

## Investigations on carotenoids in lichens. XXII. Lichens from the Upper Tracja Valley (Bulgaria)

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### Abstract

Column and thin-layer chromatography were used to study the occurrence of various carotenoids in 17 lichen species collected in the Upper Tracja Valley in Bulgaria. The following carotenoids were found: lycoxanthin, lycophyll,  $\alpha$ -,  $\beta$ -carotene, rubixanthin,  $\alpha$ -,  $\beta$ -cryptoxanthin, lutein, lutein epoxide,  $\beta$ -carotene epoxide, zeaxanthin, antheraxanthin, canthaxanthin, adonixanthin,  $\alpha$ -doradexanthin, phoenicoxanthin, mutatochrome, mutatoxanthin, diatoxanthin and neoxanthin. The total carotenoid contents ranged from 5.737 (*Peltigera horizontalis*) to 63.659  $\mu\text{g g}^{-1}$  dry weight (*Xanthoria perietina*).

*Key words:* lichens, carotenoids

### INTRODUCTION

As symbiotic plants, lichens are found everywhere, under every latitude. Each geographical region is, as is well known, characterized by specific climatic-environmental properties which favor those species which are best suited to them. Environmental factors, in turn, exert an important influence on organisms. This is seen, in the case of lichens, in the physiological processes occurring in the thalli (Kershaw 1972, Hooker 1980, Tegler and Kershaw 1980, Moser al. 1983, Ino 1985) and in their chemical composition (Skult 1984, Huneck et al. 1987).

On investigating the carotenoid contents of various species of lichens, we recently drew our attention to the occurrence and amount of these pigments in the thalli of lichens from various geographical regions. We have already examined in this respect some of the lichens from Lapland (Czczuga 1986), northern Siberia (Czczuga and Schelkunova 1986), Greenland (Czczuga and Alstrup 1987), Galilee (Czczuga and Bubrick 1986), the Pyrenean Peninsula (Czczuga and Cifuentes 1986), Canary Islands

(Czezcuga et al. 1988), Brazil (Czezcuga and Xavier-Filho 1987a), Argentina (Czezcuga and Ferraro de Corona 1987) as well the Antarctic (Czezcuga et al. 1986, Czezcuga and Xavier-Filho 1987b).

This paper is a continuation of this problem and deals with the lichens of the Upper Tracja Valley in Bulgaria, which is known for its specific climatic-environmental conditions (Chlebicki 1985).

#### MATERIAL AND METHODS

The material was collected in May, 1984 in the vicinity of Hisar, about 40 km south of the city of Plovdiv. Thalli of the following lichen species were studied: *Parmelia conspersa* (Ehrh.) Ach., *Parmelia glabra* (Schaer.) Nyl., *Parmelia incurva* (Pers.) Fr., *Parmelia sulcata* Th. Tayl. *Hypogymia physodes* (L.) Nyl., *Cladonia fimbriata* (L.) Fr., *Cladonia furcata* (Huds.) Schrad., *Cladonia minor* (Hag.) Vain., *Cladonia tenuis* (Flk.) Harm., *Cladonia uncialis* (L.) Web., *Xanthoria parietina* (L.) Th. Fr., *Xanthoria papilliferan* (Vain.) Poelt., *Solorina saccata* (L.) Ach., *Peltigera horizontalis* (Huds.) Baumg., *Dermatocarpon miniatum* (L.) Mann., *Ramalina pollinaria* (Westr.) Ach. and *Physcia tenella* D.C. em. Bitt.

Samples of equal weight of the thalli were homogenized, covered with acetone and kept in dark bottles under refrigeration until chromatographic analysis. Separation of the various carotenoids was done using column and thin-layer chromatography. Preceding chromatography, the material was subjected to hydrolysis in 10% KOH in a nitrogen atmosphere at room temperature for 24 h. The details of separation using column and thin-layer chromatography are given in a previous paper (Czezcuga 1980c). After hydrolysis, the extract was applied to a column packed with  $Al_2O_3$ . Column length ranged from 15 to 25 cm (Quickfit — England). The fractions were eluted using various solvent systems (Czezcuga 1980c).

In addition to column chromatography, each extract was separated into its individual components by means of thin-layer chromatography. It was carried out on glass plates covered with silica gel, using various solvent systems. The Rf values were then determined according to commonly used criteria.

Identification of the individual carotenoids was based on the following: a) the behavior on column chromatography, b) absorption maximums in various solvents determined using a Beckman 2400-Du spectrophotometer, c) partition characteristic in hexane and 95% methanol, d) comparison of Rf values on thin-layer chromatography — co-chromatography of standards from Hoffman-La Roche and Co. Ltd, Basel Switzerland, and Sigma Chemical Co., USA, was carried out to identify the individual carotenoids, e) the presence of allylic hydroxyl groups was determined using acid-chloroform, f) the epoxide test. The quantitative determinations of the carotenoids were based on



Table 1

List of the carotenoids from the investigated materials

Carotenoid	Structure (see Fig. 1)	Semisystematic name
Lycoxanthin	A - R - B	$\psi$ , $\psi$ -caroten-16-ol
Lycophyll	B - R - B	$\psi$ , $\psi$ -carotene-16,16'-diol
$\alpha$ -Carotene	C - R - D	$\beta$ , $\epsilon$ -carotene
$\beta$ -Carotene	C - R - C	$\beta$ , $\beta$ -carotene
Rubixanthin	A - R - E	$\beta$ , $\psi$ -caroten-3-ol
$\alpha$ -Cryptoxanthin	E - R - D	$\beta$ , $\epsilon$ -caroten-3-ol
$\beta$ -Cryptoxanthin	E - R - E	$\beta$ , $\beta$ -caroten-3-ol
Lutein	E - R - F	$\beta$ , $\epsilon$ -carotene-3,3'-diol
$\beta$ -Carotene epoxide	C - R - G	5,6-epoxy-5,6-dihydro- $\beta$ , $\beta$ -carotene
Lutein epoxide	F - R - H	5,6-epoxy-5,6-dihydro- $\beta$ , $\epsilon$ -carotene-3,3'-diol
Zeaxanthin	E - R - E	$\beta$ , $\beta$ -carotene-3,3'-diol
Antheraxanthin	E - R - H	5,6-epoxy-5,6-dihydro- $\beta$ , $\beta$ -carotene-3-3'-diol
Canthaxanthin	I - R - I	$\beta$ , $\beta$ -carotene-4,4'-dione
Adonixanthin	E - R - K	3,3'-dihydroxy- $\beta$ , $\beta$ -carotene-4-one
$\alpha$ -Doradexanthin	F - R - K	3,3'-dihydroxy- $\beta$ , $\epsilon$ -carotene-4-one
Phoenicoxanthin	I - R - K	3-hydroxy- $\beta$ , $\beta$ -carotene-4,4'-dione
Astaxanthin	K - R - K	3,3'-dihydroxy- $\beta$ , $\beta$ -carotene-4-4'-dione
Violaxanthin	H - R - H	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- $\beta$ , $\beta$ -carotene-3,3'-diol
Mutatochrome	C - R <sub>1</sub> - M	5,8-epoxy-5,8-dihydro- $\beta$ , $\beta$ -carotene
Mutatoxanthin	E - R <sub>1</sub> - N	5,8-epoxy-5,8-dihydro- $\beta$ , $\beta$ -carotene-3,3'-diol
Diatoxanthin	E - R <sub>1</sub> - L	7,8-didehydro- $\beta$ , $\beta$ -carotene-3,3'-diol
Neoxanthin	H - R <sub>1</sub> - O	5,6-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro- $\beta$ , $\beta$ -carotene-3,5,3'-triol

In the thalli of 5 species of the *Cladoniaceae* family (Table 3), 15 carotenoids were also found, and such carotenoids as  $\beta$ -cryptoxanthin, lutein epoxide and zeaxanthin were shown to be present in the thalli of all of the examined members of this family. The total carotenoid content in the studied members of the *Cladoniaceae* family ranged from 2.249 (*Cladonia uncialis*) to 11.811  $\mu\text{g g}^{-1}$  dry weight (*Cladonia tenuis*).

The carotenoids which were found to be present in the representatives of the first two families were also found in the remaining 7 lichen species from various families. (Table 4). Only the presence of  $\beta$ -carotene epoxide in the thalli of *Ramalina pollinaria* deserves special mention. The total carotenoid contents ranged from 5.737 (*Peltigera horizontalis*) to 63.659  $\mu\text{g g}^{-1}$  dry weight (*Xanthoria parietina*).

Table 2

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

Carotenoid	<i>Parmelia conspersa</i>	<i>P. glabra</i>	<i>P. incurva</i>	<i>P. sulcata</i>	<i>Hypogymnia physodes</i>
$\beta$ -Carotene	9.2		8.8		24.7
$\alpha$ -Cryptoxanthin	8.3	21.5			
$\beta$ -Cryptoxanthin	26.0	40.8	9.9		
Lycoxanthin			17.9	26.2	10.8
Canthaxanthin			17.9	8.8	
Lutein				8.8	24.4
Lutein epoxide	29.0	6.7	23.2	8.5	trace
Zeaxanthin	trace	19.4	10.8	7.8	7.4
Antheraxanthin				16.1	
Diatoxanthin					7.0
Rubixanthin				23.8	
$\alpha$ -Doradexanthin	14.8		11.5		
Astaxanthin	12.7	11.6			8.2
Mutatochrome					10.6
Mutatoxanthin					6.8
Total content, $\mu\text{g g}^{-1}$ dry wt.	2.756	2.438	4.271	5.351	10.565

Table 3

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

Carotenoid	<i>Cladonia fimbriata</i>	<i>C. furcata</i>	<i>C. minor</i>	<i>C. tenuis</i>	<i>C. uncialis</i>
$\beta$ -Carotene			20.1	12.8	6.9
$\alpha$ -Cryptoxanthin	22.5	34.1	7.7	5.2	
$\beta$ -Cryptoxanthin	15.0	6.8	9.0	14.1	24.7
Lycoxanthin				5.0	
Lutein		22.1		7.9	
Lutein epoxide	5.6	6.0	4.3	14.4	8.8
Zeaxanthin	17.2	26.0	10.0	8.9	trace
Antheraxanthin			24.8	26.9	
Diatoxanthin			4.6		
Adonixanthin	21.1	5.0			
Neoxanthin					20.4
$\alpha$ -Doradexanthin			4.0	4.8	
Astaxanthin	18.6		10.0		28.8
Mutatochrome			5.5		
Mutatoxanthin					10.4
Total content, $\mu\text{g g}^{-1}$ dry wt.	5.996	6.549	7.931	11.811	2.249

Table 4

The quantitative carotenoid composition of lichen species from other families (% of total carotenoids)

Carotenoid	<i>Xanthoria parietina</i> <sup>a</sup>	<i>Xanthoria papillifera</i> <sup>a</sup>	<i>Solorina saccata</i> <sup>b</sup>	<i>Peltigera horizontalis</i> <sup>b</sup>	<i>Dermatocarpon miniatum</i> <sup>c</sup>	<i>Ramalina pollinaria</i> <sup>d</sup>	<i>Physcia tenella</i> <sup>e</sup>
$\alpha$ -Carotene				9.4	6.1		
$\beta$ -Carotene	0.7	7.3			10.8		8.7
$\beta$ -Carotene epoxide						5.5	
$\alpha$ -Cryptoxanthin			7.0	20.6			7.6
$\beta$ -Cryptoxanthin	0.7	5.6	18.0	8.5	8.3	14.0	7.6
Lycophyll				24.3			
Lycoxanthin			8.5				
Canthaxanthin	1.0						
Lutein			12.4				
Lutein epoxide	4.5	3.6					
Zeaxanthin		0.7	7.3	20.0	6.8	19.6	35.8
Antheraxanthin			18.1	3.2			
Diatoxanthin						11.6	
Adonixanthin			11.2	6.3	12.3	24.9	8.5
Phoenicoxanthin					6.8		14.2
Astaxanthin		1.8			39.9	9.3	7.1
Mutatochrome			17.5	7.7	9.0	15.1	
Mutatoxanthin	93.1	81.0					
Violaxanthin							10.5
Total content, $\mu\text{g g}^{-1}$ dry wt.	63.659	11.986	7.522	5.737	5.895	9.620	6.407

a - *Teloschistaceae* family, b - *Peltigeraceae*, c - *Dermatocarpaceae*, d - *Usneaceae*, e - *Pyxinaceae*.

#### DISCUSSION

So-called common carotenoids, found in the thalli of numerous lichen species from all parts of the world, were also the main carotenoids found in the studied species from the Upper Tracja Valley. However, several rather rare carotenoids were found here. These are  $\alpha$ -carotene, diatoxanthin, adonixanthin, phoenicoxanthin,  $\alpha$ -doradexanthin, and rubixanthin. As far as  $\alpha$ -carotene is concerned, it is known that this carotene occurs much less frequently and in lesser amounts than  $\beta$ -carotene. However, in all of the species of lower and higher plants, with the exception of fungi, a derivative of that carotene, lutein and especially its epoxy form, are found in large amounts. In lichens,  $\alpha$ -carotene has hitherto been found in only a few species from the family *Peltigeraceae* (Czeżuga 1980a, 1986). Similarly as in higher plants, the

epoxy form of lutein is found in frequently large amounts in almost all lichen species.

As far as diatoxanthin as a derivative of zeaxanthin is concerned, it is often found in algae (Czeczuga 1979b). So far, it has been found in lichens only in some of the representatives of the *Parmeliaceae* family (Czeczuga and Cifuentes 1986).

The next three carotenoids such as adonixanthin, phoenicoxanthin and  $\alpha$ -doradexanthin belong to the group of ketocarotenoids and are part (the first two) of the metabolic pathway from  $\beta$ -carotene or some of its derivatives to astaxanthin, while doradexanthin is on the pathway from lutein (a derivative of  $\alpha$ -carotene) to astaxanthin. These carotenoids are often found in animals, especially crustaceans and fish (Czeczuga 1974, Czeczuga and Kiziewicz 1985, Czeczuga et al. 1986). Adonixanthin is also found in some algae (Czeczuga 1979b) and in higher plants from the genus *Adonis*, in which it was described for the first time (Goodwin 1980). In respect to lichens, all of these carotenoids have, albeit sporadically, been demonstrated in representatives of different lichen families (Czeczuga 1979c, 1983, 1985). The finding of these carotenoids in some species of lichens from the Upper Tracja Valley widens the list of lichens in which the mentioned ketocarotenoids occur. In respect to the finding of rubixanthin, and in large amounts (23.8%) in the thalli of *Parmelia sulcata*, it should be stated that this carotenoid has hitherto been found both in higher and lower fungi (Czeczuga 1978, 1979a, 1980d), and also in some higher plants, especially in petals and fruit (Goodwin 1980). It is a derivative of  $\gamma$ -carotene and is a monocyclic carotenoid. In lichens, rubixanthin has already been found in the thalli of *Ramalina farinaceae* as the representative of the family *Usneaceae* (Czeczuga 1979d), in several species belonging to *Parmeliaceae* (Czeczuga 1980b) and in 5 of 11 studied species from the genus *Peltigera* (Czeczuga 1980a). Since rubixanthin has not yet been found in algae, but only in fungi, one may assume that in lichens it comes from the fungal component.

This time too, the analysis has shown that in the thalli of lichens from the genus *Xanthoria* the dominant carotenoid is mutatoxanthin, similarly as in other species belonging to this genus (Czeczuga 1983). In the thalli of two species from the genus *Xanthoria* from the Upper Tracja Valley, mutatoxanthin made up as much as 81-93.1% of all of the carotenoids. As is known, carotenes in plants play a role of, among others, additional antennae which absorb shorter waves than do the chlorophylls. They also are a protective barrier against the photodestructive action of light waves on the plant's photosynthetic apparatus (Goodwin 1980). Because lichens from the genus *Xanthoria* are found in ecological niches with good irradiation, it may be concluded that carotenoids play a major role in protecting these species from the photodestructive effect of the sun.

The thalli of *Cladonia fimbriata*, *Cladonia furcata*, *Xanthoria parietina* and

*Dermatocarpon minutum* have already been examined for their carotenoid content. The first three species of this group are from northeastern Poland (Czeżuga 1983, 1985), while *Dermatocarpon minutum* – from the eastern part of Greenland (Czeżuga and Alstrup 1987). In the thalli of all four of these species from the Upper Tracja Valley, carotenoids were found that were not present in specimens of the same species but growing under different latitudes. These are  $\alpha$ -carotene,  $\alpha$ -cryptoxanthin and such ketocarotenoids as: canthaxanthin, adonixanthin, phoenicoxanthin and astaxanthin. In addition, only in *Xanthoria parietina* was the same carotenoid (mutatoxanthin) the dominant one in both stands, while the thalli of the remaining three species had different dominant carotenoids on different stands. The thalli of *Cladonia fimbriata* from northeastern Poland contained the most lutein epoxide, while in those from the Upper Tracja Valley,  $\alpha$ -cryptoxanthin dominated.  $\alpha$ -Cryptoxanthin also was the dominant carotenoid here in the thalli of *Cladonia furcata*, while the thalli of this species in Poland were richest in  $\beta$ -carotene. In turn, the thalli of *Dermatocarpon minutum* from Greenland contained lutein epoxide in the largest amounts, and astaxanthin when they came from the Upper Tracja Valley. Quantitative differences also occurred. Only did the thalli of *Dermatocarpon minutum* from the Upper Tracja Valley contain more carotenoids, while the thalli of the remaining three species from this area were poorer in carotenoids than when from northeastern Poland.

The carotenoid contents of the remaining examined species from the Upper Tracja Valley were average, characteristic for European species. The significantly higher amounts of carotenoids in the thalli of two species from the genus *Xanthoria* also fall within the range found for other species of this genus sampled for analysis from various other latitudes.

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*Badania karotenoidów u porostów. XXII. Porosty z Doliny Górno-Trackiej (Bułgaria)*

Streszczenie

Stosując chromatografię kolumnową i cienkowsarstwową, badano występowanie poszczególnych karotenoidów u 17 gatunków porostów zebranych w Dolinie Górno-Trackiej w Bułgarii. Stwierdzono następujące karotenoidy: likoksantyn, likofil,  $\alpha$ -,  $\beta$ -karoten, rubiksantyna,  $\alpha$ -,  $\beta$ -kryptoksantyna, luteina, epoksyd luteiny, epoksyd  $\beta$ -karotenu, zeaksantyna, anteraksantyna, kantaksantyna, adoniksantyna,  $\alpha$ -doradeksantyna, foenikoksantyna, mutatochrom, mutatoksan-tyna, diatoksan-tyna i neoksantyna. Ogólna zawartość karotenoidów wahała się od 5,737 (*Peltigera horizontalis*) do 63,659  $\mu\text{g g}^{-1}$  suchej masy (*Xanthoria perietina*).