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INVESTIGATION ON CAROTENOIDS OF FRUTICOSE LICHENS FROM *NOTHOFAGUS* FORESTS, NORTH-WESTERN PATAGONIA (ARGENTINA)

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Summary Carotenoid content in sixteen specimens of seven different species of fruticose lichens, collected from *Nothofagus* forests, North-Western Patagonia, have been investigated by means of column and thin-layer chromatography. The following carotenoids have been found: α-carotene, β-carotene, β-cryptoxanthin, lutein, 3'-epilutein, zeaxanthin, lutein epoxide, antheraxanthin, hyroxyechinenone, canthaxanthin, α-doradexanthin, astaxanthin, violaxanthin, neoxanthin, mutatoxanthin and capsochrome. Total content of carotenoids ranged from 18.92 *(Bunodophoron ramuliferum)* to 53.68 µg/g dry weight *(Bunodophoron insigne)*.

Resumen Se ha investigado el contenido de carotenoides en 16 especímenes de 7 especies diferentes de líquenes fruticosos de los bosques de *Nothofagus* del Noroeste de la Patagonia, por medio de cromatografía de columna y de capa delgada. Se han encontrado los siguientes carotenoides: α-caroteno, β-caroteno, β-cryptoxantina, luteina, 3'-epiluteina, zeaxantina, epoxiluteina, anteraxantina, hidroxiequinenona, cantaxantina, α-doradexantina, astaxantina, violaxantina, neoxantina, mutatoxantina y capsocromo. El contenido total de carotenoides en peso seco varió entre 18.92 (*Bunodophoron ramuliferum)* y 53.68 µg/g (*Bunodophoron insigne*).

INTRODUCTION

This is the second contribution on carotenoids of lichens from *Nothofagus* forests, North-Western Patagonia. In the first contribution the carotenoid content in corticolous lichens from *Nothofagus* forests along the precipitation gradient was investigated (Czeczuga and Calvelo, 1994).

In the present study the carotenoids of fruticose lichens Fam. Sphaerophoraceae: *Bunodophoron* Massal. and *Leifidium* Wedin and Fam. Stereocaulaceae: *Stereocaulon* Hoff. are investigated.

Bunodophoron occurs in both hemispheres, but the species are better represented in the Southern Hemisphere (Tibell, 1987; Wedin, 1994), while *Leifidium* has austral distribution. In Argentinian *Nothofagus* forests, specimens of these genera are found only in ingressions of the Valdivian forest (Wedin, 1992), where the more humid and shaded habitats occur. The species grow mainly on bark, frequently among mosses.

Stereocaulon species are widely distributed (Galloway, 1985). They occur mainly on rocks, in exposed areas, near streams or opening of the forests.

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MATERIALS AND METHODS

The species of lichens studied are: *Bunodophoron dodgei* (Ohlsson) Wedin, *B. insigne* (Laur.) Wedin, *B. patagonicum* (Dodge) Wedin, *B. ramuliferum* (Lamb) Wedin, *Leifidium tenerum* (Laur.) Wedin, *Stereocaulon patagonicum* Lamb and *S. ramulosum* (Sw.) Rauschel. The specimens for this study were collected by Calvelo and duplicates are kept in her private herbarium. Collecting sites and their characterization are specified in Table 1.

Carotenoids pigments were extracted with 95% acetone in a dark room. 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 (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 (Liaaen-Jensen, 1971).

The carotenoids were identified according to: a) the behaviour in column chromatography; b) the

Tabla 1.— Investigated specimens of fruticose lichens from North-Western Patagonia (Argentina, Prov. de Río Negro)

Family and species	Herb. N°	Date	Locality
Sphaerophoraceae			
Bunodophoron dodgei	258	10-III-90	Puerto Blest, way to Arroyo Los Cántaros, on *Saxegothaea conspicua*
B. insigne	259	10-III-90	Puerto Blest, way to Arroyo Los Cántaros, on *Nothofagus dombeyi*
B. insigne	263	18-II-89	Lago Felipe, North coast, on N. dombeyi
B. insigne	466	13-III-91	Puerto Frías, near El Abuelo. On *N. dombeyi*, on small branch.
B. patagonicum	21	20-XI-86	Puerto Blest, way to Arroyo Los Cántaros, on *N. dombeyi*
B. patagonicum	260	12-II-89	Lago Roca, West coast, on *N. dombeyi*
B. patagonicum	262	18-II-89	Lago Felipe, North coast, on *N. dombeyi*
B. patagonicum	467	14-III-91	Puerto Alegre, way to Paso de las Nubes, on *N. dombeyi*
B. ramuliferum	20	20-II-86	Puerto Alegre, way to Paso de las Nubes, on *N. dombeyi*
B. ramuliferum	464	5-III-86	Puerto Blest, way to Arroyo Los Cántaros, on *N. dombeyi*
Leifidium tenerum	256	10-III-90	Puerto Blest, way to Arroyo Los Cántaros, on *N. dombeyi*
Stereocaulaceae			
Stereocaulon patagonicum	355	9-VI-86	Llao-Llao, Lago Moreno North coast, on rocks, in *N. dombeyi* forest.
S. ramulosum	22	20-XI-86	Puerto Blest, way to Arroyo Los Cántaros, on rocks.
S. ramulosum	95	15-IV-87	Arroyo Casa de Piedra, on rocks, in *N. dombeyi* forest.
S. ramulosum	465	8-VI-80	Llao-Llao, Villa Tacul, on rocks, in *N. dombeyi* forest
S. ramulosum	468	18-II-89	Lago Felipe, on rocks, in *N. pumilio* forest.

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; and h) the infrared spectroscopy (Vetter *et. al.*, 1991 for basic methodology) were recorded with a Specord M-80 Carl Zeiss, Jena. Quantitative determinations of the concentrations of carotenoids 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). The structure of carotenoids was given according to Straub (1987).

RESULTS

In the material examined, the presence of 16 carotenoids was determined (Table 2). Fifteen carotenoids were found in the thalli of *Bunodophoron* species, 8 in *Leifidium* and 13 in *Stereocaulon* species. The carotenoids observed have been found frequently in the thalli of other lichen species; their structural features are shown on Fig. 1. The total carotenoid content in the thalli

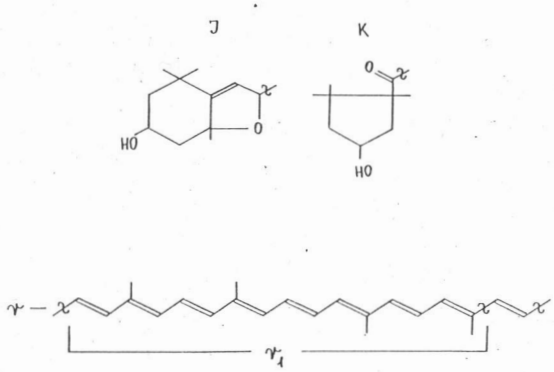

Fig. 1.—Structural features of carotenoids from investigated materials.

Tabla 2.— List of the carotenoids from the investigated materials

Nº	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.	β-cryptoxanthin	B - r - C	β, β-Caroten-3-ol
4.	lutein	C - r - D	β, ε-Carotene-3,3'-diol
5.	3' -epilutein	C - r - D	β, ε-Carotene-3,3'-diol/stereoisomeric/
6.	zeaxanthin	C - r - D	β, β-Carotene-3,3'-diol
7.	lutein epoxide	D - r - E	5,6-Epoxy-5,6-dihydro-β, ε-carotene-3,3'-diol
8.	antheraxanthin	C - r - E	5,6-Epoxy-5,6-dihydro-β, β-carotenen-3,3'-diol
9.	neoxanthin	E - r_1 - F	5,6-Epoxy-6,7-didehydro-5,6,5',6'-tetrahydro-β, β-carotene-3,5,3'-triol
10.	violaxanthin	E - r - E	5,6,5',6'-Diepoxy-5,6,5',6'-tetrahydro-β, β-carotene-3,3'-diol
11.	hydroxyechinenone	B - r - H	3-Hydroxy-β, β-caroten-4-one
12.	canthaxanthin	G - r - G	β, β-Carotene-4,4'-dione
13.	α-doradexanthin	D - r - H	3,3'-Dihydroxy-β, ε-caroten-4-one
14.	astaxanthin	H - r - H	3,3'-Dihydroxy-β, β-carotene-4,4-dione
15.	mutatoxanthin	C - r_1 - I	5,8-Epoxy-5,8-dihydro-β, β-carotene-3,3'-diol
16.	capsochrome	I - r_1 - K	5,8-Epoxy-3,3'-dihydroxy-5,8-dihydro-β, χ-caroten-6'-one

Tabla 3.— Carotenoid distribution in lichens from North-Western Patagonia (Argentina, Prov. de Rio Negro)

Family and species	Herb. Nº	Carotenoid (see Table 2)	Mayor carotenoid (%)	Total content µg/g dry weight
Sphaerophoraceae				
Bunodophoron dodgei	258	1,2,4,5,7,9,10,14	7 (38.4)	23.51
B. insigne	259	2,7,8,9,10,14,16	7 (39.7)	23.48
	263	4,7,8,9,10,14	10 (37.2)	33.85
	466	2,3,4,7,9,10,14	10 (34.2)	53.68
B. patagonicum	21	2,3,4,7,8,9,10,16	7 (28.7)	33.37
	260	1,2,4,5,7,8,9,10	10 (29,9)	24.74
	262	1,2,6,7,10,14	6 (34.7)	31.69
	467	2,6,7,8,9,10,11,13,14,16	6 (28.8)	25.93
B. ramuliferum	20	3,6,7,8,9,10,14	10 (36.7)	18.92
	464	3,4,5,7,8,10,14,15	10 (27.2)	21.11
Leifidium tenerum	256	2,3,4,7,8,10,14,15	15(35.3)	19.71
Stereocaulaceae				
Stereocaulon patagonicum	355	2,4,7,8,9,10,12,14,15	15 (19.7)	39.95
S. ramulosum	22	2,6,7,8,9,10,14	7 (27.7)	28.57
	95	4,7,9,10,12,14	10 (30.5)	23.12
	465	4,7,8,9,10,13,14	7 (21.3)	26.68
	468	2,4,7,8,9,10,11,16	10 (23.9)	22.12

of *Bunodophorum* species ranged from 18.92 to 53.68 µg/g and in those of *Stereocaulon* species from 22.12 to 39.95 µg/g dry weight (Table 3).

DISCUSSION

Green algae act as phycobionts in the species of the genera *Bunodophoron*, *Leifidium* and *Stereocaulon* (Tschermak-Woess, 1988). In most species of the genus *Stereocaulon*, cephalodia with cyanobacteria are found (*Nostoc* or *Stigonema*).

This fact seems to justify the presence of canthaxanthin, typical of cyanobacteria, in the thalli of both *Stereocaulon* species. All carotenoids found in the thalli studied, except for capsochrome, have been already found in green algae (Czeczuga, 1979;

Weber and Wettern, 1980; Liaaen-Jensen, 1989). Capsochrome (Camara and Moneger, 1981) is derived from antheraxanthin through capsanthin. In the thalli with capsochrome we also found antheraxanthin.

Common carotenoids in all five examined lichen species of the genera *Budonophoron* and *Leifidium* were found to be lutein epoxide and violaxanthin, while in those of *Stereocaulon* lutein epoxide, neoxanthin and violaxanthin were identified. Regular carotenoids, which differ, according to the specimens of *Bunodophoron, Leifidium* and *Stereocaulon*, collected from various sites, were noted: lutein epoxide, neoxanthin, violaxanthin and astaxanthin *(Bunodophoron insigne)*: β-carotene, lutein epoxide and violaxanthin (*B. patagonicum* collected from 4 sites in Patagonia); β-cryptoxanthin, lutein epoxide, antheraxanthin, violaxanthin and astaxanthin *(B. ramuliferum* collected from 2 sites); lutein epoxide, neoxanthin and violaxanthin *(Stereocaulon ramulosum* from 4 sites).

The data obtained can be applied to taxonomic studies, such as in the case of algae and fungi forming the lichen thalli. The knowledge of regular carotenoids in the respective species is essential in taxonomic studies. The fact that environmental factors considerably affect the total carotenoid number and their occurence make this knowledge necessary (Czeczuga, 1988; 1993; Czeczuga *et al.*, 1991).

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