Investigations on carotenoids in lichens. XXXII. Carotenoids occurring in the thalli of lichens from Kenya (Equatorial Africa)

BAZYLI CZECZUGA^a, ROLAND MOBERG^b, VAGN ALSTRUP^c

^a Departament of General Biology, Medical Academy, Kilińskiego 1, PL-15-230 Białystok 8, Poland

^b Institute of Systematic Botany, University of Uppsala, P.O. Box 541, S-75121 Uppsala, Sweden

^c Institute of Plant Ecology, University of Copenhagen, Øster Farimagsgade 2D, DK-1353 Copenhagen K, Denmark

(Received: November 6, 1990. Accepted: November 20, 1991)

Abstract

The presence of carotenoids in nineteen species of lichens from Kenya (Equatorial Africa) was studied by column and thinlayer chromatography. This investigation revealed the presence of the following carotenoids: neurosporene, α -carotene, β -carotene, rubixanthin, α -cryptoxanthin, β -cryptoxanthin, zeaxanthin, lutein, 3'-epilutein, torularhodin, diatoxanthin, neoxanthin, echinenone, 3'-hydroxyechinenone, canthaxanthin, α -doradexanthin, astaxanthin, β -carotene epoxide, antheraxanthin, lutein epoxide, violaxanthin, mutatoxanthin, flavoxanthin, capsochrome, β -apo-8'-carotenal, β -apo-10'-carotenal and apo-12'-violaxanthin. Five of these, torularhodin, 3'-hydroxyechinenone, capsochrome, β -apo-8'-carotenal and β -apo-10'-carotenal, are reported for the first time from lichens. The total carotenoid content of the material ranged from 15.88 (*Pyxine coccinea*) to 135.44 $\mu\text{g g}^{-1}$ dry weight (*Teloschistes chrysophthalmus*).

Key words: lichens, carotenoids, Kenya, Africa

INTRODUCTION

In recent years the attention of many lichenologists has turned to the regions of East and Equatorial Africa (Spence and Mahaney 1988, Swinscow and Krogh 1988, Almqvist 1989). These studies have provided new data on the distribution and biology of a number of species (Swinscow and Krogh

1979, Moberg 1986, 1987) and have made it possible to establish a number of new species of interest to lichenologists (Ahti et al. 1987).

The publication of data on the presence of carotenoids in the thalli of the various lichen species from this region of Africa will not only enrich our knowledge of the biochemistry of these plants but may also prove to be of value in taxonomic research since reports on the occurrence of various carotenoids in fungi (Valadon 1976) and in algae (Liaaen-Jensen 1977) have been useful in the chemotaxonomy of these organisms (Czeczuga 1988) which, as we know, from the different species of lichens as a result of symbiosis.

MATERIALS AND METHODS

The investigations were carried out on the thalli of nineteen lichen species from Kenya (Equatorial Africa) (see Table 1.) 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 (TLC) (Czeczuga 1980) were used for the separation of various carotenoids. A 15-20 cm × 1 cm glass column (Quickfit, England) packed with Al₂O₃ 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 carotenoids the 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 (Krinksky and Goldsmith 1960); (g) the mass spectrum; and (h) infrared spectroscopy for torulorhodin, 3'-hydroxyechinenone, capsorhrome, β -apo-8'-carotenal and β -apo-10'-carotenal (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).

Structure of carotenoids was given according Straub (1971, 1987).

Table 1

Species of lichens from Kenya that were investigated

Species	Locality	Habitat	Altitude in m
<i>Collema rugosum</i> Krempelh.	Nairobi District-Ngong Hills	trunk- <i>Schinus molle</i>	1600
<i>Heterodermia leucomelos</i> (L.) Poelt			
subsp. <i>boryi</i> (Fee) Swinscow et Krog	Nairobi District-Ngong Hills	trunk- <i>Schinus molle</i>	1550
<i>Heterodermia speciosa</i> (Wulf.) Trevis.	Riff Valley Province	trunk- <i>Schinus molle</i>	1900
<i>Hypogymnia bitteri</i> (Lynge) Ahti	Mt. Kenya National Park	rocks, alpine zone	3550
<i>Lasallia pustulata</i> (Ach.) Llano	Mt. Kenya National Park	boulder, alpine zone	3900
<i>Lobaria pulmonaria</i> (L.) Hoffm.	Mt. Kenya National Park	trunk- <i>Podocarpus milanjianus</i>	2900
<i>Parmotrema abessinicum</i> (Krempelh.) Hale	Nairobi District-Ngong Hills	trunk- <i>Schinus molle</i>	1700
<i>Phaeophyscia adiantola</i> (Essl.) Essl.	Aberdare National Park	trunk- <i>Hagenia abyssinica</i>	3350
<i>Physcia albata</i> (Wils.) Hale	Aberdare National Park	rocks-steep northern	3650
<i>Physcia albo-plumbea</i> (Tayl.) Nyl.	Mt. Kenya National Park	<i>Podocarpus milanjianus</i>	2900
<i>Pseudocyphellaria clathrata</i> (de Not)			
Malme	Nairobi District-Ngong Hills	on <i>Schinus molle</i>	1600
<i>Pseudocyphellaria cracata</i> (L.) Vainio	Nairobi District-Ngong Hills	on <i>Schinus molle</i>	1700
<i>Pyxine cocoes</i> (Swinscow) Nyl.	Tsavo National Park East	trunk of a tree	500
<i>Pyxine reticulata</i> (Vainio) Vainio	Riff Valley Province	trunk- <i>Schinus molle</i>	1900
<i>Ramalina celastri</i> (Sprengel) Krog et Swinsow			
	Nairobi District-Ngong Hills	trunk- <i>Schinus molle</i>	1700
<i>Teloschistes chrysophthalmus</i> (L.) Th.Fr.	Nairobi District-Ngong Hills	trunk- <i>Schinus molle</i>	1600
<i>Teloschistes perrugosus</i> Müll. Arg.	Nairobi District-Ngong Hills	trunk- <i>Schinus molle</i>	1700
<i>Umbilicaria haumaniana</i> (L.) Ach. em. Frey	Mt. Kenya National Park	rocks-alpine zone	3750
<i>Usnea exasperata</i> (Müll. Agr.) Motyka	Nairobi District-Ngong Hills	trunk- <i>Schinus molle</i>	1600

RESULTS

In the thalli of 19 lichen species, 26 carotenoids were identified including several species comparatively rarely found previously (Table 2, Fig. 1). These are neurosporene (*Usnea exasperata*), rubixanthin (*Pseudocyphellaria clathrata*), α -cryptoxanthin (*Hypogymnia bitteri*, *Phaeophyscia adiastrata*, *Physcia albo-plumbea*, *Umbilicaria haumaniana*), torularhodin (*Pseudocyphellaria crocata*), diatoxanthin (*Physcia albata*, *Umbilicaria haumaniana*), echinenone (*Collema rugosum*), 3'-hydroxyechinenone (*Collema rugosum*), flavoxanthin (*Pyxine reticulata*), capsochrome (*Lobaria pulmonaria*, *Physcia albo-plumbea*, *Pyxine coccinea*), and three carotenoids of the apocarotenal group; β -apo-8'-carotenal (*Pseudocyphellaria clathrata*), β -apo-10'-carotenal (*Hypogymnia bitteri*, *Pseudocyphellaria clathrata*) and apo-12'-violaxanthal (*Umbilicaria haumaniana*) (Table 3).

Fig. 1. Structural features of carotenoids from investigated materials

Table 2

List of the carotenoids found in lichens from Kenya

Carotenoid	Structure (see Fig. 1)	Semi-systematic name
1. Neurosporene	A — R — A	7,8-dihydro- ϕ , ϕ -carotene
2. β -Carotene	C — R — C	β , β -carotene
3. α -Carotene	B — R — C	β , ϵ -carotene
4. Rubixanthin	A — R — D	β , ϕ -caroten-3-ol
5. β -Cryptoxanthin	C — R — D	β , β -caroten-3-ol
6. α -Cryptoxanthin	B — R — D	β - ϵ -caroten-3-ol
7. Zeaxanthin	D — R — D	β - β -carotene-3,3'-diol
8. Lutein	D — R — E	β - ϵ -carotene-3,3'-diol
9. 3'-Epilutein	D — R — E	β - ϵ -carotene-3,3'-diol (stereoisomeric)
10. Torularhodin	C — R — F	3',4'-didehydro- β , ϕ -caroten-16'-oic acid
11. Diatoxanthin	D — R ₁ — C	7,8-didehydro- β , β -carotene-3,3'-diol
12. Neoxanthin	H — R ₁ — I	5',6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro- β , β -carotene-3,5,3'-triol
13. Echinenone	C — R — K	β , β -caroten-4-one
14. 3'-Hydroxyechinenone	D — R — K	3'-hydroxy- β , β -caroten-4-one
15. Canthaxanthin	K — R — K	β , β -carotene-4,4'-dione
16. α -Doradexanthin	E — R — L	3,3'-dihydroxy- β , ϵ -carotene-4-one
17. Astaxanthin	L — R — L	3,3'-dihydroxy- β , β -carotene-4,4'-dione
18. β -Carotene epoxide	C — R — M	5,6-epoxy-5,6-dihydro- β , β -carotene
19. Antheraxanthin	D — R — H	5,6-epoxy-5,6-dihydro- β , β -carotene-3,3'-diol
20. Lutein epoxide	E — R — H	5,6-epoxy-5,6-dihydro- β , ϵ -carotene-3,3'-diol
21. Violaxanthin	H — R — H	5,6,5',6'-diepoxy-5,6,5'-tetrahydro- β , β -carotene-3,3'-diol
22. Mutatoxanthin	D — R ₁ — N	5,8-epoxy-5,8-dihydro- β , β -carotene-3,3'-diol
23. Flavoxanthin	E — R ₁ — N	5,8-epoxy-5,8-dihydro- β - ϵ -carotene-3,3'-diol
24. Capsochrome	N — R ₁ — O	5,8-epoxy-3,3'-dihydroxy-5,8-dihydro- β , χ -caroten-6'-one
25. β -Apo-8'-carotenal	C — R ₁ — P	8'-apo- β -caroten-8'-al
26. β -Apo-10'-carotenal	C — R ₂ — P	10'-apo- β -caroten-10'-al
27. Apo-12'-violaxanthal	H — R ₂ — P	5,6-epoxy-3-hydroxy-5,6-dihydro-12'-apo- β -caroten-12'-al

Table 3

Carotenoid distribution in lichens from Keyna

Species	Carotenoid detected (see Table 2)	Major carotenoid (%)	Total content ($\mu\text{g g}^{-1}$ dry weight)
<i>Collema rugosum</i> Krempelh.	2, 8, 9, 12, 13, 14, 17, 20, 21	2 (43.4)	77.57
<i>Heterodermia leucomelos</i> (L.) Poelt subsp. <i>boryi</i> (Fee) et Krog	2, 7, 8, 16, 19, 20, 21	20 (30.3)	31.08
<i>Heterodermia speciosa</i> (Wulf.) Trevis.	2, 3, 5, 7, 18, 19, 20, 22	22 (56.6)	18.92
<i>Hypogymnia bitteri</i> (Lynge) Ahti	2, 5, 6, 7, 12, 18, 20, 21, 26	20 (16.2)	21.24
<i>Lasallia pustulata</i> (Ach.) Llano	2, 3, 7, 12, 19, 20, 21, 22	22 (35.6)	25.86
<i>Lobaria pulmonaria</i> (L.) Hoffm.	2, 3, 7, 8, 19, 20, 21, 24	24 (22.5)	16.18
<i>Parmotrema abessinicum</i> (Krempelh.) Hale	2, 5, 8, 17, 19, 20, 21	17 (30.2)	31.54
<i>Phaeophyscia adiastrata</i> (Essl.) Essl.	2, 3, 6, 8, 12, 20, 21, 22	22 (40.2)	30.14
<i>Physcia albata</i> (Wils.) Hale	2, 3, 8, 11, 12, 19, 20, 21	21 (26.6)	16.52
<i>Physcia albo-plumbea</i> (Tayl.) Nyl.	2, 3, 5, 6, 19, 20, 21, 24	20 (49.4)	26.16
<i>Pseudocyphellaria clathrata</i> (de Not) Malme	2, 4, 16, 17, 20, 25, 26	17 (29.2)	24.50
<i>Pseudocyphellaria cracata</i> (L.) Vainio	2, 10, 15, 16, 17, 18, 20	17 (37.3)	28.60
<i>Pyxine cocoes</i> (Swinscow) Nyl.	2, 3, 5, 8, 20, 21, 24	21 (47.3)	15.89
<i>Pyxine reticulata</i> (Vainio) Vainio	2, 3, 8, 9, 12, 19, 20, 21, 23	20 (28.4)	34.02
<i>Ramalina celastri</i> (Sprengel) Krog et Swinsow	2, 7, 8, 17, 20, 21	20 (43.4)	44.08
<i>Teloschistes chrysophthalmus</i> (L.) Th.Fr.	2, 3, 5, 8, 9, 19, 20, 21, 22	22 (79.3)	135.44
<i>Teloschistes perrugosus</i> Müll. Arg.	2, 3, 5, 8, 9, 19, 20, 22	22 (51.0)	98.13
<i>Umbilicaria haumaniana</i> (L.) Ach. em. Frey	2, 3, 6, 7, 8, 9, 11, 20, 21, 27	21 (21.3)	17.14
<i>Usnea exasperata</i> (Müll. Agr.) Motyka	1, 2, 8, 9, 17, 20, 21, 22	20 (29.9)	35.05

β -Carotene and lutein epoxide were found in the thalli of all the lichen species studied. The predominant carotenoids in these species were, with the exception of one species (*Collema rugosum*) in which β -carotene predominated, found to be the more oxidized carotenoids such as astaxanthin (in 3 species), lutein epoxide (in 6 species), violaxanthin (in 3 species), mutatoxanthin (in 5 species) and capsochrome (in 1 species). The total carotenoid content of the material investigated ranged from 15.88 (*Pyxine cocomes*) to 133.44 $\mu\text{g g}^{-1}$ dry weight (*Teloschistes chrysophthalmus*).

DISCUSSION

As the results of these studies show, the thalli of the lichens from Kenya contain a comparatively wide variety of carotenoids which, on the whole, have not been frequently found in lichens to date. One of this group of carotenoids is neurosporene. In our studies of carotenoids in lichens to date, neurosporene was found, among others, in the thalli of *Cetraria nivalis* from Greenland (Czeczuga and Alstrup 1987). Rubixanthin, on the other hand, was determined in the thalli of *Ramalina pollinaria* from Upper Galilee (Czeczuga and Bublick 1986) and in *Cetraria laevigata* from the Kolyma Mountains on the Kamchatka Peninsula (Czeczuga et al. 1989b). In the thalli of the *Alectoria ochroleuca*, also from the Kamchatka, we demonstrated the presence of α -cryptoxanthin (Czeczuga et al. 1989b). On the other hand as regards the torulene derivative, torularhodin, this is the first time that this carotenoid has been noted in lichens though it has been found in lower and higher fungi (Goodwin 1980). The carotenoids, diatoxanthin and echinenone have been found in the thalli of *Cetraria islandica* from Lapland (Czeczuga 1986). The echinenone derivative, 3'-hydroxyechinenone, has for the first time been noted in lichens. To date it has been found in some representatives of the *Euglenophyta* and in fungi of the *Hyphomycetes* (Goodwin 1980). The next rare carotenoid, flavoxanthin, was noted in *Parmelia tubulosa* thalli collected from Sicily (Czeczuga et al. 1989a). As regards capsochrome, this is also the first time this carotenoid has been reported in lichens. It is, as we know, a derivative of capsanthin (Strub 1971) and is quite often found in flowers and ripe fruit of higher plants. It originates from epoxide forms of β -carotene (Goodwin 1980).

Of the three carotenoids of the apocarotenal group, β -apo-8'-carotenal and β -apo-10'-carotenal have been found for the first time in lichens whereas apo-12'-violaxanthal has been noted previously in several, lichen species from the Antarctica (Czeczuga and Xavier-Filho 1987b). β -Apo-8'-carotenal and β -apo-10'-carotenal have been found in some species from the Antarctica (Czeczuga et al. 1982, Czeczuga and Xavier-Filho 1988).

While carrying out our studies of carotenoids in certain species of lichen from South Africa (Czeczuga et al. 1988), we also included in these investigations

some species of genera from Kenya, the *Heterodermia*, *Pseudocyphellaria* and *Teloschistes*. In the species of the *Heterodermia* genus in the thalli of both species from South and Equatorial Africa such carotenoids as β -carotene, zeaxanthin and lutein epoxide occurred whereas in the *Pseudocyphellaria* species lutein epoxide and astaxanthin were found to be common to all the species. Lutein epoxide was noted in all the species of the *Teloschistes* genus. Lutein epoxide and astaxanthin also occurred in the thalli of the *Pseudocyphellaria aurata* from Brazil (Czeżuga and Xavier-Filho 1987a). On the whole, lutein epoxide and astaxanthin were usually the predominant carotenoids in the species from South Africa while in the species studied from Equatorial Africa lutein epoxide and mutatoxanthin were predominant, astaxanthin being predominant in only 3 species of the 19 studied.

REFERENCES

- Ahti T., Krog H., Swinscow T.D.V., 1987. New or otherwise interesting *Cladonia* species in East Africa. *Ann. Bot. Fennici* 24: 85-94.
- Almóbrn O., 1989. Revision of the lichen genus *Teloschistes* in central and southern Africa. *Nord. J. Bot.* 8: 521-527.
- Czeżuga B., 1980. Investigations on carotenoids in *Embryophyta*. I. *Bryophyta*. *Bryologist* (U.S.A.) 83: 21-28.
- Czeżuga B., 1986. Investigations on carotenoids in lichens. XI. Lichens from Lapland. *Ann. Bot. Fennici* 23: 251-254.
- Czeżuga B., 1988. Carotenoids. In: *Handbook of Lichenology*. M. Galun (ed.). Vol. III. CRC Press, Boca Raton, Florida, pp. 25-34.
- Czeżuga B., Alstrup V., 1987. Investigations on carotenoids in lichens. XIV. The carotenoid content of lichens from Greenland. *Biochem. Syst. Ecol.* 15: 297-301.
- Czeżuga B., Beckett R.P., Pienaar R.N., 1988. Ditto. XVIII. Carotenoid content in certain lichens from southern Africa. *S. Afr. J. Bot.* 54: 571-574.
- Czeżuga B., Bubrick P., 1986. Ditto. IX. Carotenoids in lichens from Israel. *Isr. J. Bot.* 35: 41-46.
- Czeżuga B., Caccamese S., Passadore M.V., 1989a. Ditto. XX. Carotenoids in lichens from various Italian environments. *Phyton (Austria)* 29: 15-22.
- Czeżuga B., Gutkowski R., Czerpak R., 1982. Investigations on carotenoids in *Embryophyta*. II. Musci from Antarctica. *Nova Hedwigia* 36: 695-701.
- Czeżuga B., Skirina I.F., Maximov O.B., Stepanenko L.S., 1989b. Investigations on carotenoids in lichens. XIX. Carotenoids in lichens of the tundra of Kamchatka region (Far East). *Phyton (Austria)* 29: 7-13.
- Czeżuga B., Xavier-Filho L., 1987a. Ditto. VII. Some lichens from Brasil. *Rev. Brasil. Biol.* 47: 243-246.
- Czeżuga B., Xavier-Filho L., 1987b. Ditto. X. Luteoxanthin and apo-12'-violaxanthin in lichens from the Antarctica. *Ser. Cient. INACH* 36: 151-155.
- Czeżuga B., Xavier-Filho L., 1988. Investigations on carotenoids in *Embryophyta*. XI. The presence of torulene in mosses from the Antarctica. *Ser. Cient. INACH* 37: 111-116.
- Davies B.H., 1976. Carotenoids. In: *Chemistry and biochemistry of plant pigments*. I.W. Goodwin (ed.). Academic Press, London-New York-San Francisco, pp. 38-165.
- Goodwin T.W., 1980. *The biochemistry of the carotenoids*. Volume 1, Plants. Chapman and Hall, London and New York, 377 p.

- Krinsky N.J., Goldsmith T.H., 1960. The carotenoids of the flagellated alga, *Euglena gracilis*. Arch. Biochem. Biophys. 91: 271-279.
- Liaaen-Jensen S., 1977. Algal carotenoids and chemosystematics. Mar. Natur. Prod. Chem. 1: 239-259.
- Moberg R., 1986. The genus *Physcia* in East Africa. Nord. J. Bot. 6: 843-864.
- Moberg R., 1987. The genera *Hyperphyscia* and *Physconia* in East Africa. Nord. J. Bot. 7: 419-728.
- Spence J.R., Mahaney W.C., 1988. Growth and ecology of *Rhizocarpon* section *Rhizocarpon* on Mount Kenya, East Africa. Arct. Alp. Res. 20: 237-242.
- Straub O., 1971. Lists on natural carotenoids. In: Carotenoids. O. Isler (ed.). Birkhauser Verlag, Basel und Stuttgart, pp. 771-850.
- Straub O., 1987. Key to carotenoids, Birkhauser Verlag, Basel-Boston, 296 p.
- Swinscow T.D.V., Krog H., 1979. The lichen genera *Heppia* and *Peltula* in East Africa. Norw. J. Bot. 26: 213-224.
- Swinscow T.D.V., Krog H., 1988. Macrolichens of East Africa. British Museum (Natural History), London, 390 p.
- Valadon L.R.G., 1976. Carotenoids as additional taxonomic characters in fungi a review. Trans. Brit. Mycol. Soc. 67: 1-15.
- Vetter W., Englert G., Rigassi N., Schwieter U., 1971. Spectroscopic methods. In: Carotenoids. O. Isler (ed.). Birkhauser Verlag, Basel und Stuttgart, pp. 189-266.

Badania karotenoidów u porostów. XXXII. Karotenoidy w plechach porostów z Kenii (Afryka Równikowa)

Streszczenie

Stosując chromatografię kolumnową i cienkowsłupową badano występowanie poszczególnych karotenoidów u 19 gatunków porostów zebranych w Kenii. Stwierdzono następujące karotenoidy: neurosporen, α -, β -karoten, rubiksantyna, α -, β -kryptoksantyna, zeaksantyna, echinenon, 3'-hydroksyechinenon, kantaksantyna, α -doradeksantyna, astaksantyna, epoksyd luteiny, wiolaksantyna, mutatoksanantyna, flawoksantyna, kapsochrom, β -apo-8'-karotenal, β -apo-10'-karotenal i apo-12'-wiołaksantal. Ogólna zawartość karotenoidów wahała się od 15,88 (*Pyxine coccinea*) do 135,44 $\mu\text{g g}^{-1}$ suchej masy (*Teloschistes chrysophthalmus*).