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Carotenoids in certain higher plants from various ecological niches of Egypt

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Abstract

The carotenoids content in *Posidonia oceanica*, *Nelumbium nuciferum*, *Opuntia ficus-indica* and *Zygophyllum album* from different ecological niches in Egypt was studied.
Considerable differences, both qualitative and quantitative among four investigated plant species were found.

In addition to the green chlorophyll type of pigments, the carotenoid type also occur in plants. These pigments are present in the green parts of plants, in the flowers and in their fruit. A manifold significance is ascribed to them (Genevois et al. 1966). In his review of the literature on the importance of carotenoids in living organisms Krinsky (1971) drew attention to their luminous, non-luminous and thier metabolic functions. As regards their metabolic function, most of the carotenoids are provitamins of vitamin A or in thier activity are similar to that vitamin (Bauernfeind 1972). In their non-luminous function, the carotenoids take part, among other functions, in oxydoreduction processes. The luminous function of carotenoids includes the part played in the photosynthesis process (Sapozhnikov 1973) and protection of the photosythetic apparatus from the harmful effects of light rays (Mathis 1971, Bodea and Nicoara 1972).

Taking all these factors into cosideration, we decided it would be of interest to determine whether there are differences in the content of the various carotenoids in plants from different Egyptian ecological niches.

MATERIAL AND METHODS

Four plant species characteristc of the different ecological niches of Egypt were chosen for the investigations in July 1972. The first plant, *Posidonia oceanica*, is completely immersed in the Mediteranean Sea. The second plant, the lotus, *Nelum-*

bium nuciferum, have their leaves above the surface of the water; for the investigations leaves of this lotus from the Nile Delta were used. As a third plant, the ripe fruit of the cactus, *Opuntia ficus-indica,* picked on the Cairo-Alexandria roadside were collected, and the last plant investigated was the *Zygophyllum album.* For the investigations the shoots of this plant together with the flowers were collected on the Libian desert near El-Alamein.

Pigments

The carotenoids pigments were extracted by means of 95% acetone in a dark room. Saponification was carried out by means of 6% KOH in ethanol at a temperature of about 40°C for several minutes in the dark in a nitrogen atmosphere.

Chromatography

Columnar and thin-layer chromatography, described in detail in our previous papers (Czeczuga 1971, 1972), were used for the separation of the various carotenoids. A glass column approximately 1 cm dia. and 15-20 cm in length, filled with Al_2O_3, was used in column chromatography. The extract from plants was passed through the column after which the different fractions were eluted with the solvent systems. Silica gel was used for thin-layer chromatography, with the appropriate solvent systems, the R_f values being determined for each spot. For identification of β-carotene and lutein co-chromatography was applied using identical carotenoids.

Identification of pigments

The pigments were identified by the following methods: (a) behaviour on column chromatography; (b) absorption spectra of the pigments in various solvents were recorded by a Beckman spectrophotometer model 2400 DU; (c) the partition characteristics of the carotenoid between and 95% methanol using the method of Petracek, Zechmeister (1956); (d) comparison of R_f on thin-layer chromatography (with authentic β-carotene and luteine); (e) the presence of allylic hydroxyl groups was determined by the acid chloroform test (Karrer and Leumann 1951); and (f) the epoxide test (Krinsky and Goldsmith, 1960; Curl and Bailey, 1961).

Quantitative determination

Quantitative determination of the concentrations of carotenoid solutions were made from the quantitative absorption spectra. These determinations were based of the extinction coefficient $E^{1\%}_{1\,cm}$ at the wavelength of maximal absorbance in petroleum ether or hexane (Davies, 1965; Foppen, 1971).

RESULTS

The carotenoids found in the material, their absorption maxima in different solvents and the epiphase-hypophase ratio are presented in Table 1.

In the *Posidonia oceanica* (Table 2) the following carotenoids were found: α-carotene (5.8 %), β-carotene (13.6 %), cryptoxanthin (3.2 %), lutein (10.4 %), lutein-5,6-epoxide (64.5 %) and zeaxanthin (2.5 %). As the above data shows the dominant carotenoide in the *Posidonia oceanica* is lutein-5,6-epoxide comprising over half of the carotenoids found.

Table 1

Absorption maxima in other solvents and partition ratios of the carotenoid identified from extracts of plants.

Carotenoid	Solvents				Partition ratios
	petroleum ether	hexane	ethanol	benzene	
α-carotene	421, 445, 474				100:0
β-carotene	421, 451, 478				100:0
γ-carotene derivative	435, 467, 488				20:80
cryptoxanthin		425, 450, 478			87:13
isocryptoxanthin	447, 476				88:12
lutein		420, 445, 475	420, 445, 475		10:90
lutein-5,6-epoxide	420, 442, 470		419, 444, 472		32:68
zeaxanthin		425, 450, 478	425, 451, 482		11:89
isozeaxanthin			451, 478		20:80
antheraxanthin		421, 443, 470			5:95
eschscholtzxanthin			448, 475, 505	458, 485, 516	4:96
violaxanthin			419, 441, 471		3:97
neoxanthin			415, 438, 467		0:100

Table 2

The carotenoid composition of plants (% of total carotenoids)

Carotenoid	*Posidonia oceanica*	*Nelumbium nuciferum*	*Opuntia ficus-indica*	*Zygophyllum album*
α-carotene	5.8	2.9	—	3.7
β-carotene	13.6	23.0	—	33.0
γ-carotene derivative	—	—	1.9	—
cryptoxanthin	3.2	—	—	—
isocryptoxanthin	—	0.9	—	—
lutein	10.4	23.8	13.5	3.3
lutein-5,6-epoxide	64.5	48.1	24.2	5.0
zeaxanthin	2.5	0.4	10.2	1.1
isozeaxanthin	—	—	17.0	—
antheraxanthin	—	—	—	51.2
eschscholtzxanthin	—	—	13.7	2.7
violaxanthin	—	0.3	—	—
neoxanthin	—	0.6	—	—
unknown	—	—	19.5	—

In the lotus leaves, table 2, the presence of the following carotenoids was found: α-carotene (2.9 %), β-carotene (23.0 %), isocryptoxanthin (0.9 %), lutein (23.8 %), lutein-5,6-epoxide (48,1 %), zeaxanthin (0.4 %), violaxanthin (0.3 %) and neoxanthin (0.6 %). Here too the dominant carotenoid was lutein-5,6-epoxide. Considerable amounts of β-carotene and lutein were also found.

Chromatographic analysis of the ripe fruit of the cactus, *Opuntia ficus-indica*, revealed that they contain γ-carotene derivative (1.9 %), lutein (13.5 %), lutein-5,6--epoxide (24.2 %), zeaxanthin (10.2 %), isozeaxanthin (17.0 %) and eschscholtzxanthin (13.7 %). In the fruit of this cactus, an unidentified carotenoid was found; it gave maximum absorption at 465, 497, 545 and was of a hypophase character. This carotenoid comprised (19.5 %) of the total amount of carotenoids.

The desert plant, *Zygophyllum album* was found to have α-carotene (3.7 %), β-carotene (33.0 %), lutein (3.3 %), lutein-5,6-epoxide (5.0 %), zeaxanthin (1.1 %), antheraxanthin (51.2 %) and eschscholtzxanthin (2.7 %). This species differend from the other species investigated in that antheraxanthin dominated, comprising one have of the carotenoid content and β-carotene over 30 %.

DISCUSSION

As the data presented above show, the plant species investigated differ in their carotenoid content and in the quantitative relations of the carotenoids. α- and β-carotene were found in all the species except the fruit of the *Opuntia ficus-indica*. The α-carotene content of the remaining species ranged between 2.9-5.8 %. The greatest differences in the content of the various carotenoids were noted in the β-carotene content. The smallest amount was found in the aquatic plant, *Posidania oceanica* (13.6 %), more in the lotus leaves (23.0 %) and the largest amount in the desert plant, *Zygophyllum album* (33.0 %).

Cryptoxanthin, and its carotenoid derivative-isocryptoxanthin, were found only in the *Posidania oceanica* and *Nelumbium nuciferum*. Lutein was found in all four species the largest amount, 23.8 % in the lotus leaves, the smallest, 3.3 %, in the desert plant, *Zygophyllum album*. The amounts of lutein-5,6-epoxide decreased gradually in each species; in the aquatic plant, *Posidonia oceanica*, 64.5 % of all the carotenoids consisted of lutein 5.6-epoxide whereas in the desert plant, *Zygophyllum album*, it comprised only 5.0 %. It should here be noted that such a large amount of lutein-5,6-epoxide as that found in the *Posidonia oceanica* is similar to that found in certain higher plants (Valadon and Mummery 1968, Hanny et al. 1972, Quackenbush and Miller 1972, Ignasik and Lesins 1973). Is should also be emphasized that comparatively small amounts of lutein and lutein-5,6-epoxide were found in the desert plant, *Zygophyllum album*. Zeaxanthin on the other hand was found in all four species of plants investigated. Apart from the cactus fruit in which this carotenoid together with isozeaxanthin comprised about 30 % but in the other species only a low percentage.

Of particular interest are the large amount (51.2%) of antheraxanthin found only in the desert plant. *Zygophyllum album*. Goodwin (1955) mentions the presence of this carotenoid in fruits and plant seeds. Hanny et al. (1972) reported finding antheraxanthin in *Hibiscus syriacus* leaves, and Valadon and Mummery (1968), Quackenbush and Miller (1972) and Navarro et al. (1972) in the flowers of certain plants. It should be noted that the amount of carotenoid found in these plants by the above-mentioned authors was of the order of 1%. Eschscholtzxanthin was found in the cactus fruits and the *Zygophyllum album* only; in the cactus 13.8% of the carotenoid content consisted of this carotenoid. In addition violaxanthin and neoxanthin were found in small amounts (0.3-0.6%) in the lotus leaves only.

The data presented here show that there are considerable differences, both qualitative and quantitative, in the carotenoid content of the plant species investigated. Particularly significant differences were found in the *Opuntia ficus-indica* fruit and the *Zygophyllum album* shoots.

SUMMARY AND CONCLUSIONS

Investigations have been carried out on the carotenoids in plants from different Egyptian ecological niches

The following carotenoids were found:

a) *Posidonia oceania*: α-carotene (5.8%), β-carotene (13.6%), cryptoxanthin (3.2%), lutein (10.4%), lutein-5,6-epoxide (64.5%) and zeaxanthin (2.5%):

b) *Nelumbium nuciferum*: α-carotene (2.9%), β-carotene (23.0%) isocryptoxanthin (0.9%), lutein (23.8%), lutein-5,6-epoxide (48.1), zeaxanthin (0.4%), violaxanthin (0.3%) and neoxanthin (0.6%):

c) *Opuntia ficus-indica* (fruit): γ-carotene derivative (1.9%), lutein (13.5%), lutein -5,6-epoxide (24.2%), zeaxanthin (10.2%), isozeaxanthin (17.0%), eschscholtzxanthin (13.7%) and unknown carotenoid (19.5%);

d) *Zygophyllum album*: α-carotene (3.7%), β-carotene (33.0%), lutein (3.3%), lutein-5,6-epoxide (5.0%), zeaxanthin (1.1%), antheraxanthin (51.2%) and eschscholtzxanthin (2.7%).

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Karotenoidy u niektórych wyższych roślin z różnych środowisk ekologicznych Egiptu

Streszczenie

Zbadano zawartość karotenoidów u *Posidonia oceanica*, *Nelumbium nuciferum*, *Opuntia ficus-indica* i *Zygophyllum album* jako przedstawicieli z różnych nisz ekologicznych Egiptu.

Stwierdzono istotne różnice ilościowe, jak i jakościowe karotenoidów między czteroma badanymi gatunkami roślin:

1. *Posidonia oceania*: α-karoten (5,8%), β-karoten (13,6%), kryptoksantyna (3.2%), luteina (10,4%), 5.6-epoksydluteina (64,5%) i zeaksantyna (2,5%).
2. *Nelumbium nuciferum*: α-karoten (2,9%), β-karoten (23,0%), izokryptoksantyna (0,9%), luteina (23,8%), 5,6-epoksydluteina (48.1%), zeaksantyna (0,4%), violaksantyna (0,3%) i neoksantyna (0,6%).
3. *Opuntia ficus-indica* (owoce): pochodne γ-karotenu (1,9%), luteina (13,5%), 5,6-epoksyluteina (24,2%), zeaksantyna (10,2%), izozeaksantyna (17,0%), eschscholtzksantyna (13,7%) i karetonoidy nierozpoznane (19,5%).
4. *Zygophyllum album*: α-karoten (3,7%), β-karoten (33,0%), luteina (3,3%), 5,6-epoksydluteina (5,0%), zeaksantyna (1,1%), antheraksantyna (51,2%) i eschscholtzksantyna (2,7%).