

## Carotenoids in Certain Lichens from the State of Illinois<sup>1</sup>

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### ABSTRACT

Column and thin-layer chromatography revealed the presence of the following carotenoids in the thalli of 21 lichen species from Illinois:  $\alpha$ -,  $\beta$ -carotene, rubixanthin,  $\beta$ -cryptoxanthin, lutein, 3'-epilutein, zeaxanthin, lycoxanthin, lycopene-5,6-epoxide,  $\beta$ -carotene epoxide,  $\beta$ -carotene diepoxide, lutein epoxide, antheraxanthin, violaxanthin, mutatoxanthin,  $\alpha$ -doradexanthin, astaxanthin, neoxanthin, and rhodoxanthin. The total content of carotenoids ranged from 26.15 (*Punctelia bolliana*) to 106.21  $\mu\text{g g}^{-1}$  dry weight (*Phaeophyscia rubropulchra*).

### INTRODUCTION

During the last 20 years, considerable progress has been made in studies of the species composition of lichens in Illinois (Skorepa & Snider 1967, Wiedman & Whiteside 1975, McClain 1978, Skorepa 1970, 1973a, 1973b, 1977, Wilhelm and Ladd 1985, Wilhelm and Lampa 1987, McKnight et al. 1987, and Wilhelm & Parker 1988). In some of these studies attention was paid, not only to the geographical distribution of the various species, but also to their biology and the biochemical content of some lichen products which are important factors in taxonomic research. While reference has been made to the organic acids, terpenes, anthraquinones, etc., none of these works addressed the presence of carotenoids. In view of the fact that in the chemotaxonomy of fungi (Valadon 1976) and algae (Weber and Wettern 1980, Liaacn-Jensen 1989) the presence of carotenoids is taken into consideration, we decided to investigate the carotenoid content of the thalli of lichens from Illinois in order to find new data which would give a fuller picture of the characteristics of this group of plants.

## MATERIALS AND METHODS

The 21 lichen species studied from Illinois, U.S.A. were collected in November-December, 1988. The location, voucher number, and substrate of each of these species are presented in Table 1.

Carotenoid pigments were extracted with 95% acetone in a darkroom. Saponification was carried out with 10% KOH in ethanol in a nitrogen atmosphere at approximately 20°C for 24 hours in the dark. Column and thin-layer chromatography (Czeczuga 1980) were used for the separation of various carotenoids. A 15-20 cm X 1 cm glass column (Quickfit, England) packed with  $Al_2O_3$  was used for column chromatography. The extract was passed through the column and the different fractions were eluted with petroleum ether and acetone. Silica gel was used for thin-layer chromatography (TLC), with benzene-petroleum ether-acetone (10:2.5:2) as the solvent system.  $R_f$  values were determined for each spot. For identification of the thallus carotenoids, standards (Hoffman-La Roche and Co., Ltd., Basel, Switzerland, and Sigma Chemical Co., USA) were co-chromatographed with the lichen extracts.

The carotenoids were identified according to: (a) their behavior in column chromatography; (b) their absorption spectra in various solvents as recorded on a Beckman 2400 DU spectrophotometer; (c) the partition characteristics between hexane and 95% methanol; (d) a comparison of  $R_f$  values in TLC; (e) the presence of allylic hydroxyl groups as determined by the acid-chloroform test; (f) the epoxide test; (g) the mass spectrum; and (h) their infrared spectrum (Vetter et al. 1971 for basic methodology) were recorded by a Specord M-80 Carl Zeiss, Jena. Quantitative determinations of the concentrations of carotenoid solutions were made from the absorption spectra. These determinations were based on the extinction coefficient ( $E 1\% \text{ cm}^{-1}$ ) at the wavelengths of maximal absorbance of petroleum ether or hexane (Davies 1976).

Structure of carotenoids was given (Figure 1) according to Straub (1987). Nomenclature follows Egan (1987). Voucher specimens are identified by the Wilhelm collection number in Table 1 and housed at the Morton Arboretum Herbarium (MOR), Lisle, Illinois.

## RESULTS

In the thalli of 21 lichen species, the presence of 19 carotenoids was determined. Apart from the commonly found carotenoids, some of particular interest were noted: rubixanthin (*Physcia millegrana*), lycopene-5,6-epoxide (*Phaeophyscia rubropulchra*, *Physcia stellaris*, and *Punctelia perreticulata*),  $\beta$ -carotene diepoxide (*Phaeophyscia rubropulchra*),  $\beta$ -carotene epoxide and rhodoxanthin (*Xanthoparmelia somloënsis*). The predominant carotenoids were zeaxanthin (3 species),  $\beta$ -carotene diepoxide (1 species), lutein epoxide (7 species), violaxanthin (4 species), mutatoxanthin (5 species), and  $\alpha$ -doradexanthin (2 species). The total carotenoid content in the thalli of the species studied ranged from 26.15 (*Punctelia bolliana*) to 106.21  $\mu\text{g/g}$  dry weight (*Phaeophyscia rubropulchra*). A list of all the carotenoids found and their distribution in the 21 lichen species is given in Table 2.

## DISCUSSION

Of the findings in this study, the most noteworthy was that of the presence of  $\beta$ -carotene diepoxide which was the predominant carotenoid in thalli of *Phaeophyscia rubropulchra*. This is the first report of the presence of this carotenoid in lichens (Czeczuga 1988a). Previously, it had been found in the anthers, filaments, and petals, as well as the fruit of numerous species of flowering plants (Goodwin 1980). The carotenoids rubixanthin,  $\beta$ -carotene epoxide and rhodoxanthin have been found rarely in lichenized fungi. Rubixanthin, among others, has been found in the thalli of the *Cetraria laevigata*, from the Kamchatka Peninsula (Czeczuga et al. 1989a), whereas  $\beta$ -carotene epoxide has been found in the thalli of *Ramalina pollinaria*, from Bulgaria (Czeczuga 1988b) and in *Nephroma arcticum*, *Solorina crocea*, *Sphaerophorus globosus*, *Thamnolia vernicularis*, and *Usnea sulphurea* from Austria (Czeczuga & Olech 1990). Rhodoxanthin, among others, was noted in the thalli of a few species of *Parmelia* (s.l.) from Italy (Czeczuga et al. 1989b).

In this study,  $\beta$ -carotene and lutein epoxide were present in the thalli of all of the species evaluated. In general, it would appear that the genera and species of lichenized fungi have suites of carotenoids in common, but that these common suites are by no means exclusive to either genera or species and common carotenoids do not necessarily imply a close relationship. The specimens of *Parmelia sulcata* and *Peltigera canina* examined in this study, for example, had the same suite of carotenoids, even though the phycobiont of *Parmelia* is green and that of *Peltigera* is blue green.

The carotenoids common to the seven species of *Cladonia* in this study were  $\beta$ -carotene and lutein epoxide, and antheraxanthin, zeaxanthin, and mutatoxanthin, though the latter three were absent in two *Cladonia* specimens from Austria (Czeczuga & Olech 1990). Where two *Cladoniae* from the Canary Islands (Czeczuga et al. 1988) were compared to the seven *Cladoniae* from Illinois, only  $\beta$ -carotene and lutein epoxide were in common.

The carotenoids common to the three species of *Physcia* were  $\beta$ -carotene, lutein epoxide, lutein, zeaxanthin, and violaxanthin. *Peltigera canina* from Illinois had six carotenoids in common with *P. horizontalis* collected in Ireland (Czeczuga & Richardson 1989):  $\beta$ -carotene, lutein epoxide,  $\beta$ -cryptoxanthin, lutein, antheraxanthin, and violaxanthin, though only  $\beta$ -carotene, lutein epoxide, and violaxanthin were in common with two species from Austria (Czeczuga & Olech 1990).

There is also variation with respect to carotenoid composition within a species. *Parmelia sulcata* from Illinois, for example, contained the same four carotenoids found in *P. sulcata* collected in Finland (Czeczuga and Skult 1988), but with  $\beta$ -carotene, lutein, and antheraxanthin in addition, perhaps as accessory pigments.  $\beta$ -carotene, lutein epoxide, lutein, antheraxanthin, and violaxanthin occurred in the thalli of four species of *Punctelia*, a segregate of *Parmelia*. In another segregate of *Parmelia*, the carotenoids  $\beta$ -carotene, lutein epoxide, lutein, 3'-epilutein, antheraxanthin, violaxanthin, and mutatoxanthin were all noted in the thalli of the two species of *Flavoparmelia*, but zeaxanthin was absent from *F. caperata*.

It is apparent that much more survey work among the genera and species, replicated by analysis of specimens of the same species from different areas, must be done before it is clear how important carotenoid analysis will be in lichen taxonomy. The *Parmeliaceae*,

as well as most other groups of lichenized fungi, is in an active state of generic evaluation (Adler 1990). As genera and subgeneric relationships are explored, it is possible that carotenoid content might be of value in making these evaluations.

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Table 1. Investigated species of Illinois lichens

Species	Coll. No.	County	Substrate
<i>Canoparmelia texana</i> (Tuck.) Elix & Hale	16567	Saline	<i>Carya tomentosa</i>
<i>Cladonia subtenuis</i> (des Abb.) Hale & Culb.	16590	Saline	sandstone
<i>Cladonia caroliniana</i> Schwein. ex Tuck.	16586	Saline	sandstone
<i>Cladonia furcata</i> (Huds.) Schrad.	16589	Saline	sandstone
<i>Cladonia grayi</i> G. K. Merr. ex Sandst.	16549	Saline	sandstone
<i>Cladonia robbinsii</i> A. Evans	16584	Saline	sandy soil
<i>Cladonia squamosa</i> (Scop.) Hoffm.	16580	Saline	sandstone
<i>Cladonia strepsiliis</i> (Ach.) Vainio	16600	Saline	sandstone
<i>Flavoparmelia baltimorensis</i> (Gyeln. et Föriss) Hale	16606	Saline	sandstone
<i>Flavoparmelia caperata</i> (L.) Hale	16554	Saline	<i>Juniperus virginiana</i>
<i>Parmelia sulcata</i> Tayl.	16623	DuPage	<i>Fraxinus</i>
<i>Peltigera canina</i> (L.) Willd.	16583	Saline	sandstone
<i>Phaeophyscia rubropulchra</i> (Degel.) Moberg	16624	DuPage	<i>Fraxinus</i>
<i>Physcia adscendens</i> (Fr.) M. Oliv.	16622	DuPage	<i>Fraxinus</i>
<i>Physcia millegrana</i> Degel.	16621	DuPage	<i>Fraxinus</i>
<i>Physcia stellaris</i> (L.) Nyl.	16625	DuPage	<i>Fraxinus</i>
<i>Punctelia bolliana</i> (Müll. Arg.) Krog	16626	DuPage	<i>Quercus alba</i>
<i>Punctelia perreticulata</i> (Räs.) Wilhelm et Ladd in ed.	16617	Saline	<i>Juniperus virginiana</i>
<i>Punctelia rufecta</i> (Ach.) Krog	16552	Saline	<i>Juniperus virginiana</i>
<i>Punctelia subrufecta</i> (Nyl.) Krog	16553	Saline	<i>Juniperus virginiana</i>
<i>Xanthoparmelia somloënsis</i> (Gyeln.) Hale	16571	Saline	sandstone

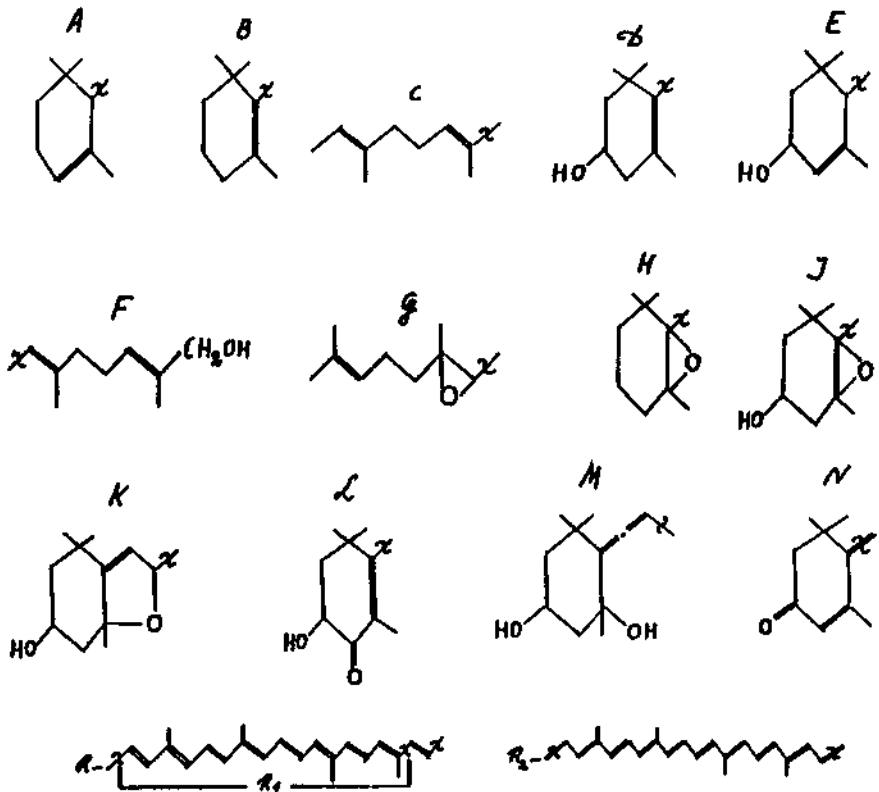
Figure 1. A-N, + R, R<sub>1</sub>, and R<sub>2</sub>

Table 2. List of the carotenoids and their distribution

Carotenoid	Structure (Figure 1)	Semisystematic name	Carotenoid																	Major carot. (%)	Total µg/g			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19			
1 $\alpha$ -carotene	A-R-B	$\beta,\beta$ -carotene																						
2 $\beta$ -carotene	B-R-B	$\beta,\beta$ -carotene																						
3 rubixanthin	C-R-D	$\beta,\psi$ -carotene-3-ol																						
4 $\beta$ -cryptoxanthin	B-R-D	$\beta,\beta$ -carotene-3-ol																						
5 lutein	D-R-E	$\beta,\epsilon$ -carotene-3,3'-diol																						
6 3'-epilutein	D-R-E	$\beta,\epsilon$ -carotene-3,3'-diol (stereoisomeric)																						
7 zeaxanthin	D-R-D	$\beta,\beta$ -carotene-3,3'-diol																						
8 lycopanthin	C-R-F	$\psi,\psi$ -carotene-16-ol																						
9 lycopene-5,6-epoxide	C-R-G	5,6-epoxy-5,6-dihydro- $\psi,\psi$ -carotene																						
10 $\beta$ -carotene epoxide	B-R-H	5,6-epoxy-5,6-dihydro- $\beta,\beta$ -carotene																						
11 $\beta$ -carotene diepoxide	H-R-H	5,6,5',6'-diepoxo-5,6,5',6'-tetrahydro- $\beta,\beta$ -carotene																						
12 lutein epoxide	E-R-I	5,6-epoxy-5,6-dihydro- $\beta,\epsilon$ -carotene-3,3'-diol																						
13 antheraxanthin	D-R-I	5,6-epoxy-5,6-dihydro- $\beta,\beta$ -carotene-3,3'-diol																						
14 violaxanthin	I-R-I	5,6,5',6'-diepoxo-5,6,5',6'-tetrahydro- $\beta,\beta$ -carotene-3,3'-diol																						
15 mutatoxanthin	D-R <sub>1</sub> -K	5,6-epoxy-5,8-dihydro- $\beta,\beta$ -carotene-3,3'-diol																						
16 $\alpha$ -doradexanthin	E-R-L	3,3'-dihydroxy- $\beta,\epsilon$ -carotene-4-one																						
17 astartaxanthin	L-R-L	3,3'-dihydroxy- $\beta,\beta$ -carotene-4,4'-dione																						
18 neoxanthin	I-R <sub>1</sub> -M	5,6-epoxy-6,7-dihydro-5,6,5',6'-tetrahydro- $\beta,\beta$ -carotene-3,5,3'-triol																						
19 rhodoxanthin	N-R <sub>2</sub> -N	4',5'-didehydro-4,5'-retro- $\beta,\beta$ -carotene-3,3'-dione																						
Lichen species			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Major carot. (%)	Total µg/g	
<i>Canoparmelia texana</i>			x		x	x	x	x						x		x	x	x				12 (40.8)	44.84	
<i>Cladonia subtenuis</i>			x		x	x	x	x						x	x	x	x					12 (31.7)	50.28	
<i>Cladonia caroliniana</i>			x					x						x	x	x	x					12 (30.9)	32.77	
<i>Cladonia furcata</i>			x					x						x	x	x	x					12 (33.2)	50.33	
<i>Cladonia greyi</i>			x		x	x	x	x						x	x	x				x		15 (39.6)	42.89	
<i>Cladonia robbinsii</i>			x			x	x	x	x					x	x	x	x					7 (38.9)	41.42	
<i>Cladonia squamosa</i>			x	x		x	x	x						x	x	x	x					7 (38.4)	45.98	
<i>Cladonia strepsilis</i>			x			x	x	x						x	x	x	x	x				16 (26.9)	35.48	
<i>Flavoparmelia baltimorensis</i>			x			x	x	x						x	x	x	x					15 (28.9)	55.36	
<i>Flavoparmelia caperata</i>			x			x	x	x						x	x	x	x			x		15 (27.4)	61.01	
<i>Farmelia sulcata</i>			x		x	x	x							x	x	x	x					14 (22.9)	61.17	
<i>Peltigera canina</i>			x		x	x								x	x	x	x					14 (36.8)	50.45	
<i>Phaeophyscia rubropulchra</i>			x			x	x			x		x	x	x	x	x						11 (48.2)	106.21	
<i>Physcia adscendens</i>			x			x		x			x			x	x	x		x				12 (25.6)	85.04	
<i>Physcia millegrana</i>			x	x		x		x						x		x	x				x	14 (33.7)	33.47	
<i>Physcia stellaris</i>			x		x	x	x	x		x	x			x	x	x	x					15 (21.1)	43.62	
<i>Punctelia boilliana</i>			x	x		x	x	x						x	x	x				x		16 (20.2)	26.15	
<i>Punctelia perreticulata</i>			x		x	x	x	x		x				x	x	x						7 (23.8)	60.26	
<i>Punctelia rudecta</i>			x		x									x	x	x				x	x	12 (27.0)	36.38	
<i>Punctelia subrudecta</i>			x		x		x							x	x	x	x					15 (29.3)	85.99	
<i>Xanthoparmelia somloënsis</i>			x			x	x	x						x		x	x			x	x	12 (25.4)	48.54	