body oil are rarely encountered, and except for the fact that their fat is permeated with pigment they appear normal. The condition is regarded as pathological but the present authors know of no detailed evidence concerning this. The oil shows a single broad absorption band with a maximum near 484 $m\mu$ in chloroform, strikingly similar to the characteristic astacene band ($E_{1\text{ cm.}}^{1\%}=0.112$).

The pigment was separated from the bulk of the oil (3000 ml.) by passing the latter in 20–30 % solution in light petroleum (B.P. 40–60°) through a tower packed with fuller's earth admixed with inactive alumina. It was completely held in a narrow zone (2–10 mm.) at the top of the adsorption column and was eluted with methyl alcohol. The oily material was recovered practically quantitatively from the filtrate. By partition between methyl alcohol–light petroleum it proved to be hypophasic (contrasting with the cerise band from the angler oil). The alcoholic phase after concentration (200 ml.) was covered with light petroleum and saponified for twelve hours in the cold with aqueous alcoholic sodium hydroxide (60 ml., 2*N*), a red sodium salt separated at the interphase. Acidification of this salt with acetic acid (50 %) gave a sticky dark red mass, which was washed free from acetic acid and taken up in chloroform. By precipitation of the dried solution with light petroleum a brownish red amorphous solid was obtained. Its absorption spectrum in the visible showed no maximum, the substance being presumably an oxidation product of the pigment. The mother-liquor was evaporated and the minute deep red residue redissolved in light petroleum. This solution exhibited a single broad absorption band near 470 $m\mu$. Addition of aqueous sodium hydroxide reprecipitated the red sodium salt; this was removed, suspended in alcohol (70 %) and acidified with acetic acid (50 %); violet-black needles slowly separated, but the amount was too small for further investigation. From the appearance of these crystals together with the position and general character of the absorption bands of the original oil and of the separated "acid" we conclude that the main pigment of the whale oil is probably a hypophasic astacene ester¹.

Oil from the prawn (*Nephrops norvegicus*). *Nephrops*, a species of large prawn, differs from the common lobster in that the claws and shell are pink in the natural state and do not change colour on boiling. The oil was prepared by taking the whole animal, mincing, desiccating with anhydrous sodium sulphate and extracting with ether. The pigment is probably derived solely from the shells, as the flesh is white. A chloroform solution of the oil showed a single broad band with maximum near 490 $m\mu$ ($E_{1\text{cm.}}^{1\%} = 3.83$) indicating a pigment content approximately 35 times that of the whale oil.

The original oil (100 ml.) was taken up in methyl alcohol (500 ml.) and cooled to –10°; a deep red thick oil separated. On concentration, the mother-liquor deposited flat colourless needles, M.P. 146–147° (acetate M.P. 113–114°) giving no depression in M.P. on admixture with cholesterol. The filtrate on concentrating further provided more heavy red oil, which was combined with the above and the whole adsorbed from a light petroleum solution on alumina. The following complex chromatograph was obtained:

From the top of the column	Band or zone	From the top of the column	Band or zone
cm.		cm.	
3.0	(A) Pink zone	6.8	(C) Sharp red band
3.5	(B) Deep red zone	8.8	— Practically colourless
6.5	— Practically colourless	8.9	(D) Orange band

It is not yet clear whether this series of zones arises from an intrinsic complexity of pigments or whether it is due to interference with the normal process of adsorption by the oily constituents present. Elutions were carried out with methyl alcohol, but in the case of fractions A, B and C a small proportion of pigment remained on the adsorbent and could only be removed by glacial acetic

¹ We are, however, continuing the examination of this pigment, and the corresponding one from the angler, with a view to determining whether the small differences between their spectra and that of pure astacene indicate differences in structure or arise from impurities in the products from the oils.

acid. Band C, which contained the major part of the pigment, was selected for further examination. The eluted fraction was evaporated and the residue crystallised from light petroleum-ethyl alcohol; a small quantity of a colourless solid, m.p. 65–66°, was deposited. This proved to be hentriacontane, $C_{31}H_{64}$, giving no depression in m.p. on admixture with a specimen of the hydrocarbon obtained from *Fucus vesiculosus* [Heilbron *et al.*, 1934]. On concentration of the filtrate a further small crop of cholesterol was obtained. The mother-liquor (3 ml.) was diluted with light petroleum and saponified in the cold for two hours with 2*N* aqueous alcoholic NaOH (5 ml.). The separated red sodium salt was removed, suspended in ethyl alcohol (70 %) and acidified with acetic acid (50 %); the colour of the salt rapidly changed almost to black. The free pigment was removed, washed with light petroleum (b.p. 40–60°) and recrystallised from aqueous pyridine, giving violet-black crystals, m.p. 235° (m.p. pure astacene 240–243° [Kuhn and Lederer, 1933]¹).

In solution in pyridine the free compound exhibited a single broad absorption band with a maximum near 488 $m\mu$. A comparison of the absorption curve of pure astacene from lobster, as given by the above authors, with that of our specimen shows the two curves to be very similar, although the maximum recorded by Kuhn and Lederer is near 500 $m\mu$ in pyridine. In carbon disulphide the maximum for our sample lies near 509 $m\mu$, which is in fair agreement with that recorded for the red angler pigment in the same solvent [Sørensen, 1934]. The difference in the melting-points and the location of the absorption maxima suggest that the solid obtained by us was slightly impure. We consider, however, that it is almost certainly astacene.

SUMMARY.

Red oils from the angler fish (*Lophius piscatorius*), the prawn (*Nephrops norvegicus*) and the whale have been examined chromatographically. In each case a violet-black solid, almost certainly identical with astacene, has been isolated.

In the case of the angler-liver oil a yellow pigment is also present, which in solution shows absorption maxima characteristic of taraxanthin [*cf.* Sørensen, 1934]. The occurrence of taraxanthin and astacene together in the angler-liver oil is probably more than coincidence in view of the recent discovery that both pigments contain the same number of carbon and oxygen atoms.

We desire to express our thanks to the Department of Scientific and Industrial Research for a maintenance grant to one of us (E. G. P.) and to Mr A. E. Gillam, who carried out the spectrographic determinations.

¹ Since this work was completed we have seen a paper by Fabre and Lederer [1934, *Bull. Soc. Chim. Biol.* **16**, 105], in which the isolation of astacene (m.p. 228–230°, absorption maximum in CS_2 at 510 $m\mu$) from a species of *Nephrops* is reported.

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