298. *The Chemistry of the Algæ. Part II. The Carotenoid Pigments of* Oscillatoria Rubrescens.

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The existence of specific phytocarotenoids in the blue-green algæ (*Myxophyceæ*) was qualitatively demonstrated by Kylin (*Z. physiol. Chem.*, 1927, **166**, 50), who, by capillary analysis of extracts of *Calothrix scopulorum*, claimed to have detected the presence of three pigments to which the names myxorhodin-α and -β and kalorhodin were given. The first isolation of an individual polyene pigment from the *Myxophyceæ* was recorded by Heilbron, Lythgoe, and Phipers when *myxoxanthin* was obtained in crystalline form from *Rivularia nitida* (*Nature*, 1935, **136**, 989). We have subsequently obtained this pigment from other members of the *Myxophyceæ* (unpublished work), and we conclude that it is characteristic of this class just as fucoxanthin is typical of the *Phæophyceæ*.

A convenient source of myxoxanthin was found in the fresh-water species *Oscillatoria rubrescens*, occurring as a scum on one of the Hampton reservoirs of the Metropolitan Water Board. The carotenoid pigments were extracted from the alga by successive treatment with alcohol and ether. The combined extracts were saponified to remove chlorophyll, and the unsaponifiable fraction partitioned between light petroleum and 90% methyl alcohol. Chromatographic analysis of the epiphasic (light petroleum) carotenoids gave three pigmented zones, from the lowest of which β-carotene was isolated. Although no crystalline pigment could be obtained from the narrow uppermost zone, the violet middle zone furnished myxoxanthin, contaminated with an unidentified colourless impurity. By repeated crystallisation, the m. p. was raised from 117—118° (Heilbron, Lythgoe, and Phipers, *loc. cit.*) to the constant value of 168—169°.

Myxoxanthin has the formula $C_{40}H_{54}O$; the oxygen atom is present as a carbonyl group (an *oxime* was prepared), which shows no tendency to enolise. Quantitative microhydrogenation indicates the presence of 12 readily reducible linkages, which must be ethenoid, since myxoxanthin oxime is saturated by 13 molecules of hydrogen, the absorption of one of which is to be ascribed to the oximino-group. It follows that myxoxanthin is monocyclic and is therefore to be classified with γ-carotene and rubixanthin. Like γ-carotene, myxoxanthin contains an unsubstituted β-ionone ring, since biological experiments show that it possesses growth-promoting properties.

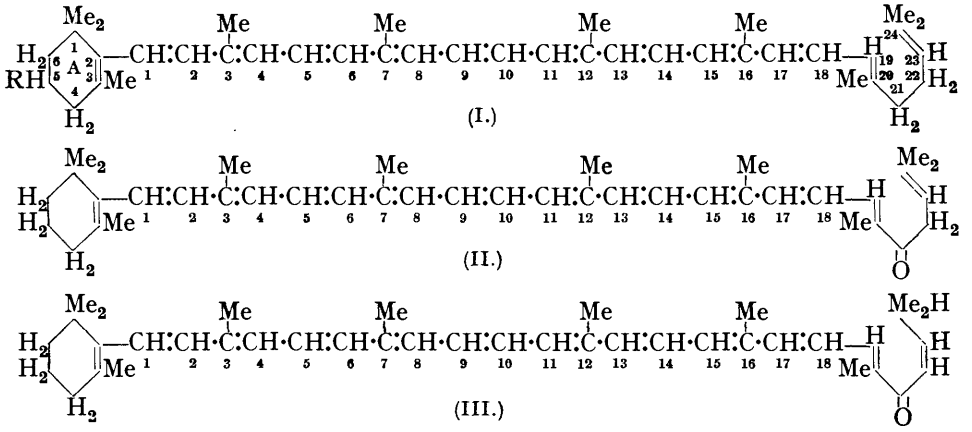
That the carbonyl group in myxoxanthin is conjugated with the polyene chain is indi-

cated both by the colour difference between solutions of the same concentration in light petroleum (yellow) and in alcohol (pink) and also by comparative spectrographic examination of myxoxanthin and its oxime; in the latter, the optical maximum in chloroform is situated 100 A. nearer the violet than in the former (compare Kuhn and Brockmann, *Ber.*, 1933, **66**, 828).

Reduction of myxoxanthin by aluminium *iso*propoxide gives the alcohol myxoxanthol, which is spectroscopically identical with both γ-carotene (I, R = H) and rubixanthin (I, R = OH) (see table, in which wave-lengths are in A.). Myxoxanthol therefore possesses a chromophoric grouping of one cyclic and ten acyclic ethenoid linkages in unbroken conjugation, and consequently in myxoxanthin a carbonyl group must be situated at C_{21} in a

Solvent.	γ-Carotene.	Rubixanthin.	Myxoxanthol.
Carbon disulphide	5335, 4960, 4630	5330, 4940, 4610	5290, 4940, 4640
Chloroform	5085, 4750, 4460	5090, 4740, 4390	5080, 4740, 4410
Light petroleum (b. p. 70—80°)	4950, 4620, 4310	4940, 4640, 4320	4950, 4650, 4310

γ-carotene skeleton, the only possible alternative position (C_4, ring A) being excluded by the biological activity of the pigment.* The unlocated ethenoid linkage of myxoxanthin may



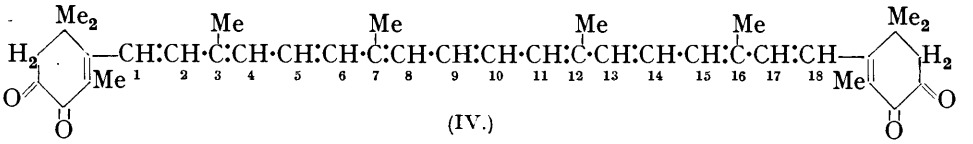
occupy one of two positions leading to two possible structures (II) and (III) for the pigment, either of which is compatible with its observed optical inactivity.

A decision in favour of (III) has been reached from spectroscopic observations; myxoxanthin shows a *single broad band* with head at 4880 A. in carbon disulphide, whilst a pigment of structure (II) would, by analogy with other aldehydic and ketonic carotenoids having a carbonyl group terminating the chromophoric system (*e.g.*, lycopenal, bixin dialdehyde, β-carotenone aldehyde, rhodoxanthin, capsanthin, capsorubin, and the carotenones), exhibit the normal triplet spectrum. Other carotenoid pigments of known constitution exhibiting a single-banded spectrum are astacene (IV) (Karrer and Benz, *Helv. Chim. Acta*, 1934, **17**, 412; Karrer and Loewe, *ibid.*, p. 745; Karrer, Loewe, and Hübner, *ibid.*, 1935, **18**, 96) and euglenarhodone (V) (Tischer, *Z. physiol. Chem.*, 1936, **239**, 257). The ease with which by enolisation these can pass into (VI) and (VII) respectively, and the close similarity of the chromophores of these enolic forms to that of (III), not only show that the last-named accurately portrays the structure of myxoxanthin but lead to the conclusion that *the single-banded spectrum of such pigments is due to the simultaneous conjugation of the polar carbonyl group with two sets of unsaturated linkages.*

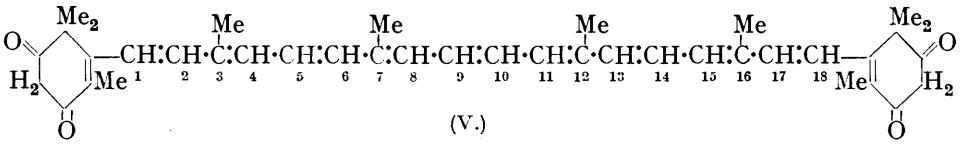
Chromatographic analysis of the hypophasic (methyl alcohol) pigments gave two crystalline carotenoids, one of which has been identified as lutein. The other is a new pigment *myxoxanthophyll*, $C_{40}H_{56}O_7$ (± 2H), m. p. 169—170°, with optical maxima at 5180,

* The spectroscopic identity of the triol capsanthol, obtained by the action of aluminium *iso*propoxide on capsanthin (Karrer and Hübner, *Helv. Chim. Acta*, 1936, **19**, 474), with α-carotene likewise affords proof of the structure deduced for capsanthin by Zechmeister and v. Cholnoky (*Annalen*, 1935, **516**, 30).

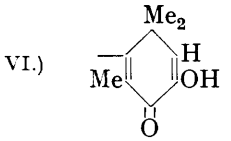
4845, and 4500 A. in chloroform. In contrast with all known xanthophylls, myxoxanthophyll is strongly lævorotatory ($[\alpha]_{Cd}$ — 255° in alcohol). From the tenacity with which it is



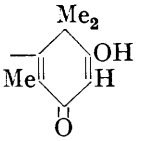
(IV.)



(V.)



VI.)



(VII.)

retained by adsorbents, it obviously contains a multiplicity of hydroxyl groups, while the absence of polar groups conjugated with the polyene system is suggested by the fact that alcoholic solutions are coloured only yellow to orange-red. Unfortunately, lack of material has precluded a detailed examination of the pigment.

Experimental.

Extraction of the Total Carotenoids of Oscillatoria rubrescens.—A mixture of the algal scum (90 l.) and methylated spirits (90 l.; 95%) was set aside for 7 days. After removal of the alcohol, the dehydrated alga (dry weight 7 kg.) was extracted first with absolute methylated spirits (30 l.) during 3 days and thrice with ether (3 × 30 l.). The combined extracts were concentrated to 12 l. (this and all subsequent evaporation processes being effected below 50° in an atmosphere of nitrogen and under reduced pressure) and saponified with aqueous potassium hydroxide by heating under reflux for 6 hours. The non-saponifiable fraction isolated by means of ether in the usual manner was partitioned between light petroleum (8 l.; b. p. 40—60°) and methyl alcohol (8 l.; 90%).

Epiphasic Pigments.

The concentrated light petroleum solution (1000 c.c.) deposited on standing β-carotene (200 mg.), after removal of which the filtrate was evaporated to dryness. The semi-crystalline residue was taken up in benzene (200 c.c.) and light petroleum (200 c.c.) and filtered through aluminium oxide (Merck), the chromatogram being developed by washing with benzene until a complete separation of the three zones was effected. Elutriation of the upper violet zone with benzene–methyl alcohol and evaporation of the elutriate gave the pigment as an uncrystallisable oil with optical maxima at 4820 and 4500 A. in carbon disulphide. The lowest zone (orange) furnished by similar treatment a further quantity of β-carotene (750 mg.), separating from pyridine in plates, m. p. 182°, with optical maxima at 5140 and 4840 A. in carbon disulphide.

Myxoxanthin.—The central zone was elutriated with benzene–methyl alcohol, and the elutriate washed free from methyl alcohol; the dried benzene solution was again chromatographed on aluminium oxide. After elutriation as above and removal of the solvent, the crude pigment was taken up in ether, and the solution diluted with methyl alcohol; on refrigeration, successive crops of a colourless contaminant were removed. The pigment obtained on concentration of the mother-liquor, followed by further dilution with methyl alcohol, was repeatedly crystallised from ether–methyl alcohol and finally from pyridine–methyl alcohol, yielding pure *myxoxanthin* in deep violet prisms with a high surface lustre (orange in thin layers), m. p. 168—169° (Berl-block, evacuated tube). Optical maxima : 4880 in carbon disulphide, 4730 in chloroform, 4700 in alcohol, 4650 A. in light petroleum (b. p. 70—80°). Myxoxanthin is readily soluble in chloroform, ether, and light petroleum, but only sparingly soluble in methyl alcohol. It is completely epiphasic on partition between light petroleum and 90% methyl alcohol; it is not adsorbed from light petroleum solution by calcium carbonate, but is strongly retained by both

calcium and magnesium hydroxides. The deep red zone formed on aluminium oxide is changed to a characteristic violet colour by washing with benzene. Colour reactions: chloroform and concentrated sulphuric acid → deep blue; chloroform and concentrated nitric acid → blue → green → yellow; formic acid (90%) → green; ether and concentrated hydrochloric acid, no colour change; methyl alcohol saturated with hydrogen chloride → greenish-blue; fused trichloroacetic acid → blue; antimony trichloride in chloroform → violet (Found: C, 86·7, 86·8; H, 10·0, 9·9; *M*, 560. $C_{40}H_{55}O$ requires C, 87·1; H, 9·9%; *M*, 550).

Microhydrogenation. 3·289 Mg. of myxoxanthin in decalin–acetic acid required 1·700 c.c. of hydrogen at 764·5 mm. and 18·2°, corresponding to 12·0 |=.

Biological test. Daily doses of 10 γ of myxoxanthin administered to vitamin-*A*-starved rats produced an average weight increase of 0·75 g. per rat per day.

Oxime. A solution of myxoxanthin (50 mg.) in pyridine (50 c.c.) was heated under reflux with hydroxylamine (1 g.) in alcohol (40 c.c.) for 12 hours; after dilution with water, the product was extracted with light petroleum, and the dried solution passed through aluminium oxide. The chromatogram was developed with benzene (300 c.c.), and the main red zone elutriated with benzene–methyl alcohol. After removal of solvent, the residue was crystallised four times from pyridine–methyl alcohol, from which *myxoxanthin oxime* (20 mg.) separated in glistening vermilion plates, m. p. 195—196° (Berl-block, evacuated tube). It possesses a single broad band with head at 4630 A. in chloroform (Found: N, 2·4. $C_{40}H_{55}ON$ requires N, 2·5%). It is less soluble than myxoxanthin in ether and light petroleum, and only sparingly soluble in methyl alcohol; it is completely epiphasic when partitioned between light petroleum and 90% methyl alcohol, and is strongly retained from light petroleum solution both by calcium carbonate and by aluminium oxide.

Microhydrogenation. 1·846 Mg. of the oxime in decalin–acetic acid required 1·01 c.c. of hydrogen at 765·7 mm. and 14·6°, corresponding to 13·2 |=.

Myxoxanthol.—A solution of myxoxanthin (40 mg.) in benzene (20 c.c.) and *iso*propyl alcohol (40 c.c.) was heated under reflux with aluminium *iso*propoxide (5 g.) for 24 hours. After dilution with aqueous potassium hydroxide (200 c.c.; 10%), the pigment was extracted with ether and, after removal of solvent, transferred to light petroleum and adsorbed on aluminium oxide. The chromatogram was developed by benzene–light petroleum (single orange band), and the pigment removed by means of benzene–methyl alcohol. After a single crystallisation from pyridine–methyl alcohol, myxoxanthol (2·5 mg.) was obtained in dense, deep-red crystals, m. p. 169—172°; lack of material precluded further purification. In contrast with that of myxoxanthin, the red band formed by myxoxanthol on aluminium oxide is not changed in colour on washing with benzene. Myxoxanthol is completely epiphasic on partition between light petroleum and 90% methyl alcohol.

Hypophasic Pigments.

Myxoxanthophyll.—The 90% methyl-alcoholic solution was concentrated (4 l.), and the pigments isolated by means of ether; after removal of solvent, the residue was dissolved in chloroform, and the solution filtered through calcium carbonate; after development with chloroform (filtrate A) two zones were formed. The main salmon-pink lower zone was elutriated with methyl alcohol, and the residue obtained after removal of solvent taken up in pyridine and strongly cooled, successive crops of a colourless wax-like solid being removed. Addition of benzene–light petroleum to the filtrate precipitated *myxoxanthophyll* (36 mg.), which, after two crystallisations from acetone, separated in violet needles, m. p. 169—170° (Berl-block, open tube) (Found: C, 74·5, 74·1; H, 8·8, 9·1. $C_{40}H_{56}O_7$ requires C, 74·1; H, 8·7%). Optical maxima: 5260, 4890, 4580 (pyridine); 5180, 4845, 4540 (chloroform); 5030, 4710, 4450 A. (alcohol). The pigment is readily soluble in pyridine and alcohol, moderately soluble in chloroform and acetone, and insoluble in light petroleum, ether, benzene, and carbon disulphide. Myxoxanthophyll is completely hypophasic on partition between light petroleum and 70% methyl alcohol, and is strongly adsorbed by aluminium oxide, from which it cannot be wholly removed. Colour reactions: chloroform and concentrated sulphuric acid → blue; chloroform and concentrated nitric acid → deep blue → green; alcohol–ether and concentrated hydrochloric acid, no colour; formic acid (90%) → yellow → green on heating; fused trichloroacetic acid → blue; chloroform and antimony trichloride → blue.

Lutein.—The chloroform filtrate A was passed through aluminium oxide, and the chromatogram developed by washing with chloroform. This consisted of a multiplicity of bands, the main zone being of an intense orange colour. Elutriation of this with methyl alcohol, followed by repeated crystallisation of the pigment from benzene–light petroleum, gave lutein (50 mg.),

m. p. 190—191°, with maxima at 5060, 4730 A. in carbon disulphide (Found : C, 84·15; H, 9·9. Calc. for $C_{40}H_{56}O_2$: C, 84·45; H, 9·9%).

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