Boim Botânica, Univ. São Paulo, 13:23-29, 1992

CAROTENOIDS IN SOME LICHEN SPECIES FROM SOUTH BRAZIL AND PARAGUAY (1)

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ABSTRACT - (Carotenoids in some lichen species from South Brazil and Paraguay). Column, thin-layer chromatography and spectrometric methods, revealed the presence of the following carotenoids in the thalli of 19 lichen species from Brazil and Paraguay: (σ)-, (α)-, (β)- carotene, (β)- cryptoxanthin, lutein, 3'- epilutein, zeaxanthin, (α)- doradexanthin, canthaxanthin, astaxanthin, lutein epoxide, antheraxanthin, violaxanthin, neoxanthin, mutatoxanthin, auroxanthin, rhodoxanthin, capsochrome and apo-12'- violaxanthal. The total content of carotenoids ranged from 19.40 (*Usnea amaliae*) to 96.83 (μ)g. g-1 dry wt (*Parmelina linoknanii*).

RESUMO - (Carotenóides em algumas espécies de líquens do Sul do Brasil e Paraguai). Os métodos empregando cromatografia em coluna e camada delgada e em espectrometria revelaram a presença dos seguintes carotenóides no talo de 19 espécies de líquens do Brasil e do Paraguai: (σ)-, (α)-, (β)-caroteno, (β)-criptoxantina, luteína, 3'-epiluteína, zeaxantina, (α)-doradexantina, cantaxantina, astaxantina, epóxido de luteína, anteraxantina, violaxantina, neoxantina, mutatoxantina, auroxantina, rodoxantina, capsocromo e apo-12'-violaxantal. O conteúdo total de carotenóides varia de 19,40 (*Usnea amaliae*) a 96,83 (μ)g. g-1 em peso seco (*Parmelina linoknanii*).

Key words: lichens, carotenoids

INTRODUCTION

In recent years, chemical methods have been increasingly used since, together with the classical methods, they provide better taxonomic possibilities. In taxonomic studies of fungi (Valadon 1976) and algae (Weber & Wettern 1980, Liaaen-Jensen 1989) in recent years, data on the presence or absence, of the various carotenoids have been used.

In the lichenoflora of South America there is an abundance of species peculiar only to that continent. This applies both to Brazil (Xavier-Filho & Rizzini 1976) and to Paraguay (Osorio 1970). During our studies of carotenoids in lichens from various latitudes (see Czeczuga 1988) we turned our attention to the lichenoflora of this continent. Our investigations have included some species occurring in Argentina (Czeczuga & Ferraro de Corona 1987), Uruguay (Czeczuga & Osorio 1989), North Brazil (Czeczuga & Xavier-Filho 1988) and Chile (Czeczuga & Koch 1991).

The present paper is a continuation of the work on this problem and provides additional information on the results obtained previously about the presence of carotenoids in the thalli of lichens from South America.

(1) Part 47 in the series "Investigations on Carotenoids in Lichens".

MATERIALS AND METHODS

Lichens were collected in August 1988 (5-10 g dry weight) from Brazil (9) and Paraguay (10 species) (Table 1).

Table 1 - Investigated species of lichens

Family and species	Locality
Graphidaceae	
Chiodecton sanguineum (Sw.) Tober	Brasil-Paraná, en selva sobre troncos
Coenogoniaceae	
Coenogonium linkii Ehrenb.	Paraguay-Depto. Alto Paraná, RBY[1], sendero Tangara interior de selva, corticola
Collemataceae	
Leptogium marginellum (Sw.) S. Gray	Paraguay-Depto. Amambay, PNCC[2], sobre cortezas en la selva de la ladera
Stictaceae	
Sticta damaecornis (Sw.) Ach.	Brasil-Paraná, sobre troncos
Cladoniaceae	
Cladonia confusa R. Sant.	Brasil-Paraná, sobre terruno
Stereocaulaceae	
Stereocaulon ramuloson (Sw.) Räusch.	Brasil-Paraná, sobre terruno
Pertusariaceae	
Pertusaria sp.	Paraguay-Depto. Presidente Hayes, Ruta Trans-Chaco
Parmeliaceae	
Parmelina linokanii (Lynge) Hale	Paraguay-Depto. Presidente Hayes, Ruta Trans-Chaco
Parmotrema araucariarum (Zahlbr.) Hale	Brasil-Paraná, sobre terruno
Parmotrema aff. *andinum* (Müll. Arg.) Hale	Paraguay-Depto. Alto Paraná, PNCC[2], alredores de la administración
Parmotrema mesotropum (Müll. Arg.) Hale	Paraguay-Depto. Presidente Hayes, en bosque chaqueno alterado
Parmotrema praesorediosum (Nyl.) Hale	Paraguay-Depto. Cordillera, arroyo Tres Bocas, sobre troncos
Parmotrema sancti-angelii (Lynge) Hale	Brasil-Rio Grande do Sul, sobre el rio do Mel
Parmotrema sancti-angelii (Lynge) Hale	Paraguay-Depto. Cordillera, arroyo Tres Bocas
Parmotrema tinctorum (Nyl.) Hale	Brasil-Rio Grande do Sul, sobre el rio do Mel
Parmotrema tinctorum (Nyl.) Hale	Paraguay-Depto. Missiones, sobre arbol caido en el borde del monte
Parmotrema tinctorum (Nyl.) Hale	Paraguay-Depto. Amambay, PNCC[2], alredores de la administración
Punctelia microsticta (Müll. Arg.) Krog	Paraguay-Depto. Cordillera, arroyo Tres Bocas, corticola
Usneaceae	
Ramalina celastri (Spreng.) Mey et Flot.	Brasil-Santa Catarina, camino a Cerro Negro
Usnea amaliae Mot.	Brasil-Paraná, sobre cortezas
Thelephoraceae	
Dictyonema pavonia (Sw.) Parm.	Brasil-Paraná, sobre terruno
Physciaceae	
Heterodermia vulgaris (Vain.) Hale	Brasil-Paraná, sobre arbol

1RBY - Reserva Biológica Ytabó - 2PNCC - Parque Nacional Cerro Corá

The thalli were cleaned of all organic debris, macerated and homogenized, placed in dark glass bottles, and covered with acetone. The air above the fluid in the bottle was replaced by nitrogen to ensure an anaerobic atmosphere. Samples were refrigerated until used for chromatographic analysis of the carotenoid content.

Carotenoid 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 h in the dark. Column and thin-layer chromatography were used for the separation of various carotenoids (Czeczuga 1989). A 15-20 cm x 1 cm glas column (Quickfit, England) packed with Al_2O_3, was used for column chromatography. 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), and R_f values were determined for each spot. For identification of the 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) the behaviour 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 and (h) infrared spectroscopy for keto carotenoids were recorded in a Specord M-80, Jena (see Vetter et al. 1971 for basic methodology).

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). The structures of the carotenoids were reported previously (Straub 1987).

RESULTS

In the thalli of the lichen species studied 19 carotenoids were identified (Table 2 and Fig. 1). Of these carotenoids lutein epoxide was most frequently found to be predominant. Another interesting finding was the presence of (σ)-carotene in *Parmotrema araucariarum* and astaxanthin in the thali of many species (Table 3). The total carotenoid content in the material studied varied between 19.40 (*Usnea amaliae*) and 96.83 μg g^{-1} dry wt (*Parmelina linoknanii*)

DISCUSSION

The carotenoid (σ)-carotene was first found in lichens in the thalli of *Ramalina usnea* (L.) Howe from Argentina (Czeczuga & Ferraro de Corona 1987). It was later found in the thalli of 4 New Zealand lichens (Czeczuga & Taylor 1991).

As our previous studies demonstrated (Czeczuga 1988) there are some carotenoids which are characteristic of the species of some lichen genera and occur irrespective of the environment in which the lichen grows. A typical example is the presence of mutatoxanthin in species of the genus *Xanthoria* (Czeczuga 1983). In the present material there were 6 species of the genus *Parmotrema*. On comparing the results of the analysis of the thalli of the lichen species of this genus it was found that the carotenoids common to all the species were (β)-carotene, astaxanthin, and antheraxanthin. The thalli of *Parmotrema sancti-angelii* and *Parmotrema tinctorum* collected from different ecological niches differed in the composition of the carotenoids contained. As regards the thalli of the former, these differences applied to such carotenoids as lutein, zeaxanthin, violaxanthin and

mutatoxanthin, and in the thalli of the letter, (β)-cryptoxanthin, 3'-epilutein, canthaxanthin, violaxanthin and capsochrome. These are carotenoids whose presence would seem to be affected by environmental factors.

Table 2 - List of the carotenoids from the investigated materials

Carotenoid	Structure	Semisystematic name
01. ε-carotene	A-R-A	ε, ε-carotene
02. α-carotene	A-R-B	β, ε-carotene
03. β-carotene	B-R-B	β, β-carotene
04. β-cryptoxanthin	B-R-D	β, β-carotene-3-ol
05. lutein	C-R-D	β, ε-carotene-3,3'-diol
06. 3'-epilutein	C-R-D	β, ε-carotene-3,3'-diol (steroisomeric)
07. zeaxanthin	D-R-D	β, β-carotene-3,3'-diol
08. α-doradexanthin	C-R-F	3,3'-dihydroxy-β,ε-caroten-4-one
09. canthaxanthin	E-R-E	β, β-carotene-4,4'-dione
10. astaxanthin	F-R-F	3,3'-dihydroxy-β, β-carotene-4,4'-dione
11. lutein epoxide	C-R-G	5,6-epoxy-5,6-dihydro-β, ε-carotene-3,3'-diol
12. antheraxanthin	D-R-G	5,6-epoxy-5,6-dihydro-β, β-carotene-3,3'-diol
13. violaxanthin	G-R-G	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro-β, β-carotene-3,3'-diol
14. neoxanthin	G-R_1-H	5,6-epoxy-6,7-didehydro-5,6,5',6'-β, β-carotene-3,5,3'-triol
15. mutatoxanthin	D-R_1-I	5,8-epoxy-5,8-dihydro-β, β-carotene-3,3'-diol
16. auroxanthin	I-R_2-I	5,8,5',8'-diepoxy-5,8,5',8'-tetrahydro-β, β-carotene-3,3'-diol
17. rhodoxanthin	K-R_3-K	4',5'-didehydro-4,5'-retro-β,β-carotene-3,3'-diol
18. capsochrome	I-R_1-L	5,8-epoxy-3,3'-dihydroxy-5,8-dihydro-β, χ-caroten-6'-one
19. apo-12'-violaxanthal	G-R_4-M	5,6-epoxy-3-hydroxy-5,6-dihydro-12'-apo-β-caroten-12'-al

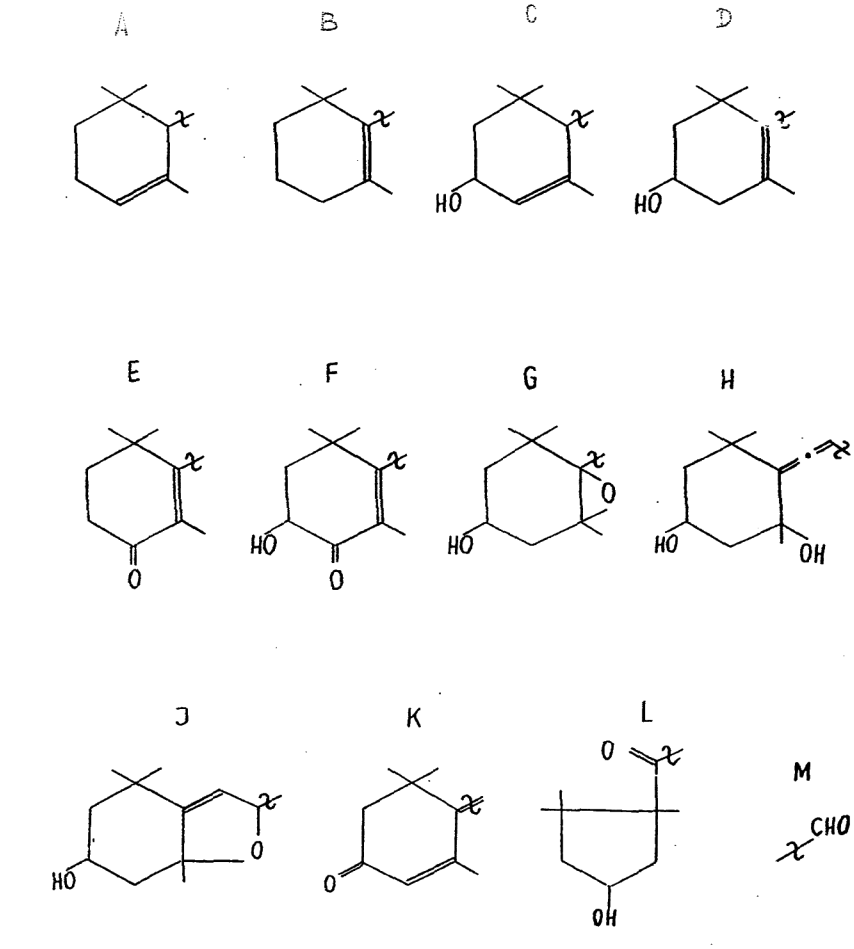
It is interesting to note that capsochrome was found in 13 of the 19 species studied. This carotenod is a derivative of capsanthin which was first isolated from the fruit of *Capsicum anuum* by Camara and Moneger (1980). The oxidation of capsanthin by capsanthin monoepoxide results in the formation of capsochrome. As further studies of Camara and Moneger (1981) revealed capsanthin is formed by the conversion of antheraxanthin. As the analysis of the material studied here indicates, antheraxanthin was present in most of the thalli of the species studied. Furthermore capsochrome has been found in the thalli of some lichen species of the genus *Peltigera* collected in the mountains of India (Czeczuga & Upreti 1992). In the plant world as a whole only capsanthin and some of its derivatives, with the exception of capsochrome, have been found in the flowers and ripe fruit of some species (Goodwin 1980).

The carotenoids here investigated can be said to be classified in two groups; primary and secondary carotenoids.

There exist primary and secondary carotenoids in green algae and in blue bacteria which are the regular constituents of the lichens. The primary carotenoids of green algae comprise carotene, lutein, zeaxanthin, violaxanthin and neoxanthin with minor amounts of antheraxanthin, lutein epoxide etc. These are the components of the photosynthetic biomembranes and needed for the performance of the photosynthetic light reactions. Cyanobacteria have a similar, though slightly different composition of primary carotenoids. Secondary carotenoids are formed in free living algae only un-

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

Fig. 1- Características estruturais de carotenóides dos materiais estudados.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der particular strees conditions e.g. high light stress, N-deficiency (Lichtenthaler & Verbeek 1973). To second carotenoids belong first of all the keto carotenoids and capsochrome.

In investigated materials from Brazil and Paraguay the following carotenoids belong to keto carotenoids: canthaxanthin, (α)- doradexanthin, astaxanthin and rhodoxanthin. The keto carotenoids were found in the thalli of all investigated species of Parmeliaceae.

Table 3 - Carotenoid distribution in lichens

Family and species	Carotenoid (see table 2 and Fig. 1)	Major carotenoids (%)	Total content (micro-g^{-1} weight)
Graphidaceae			
Chiodecton sanguineum (Sw.) Tober	2,3,5,11,12,15,16	16(41.1)	49.74
Coenogoniaceae			
Coenogonium linkii Ehrenb.	3,4,9,10,11,13,15,18	11(23.6)	55.54
Collemataceae			
Leptogium marginellum (Sw.) S. Gray	3,4,5,10,11,12,13,15,19	11(25.7)	54.16
Stictaceae			
Sticta damaecornis (Sw.) Ach.	2,3,4,5,7,11,12,15,18	15(29.0)	29.07
Cladoniaceae			
Cladonia confusa R. Sant.	2,3,4,5,6,7,14,15	15(32.3)	29.56
Stereocaulaceae			
Stereocaulon ramuloson (Sw.) Räusch.	2,3,4,5,7,11,13,18	7(27.0)	33.32
Pertusariaceae			
Pertusaria sp.	3,4,5,6,11,12,13	13(33.4)	81.38
Parmeliaceae			
Parmelina linokanii (Lynge) Hale	3,4,5,6,10,11,12,13,15	10(9.9)	96.38
Parmotrema araucariarum (Zahlbr.) Hale	1,2,3,4,7,10,11,12,13,18	18(33.8)	37.86
Parmotrema aff. *andinum* (Müll. Arg.) Hale	3,4,8,10,11,12,13,15	4(21.4)	52.29
Parmotrema mesotropum (Müll. Arg.) Hale	3,9,10,11,12,13	11(57.8)	21.97
Parmotrema praesorediosum (Nyl.) Hale	3,9,10,11,12,18	11(38.1)	39.99
Parmotrema sancti-angelii (Lynge) Hale	3,5,10,11,12,15,18	11(32.1)	67.98
Parmotrema sancti-angelii (Lynge) Hale	3,4,7,10,11,12,13,18	13(26.5)	43.08
Parmotrema tinctorum (Nyl.) Hale	3,4,5,9,10,11,12,15,18	11(35.5)	62.33
Parmotrema tinctorum (Nyl.) Hale	3,5,6,10,11,12,13,15	11(37.0)	55.81
Parmotrema tinctorum (Nyl.) Hale	3,4,5,6,10,11,12,13,15,18	15(35.0)	64.83
Punctelia microsticta (Müll. Arg.) Krog	3,4,5,6,10,11,12,15	11(24.6)	50.14
Usneaceae			
Ramalina celastri (Spreng.) Mey et Flot.	5,9,10,11,12,13,17,18	11(33.6)	62.35
Usnea amaliae Mot.	2,3,4,5,6,7,11,12,15,18	18(23.7)	19.40
Thelephoraceae			
Dictyonema pavonia (Sw.) Parm.	2,3,4,5,7,11,13,14,18	7(7.4)	24.05
Physciaceae			
Heterodermia vulgaris (Vain.) Hale	2,3,4,7,13,18	7(41.4)	30.37

Keto carotenoids have been found not only in green algae and cyanobacteria but also in fungi (Goodwin 1980).

In addition, as stated by Czeczuga (1983), in shady habitats the lichen thalli tend to contain higher concentrations of carotenoids but fewer carotenoid compounds than in habitats with intense insolation. In open places a major increase has been noted in, for instance, the amount of epoxide forms. Environmental changes such as variation in the mineral status of the substrate and temperature fluctuations have well-established effects on terpenoid levels (carotenoids are tetraterpenoids).

The biosynthesis of carotenoids is also markedly dependent on the season of the year, usually being most vigorous in spring (in areas with seasoal climats), as is the case of the lichen metabolism in general (Czeczuga *et al.* 1991). This is in agreement with reports that terpenoids in plants as a rule show considerable developmental variation.

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