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ON THE NON-EXISTENCE OF ELOXANTHIN

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Key Word Index—*Elodea canadensis*; *E. densa*; *E. gigantea*; Hydrocharitaceae; distrubution of carotenoids; eloxanthin; lutein epoxide.

Abstract—Abandonment of the name eloxanthin is proposed. The principal carotenoids in various species of *Elodea* were (3*R*, 3′*R*, 6′*R*)-lutein (β, ε-carotene-3, 3′-diol) and β, β-carotene. The minor pigments were neoxanthin-X (5′, 6′-epoxy-6, 7-didehydro-5, 6, 5′, 6′-tetrahydro-β, β-carotene-3, 5, 3′-triol), 9′-*cis*-neoxanthin-X, 9- and 13-*cis*-violaxanthin (5, 6, 5′, 6′-diepoxy-5, 6, 5′, 6′-tetrahydro-β, β-carotene-3, 3′-diol), antheraxanthin (5, 6-epoxy-5, 6-dihydro-β, β-carotene-3, 3′-diol), neolutein A (13- or 13′-*cis*-lutein) and neolutein B (9- or 9′-*cis*-lutein). All attempts to isolate eloxanthin failed.

INTRODUCTION

As early as 1937 Hey [1] reported the isolation from the leaves of *Elodea canadensis* of a new xanthophyll, which he called 'eloxanthin' [2]. Later Karrer and Rutschmann [3, 4] suggested that eloxanthin might be identical with lutein epoxide. However, in spite of the relatively large amount of *E. canadensis* used, they failed to isolate lutein epoxide in a crystalline state. The conclusion, therefore, that "the properties of eloxanthin are so similar to those of xanthophyll epoxide (lutein epoxide), that the identity of the two pigments appeared very probable" [5] was also rather vague. Thus the question of what pigments are actually present in *E. canadensis* and which stereoisomer (3*R*, 5*R*, 6*S*, 3′*R*, 6′*R* or 3*R*, 5*S*, 6*R*, 3′*R*, 6′*R*) of lutein epoxide, if any, is identical with eloxanthin has been considered in our laboratory.

RESULTS AND DISCUSSION

The leaves of *E. canadensis*, *E. gigantea* and *E. densa* contain about the same carotenoids, namely neoxanthin-X, 9′-*cis*-neoxanthin-X (neoxanthin), violaxanthin, 9- and 13-*cis*-violaxanthin, lutein, neolutein A and B and β-carotene. The consistency of the distribution of carotenoids in *Elodea* species (Table 1) with that in other higher plants is remarkable [6]. The principal carotenoids are lutein (*ca* 36%) and β-carotene (*ca* 30%). The CD curve of lutein isolated from the leaves of *E. densa* showed that it had the same 3*R*,3′*R*,6′*R* configuration as luteins isolated from other sources [7]. The distribution of carotenoids depends only slightly on climatic and geographical factors (Table 1), and eloxanthin or lutein epoxide has never been detected in *Elodea* species. The isolation of the individual carotenoids was carried out by column (zone) chromatography, but the pigment composition was also confirmed by HPLC (Fig. 1).

Therefore, we suggest that Hey's finding was a case of misinterpretation due to the early, undeveloped chromatographic methods, and Karrer's school mistook lutein epoxide for the then still unknown neoxanthin-X, whose properties resemble those of lutein epoxide in many respects (λ_{max}, acid test, partition, etc.). Furthermore, as no new evidence for the existence of eloxanthin has been published since 1972, when the problem of eloxanthin had already been raised [8] we propose that the name eloxanthin should be abandoned.

EXPERIMENTAL

Biological materials and methods. E. canadensis and *E. gigantea* were collected in Toronto, May 1976 and sent to Pécs between wet sheets of filter paper in a light-tight plastic bag. *E. densa* (Tata) was grown at Lake Tata in mid-June 1971, and *E. canadensis* (Pécs) and *E. densa* (Pécs) in aquariums. The procedures were those normally employed in our laboratory and are summarized elswhere [9].

Pigment extraction and separation. The leaves of the *Elodea* species (10 g) were extracted with MeOH at 4–6° for 24 hr. The dark green methanolic soln was filtered and the material was homogenized with MeOH in an electric blender. The extraction was repeated with MeOH and Et_2O until complete decolouration of the powder. The combined MeOH and Et_2O extracts were washed with H_2O, dried (Na_2SO_4) and saponified with 30% methanolic KOH for 12 hr. Because of gel formation, the saponification procedure was repeated in the same way. The ethereal soln was washed free from chlorophyll and alkali with H_2O. Finally the dried ethereal soln was evapd and the residue dissolved in a mixture of C_6H_6–petrol (2:1) for chromatography. Zone chromatography on $CaCO_3$ (Biogal, Hungary) with C_6H_6–petrol (2:1) as eluant gave the chromatogram listed in Table 1. The most polar band (a mixture of 13-*cis*-violaxanthin, neoxanthin, violeoxanthin and neoxanthin-X) was subjected to re-chromatography on $CaCO_3$ with C_6H_6 containing 0.5%

Table 1. Quantitative distribution of carotenoids in various species of *Elodea*

Pigment	Percentage of total carotenoids present in: *E. canadensis* Pécs 1972	*E. canadensis* Toronto 1976	*E. canadensis* Pécs 1978	*E. gigantea* Toronto 1976	*E. densa* Pécs 1978
13-*cis*-Violaxanthin	*t*	*t*	1.0	*t*	*t*
Neoxanthin	3.8		5.3		3.9
Violeoxanthin	2.3	14.6	2.0	13.6	1.7
Neoxanthin-X	1.8		0.7		0.6
Neolutein A	5.3	9.4	4.2	8.7	4.7
Neolutein B	11.8	8.4	2.3	15.1	4.8
Violaxanthin	10.0	6.6	11.1	6.3	6.3
Antheraxanthin	3.1	*t*	2.2	*t*	1.0
Lutein	34.0	31.2	41.0	31.3	41.9
β-Carotene	27.9	29.8	30.2	25.0	35.1
Concentration mg/g dry wt	0.827	3.303	1.150	1.555	1.272

The carotenoids are shown in order of decreasing adsorption affinities. *t* = trace.

Me_2CO. After the usual procedures, the pigments were identified in soln by λ_{max}, partition test, mixed chromatography, furanoid oxide test, and isomerization. The carotenoids were quantitatively determined by measuring the extinction at λ_{max}. *E. densa* (300 g dry material) was also worked up on a preparative scale.

13-cis-*Violaxanthin*. $\lambda^{C_6H_6}_{max}$ nm: 478, 448 and 422; Q = 2.1; with 77% MeOH it was hypophasic (RP = 2.71); epoxide-furanoid test resulted in a hypsochromic shift of 43 nm ($\lambda^{C_6H_6}_{max}$ nm: 435, 408 and 386); iodine stereomutation gave all-*trans* violaxanthin, which was inseparable from authentic violaxanthin (ex *Viola tricolor*) on a mixed chromatogram ($CaCO_3$, C_6H_6).

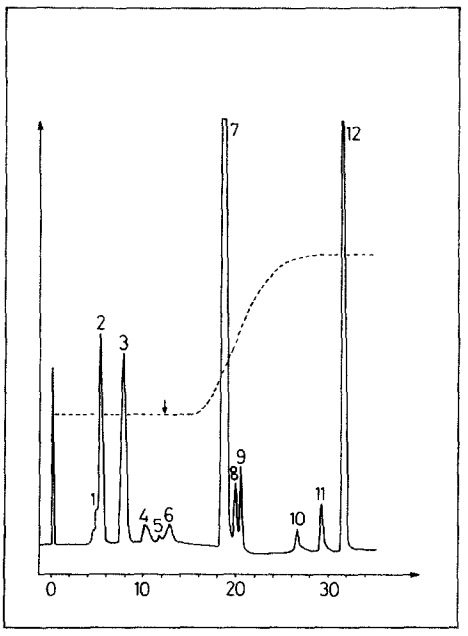

Fig. 1. Chromatogram of *Elodea canadensis* extract on a column of 200×4 mm i.d., packed with Nucleosil 10 C_{18}. Mobile phase: gradient of acetone–water (100:40–100:5), flow rate: 1.27 ml/min, pressure: 6.8–3.3 M Pa. Detection: UV–vis at 480 nm. 1, Neoxanthin-X; 2, neoxanthin; 3, violaxanthin; 4, 9-*cis*-violaxanthin; 5, 13-*cis*-violaxanthin; 6, antheraxanthin,; 7, lutein; 8, neolutein B; 9, neolutein A; 10, 11, *cis*-isomers of β-carotene; 12, β-carotene.

Neoxanthin. $\lambda^{C_6H_6}_{max}$ nm: 479, 448 and 423; with 65% MeOH it was hypophasic (RP = 3.26); the epoxide–furanoid test led to a hypsochromic shift of 19 nm ($\lambda^{C_6H_6}_{max}$ nm; 460, 432 and 408) inseparable from authentic neoxanthin (ex maple leaves) on a mixed chromatogram ($CaCO_3$, C_6H_6–Me_2CO, 97:3); iodine isomerization gave neoxanthin-X. From *E. densa*, 8.6 mg neoxanthin was also isolated, crystals, mp 135°.

Violeoxanthin (9-cis-*violaxanthin*). $\lambda^{C_6H_6}_{max}$ nm: 479, 448 and 423; $\varepsilon_{max\,cis\text{-}peak}$ = 11 600; with 77% MeOH it was hypophasic (RP = 2.68); the epoxide–furanoid test gave a hypsochromic shift of 24 nm ($\lambda^{C_6H_6}_{max}$ nm; 435, 407 and 386); iodine stereomutation resulted in all-*trans* violaxanthin, which was inseparable from authentic *trans*-violaxanthin (ex *Viola tricolor*).

Neoxanthin-X. $\lambda^{C_6H_6}_{max}$ nm; 483, 452 and 426; with 65% MeOH it was hypophasic (RP = 3.30); epoxide–furanoid test resulted in a hypsochromic shift of 22 nm ($\lambda^{C_6H_6}_{max}$ nm: 460, 431 and 408); inseparable from authentic neoxanthin-X (ex maple leaves) on a mixed chromatogram ($CaCO_3$, C_6H_6–Me_2CO, 97:3).

Neolutein A. $\lambda^{C_6H_6}_{max}$ nm; 483, 453 and 428; Q = 2.2; with 90% MeOH it was hypophasic (RP = 2.06); iodine stereomutation led to all-*trans* lutein, which was inseparable from an authentic sample (ex maple leaves).

Neolutein B. $\lambda^{C_6H_6}_{max}$ nm: 483, 453 and 430; $\varepsilon_{max\,cis\text{-}peak}$ = 11 900; with 90% MeOH it was hypophasic (RP = 2.01); stereomutation with iodine gave all-*trans* lutein, which was inseparable from authentic lutein (ex maple leaves).

Violaxanthin. $\lambda^{C_6H_6}_{max}$ nm: 483, 453 and 430; with 77% MeOH it was hypophasic (RP = 2.55); the epoxide–furanoid test resulted in a hypsochromic shift of 46 nm ($\lambda^{C_6H_6}_{max}$ nm: 437, 410 and 389). From *E. densa* 4.5 mg crystalline violaxanthin was isolated, mp 170°.

Lutein. $\lambda^{C_6H_6}_{max}$ nm: 488, 457 and 435; with 90% MeOH it was hypophasic (RP = 1.95); inseparable from authentic lutein (ex maple leaves) on a mixed chromatogram ($CaCO_3$, C_6H_6–petrol, 70:30). From *E. densa*, 85 mg lutein was isolated, crystals, mp 173°.

β-Carotene. $\lambda_{max}^{C_6H_6}$ nm: 492 and 463; with 95% MeOH it is epiphasic; inseparable from authentic β-carotene (ex *Daucus carota*). From *E. densa* 5.5 mg of crystalline β-carotene was obtained, mp 173°.

Antheraxanthin. $\lambda_{max}^{C_6H_6}$ nm: 488, 457 and 433; with 85% MeOH it was hypophasic (RP = 2.26); the epoxide–furanoide test showed a hypsochromic shift of 23 nm ($\lambda_{max}^{C_6H_6}$ nm: 465, 437 and 416; inseparable from authentic antheraxanthin (ex *Cucurbita pepo*) on mixed chromatography ($CaCO_3$, C_6H_6).

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