Leaf pigments of *Lycium europaeum*: seasonal effect on zeaxanthin and lutein formation

The genus *Lycium*, which belongs to the *Solanaceae*, represents a small group of plants, which can be found at the seaside of the Mediterranean, growing on dry and sandy strands. Five species are known of which *Lycium halimifolium* has been studied by ZECHMEISTER & CHOLNOKY [1]. They isolated physaliene, from its fresh fruits. This pigment was also obtained from fruits of *Lycium barbarum* [2].

In the present work the flavonoid, carotenoid and chlorophyll pigments extracted from leaves of *Lycium europaeum* are described, while the changes in content of xanthophylls and chlorophylls during a whole year are discussed.

Results and discussion

Flavonoids. — The crude ethanol extract from leaves of *Lycium europaeum* gave a yellow crystalline compound which for its melting point and absorption spectrum showed a great resemblance with the flavonoid glycoside rutin.

Acid hydrolysis of this pigment gave another yellow compound, which had its melting point, 312°C, and ultraviolet absorption spectrum identical with quercitin. This identity was confirmed by thin-layer co-chromatography with authentic quercetin, and the infrared absorption spectra in chloroform. A mixed melting point demonstrated definitively the identity.

Paper-chromatography on Whatman 1 paper showed that glucose and rhamnose were present in the acid fraction after hydrolysis. The identification of quercetin, glucose and rhamnose as hydrolysis products of the extracted yellow pigment confirmed that the flavonoid glycoside was identical with rutin. Its identity was confirmed by co-chromatography, mixed melting point and ultraviolet and infrared absorption spectra (Table 1) with an authentic sample of rutin. Also the acetyl derivative of the yellow pigment in its melting point, ultraviolet and infrared absorption spectra was identical with that of rutin [3].

The protective activity against capillar fragility and eye diseases of extracts of *Lycium europaeum* [4], like that of rutin [5], may be ascribed to this flavonoid.

TABLE 1.

Spectral characteristics of pigments isolated from « Lycium europaeum ».

	λ_{max} in nm				
	Ethanol	Hexane	Diethyl ether	Chloroform	Carbon disulpide
P_1 β-Carotene	—	~ 425,451,482	—	467,497	~ 450,485,520
P_2 Lutein	—	421,447,477	—	428,456,487	445,476,509
P_3 Zeaxanthin . . .	—	423,452,482	—	428,463,494	450,483,518
P_4 Chlorophyll *a* . .	—	—	436,660	—	—
P_5 Chlorophyll *b* . .	—	—	454,642	—	—
P_6 Rutin	258,360	—	—	—	—

~ Denotes an inflexion.

Ann. Ist. Super. Sanità (1969) **5**, 51-53.

Chlorophylls and carotenoids. — The visible and ultraviolet spectra of the fraction eluted before the pigments excluded the presence of uncolored C_{40}-polyenes.

Pigment P_1, which gave absorption spectra in different solvents identical with those of β-carotene, was identical with this pigment, as confirmed by thin-layer co-chromatography with an authentic sample of β-carotene.

Pigment P_2 showed its absorption spectra in different solvents identical with those of lutein (3,3'-dihydroxy-α-carotene). Its position on columns of MgO-Celite 503 (1 : 1,w/w) is identical with that of authentic lutein. The partition coefficient in hexane saturated with methanol-water (85 : 15,v/v) was 43 : 57, and co-chromatography on kieselgel G thin-layer in the solvent system methylene chloride-ethyl acetate (4 : 1, v/v) showed R_F 0.35. This R_F-value did not change after saponification.

Pigment P_3 showed its absorption spectra in different solvents identical with zeaxanthin (3,3'-dihydroxy-β-carotene). It took the same position on columns of cellulose and of MgO-Celite 503 (1 : 1,w/w) as authentic zeaxanthin, while co-chromatography on kieselgel G thin-layer in solvent system methylene chloride-ethyl acetate (4 : 1,v/v) showed identical R_F-values. Its R_F-value (0.24) did not change after saponification. The partition coefficient in hexane saturated with methanol-water 95 : 5 (v/v), was 11 : 89; 40 : 60 with 85 : 15 (v/v), and 87 : 13 with 75 : 25 (v/v), demonstrating the identity of pigment 3 with zeaxanthin.

Pigment P_4 had its absorption maxima in diethyl ether at 430 and 660 nm and characteristic spectra identical with those of chlorophyll *a*, and the pigment P_5 had spectra and absorption maxima in diethyl ether at 454 and 642 nm identical with those of chlorophyll *b* (Table 1).

Only trace amounts were found of carotenoid pigments which gave positive colour reactions for epoxy-groups [6].

Quantitative determinations of photosynthetic pigments were carried out in December. Only trace amounts of lutein were found, whereas zeaxanthin, in the non-esterified form, accounted for about 36 % of total carotenoids. For this unexpected high value it was decided to follow the carotenogenesis in the course of one year. The amount of lutein varied in proportion to the chlorophylls, whereas zeaxanthin showed different development (Fig. 1 and 2). From the end of July till December, the diminution of lutein corresponded almost exactly to the increase of zeaxanthin and from January till March it was lutein which appeared at the cost of zeaxanthin. This evident relationship led to a hypothesis of possible interconversion between these xanthophylls.

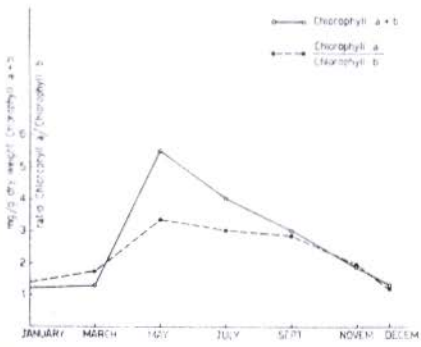

Fig. 1. — Changes in chlorophyll a and b in ratio $\frac{Chlorophyll\ a}{Chlorophyll\ b}$ in the course of one year in leaves of *Lycium europaeum*.

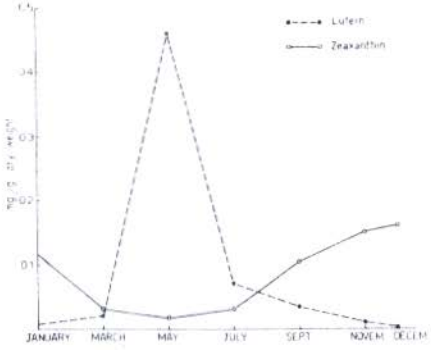

Fig. 2. — Changes in lutein and zeaxanthin in the course of one year in leaves of *Lycium europaeum*.

Several hypotheses on the formation and transformation of the xantophylls with α and β-ionone ring systems have been envisaged as in the higher plants as in the algae. CHOLNOKY *et al.*[7] retained valid the system :

zeaxanthin ⟶ antheraxanthin ⟶ lutein

but an auxiliary system :

β-carotene ⟶ epoxy-β-carotene ⟶ α-carotene

might exist.

According to SAPOZHNIKOV *et al.*[8] a conversion of lutein into violaxanthin and according to YAMAMOTO, NAKAYAMA & CHICHESTER[9] an interconversion of violaxanthin and zeaxanthin with antheraxanthin as intermediate might be possible. A part of the latter reaction sequence has been studied in greater detail by KRINSKY & BORDON[10] in intact or broken cell preparations of *Euglena gracilis*. COSTES[11] has reported the conversion of violaxanthin ^{14}C to β-carotene, lutein and lutein epoxide in isolated tomato and mais leaves' chloroplasts.

The present data show the proportionality between the variation of lutein and zeaxanthin and the presence of small amounts of epoxy-carotenoids. This has induced us to propose a possible interconversion of lutein and zeaxanthin via the same reaction mechanism (Fig. 3). On the other hand the increase of zeaxanthin is

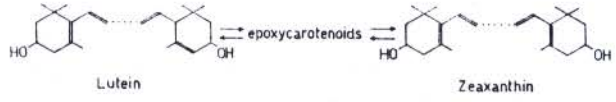

Fig. 3. — Probable interconversion between lutein and zeaxanthin with epoxy-carotenoids as intermediates in leaves of *Lycium europaeum*.

doubtless due to a process different from the aging reactions occurring in the leaves. In fact the percentage of xanthophylls increases during this period. But additionally, a reaction that appears to be general is their esterification as a consequence of changement in chloroplasts[12, 13].

The fact that zeaxanthin is always present in the leaves of *Lycium* in the non-esterified form supports the conclusion that its important increase does not depend on the final phase of aging, but on an interconversion in which the shifts depend on the physiological conditions of the plant.

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