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Investigations on carotenoids in lichens. XII. Some species from the Pyrenean Peninsula

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Abstract

By means of column and thin-layer chromatography, the authors investigated the presence of various carotenoids in thalli of 12 species of lichens from the Pyrenean Peninsula. The following carotenoids were found: lycophyll, lycoxanthin, β-carotene, α-, β-cryptoxanthin, lutein, lutein epoxide, zeaxanthin, antheraxanthin, canthaxanthin, phoenicoxanthin, adonixanthin, α-doradexanthin, astaxanthin, diatoxanthin, neoxanthin, violaxanthin, mutatochrome, mutatoxanthin and rhodoxanthin. The total content of carotenoids ranged from 2.299 (*Cetraria cucullata*) to 39.402 $mg \cdot g^{-1}$ dry weight (*Pseudoevernia furfuraceae*).

Key words: lichens, carotenoids

INTRODUCTION

During our investigations on the presence of carotenoids in lichens (Czeczuga 1979a, b, 1980b, c, 1985b) we found that some carotenoids are characteristic for species of certain genera. These investigations also showed that environmental factors have a marked effect on the total carotenoid content and on the presence of the various carotenoids in the species. It was with this in view that the present studies were undertaken to investigate the presence of carotenoids and their total content in the thalli of some lichen species growing in the Pyrenean Peninsula. The investigations were felt to be warranted since recently, increasing attention has been paid to carotenoids as pigments which can be of value in the taxonomy of plants. Such studies have been published on algae (Liaaen-Jensen 1977, Weber and Wettern 1980) and on fungi (Valadon 1976). It is therefore possible that the results of these investigations on various species of lichens may be applied in chemotaxonomy.

MATERIAL AND METHODS

Twelve species of lichens belonging to 4 families were investigated (Table 1). They were collected from the Pyrenean Peninsula, Spain.

The thalli were cleaned of all organic debris, macerated, placed in dark bottles and covered with acetone. The air above the fluid in the bottle

Table 1

Investigated species of lichens

Family and species	Collected from	Locality
Stictaceae		
Stica pulmonaceae Ach.	*Fagus sylvatica* L.	Madrid
Lecanoraceae		
Placodium teicholitum Ach.	*Pinus pinaster* L.	Madrid
Parmeliaceae		
Cetraria cucullata Bell.	Land	Ciudad Real
Parmelia borrei Turn.	*Pinus pinaster* L.	Madrid
Parmelia physodes Ach.	*Pinus pinaster* L.	Madrid
Platysma glaucum L.	*Pinus pinaster* L.	Madrid
Pseudoevernia furfuraceae Ach.	*Querqus pirenaica* Wit.	Segovia
Usneaceae		
Evernia divaricata Ach.	*Pinus pinaster* L.	Segovia
Evernia prunastri Ach.	*Querqus pirenaica* Wit.	Segovia
Ramalina farinaceae L.	*Querqus pirenaica* Wit.	Segovia
Ramalina scopolorum Dicks.	*Querqus pirenaica* Wit.	Segovia
Usnea lariciana Vain.	*Pinus pinaster* L.	Madrid

was changed to nitrogen to ensure an anaerobic atmosphere. The samples were kept in a refrigerator until used for chromatographic analysis of the carotenoid content.

The carotenoid pigments were extracted by means of 95% acetone in a dark room. Saponification was carried out by means of 10% KOH in ethanol at a temperature of about 20°C for 24 hours in the dark in the nitrogen atmosphere.

Column and thin-layer chromatography, described in detail in our previous paper (Czeczuga 1980a) were used for separation of various carotenoids. A glass column (Quickfit-England) approximately 1 cm in diameter and 15-20 cm in length, filled with Al_2O_3 was used in column chromatography. The extract was passed through the column and the different fractions were eluted with the solvent. 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 carotenoids, co-chromatography was employed with the use of standard carotenoids (Hoffman-La Roche and Co. Ltd. — Basle, Switzerland and Sigma Chemical Company — USA).

The pigments were identified by the following methods: a) behavoiur in column chromatography, b) absorption spectra of the pigments in various solvents recorded on a Beckman spectrophotometer model 2400 Du, c) partition characteristics of the carotenoid between hexane and 95% methanol, d) comparision of R_f values in thin-layer chromatography, e) the presence of allylic hydroxyl groups determined by the acid-chloroform test, f) the epoxide test, and g) mass spectrum (Vetter et al. 1971).

Quantitative determinations of the concentrations of carotenoid solutions were made from the absorption spectra. These determinations were based on the extinction coefficient E 1% cm^{-1} at wavelengths of maximal absorbance in petroleum ether or hexane (Davies 1976).

RESULTS

The results of the chromatographic analysis of the lichen thalli are presented in Table 2 and Fig. 1. As can be seen from Table 3, 15

Table 2

List of the carotenoids from the investigated material

Carotenoid	Structure (see Fig. 1)	Semisystematic name
Lycoxanthin	A-X-B	ψ,ψ-caroten-16-ol
Lycophyll	B-X-B	ψ,ψ-carotene-16,16'-diol
β-Carotene	D-X-D	β,β-carotene
α-Cryptoxanthin	C-X-F	β,ε-caroten-3'-ol
β-Cryptoxanthin	D-X-F	β,β-caroten-3-ol
Canthaxanthin	G-X-G	β,β-carotene-4,4'-dione
Lutein	E-X-F	β,ε-carotene-3,3'-diol
Lutein epoxide	E-X-I	5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol
Zeaxanthin	F-X-F	β,β-carotene-3,3'-diol
Antheraxanthin	F-X-I	5,6-epoxy-5,6-dihydro-β,β-carotene-3,3'-diol
Diatoxanthin	F-Y_1-F	7,8-didehydro-β,β-carotene-3,3'-diol
α-Doradexanthin	E-X-H	3,3'-dihydroxy-β,ε-caroten-4-one
Adonixanthin	F-X-H	3,3'-dihydroxy-β,β-caroten-4-one
Phoenicoxanthin	G-X-H	3-hydroxy-β,β-carotene-4,4'-dione
Astaxanthin	H-X-H	3,3'-dihydroxy-β,β-carotene-4,4'-dione
Neoxanthin	I-Y-M	5,6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro-β,β-carotene-3,5,3'-triol
Violaxanthin	I-X-I	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro-β,β-carotene-3,3'-diol
Mutatochrome	D-Y-K	5,8-epoxy-5,8-dihydro-β,β-carotene
Mutatoxanthin	F-Y-L	5,8-epoxy-5,8-dihydro-β,β-carotene-3,3'-diol
Rhodoxanthin	N-X_1-N	4',5'-didehydro-4,5'-retro-β,β-carotene-3,3-dione

Table 3

The carotenoid composition of lichen species from the *Parmeliaceae* family (% of total carotenoids)

Carotenoid	*P. furfuraceae*	*P. borrei*	*P. physodes*	*C. cucullata*	*P. glaucum*
β-Carotene	7.5				
α-Cryptoxanthin	2.4				5.5
β-Cryptoxanthin	4.9	17.2	12.2	trace	20.7
Canthaxanthin	25.2			9.5	
Lycophyll		7.5			
Lycoxanthin				27.2	
Lutein	28.0	3.1	37.3		
Lutein epoxide	14.8	2.6			7.1
Zeaxanthin	10.8	2.9	31.3	46.3	17.4
Antheraxanthin					14.0
Phoenicoxanthin			4.3		
Diatoxanthin		5.2			
Neoxanthin		11.1			8.8
Astaxanthin	6.2	4.3	14.8	16.9	26.4
Mutatochrome		3.0			
Total content, mg·g^{-1} dry wt.	39.402	16.953	19.629	2.299	26.647

Table 4

The carotenoid composition of lichen species from the *Usneaceae* family (% of total carotenoids)

Carotenoid	*E. divaricata*	*E. prunastri*	*R. farinaceae*	*R. scopolorum*	*U. lariciana*
β-Carotene	4.0			5.5	
α-Cryptoxanthin		3.3	12.4		17.9
β-Cryptoxanthin	2.8	trace	16.2	5.9	15.2
Canthaxanthin		13.9	39.6		
Lycoxanthin		3.5			
Lutein	7.7		1.7		16.8
Lutein epoxide	4.1		26.9		
Zeaxanthin	2.9	7.6		29.2	
Antheraxanthin		24.7	10.2		
Phoenicoxanthin		5.2			
Adonixanthin					32.5
Violaxanthin					5.9
α-Doradexanthin			6.7	4.7	
Astaxanthin	58.8	44.8	12.2	14.9	14.4
Mutatochrome	13.9				
Mutatoxanthin			13.7		3.3
Total content, mg·g^{-1} dry wt.	9.564	14.230	11.871	21.358	5.740

carotenoids were determined in the 5 species of lichens of the *Parmeliaceae* family. Of the 15 carotenoids found, β-cryptoxanthin, zeaxanthin and astaxanthin occurred in the thalli of all of the representatives of the *Parmeliaceae* studied. Another interesting finding was that of the presence of lycophyll

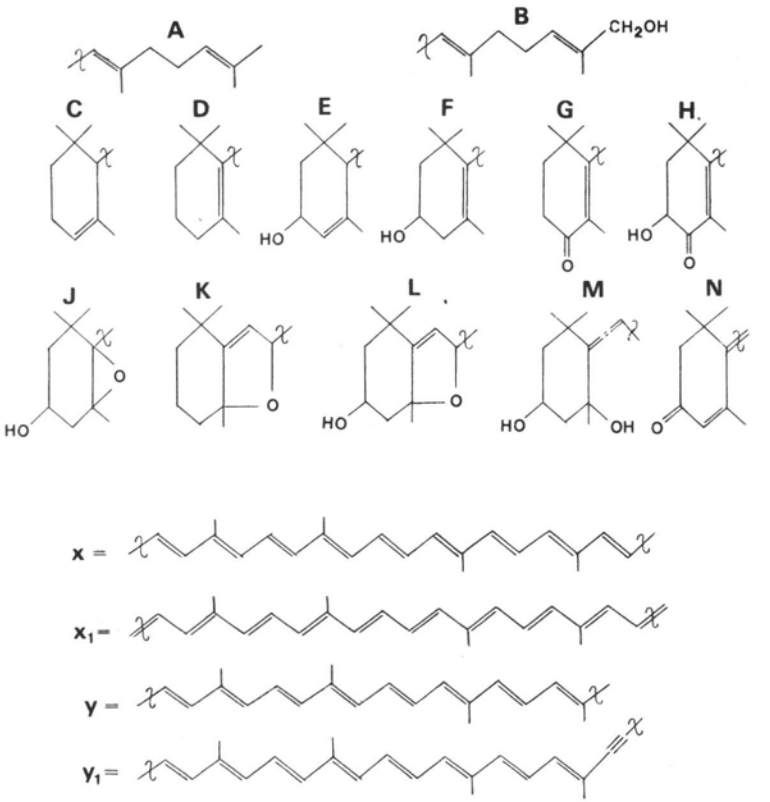

Fig. 1. Structural features of carotenoids from lichens

and diatoxanthin (*Parmelia borrei*), as well as lycoxanthin (*Cetraria cucullata*). The total carotenoid content in the representatives of this family ranged from 2.299 to 39.402 $mg \cdot g^{-1}$ dry wt.

In the representatives of the *Usneaceae* family (Table 4), 16 carotenoids were determined of which β-cryptoxanthin and astaxanthin were found in all the *Usneaceae* species. The total carotenoid content of this material varied between 5.740 $mg \cdot g^{-1}$ (*Usnea laricina*) and 21.358 $mg \cdot g^{-1}$ of dry mass (*Ramalina scopolorum*).

In the representatives of the *Stictaceae* family (Table 5), 7 carotenoids were established of which lutein epoxide (24.3%) and zeaxanthin (23.9%) constituted the largest amounts. The total carotenoid content was 15.531

Table 5

The carotenoid composition of lichen species from the *Stictaceae* and *Lecanoraceae* families (% of total carotenoids)

Carotenoid	*S. pulmonaceae*	*P. teicholitum*
β-Carotene	17.3	
β-Cryptoxanthin	3.9	22.5
Canthaxanthin	2.4	
Lutein	8.7	5.4
Lutein epoxide	25.3	
Zeaxanthin	23.9	14.1
Rhodoxanthin		43.1
Astaxanthin	18.6	15.0
Total content, $mg \cdot g^{-1}$ dry wt.	15.531	8.541

$mg \cdot g^{-1}$ dry wt. In the representative of the *Lecanoraceae* family (*Placodium teicholitum*), 5 carotenoids were identified, their total content being 8.541 $mg \cdot g^{-1}$ dry wt. An interesting finding was that of rhodoxanthin which occurred in large amounts (43.1%).

DISCUSSION

Of the carotenoids found in the species studied from the Pyrenean Peninsula, the most noteworthy are the 2 carotenoids of the acyclic group (lycophyll and lycoxanthin), 4 carotenoids of the ketocarotenoid group (phenicoxanthin, adonixanthin, α-doradexanthin and rhodoxanthin) and one of the acetylenic group, diatoxanthin. The other carotenoids have been found in many other lichens (Czeczuga 1979a, b, 1980b, c, 1983, 1985b).

Lichens are composed of algae and fungi in symbiosis so that the carotenoids occurring in them are usually produced by the two symbionts. As regards the acyclic carotenoids found in the lichens studied, they appear to have been produced by the fungus component since such carotenoids have not been noted to date in algae (Wettern and Weber 1979). Only lycopene from which these two carotenoids are formed has been found in algae. These carotenoids have been found in some species of lichens from the *Peltigera* and *Usnea* families (Czeczuga 1979a, b). As for the carotenoids of the ketocarotenoid group, as phoenicoxanthin, adonixanthin and α-doradexanthin, they are in the pathway of the conversion of β-carotene or some of its derivatives into astaxanthin (Katayama et al. 1970). Adonixanthin has been found in plants, usually the higher plants, above all, in those of the *Adonis* genus (Egger 1965, Neamtu and Bodea 1968, Czygan 1969) and in the marine alga. *Acetabularia mediter-*

ranea (Kleinig and Egger 1967). Phoenicoxanthin is formed from canthaxanthin which has frequently been noted in algae (Czeczuga 1979c), fungi (Czeczuga 1980d) and lichens (Czeczuga 1985b). As regards α-doradexanthin, it is, according to Buchecker et al. (1978), formed from 3'-epilutein which has been described in plants as being a lutein isomer under the name of calthaxanthin (Dabbagh and Egger 1974). The presence of rhodoxanthin, in large amounts, in the thalli of *Placodium teicholitum* is noteworthy. This carotenoid, a derivative of zeaxanthin, has been found mainly in some representatives of mosses (Czeczuga 1985a) and the needles of coniferous trees (Ida 1981), although it has been found in the fungus *Epicoccum nigrum* (Foppen and Gribanovski-Sassu 1968). Diatoxanthin is also formed from zeaxanthin and has been found in algae of various families, among others, the *Chlorophyceae* (Liaaen-Jensen 1977, Czeczuga 1979c).

The total carotenoid content in the thalli of the studied species varied between 2.99 mg and 39.402 $mg \cdot g^{-1}$ dry wt., which in comparison with the data from other species, fall in the range of average values noted in other previously studied species (Czeczuga 1979a).

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Badania karotenoidów u porostów. XII. Niektóre gatunki z półwyspu Pirenejskiego

Streszczenie

Stosując chromatografię kolumnową i cienkowarstwową badano występowanie poszczególnych karotenoidów w plechach 12 gatunków porostów z półwyspu Pirenejskiego. Ustalono występowanie następujących karotenoidów: likofil, likoksantyna, β-karoten, α-, β-kryptoksantyna, luteina, epoksyd luteiny, zeaksantyna, anteraksantyna, kantaksantyna, feonikoksantyna, adoniksantyna, α-doradeksantyna, astaksantyna, diatoksantyna, neoksantyna, wiolaksantyna, mutatochrom, mutatoksantyna oraz rodoksantyna. Ogólna zawartość karotenoidów wahała się od 2,299 (*Cetraria cucullata*) do 39,402 mg·g^{-1} suchej masy (*Pseudoevernia furfuraceae*).