CAROTENOIDS IN CERTAIN LICHENS OF BIAŁOWIEŻA FOREST *

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ABSTRACT

Column-, and thin-layer chromatography revealed the presence of the following carotenoids in the thalli of 29 lichen species from Białowieża Forest: α -carotene, β -carotene, α -cryptoxanthin, β -cryptoxanthin, lutein, 3'-epilutein, zeaxanthin, β -carotene monoepoxide, lutein epoxide, antheraxanthin, 3'-hydroxyechinenone, α -doradexanthin, canthaxanthin, astaxanthin, neoxanthin, violaxanthin and mutatoxanthin. The total content of carotenoids ranged from 16.83 (*Cladonia rangiferina*) to 92.98 $\mu\text{g g}^{-1}$ dry wt (*Xanthoria parietina*). There were differences in carotenoid composition, concentration of each carotenoid, and in the total content in the thalli of four species collected from niches with different insolation.

KEY WORDS: lichens, carotenoids, Białowieża Forest

INTRODUCTION

The Białowieża Forest constitutes a noteworthy type of tree stand in the European Lowlands. In addition to the remains of the old stands which are still preserved there are numerous types of green flora very rarely encountered elsewhere. The Białowieża Forest also contains a very rich lichen flora (Błoński et al. 1888, Błoński and Drymer 1889, Krawiec 1938, Lecewicz 1954, Tobolewski 1955, Rydzak 1962, 1969 and Fabiszewski 1968).

For this reason, while carrying out studies of carotenoids in lichens from various ecological niches, we decided to analyse the content of these pigments in the thalli of species from the very specific ecological niches still prevalent in the Białowieża Forest.

MATERIALS AND METHODS

Lichens (29 species, see Table 2) were collected in August 1990 (5-15 g dry weight) from Rezerwat Przyszosowy on the road from Hajnówka to Białowieża.

The thalli were cleaned of all organic debris, placed in dark glass bottles and macerated with acetone. The air above the fluid in the bottle was replaced with nitrogen to ensure an anaerobic atmosphere. Samples were kept in a refrigerator until analysed for their carotenoid content by column and thin-layer chromatography. Carotenoid pigments were extracted with 95% acetone in a dark room. Saponification was carried out with 10% KOH in ethanol at about 20 °C for 24 h in the dark in a nitrogen atmosphere.

Column and thin-layer chromatography, described in detail in Czeczuga (1980), were used to separate various carotenoids. These were identified by performing replicate chromatography with standard carotenoids (Hoffman-La Roche and

Co. Ltd., Basel, Switzerland, and Sigma Company, USA). Pigments were identified on the basis of: (a) their behaviour on column chromatography; (b) their absorption spectra in various solvents (Beckman spectro-photometer model 2400); (c) their partition between hexane and 95% methanol; (d) their R_f values (TLC); (e) the presence of allylic hydroxyl groups, determined by the acid-chloroform test; (f) the epoxide test, and (g) the mass spectrum of end groups (see Vetter et al. 1971 for basic methodology). Concentration of carotenoid solutions were determined from the absorption spectra, on the basis of the extinction coefficient $E 1\% \text{ cm}^{-1}$ at wavelengths absorbance in petroleum ether or hexane (Davies 1976). The structures of the carotenoids have been reported previously by Straub (1987).

RESULTS

In the thalli of the lichen species studied 17 carotenoids were identified, of which a finding worthy of note was the presence of carotenoids which have to date rarely been found in lichens, that is α -cryptoxanthin, β -carotene monoepoxide and 3'-hydroxyechinenone (Table 1). Another interesting finding was the presence of β -carotene and lutein in the thalli of all the species studied, and lutein epoxide and astaxanthin in the thalli of most of these species (Table 2). There were differences in the carotenoid composition, concentration of each carotenoid, and in the total content in thalli of four species collected from niches with different insolation (Table 3). The total carotenoid content in the material varied from 16.83 (*Cladonia rangiferina*) to 92.98 $\mu\text{g g}^{-1}$ dry wt (*Xanthoria parietina*).

DISCUSSION

The rare carotenoids noted in these studies have been found occasionally in some lichen species previously. The carote-

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TABLE 1. List of carotenoids from the investigated thalli lichens

Carotenoid	Structure (see Fig. 1)	Semisystematic name
1. α -carotene	A – R – B	β , ϵ -carotene
2. β -carotene	B – R – B	β , β -carotene
3. α -cryptoxanthin	A – R – D	β , ϵ -caroten-3-ol
4. β -cryptoxanthin	B – R – D	β , β -caroten-3-ol
5. lutein	C – R – D	β , ϵ -carotene-3,3'-diol
6. 3'-epilutein	C – R – D	β , ϵ -carotene-3,3'-diol (stereoisomeric)
7. zeaxanthin	D – R – D	β , β -carotene-3,3'-diol
8. β -carotene monoepoxide	B – R – E	5,6-epoxy-5,6-dihydro- β , β -carotene
9. lutein epoxide	C – R – F	5,6-epoxy-5,6-dihydro- β , ϵ -carotene-3,3'-diol
10. antheraxanthin	D – R – F	5,6-epoxy-5,6-dihydro- β , β -carotene-3,3'-diol
11. 3'-hydroxyechinenone	D – R – G	3'-hydroxy- β , β -caroten-4-one
12. α -doradexanthin	C – R – H	3,3'-dihydroxy- β , ϵ -caroten-4-one
13. canthaxanthin	G – R – G	β , β -carotene-4,4'-dione
14. astaxanthin	H – R – H	3,3'-dihydroxy- β , β -carotene-4,4'-dione
15. neoxanthin	F – R ₁ – I	5,6-epoxy-6,7-didehydro-5,6,5',6'- β , β -carotene-3,5,3'-triol
16. violaxanthin	F – R – F	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β , β -carotene-3,3'-diol
17. mutatoxanthin	D – R ₁ – K	5,8-epoxy-5,8-dihydro- β , β -carotene-3,3'-diol

TABLE 2. Carotenoid distribution in lichens from Białowieża Forest

Family and species	Carotenoid (see Table 1 and Fig. 1)	Major carotenoids (%)	Total content ($\mu\text{g g}^{-1}$ dry wt)
Caliciaceae			
Chaenotheca chrysocephala (Turn.) Th. Fr.	1,2,5,7,9,10,14,15	9(22.3)	44.29
Chrysotrichaceae			
Crocynia membranacea (Dicks.) A. Zahlbr.	1,2,4,5,7,9,15,16	7(25.9)	52.78
Peltigeraceae			
Peltigera canina (L.) Willd.	2,4,5,7,9,14,16,17	2(46.9)	40.09
Lecideaceae			
Lecidea scalaris (Ach.) Ach.	2,5,9,14,16	2(27.1)	29.51
Cladoniaceae			
Cladonia cenotea (Ach.) Schaer.	1,2,5,7,9,14,16	14(39.5)	21.01
Cladonia chlorophaea (Flk.) Spreng.	1,2,4,5,7,9,10,14	9(34.8)	32.42
Cladonia deformis (L.) Hoffm.	1,2,5,6,8,9,10,12,14,15,16	9(28.53)	28.64
Cladonia elongata (Ach.) Nyl. var. squamosa Anders	1,2,5,7,9,10,14,16	2(27.0)	25.07
Cladonia fimbriata (L.) Fr.	1,2,4,5,9,14,16	5(33.)	33.94
Cladonia furcata (Huds.) Schrad. for. fissa Flk.	1,2,4,5,7,9,14,15,16	9(24.8)	28.06
Cladonia macilenta (Hoffm.) Nyl. var. squamosa Vain.	1,2,4,5,7,9,10,13,14,17	17(34.0)	21.45
Cladonia minor (Hag.) Vain.	1,2,4,5,7,9,12,13,14,16	9(23.8)	35.66
Cladonia polydactyla (Flk.) Spreng.	1,2,5,7,9,12,14,16	9(32.6)	53.55
Cladonia rangiferina (L.) Web.	1,2,4,5,9,12,13,14	14(18.6)	16.83
Cladonia rangiformis Hoffm. var. foliosa (Duf.) Flk.	1,2,5,6,9,10,14,16	9(39.6)	41.74
Pertusariaceae			
Pertusaria discoidea (Pers.) Malme	1,2,4,5,6,7,9,10,14,16	16(27.1)	39.68
Permeliaceae			
Cetraria islandica (L.) Ach.	1,2,5,6,7,9,10,15,16,17	9(38.6)	27.32
Hypogymnia physodes (L.) Nyl. var. labrosa (Ach.) Wats	1,2,5,6,7,11,12,13,14	12(39.3)	20.53
Parmelia cetrarioides Del.	2,5,6,7,9,10,14,16	9(48.0)	46.87
Parmelia fuliginosa (Wib.) Nyl.	2,4,5,6,8,9,10,16,17	17(29.8)	61.75
Parmelia sulcata Th. Tayl.	2,3,5,6,9,10,12,14	14(23.1)	18.77
Usneaceae			
Usnea comosa (Ach.) Rohl.	1,2,4,5,7,9,14,16	5(27.8)	32.76
Ramalina farinacea (L.) Ach.	1,2,5,7,9,13,14,16	5(38.0)	51.10
Ramalina fraxinea (L.) Ach.	2,4,5,6,9,10,12,13,14	2(21.7)	35.15
Ramalina pollinaria (Westr.) Ach.	2,5,9,13,14,16	14(34.0)	48.23
Evernia prunastri (L.) Ach.	1,2,4,5,7,8,9,10,14,16	7(29.2)	34.92
Teloschistaceae			
Xanthoria parietina (L.) Th. Fr.	2,4,5,9,14,15,17	17(79.5)	92.98
Physciaceae			
Physcia pulverulenta (Schreb.) Hampe emend. Sandst.	1,2,4,5,7,9,10,11,15,16	9(28.6)	43.82
Anaptychia ciliaris (L.) Koreb.	1,2,5,7,9,16,17	9(36.7)	76.26

TABLE 3. Carotenoid content in lichens grown in different intensities of sunlight

Carotenoid	<i>Cetraria islandica</i>		<i>Cladonia deformis</i>		<i>Cladonia rangiferina</i>		<i>Hypogymnia physodes</i>	
	sunlight ^x	shade ^{xx}	sunlight	shade	sunlight	shade	sunlight	shade
a-carotene		1.4		6.9				
b-carotene	12.2	27.9	6.6	13.2	17.3	28.3	2.8	6.1
b-cryptoxanthin							4.0	9.0
lutein + 3'-epilutein	1.4	21.3	7.5	9.1	trace	27.0	1.6	2.2
zeaxanthin	11.1	trace						
b-carotene monoepoxide			4.5	trace	2.2	trace		
lutein epoxide	32.5	14.7	39.3	26.3	55.4	11.8	19.4	5.0
antheraxanthin	5.3	1.7	2.2	trace	5.8	12.8	34.7	trace
a- doradexanthin			17.4					
astaxanthin			8.4	13.7	10.7	8.3		1.2
neoxanthin	6.2	trace	2.7	10.3			34.3	33.7
violaxanthin	21.3	30.0	11.4	20.5	9.5	11.8	3.2	34.3
mutatoxanthin	10.0	3.0						
Total content µg g ⁻¹ dry wt n ⁻³	25.73±0.23	29.97±0.31	25.08±0.82	33.41±0.74	19.85±0.18	23.88±0.54	26.18±0.32	29.82±0.54

x – intensity of sunlight in the exposed sites ranged from 102.0 to 69.0 W m⁻²
xx – shady sites intensity of sunlight ranged from 42.0 to 13.8 W m⁻²

Fig. 1. Structural features of carotenoids from investigated materials (see Table 1)

noid α -cryptoxanthin has been noted in the thalli of *Alectoria ochroleuca* from Kamchatka (Czeczuga et al. 1989), whereas β -carotene monoepoxide has been reported in the thalli of the *Thamnolia vernicularis* from Spitsbergen (Czeczuga and Olech 1990). As regards 3'-hydroxyechinenone, this carotenoid has been noted to date in the thali of *Ramalina eckonii* from the Antarctica (Czeczuga and Olech 1989).

The analysed material contained a comparatively large number of poorly oxidized carotenoids, among others, β -carotene and lutein found in the thalli of all the species investigated.

Our previous studies demonstrated that the presence of β -carotene or lutein in large amounts is associated with poor lighting, that is, in shaded areas (Czeczuga 1986). In the case of conditions in the Białowieża Forest, the insolation corresponds to a considerable extent to that of shaded areas. The presence of large amounts of these two carotenoids was observed in all the species studied from Ireland (Czeczuga and Richardson 1989), Iceland (Czeczuga and Kristinsson 1992), and the forests of Tasmania (Czeczuga and Kantvilas 1990).

This is also confirmed by the differing content of these carotenoids in the thalli of the same species taken from well-lit and poorly-lit sites in the Białowieża Forest. In the thalli from the shadier sites the total carotenoid content and violaxanthin was also higher. A similar observation was made in other lichen species and applied not only to carotenoids but also to chlorophylls (Czeczuga 1981).

A particularly marked increase in these pigments occurs under the influence of blue rays which, as we know, are dominant in areas of forest shade (Minnaert 1958).

It is known that for species of the same genus a set of carotenoids is often characteristic. Thus for 10 species of the *Cladonia* genus, the characteristic carotenoids were found to be α -, β -carotene, lutein, lutein epoxide and astaxanthin. Again, for 3 species of the *Parmelia* genus from the Biało-

wieża Forest, the carotenoids common to each were β -carotene, lutein, 3'-epilutein, lutein epoxide and antheraxanthin, and those common to 3 species of the *Ramalina* were β -carotene, lutein, lutein epoxide and astaxanthin. It should be mentioned here that the carotenoids common to 10 species of the *Cladonia* genus collected from Mecklenburg (Germany) were β -carotene, zeaxanthin, lutein epoxide and violaxanthin (Czacuga and Doll 1991). On the other hand, the carotenoids common to the thalli of 20 species of the *Cladonia* genus from the United States of America were found to be only lutein epoxide and violaxanthin (Czacuga 1993). Furthermore, in the thalli of *Cladonia rangiferina* collected from different continents the constant carotenoids proved to be β -carotene and lutein epoxide (Czacuga et al. 1991). It is also interesting to note that many of the lichen species from the Białowieża Forest contained the ketocarotenoid astaxanthin.

In recent years, chemical methods have been increasingly used since together with the classical methods they provide better taxonomic possibilities. In recent taxonomic studies of fungi (Valadon 1976) and algae (Weber and Wettern 1980), data on presence or absence of the various carotenoids have been used.

The total carotenoid content in the present material is somewhat higher than that noted so far in the lichen thalli from other environments in this latitude (Czacuga 1988).

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KAROTEIDY U NIEKTÓRYCH POROSTÓW PUSZCZY BIAŁOWIESKIEJ

STRESZCZENIE

Stosując chromatografię kolumnową i cienkowarstwową badano występowanie poszczególnych karotenoidów u 29 gatunków porostów zebranych w Puszczy Białowieskiej. Stwierdzono następujące karotenoidy: α -carotene, β -carotene, α -cryptoxanthin, α -cryptoxanthin, lutein, 3'-epilutein, zeaxanthin, β -carotene monoepoxide, lutein epoxide, antheraxanthin, 3'-hydroxyechinenone, α -doradexanthin, canthaxanthin, astaxanthin, neoxanthin, violaxanthin i mutatoxanthin. Ogólna zawartość karotenoidów wahała się od 16.83 (*Cladonia rangiferina*) do 92.08 $\mu\text{g g}^{-1}$ suchej masy (*Xanthoria parietina*). Ustalono różnice w zawartości karotenoidów w plechach porostów ze stanowisk o różnej intensywności insolacji słonecznej (Tabela 3).

SŁOWA KLUCZOWE: porosty, karotenoidy, Puszcza Białowieska.