LXXIV. ELOXANTHIN—A NEW CAROTENOID PIGMENT FROM THE PONDWEED *ELODEA CANADENSIS*

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ALTHOUGH the carotenoid pigments of land plants and of algae and other aquatic cryptogams have been the subject of numerous investigations, little attention has been given to the pigments of aquatic phanerogams. Rhodoxanthin, isolated from the berries of the yew tree (*Taxus baccata*) and thoroughly characterized by Kuhn & Brockmann [1933], was first detected by Monteverde [1893] in a pondweed (*Potamogeton natans*) and subsequently obtained in crystalline form from this source by Monteverde & Lubimenko [1913]. *Elodea canadensis*, the subject of the present work, was examined by microchemical methods by Tammes [1908], and by van Wisselingh [1914], and the presence of some carotenoid pigment in the leaves of the plant was demonstrated but no conclusion was reached regarding its nature.

A search of the literature revealed no other studies of the pigments of flowering water plants, so that the present investigation appears to open up a new field for research in connexion with the survey of plant carotenoids.

In the course of a large-scale investigation of the carotenoids from the leaves of *Elodea canadensis* there has been isolated, in addition to carotene, a new xanthophyll (for which the name *eloxanthin* is proposed) isomeric with flavoxanthin [Kuhn & Brockmann, 1932]. Elementary analysis agrees with the formula $C_{40}H_{56}O_3$, in harmony with Zerewitinov determinations indicating three active hydrogen atoms per mol. Catalytic hydrogenation indicates the presence of eleven double bonds, of which nine must be in conjugation since the absorption spectrum maxima (502, 472, 444 $m\mu$ in carbon disulphide) lie close to those of taraxanthin (501, 469, 441 $m\mu$ [Kuhn & Lederer, 1931; 1932]) and violaxanthin (500.5, 469, 440 $m\mu$ [Kuhn & Winterstein, 1931]). Unlike flavoxanthin, the pigment gives no colour reaction with 25 % hydrochloric acid. When crystallized from methyl alcohol it appears to retain alcohol of crystallization but this is removed on drying at 100° for half an hour *in vacuo*. The optical rotation is strongly positive, $[\alpha]_{D}^{18} = +225^\circ$ (in benzene).

EXPERIMENTAL

The fresh pondweed (5 cwt. wet) was air-dried and extracted with ether. The pigment, transferred to light petroleum (b.p. 70–80°), was saponified by refluxing for 2 hours with 2 % methyl alcoholic KOH. The non-saponifiable matter was ether-extracted, the ether was removed and replaced by methyl alcohol; on concentrating heavily sitosterol and hentriacontane separated and were removed. The filtrate was evaporated on the steam-bath and the dark red oily residue was washed with successive small quantities of cold methyl alcohol. The xanthophyll constituents dissolved, leaving a dark coloured semi-solid mass consisting of carotene and hentriacontane.

Carotene fraction. This residual pigmented mass, after adsorption on alumina from a solution in light petroleum (B.P. 70–80°), yielded crystalline carotene (M.P. 175° uncorr., maxima in light petroleum 481, 451 $m\mu$) and traces of the new xanthophyll (maxima in carbon disulphide 502, 472, 446 $m\mu$; in petroleum 473, 444 $m\mu$).

Xanthophyll fraction. The methyl alcohol solution was evaporated to dryness. The pigment, redissolved in a mixture of benzene and light petroleum (1 : 5) and adsorbed on alumina, formed a three-zoned chromatogram.

The lowest zone yielded a little crystalline carotene (M.P. 176° uncorr., maxima in light petroleum (B.P. 70–80°) 481, 451 $m\mu$), while the pigment of the very weak middle zone showed only an indefinite spectrum.

The carotenoid from the main (upper) zone, eluted with methyl alcohol, was precipitated under a layer of light petroleum by careful addition of water, filtered off and washed several times with a little warm petroleum (B.P. 70–80°). This redissolved very little of the solid. When the crude pigment (M.P. 105°) was recrystallized twice from methyl alcohol, the M.P. rose to 181–182° (uncorr.). This purified material was again recrystallized from methyl alcohol with a trace of ether, and well-formed glistening bronze leaflets were obtained, M.P. 182.5–183° (corr. Berl block, evacuated tube). The crystallization was carried out in stages; the successive fractions were analysed separately to test the homogeneity of the product and gave the following results.

Fraction	Wt. (mg.)	C (%)	H (%)
(i)	40	82.1	9.7
(ii)	150	82.3	9.3
(iii)	70	82.1	9.5
Calc. for $C_{40}H_{56}O_3$	—	82.1	9.7

The three oxygen atoms are all hydroxylic. (Found (Zerewitinov) 3.03, 3.03 active H atoms.) These latter determinations, together with catalytic hydrogenation experiments indicating 10.5 and 11.6 double bonds were kindly performed by Drs Roth and Möller of Heidelberg.

As a final test of homogeneity [compare Kuhn & Lederer, 1931] a portion of the pigment was adsorbed on calcium carbonate from petroleum containing a little benzene; it formed a single zone firmly held in the first cm. of the tower. By washing with benzene-petroleum (1 : 4) the zone was spread out over a depth of 15 cm.; sections (1 cm. deep) from the top and bottom were separately eluted and the two solutions were compared spectroscopically, the following maxima being obtained (in carbon disulphide):

Solution before adsorption	...	...	503, 472, 444 $m\mu$
Top fraction	...	...	503, 472, 445 $m\mu$
Bottom fraction	...	...	502, 472, 443 $m\mu$

Similar phenomena were observed on re-adsorbing a second portion of the pigment on calcium carbonate from carbon disulphide solution. There is thus no evidence of heterogeneity.

SUMMARY

A new xanthophyll, isomeric with flavoxanthin, has been isolated from the leaves of the flowering pondweed *Elodea canadensis*. It is accompanied by carotene; lutein, the normal leaf xanthophyll, was not detected.

Elementary analysis agrees with the formula $C_{40}H_{56}O_3$, Zerewitinov determinations indicate the presence of three hydroxyl groups and catalytic hydrogenation shows the molecule to possess eleven double bonds. The light absorption data suggest that only nine of the double bonds are in conjugation.

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