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STUDIES ON CAROTENOIDS OF FOLIOSE LICHENS FAM. *PARMELIACEAE s. lat. (ASCOMYCETES)* FROM NORTH-WESTERN PATAGONIAN *NOTHOFAGUS* FORESTS (ARGENTINA)

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Summary Column and thin-layer chromatography revealed the presence of the following carotenoids, in twenty-one thalli of twelve different species of foliose lichens Fam. *Parmeliaceae s. lat. (Ascomycetes)* from North-Western Patagonian *Nothofagus* forests: ß-carotene, ß-cryptoxanthin, lutein, zeaxanthin, lutein epoxide, antheraxanthin, violaxanthin, 4-hydroxyechinenone, ß-doradexanthin, canthaxanthin, astaxanthin, neoxanthin, rhodoxanthin and capsochrome. Total content of carotenoids ranged from 19.61 for *Hypogymnia lugubris* (Pers.) Krog to 81.25 µg/g dry weight for *Hypotrachyna brevirhiza* (Kurok.) Hale. The predominant carotenoids are canthaxanthin and astaxanthin. Rhodoxanthin, a carotenoid rarely found on lichens was detected in thalli of three species: *Hypotrachyna brevirhiza* (Kurok.) Hale, *Hypogymnia lugubris* (Pers.) Krog and *Omphalodium pisacomense* Mey. & Flot.

Resumen Por medio de cromatografía de columna y de capa delgada se detectó la presencia de los siguientes carotenoides, en veintiún talos de doce especies diferentes de líquenes foliosos, Fam. *Parmeliaceae s. lat. (Ascomycetes)*, de bosques de *Nothofagus* del noroeste patagónico: ß-caroteno, ß-cryptoxantina, luteina, zeaxantina, epoxiluteina, anteraxantina, violaxantina, 4-hidroxiequinenona, ß-doradexantina, cantaxantina, astaxantina, neoxantina, rodoxantina y capsocromo. El contenido total de carotenoides en peso seco varió entre 19.61 para *Hypogymnia lugubris* (Pers.) Krog y 81.25 µg/g para *Hypotrachyna brevirhiza* (Kurok.) Hale. Los carotenoides predominantes son cantaxantina y astaxantina. Rodoxantina, un carotenoide que se ha encontrado raramente en líquenes, fue detectado en los talos de tres especies: *Hypotrachyna brevirhiza* (Kurok.) Hale, *Hypogymnia lugubris* (Pers.) Krog y *Omphalodium pisacomense* Mey. & Flot.

Key Words: Lichens, *Parmeliaceae*, Patagonia, *Nothofagus*, Carotenoids

INTRODUCTION

The Nahuel Huapi, Puelo and Lanín National Parks areas are environmentally varied. There are spread *Nothofagus* forests, where abundant variety of lichen species can be found growing on different microhabitats (Calvelo, 1992; Calvelo and Lorenzo, 1989). The species of Fam. *Parmeliaceae s. lat.* are very frequent and dominant both in number of species and individuals as well as in cover at high altitudes and/or in the drier habitats of the area (Adler and Calvelo, 1993; Calvelo, 1995; Calvelo and Adler, 1992). Our previous studies on the carotenoid content in lichen thalli revealed a significant effect of environmental factors upon their distribution (Czeczuga, 1988; Czeczuga and Kashiwadani, 1993). Taking into account the specific conditions found in these National Parks, we have initiated the analysis of the occurrence of carotenoids in the thalli of various lichen species. Up to now we have investigated along the precipitation gradient typical of the area (Barros et al., 1983) for the carotenoid content in lichens (Czeczuga and Calvelo, 1994) and we have performed the analysis of carotenoids of fruticose lichens in this region (Czeczuga and Calvelo, 1995). The present contribution deals with the carotenoids found in the thalli of foliose lichens, Fam. *Parmeliaceae* s. lat. from Nahuel Huapi, Lanín and Puelo National Parks.

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MATERIALS AND METHODS

Twenty one specimens of twelve species of foliose *Parmeliaceae* s. lat. from the area under study have been examined, namely *Cetraria chlorophylla* (Humb.) Vain., *Cetrariastrum americanum* (Mey. & Flot.) Culb. & Culb., *Hypotrachyna brevirhiza* (Kurok.) Hale, *H. densirhizinata* (Kurok.) Hale, *H. sinuosa* (Sm.) Hale, *Flavoparmelia ferax* (Müll. Arg.) Hale, *Hypogymnia lugubris* (Pers.) Krog, *Omphalodium pisacomense* Mey. & Flot., *Pannoparmelia angustata* (Pers. in Gaudichaud) Zahlbr. emend. Calvelo & Adler, *Parmelia cunninghamii* Crombie, *Parmelia sulcata* Taylor y *Parmotrema reticulatum* (Taylor) Choisy. The description of the area where the collections were made, as well as the characteristics and distributions of the species were recently published (Calvelo & Adler, 1992; Adler & Calvelo, 1993). Specimens studied are enumerated on Table 1; they were collected by Calvelo and duplicates are kept in her herbarium.

Carotenoid pigments were extracted with 95% acetone in a dark room. Saponification was carried

Table 1. Specimens of foliose lichens from Patagonian *Nothofagus* forests examined for this study

N°	Species	Herb.N°	Date	Locality
1	*Cetraria chlorophylla*	435	3-III-1991	Prov. Río Negro: Dpto. Bariloche, Arroyo Casa de Piedra, on *N. dombeyi*. Alt. 800 m
2	*Cetraria chlorophylla*	485	2-VIII-1990	Prov. Río Negro: Dpto. Bariloche, Cerro Carbón, on *N.dombeyi*. Alt. 1050 m
3	*Cetraria chlorophylla*	515	13-III-1992	Prov. Río Negro: Dpto. Bariloche, Llao- Llao, Villa Tacul, on *N.dombeyi*. Alt. 800 m
4	*Cetrariastrum americanum*	592	8-XI-1992	Prov. Chubut: Parque Nacional Lago Puelo, Río Turbio, on *Lomatia hirsuta*. Alt. 450 m.
5	*Cetrariastrum americanum*	606	8-XI-1992	Ibid. on *Colletia spinosissima*.
6	*Cetrariastrum americanum*	516	3-IV-1992	Prov. Río Negro: Dept. Bariloche, Lago Gutiérrez, on mosses. Alt. 850 m.
7	*Hypotrachyna brevirhiza*	554	17-IV-1992	Prov. Neuquén: Parque Nacional Lanín, Lago Epulaufquen, on *N.alpina*. Alt. 750 m.
8	*Hypotrachyna brevirhiza*	549	14-VI-1991	Prov. Río Negro: Dept. Bariloche, Km 9.5 Ruta a Llao-Llao, on *Acer pseudoplatanus*. Alt. 780 m.
9	*Hypotrachyna brevirhiza*	546	3-III-1991	Prov. Río Negro: Dpto. Bariloche, Arroyo Casa de Piedra, on *N.dombeyi*. Alt. 850 m
10	*Hypotrachyna densirhizinata*	586	8-XI-1992	Prov. Chubut, Parque Nacional Lago Puelo, Río Turbio, on *Austrocedrus chilensis*. Alt. 450 m
11	*Hypotrachyna densirhizinata*	616	8-XI-1992	Ibid.
12	*Hypotrachyna sinuosa*	401	4-II-1989	Prov. Río Negro: Dpto. Bariloche, Pampa de Huenuleo, on *Anarthrophyllum rigidum*, Leg. Brion. Alt. 850 m
13	*Flavoparmelia ferax*	552	22-XI-1991	Prov. Río Negro: Dpto. Bariloche, Km 9.5 Ruta a Llao-Llao, on *Acer pseudoplatanus*. Alt. 780 m
14	*Flavoparmelia ferax*	502	22-XI-1991	Ibid.
15	*Hypogymnia lugubris*	783	3-IV-1992	Prov. de Río Negro: Dpto. Bariloche, Cerro Carbón, on *N.pumilio*. 1350 m
16	*Omphalodium pisacomense*	103	8-II-1987	Prov. de Río Negro: Parque Nacional Nahuel Huapi, Cerro Chalhuaco, on rocks. Alt.1450m
17	*Pannoparmelia angustata*	542	17-IV-1992	Prov. Neuquén: Parque Nacional Lanín, near Lago Epulaufquen, on *N.dombeyi*. Alt. 550 m.
18	*Parmelia cunninghamii*	397	11-I-1990	Prov. de Río Negro: Parque Nacional Nahuel Huapi, Laguna Llum, on *N. dombeyi*. Alt.950m
19	*Parmelia sulcata*	090	8-XII-1986	Prov. de Río Negro: Parque Nacional Nahuel Huapi, Cerro Catedral, pathway to Frei, on *N.dombeyi*. Alt. 900 m
20	*Parmotrema reticulatum*	512	22-III-1990	Prov. Río Negro: Dpto. Bariloche, Llao-Llao on *Luma apiculata*. Alt. 800 m
21	*Parmotrema reticulatum*	513	13-III-1991	Prov. de Río Negro: Parque Nacional Nahuel Huapi, Puerto Blest, pathway to Lago Frías on *N.dombeyi*. Alt. 950 m.

Table 2. List of carotenoids from the investigated materials

N°	Carotenoid	Structure (See Fig. 1)	Semisystematic name
1	ß-carotene	A - r - A	ß,ß-Carotene
2	ß-cryptoxanthin	A - r - B	ß,ß-Carotene-3-ol
3	lutein	B - r - C	B-r-Carotene-3,3'-diol
4	zeaxanthin	B - r - B	ß,ß-Carotene-3,3'-diol
5	4'-hydroxyechinenone	D - r - E	4'-Hydroxy-ß,ß-caroten-4-one
6	canthaxanthin	E - r - E	ß,ß-Carotene-4,4'-dione
7	ß-doradexanthin	B - r - F	3,3'-Dihydroxy-ß,ß-caroten-4-one
8	astaxanthin	F - r - F	3,3'-Dihydroxy-ß,ß-carotene-4,4'-dione
9	lutein epoxide	C - r - G	5,6-Epoxy-5,6-dihydro-ß,ε-carotene-3,3'-diol
10	antheraxanthin	B - r - G	5,6-Epoxy-5,6-dihydro-ß,ß-carotene-3,3'-diol
11	violaxanthin	G - r - G	5,6,5',6'-Diepoxy-5,6,5',6'-tetrahydro-ß,ß-carotene-3,3'-diol
12	neoxanthin	G - r_1 - H	5,6-Epoxy-6,7-didehydro-5,6,5',6'-ß,ß-carotene-3,5,3'-triol
13	rhodoxanthin	I - r_2 - I	4',5'-Didehydro-4,5'-retro-ß,ß-carotene-3,3'-dione
14	capsochrome	K - r_1 - L	5,8-Epoxy-3,3'-dihydroxy-5,8-dihydro-ß,χ-carotene-6'-one

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 (TLC) (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 TLC with benzene-petroleum ether-acetone (10:2.5:2) as the solvent system and R_f values were determined for each spot. For identification of the thallus carotenoid 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) the behavior in column chromatography; b) the absorption spectra in various solvents as recorded on a Beckman 2400 Du spectrophotometer; c) the partition characteristics between hexane and 95% methanol; d) the 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; h) the infrared spectroscopy (Vetter et al., 1971 for basic methodology), recorded with 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% cm^{-1}, at the wavelengths of maximal absorbance of petroleum ether or hexane (Davies, 1976). Structure of carotenoids was given according to Straub (1987).

RESULTS

Fourteen carotenoids were detected in the thalli of the species examined (Table 2). The predominant carotenoids were violaxanthin and astaxanthin (Table 3). The total carotenoid content in the material studied ranged from 19.61 (*Hypogymnia lugubris*) to 81.25 µg/g dry weight mass (*Hypotrachyna brevirhiza* N° 9).

DISCUSSION

The carotenoids found in the examined foliose lichens Fam. *Parmeliaceae* s. lat. from North-Western Patagonian *Nothofagus* forests, except rhodoxanthin, have already been reported to occur in the thalli of a number of other species from this region (Czeczuga and Calvelo, 1994; 1995). In the group of the 12 lichen species examined rhodoxanthin was found in the thalli of three species, namely *Hypotrachyna brevirhiza*, *Hypogymnia lugubris* and *Omphalodium pisacomense*. This carotenoid, known as keto-carotenoid, has been found in certain bacteria species, the phylum Pteridophyta (Czeczuga, 1985), coniferous tree leaves (Czeczuga, 1986; 1987) as well as in flower petals and ripe fruits of angiospermous plants (Goodwin, 1980). With regard to lichens, rhodoxanthin was previously found only in few species of Mediterranean islands (Czeczuga et al., 1994).

Our previous studies on different thalli of the same lichen species collected at various latitudes have revealed that some carotenoids are not present

TABLA 3. Carotenoid distribution from North-Western Patagonian *Parmeliaceae* lichens.

Species of lichens	N°	Carotenoid (See Table 2 and Fig. 1)	Mayor carotenoid %	Total content µg/g dry weight
Cetraria chlorophylla	1	2,3,4,6,8,9,11	8 (23.1)	74.79
	2	1,2,3,5,6,8,9,11	8 (25.3)	76.39
	3	1,2,4,6,7,8,9	4 (22.2)	62.93
Cetrariastrum americanum	4	1,2,3,4,6,8,9,14	14 (28.7)	32.97
	5	1,2,6,8,9,11,12	6 (43.5)	38.23
	6	1,2,6,8,9,10,11,12,14	9 (20.3)	34.31
Hypotrachyna brevirhiza	7	1,2,4,6,11,12,13,14	12 (23.2)	28.44
	8	1,2,3,4,6,8,9,11,13	11 (29.9)	60.38
	9	1,2,4,6,8,9,11	11 (38.6)	81.25
Hypotrachyna densirhizinata	10	1,2,4,6,8,9,11	8 (30.3)	61.39
	11	2,3,6,7,8,9,11,12	8 (25.6)	44.22
Hypotrachyna sinuosa	12	1,2,4,6,8,9,11,12,14	11 (29.3)	73.42
Flavoparmelia ferax	13	1,2,3,4,6,8,9,11,12	8 (30.5)	30.34
	14	1,2,3,4,6,8,9,11,14	4 (31.1)	45.42
Hypogymnia lugubris	15	1,4,6,7,8,9,11,13	6 (25.5)	19.61
Omphalodium pisacomense	16	2,4,5,6,8,11,12,13,14	12 (23.8)	28.14
Pannoparmelia angustata	17	1,3,8,9,10,11,14	14 (25.5)	27.85
Parmelia cunninghamii	18	1,2,4,6,8,9,11,12,14	11 (29.4)	64.85
Parmelia sulcata	19	1,2,6,7,8,9,10,11	11 (29.9)	61.39
Parmotrema reticulatum	20	1,2,3,8,9,11,12,14	11 (28.2)	40.95
	21	1,3,4,6,8,9,11,12,14	11 (27.8)	32.07

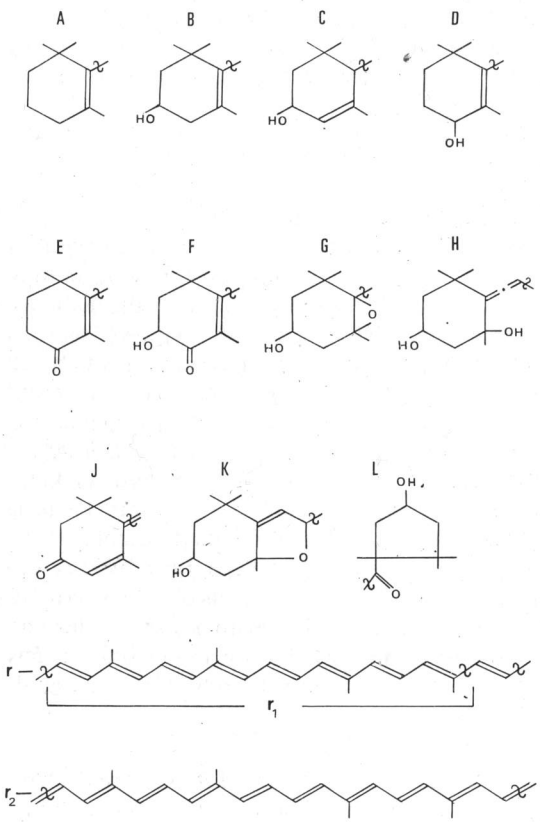

Fig. 1.– Structural features of carotenoids from investigated material

in all the thalli, their presence being environmental factor-dependent. While others are present in all the thalli of the same species, and are independent of the environmental factors. Only these constant carotenoids ought to be considered in taxonomic studies of lichens (Czeczuga, 1993).

With this idea in mind, we made a comparison of the carotenoid distribution in *Cetraria chlorophylla*, *Cetrariastrum americanum* and *Hypotrachyna brevirhiza* thalli, collected in three different environments of North-Western Patagonian forests. The result showed that four carotenoids: ß-cryptoxanthin, lutein epoxide, canthaxanthin and astaxanthin were constant for *Cetraria chlorophylla*; five carotenoids: ß-carotene, ß-cryptoxanthin, lutein epoxide, canthaxanthin and astaxanthin were constant for *Cetrariastrum americanum*; and five carotenoids: ß-carotene, ß-cryptoxanthin, lutein epoxide, canthaxanthin and astaxanthin were constant for *Hypotrachyna brevirhiza*.

The presence of canthaxanthin and astaxanthin in the thalli of nearly all lichen species found in *Nothofagus* North-Western Patagonian forests is noteworthy. Furthermore, in five species canthaxanthin was the predominant carotenoid. It should be recalled that in other lichen species from the same area, these carotenoids (astaxanthin in particular) were frequently observed (Czeczuga and Calvelo, 1994; 1995). Thus, it can be assumed that

the environmental conditions in *Nothofagus* North-Western Patagonian forests promote the biosynthesis of the two ketocarotenoids (mainly astaxanthin) by lichens found in that region.

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