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Investigations on Carotenoids in Lichens. XX. Carotenoids in Lichens from Various Italian Environments

By

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With 1 Figure

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Summary

CZECZUGA B., CACCAMESE S. & PASSADORE M. V. 1989. Investigations on carotenoids in lichens XX. Carotenoids in lichens from various Italian environments. – *Phyton* (Austria) 29 (1): 15–22, with 1 figure. – English with German summary.

Column and thin-layer chromatography revealed the presence of the following carotenoids in the thalli of 10 lichen species from various Italian environments: torulene, β -carotene, lycoxanthin, lycophyll, β -cryptoxanthin, zeaxanthin, antheraxanthin, lutein, lutein epoxide, echinenone, canthaxanthin, astaxanthin, violaxanthin, neoxanthin, mutatoxanthin, auroxanthin, luteoxanthin, flavoxanthin, rhodoxanthin, β -apo-2'-carotenal, 3-hydroxy- β -apo-10'-carotenal and apo-12'-violaxanthin.

The total content of carotenoids ranged from 15.4 (*Ramalina fraxinea*) to 263.1 $\mu\text{g g}^{-1}$ dry wt (*Xanthoria parietina* from Mt. Etna, Sicily).

Zusammenfassung

CZECZUGA B., CACCAMESE S. & PASSADORE M. V. 1989: Untersuchungen über Carotinoide in Flechten XX. Carotinoide in Flechten verschiedener Standorte in

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Italien. — Phytol (Austria) 29 (1): 15–22, mit 1 Figur. — Englisch mit deutscher Zusammenfassung.

An zehn Flechtenspezies von verschiedenen Standorten Italiens wurde mittels Säulen- und Dünnschichtchromatographie das Vorkommen folgender Carotinoide festgestellt: Torulin, β -Carotin, Lycoxanthin, Lycophyll, β -Cryptoxanthin, Zeaxanthin, Antheraxanthin, Lutein, Luteinopoxid, Echinenon, Canthaxanthin, Astaxanthin, Violaxanthin, Neoxanthin, Mutatoxanthin, Auroxanthin, Luteoxanthin, Flavoxanthin, Rhodoxanthin, β -Apo-2'-carotenal, 3-Hydroxy- β -apo-10'-carotenal und Apo-12'-violaxanthin. Der Gesamtgehalt an Carotinoiden liegt zwischen 15,4 (*Ramalina fraxinea*) und 263, 1 $\mu\text{g g}^{-1}$ dry wt. (*Xanthoria parietina* vom Aetna, Sizilien).

Introduction

The studies we have carried out so far on the presence of carotenoids in lichens showed that the thalli of the same species of lichen may contain different carotenoids and that their total content may also differ (CZECZUGA 1983, CZECZUGA & ALSTRUP 1987). These data suggested that this depends primarily on the substratum on which a given thallus grows and also on climatic conditions. It was with this view that we decided to study the presence of carotenoids and their total content in the same species of lichen growing on different types of substratum and in different regions of Italy.

Material and Methods

The investigations were carried out on the following 10 species collected from various Italian environments: *Peltigera ponojensis* GYELNIK, *Stereocaulon vesuvianum* PRES., *Parmelia acetabulum* (NECK.) DUBY, *Parmelia furfuracea* (L.) ACH., *Parmelia saxatilis* (L.) ACH., *Parmelia tubulosa* (SCHAER.) BITT., *Ramalina fraxinea* (L.) ACH., *Xanthoria parietina* (L.) BELTR., *Anaptychia ciliaris* (L.) KOERB. and *Physcia pulverulenta* (SCHREB.) HAMPE (Table 1).

The thalli were cleaned of all organic debris, macerated, placed in dark glass bottles and covered with acetone. The air above the fluid in the bottle was replaced with nitrogen, to ensure an anaerobic atmosphere. The samples were kept in a refrigerator (-4°C) until analysed by chromatography for their carotenoid content.

The 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 hours in the dark in a nitrogen atmosphere.

Column and thin-layer chromatography, described in detail in a previous paper (CZECZUGA 1980), were used to separate various carotenoids. A glass column (Quickfit-England), approximately 1 cm in diameter and 15–20 cm in length, filled with Al_2O_3 was used in the column chromatography. The extract was passed through the column and the different fractions were eluted with the solvent. Silica gel was used for thin-layer

Table 1
Investigated species of lichens from Italia

Familia and species	Collected from	Locality
<i>Peltigeraceae</i>		
1. <i>Peltigera ponojensis</i>	<i>Morus alba</i>	Urbino, Marches
<i>Stereocaulaceae</i>		
2. <i>Stereocaulon vesuvianum</i>	lava rocks	Mt. Etna, Sicily
<i>Parmeliaceae</i>		
3. <i>Parmelia acetabulum</i>	rocks	Nebrodi, Sicily
4. <i>Parmelia furfuracea</i>	<i>Pinus sylvestris</i>	Asiago, Veneto
5. <i>Parmelia furfuracea</i> var. <i>olivetorina</i>	<i>Pinus pinea</i>	Sila, Calabria
6. <i>Parmelia furfuracea</i> var. <i>olivetorina</i>	<i>Pinus sylvestris</i>	near Champex, Switzerland
7. <i>Parmelia saxatilis</i>	rocks	Nebrodi, Sicily
8. <i>Parmelia tubulosa</i>	<i>Pinus sylvestris</i>	Asiago, Veneto
9. <i>Parmelia tubulosa</i>	<i>Fagus sylvatica</i>	Nebrody, Sicily
<i>Usneaceae</i>		
10. <i>Ramalina fraxinea</i> var. <i>caliciformis</i>	<i>Fagus sylvatica</i>	Nebrodi, Sicily
<i>Teloschistaceae</i>		
11. <i>Xanthoria parietina</i>	lava rocks	Mt. Etna, Sicily
12. <i>Xanthoria parietina</i>	<i>Eucalyptus camaldulensis</i>	Catanzaro, Calabria
13. <i>Xanthoria parietina</i>	rocks	St. Antine, Sardinia
<i>Physciaceae</i>		
14. <i>Anaptychia ciliaris</i>	<i>Fagus sylvatica</i>	Nebrodi, Sicily
15. <i>Physcia pulverulenta</i>	<i>Morus alba</i>	Genga, Marches

chromatography with the appropriate solvent systems, the R_f values being determined for each spot.

For identification of carotenoids replicate chromatography was performed with standard carotenoids (Hoffman-La Roche & Co. Ltd., Basle, Switzerland and Sigma Chemical Company, USA).

The pigments were identified on the basis of: a) their behaviour in column chromatography, b) their absorption spectra in various solvents, recorded with a Beckman spectrophotometer model 2400 Du, c) their partition between hexane and 95% methanol, d) their R_f values in thin-layer chromatography, e) the presence of allylic hydroxyl groups, determined by the acid-chloroform test, f) the epoxide test, and g) the mass spectrum (VETTER & al. 1971).

The concentrations of carotenoid solutions were determined from the absorption spectra, on the basis of the extinction coefficient E $1\% \text{ cm}^{-1}$ at wavelengths of maximal absorbance in petroleum ether or hexane (DAVIES 1976).

Results

In the thalli of the lichen species studied, the presence of 22 carotenoids, was established (Table 2, Fig. 1). Those most worthy of note were; torulene (*Xanthoria parietina* from Calabria), echinenone (*Parmelia tubulosa* from Sicily), auroxanthin (*Parmelia furfuracea* var. *olivetorina* from the border with Switzerland), luteoxanthin (*Parmelia tubulosa* from

Table 2

List of the carotenoids from the investigated lichen species

Carotenoid	Structure (see Fig. 1)	Semisystematic name
1. Torulene	A - R - B	3',4'-didehydro- β , ψ -carotene
2. β -Carotene	B - R - B	β , β -carotene
3. Lycoxanthin	D - R - E	ψ , ψ -carotene-16-ol
4. Lycophyll	E - R - E	ψ , ψ -carotene-16,16'-diol
5. β -Cryptoxanthin	B - R - C	β , β -carotene-3-ol
6. Zeaxanthin	C - R - C	β , β -carotene-3,3'-diol
7. Antheraxanthin	C - R - G	5,6-epoxy-5,6-dihydro- β , β -carotene-3,3'-diol
8. Lutein	C - R - F	β , ϵ -carotene-3,3'-diol
9. Lutein epoxide	F - R - G	5,6-epoxy-5,6-dihydro- β , ϵ -carotene-3,3'-diol
10. Echinenone	B - R - K	β , β -carotene-4-one
11. Canthaxanthin	K - R - K	β , β -carotene-4,4'-dione
12. Astaxanthin	L - R - L	3,3'-dihydroxy- β , β -carotene-4,4'-dione
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'- tetrahydro- β , β -carotene-3,5,3'-triol
15. Mutatoxanthin	C - R ₁ - J	5,8-epoxy-5,8-dihydro- β , β -carotene-3,3'-diol
16. Auroxanthin	I - R ₃ - I	5,8,5',8'-diepoxy-5,8',5',8'-tetrahydro- β , β -carotene-3,3'-diol
17. Luteoxanthin	G - R ₁ - I	5,6,5',8'-diepoxy-5,6,5',8'-tetrahydro- β , β -carotene-3,3'-diol
18. Flavoxanthin	F - R ₁ - J	5,8-epoxy-5,8-dihydro- β , ϵ -carotene-3,3'-diol
19. Rhodoxanthin	M - R ₄ - M	4',5'-didehydro-4',5'-retro- β , β -carotene-3,3'-dione
20. β -Apo-2'-carotenal	B - R - N	3',4'-didehydro-2'-apo- β -caroten-2'-al
21. 3-Hydroxy- β -apo- 10'-carotenal	C - R ₂ - P	3-hydroxy-10'-apo- β -caroten-10'-al
22. Apo-12'-violaxanthal	G - R ₂ - O	5,6-epoxy-3-hydroxy-5,6-dihydro- 12'-apo- β -caroten-12'-al

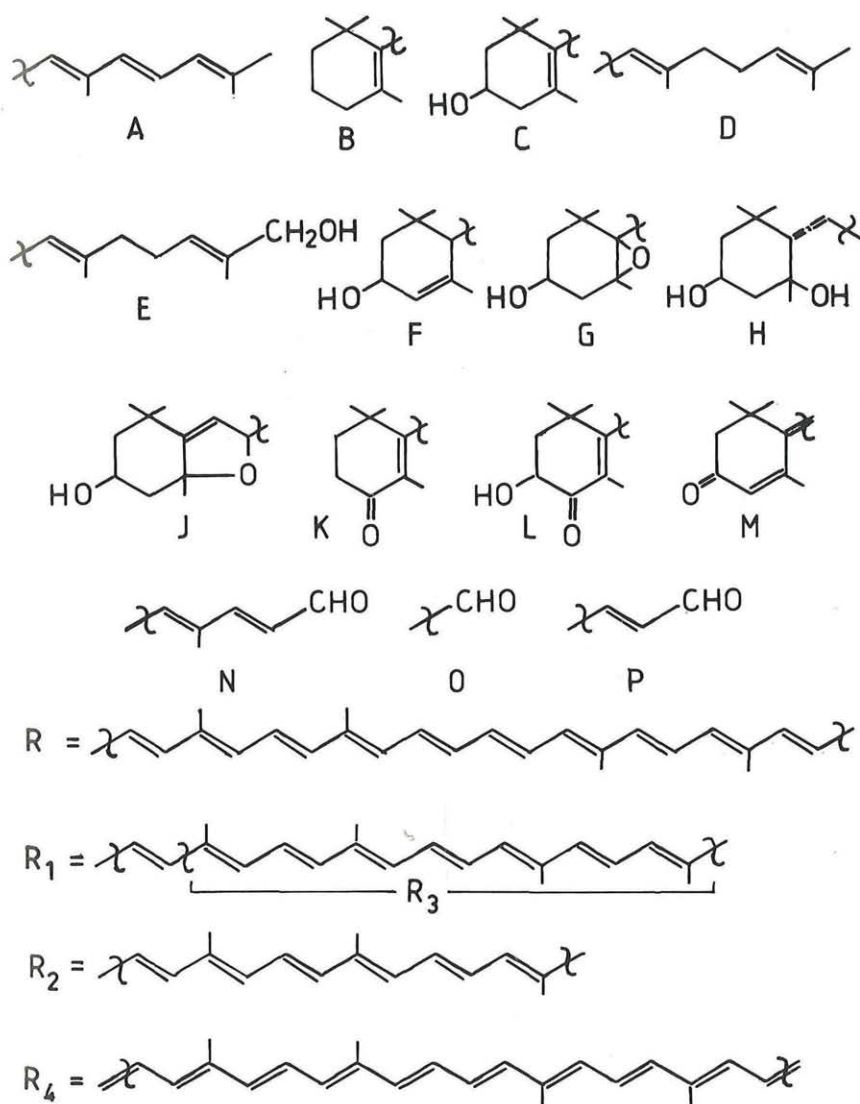


Fig. 1. Structural features of carotenoids.

Sicily), 3-hydroxy- β -apo-10'-carotenal (*Xanthoria parietina* from Sicily) and apo-12'-violaxanthal (*Peltigera ponojensis* and *Parmelia tubulosa* from Veneto). Of particular interest is the fact that in the same species of lichens taken from different sites, the carotenoids different. This was the case with *Parmelia furfuracea*, *Parmelia tubulosa* and *Xanthoria parietina*. Only in the thalli of *Xanthoria parietina* collected from all three sites was the same carotenoid (mutatoxanthin) found to predominate; in the other two species

(*Parmelia furfuracea* and *Parmelia tubulosa*) the predominant carotenoids differed in the thalli taken from different sites. The total carotenoid content in the thalli of the species studied ranged from 15.4 (*Ramalina fraxinea*) to 263.1 $\mu\text{g g}^{-1}$ dry wt (*Xanthoria parietina* from Sicily). In these same species collected from various regions of Italy the total carotenoid contents also differed.

Discussion

As was mentioned above, the presence of a number of carotenoids, not frequently found in lichens to date, was noted in these studies. One of these carotenoids is torulene. This carotenoid is commonly found in fungi (GOODWIN 1980) but has been noted in three lichen species of South Africa (CZECZUGA & BECKETT 1988). Echinenone, on the other hand, is one of the few ketocarotenoids which are rather rarely found in nature and are even rarer in lichens. The next three carotenoids, auroxanthin, luteoxanthin and flavoxanthin belong to the group of epoxide carotenoids and are quite frequently found in flowers, ripe fruits and in the autumn leaves of higher plants (CZECZUGA 1986). In the lichens, luteoxanthin has been reported in several species whereas auroxanthin has been noted in the thalli of only a few single lichen species. Auroxanthin constitutes over 80% of all the carotenoids in the thalli of the *Pseudocyphellaria aurata* and *Pseudocyphellaria clothera* lichen species from Brazil (CZECZUGA & XAVIER-FILHO 1985). On the other hand, the present investigations have revealed the presence of flavoxanthin in lichens for the first time. This is also the first report of the presence of 3-hydroxy- β -apo-10'-carotenal, which as we know is formed as a result of the natural degradation of β -carotene, in lichens (WEEDON 1971). The second apocarotenal found in the lichen species studied, apo-12'-violaxanthal, is a carotenoid typical of yellow autumn leaves (CZECZUGA 1986) but has to date been noted in only a few lichen species.

The present studies have revealed the significant effect that the environment has on the presence of the various carotenoids on the one hand and their total content on the other. This is clearly evident from the data obtained from thalli collected at various sites in Italy of such lichen species as *Parmelia furfuracea*, *Parmelia tubulosa* and *Xanthoria parietina*. Auroxanthin occurred only in the thalli of *Parmelia furfuracea* var. *olivetorina* collected from the Italian-Swiss border, whereas the *Parmelia tubulosa* thalli taken from the bark of *Fagus sylvatica* in Sicily contained flavoxanthin. Then again, the *Xanthoria parietina* thalli from the bark of *Eucalyptus camaldulensis* contained torulene whilst the thalli of that same species collected from different sites and from a different substratum did not contain this carotenoid. Only the *Xanthoria parietina* thalli from various sites contained the same predominant carotenoid (mutatoxanthin) whereas in the *Parmelia furfuracea* and *Parmelia tubulosa* thalli taken from differ-

Table 3
Carotenoid distribution in lichens from Italy

Familia and species	Carotenoids (see Table 2)	Major carotenoid (%)	Total content ($\mu\text{g g}^{-1}$ dry wt)
<i>Peltigeraceae</i>			
1. <i>Peltigera ponojensis</i>	2, 3, 4, 6, 9, 12, 13, 14, 22	12 (38.3)	17.4
<i>Stereocaulaceae</i>			
2. <i>Stereocaulon vesuvianum</i>	3, 4, 5, 6, 9, 12, 13, 15	12 (37.4)	16.6
<i>Parmeliaceae</i>			
3. <i>Parmelia acetabulum</i>	2, 3, 5, 9, 12, 13, 14, 15	15 (37.8)	21.3
4. <i>Parmelia furfuracea</i>	5, 6, 9, 11, 12, 14, 15, 19	15 (25.8)	18.7
5. <i>Parmelia furfuracea</i> var. <i>olivetorina</i>	9, 11, 12, 13, 14, 15	9 (26.5)	19.3
6. <i>Parmelia furfuracea</i> var. <i>olivetorina</i>	3, 5, 6, 7, 9, 11, 12, 15, 16, 19	16 (35.9)	15.9
7. <i>Parmelia saxatilis</i>	5, 6, 9, 12, 13, 14	9 (23.1)	26.6
8. <i>Parmelia tubulosa</i>	5, 6, 9, 14, 15, 19, 22	5 (31.1)	21.2
9. <i>Parmelia tubulosa</i>	6, 7, 9, 10, 12, 14, 17, 18	17 (44.7)	32.3
<i>Usneaceae</i>			
10. <i>Ramalina fraxinea</i>	6, 9, 12, 13, 15, 19, 20	13 (32.1)	15.4
<i>Teloschistaceae</i>			
11. <i>Xanthoria parietina</i>	3, 5, 6, 12, 14, 15, 21	15 (78.9)	263.1
12. <i>Xanthoria parietina</i>	1, 4, 5, 9, 12, 15, 19	15 (82.2)	89.4
13. <i>Xanthoria parietina</i>	2, 5, 9, 12, 15	15 (92.0)	203.9
<i>Physciaceae</i>			
14. <i>Anaptychia ciliaris</i>	6, 9, 12, 13, 14, 15, 17, 19, 20	9 (35.0)	17.6
15. <i>Physcia pulverulenta</i>	5, 6, 7, 8, 9, 12, 15, 17	8 (23.5)	26.9

ent sites, different carotenoids were found to be predominant. In all three species, too, the total carotenoid content differed. In summing up, it should be emphasized that environmental factors have a significant effect on the biosynthesis of carotenoids in lichens.

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Recensio

CONERT Hans Joachim 1987. *Gramineae* (Echte oder Süßgräser). — Lief. 4. [Pooideae, Fortsetzung] *Helictotrichon* [Fortsetzung], *Gaudinia*, *Ventenata*, *Koeleria*, *Rostraria*, *Trisetum*, *Aira*, *Deschampsia*, *Corynephorus* [Anfang]. — Lex. 8°, Seiten 241–320, Abb. 101–137, Tafeln 29–30; brosch. — In: CONERT H. J., HAMANN U., SCHULTZE-MOTEL W. & WAGENITZ G. (Eds.): Gustav HEGI, Illustrierte Flora von Mitteleuropa, Band 1, Teil 3; 3. völlig neu bearbeitete Auflage. — Verlag Paul Parey Berlin, Hamburg. — DM 35,—. — ISBN 3-489-52320-2.

Der erste Band des HEGI (*Pteridophyta-Gramineae*) erschien 1906–1908 in erster Auflage und 1936 als 2., neubearbeitete Auflage; davon gab es 1965 einen Reprint. In der dritten Auflage kommt dieser Band in Form dreier getrennter Teile heraus, wobei Teil 3 die *Gramineae* enthält; von diesem Teil, dessen Herausgabe 1979 begann, liegt nun die vierte Lieferung vor. Lief. 1 und 2 sind in *Phyton* 24 (2): 317–318; Lief. 3 in *Phyton* 26 (1): 131–132 besprochen worden. Die neue Lieferung gleicht in Umfang und Ausstattung im wesentlichen den vorhergehenden. Da auch nicht wiederholt zu werden braucht, daß die Neubearbeitung der Familie höchst wünschenswert war, kann auf die zitierten Rezensionen verwiesen werden.

Hinsichtlich der Systematik fällt die weite Fassung der Gattungen *Helictotrichon* (inkl. *Avenochloa* bzw. *Avenula*) und *Deschampsia* (inkl. *Avenella* und *Aristavena*) auf. Schade, daß die, für die Systematik von *Aira*, *Deschampsia* und verwandten Gattungen wichtige Arbeit von ALBERS 1980 (*Phyton* 20 [1–2]: 95–116), nicht berücksichtigt worden ist; CONERT vereinigt in *Deschampsia* Elemente, die nach Meinung von ALBERS verschiedenen Subtriben angehören. Im allgemeinen empfindet der Rezensent das Vermeiden allzugroßen Splittings bei Gattungen und Arten (z. B. *Koeleria*) jedoch positiv.

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