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

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Abstract

The presence of cartenoids in nineteen species of lichens from Kenya (Equatorial Africa) was studied by column and thinlayer chromatography. This investigations 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'-violaxanthal. 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 cocoes*) to 135.44 μg 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 (S p e n c e and M a h a n e y 1988, S w i n c s o w and K r o g 1988, A l m o b r n 1989). These studies have provided new data on the distribution and biology of a number of species (S w i n s c o w and K r o g

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 chemotoxonomy 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 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 indentification 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 (Krinsky and Goldsmith 1960); (g) the mass spectrum; and (h) infrared spectroscopy for torulorhodin, 3'-hydroxyechinenone, capsochrome, 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
Collema rugosum Krempelh.	Nairobi District-Ngong Hills	trunk-*Schinus molle*	1600
Heterodermia leucomelos (L.) Poelt subsp. *boryi* (Fee) Swinscow et Krog	Nairobi District-Ngong Hills	trunk-*Schinus molle*	1550
Heterodermia speciosa (Wulf.) Trevis.	Riff Valley Province	trunk-*Schinus molle*	1900
Hypogymnia bitteri (Lynge) Ahti	Mt. Kenya National Park	rocks, alpine zone	3550
Lasallia pustulata (Ach.) Llano	Mt. Kenya National Park	boulder, alpine zone	3900
Lobaria pulmonaria (L.) Hoffm.	Mt. Kenya National Park	trunk-*Podocarpus milanjianus*	2900
Parmotrema abessinicum (Krempelh.) Hale	Nairobi District-Ngong Hills	trunk-*Schinus molle*	1700
Phaeophyscia adiastola (Essl.) Essl.	Aberdare National Park	trunk-*Hagenia abyssinica*	3350
Physcia albata (Wils.) Hale	Aberdare National Park	rocks-steep northern	3650
Physcia albo-plumbea (Tayl.) Nyl.	Mt. Kenya National Park	*Podocarpus milanjianus*	2900
Pseudocyphellaria clathrata (de Not) Malme	Nairobi District-Ngong Hills	on *Schinus molle*	1600
Pseudocyphellaria cracata (L.) Vainio	Nairobi District-Ngong Hills	on *Schinus molle*	1700
Pyxine cocoes (Swinscow) Nyl.	Tsavo National Park East	trunk of a tree	500
Pyxine reticulata (Vainio) Vainio	Riff Valley Province	trunk-*Schinus molle*	1900
Ramalina celastri (Sprengel) Krog et Swinsow	Nairobi District-Ngong Hills	trunk-*Schinus molle*	1700
Teloschistes chrysophthalmus (L.) Th.Fr.	Nairobi District-Ngong Hills	trunk-*Schinus molle*	1600
Teloschistes perrugosus Müll. Arg.	Nairobi District-Ngong Hills	trunk-*Schinus molle*	1700
Umbilicaria haumaniana (L.) Ach. em. Frey	Mt. Kenya National Park	rocks-alpine zone	3750
Usnea exasperata (Müll. Agr.) Motyka	Nairobi District-Ngong Hills	trunk-*Schinus molle*	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 adiastola, 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 cocoes*), 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).

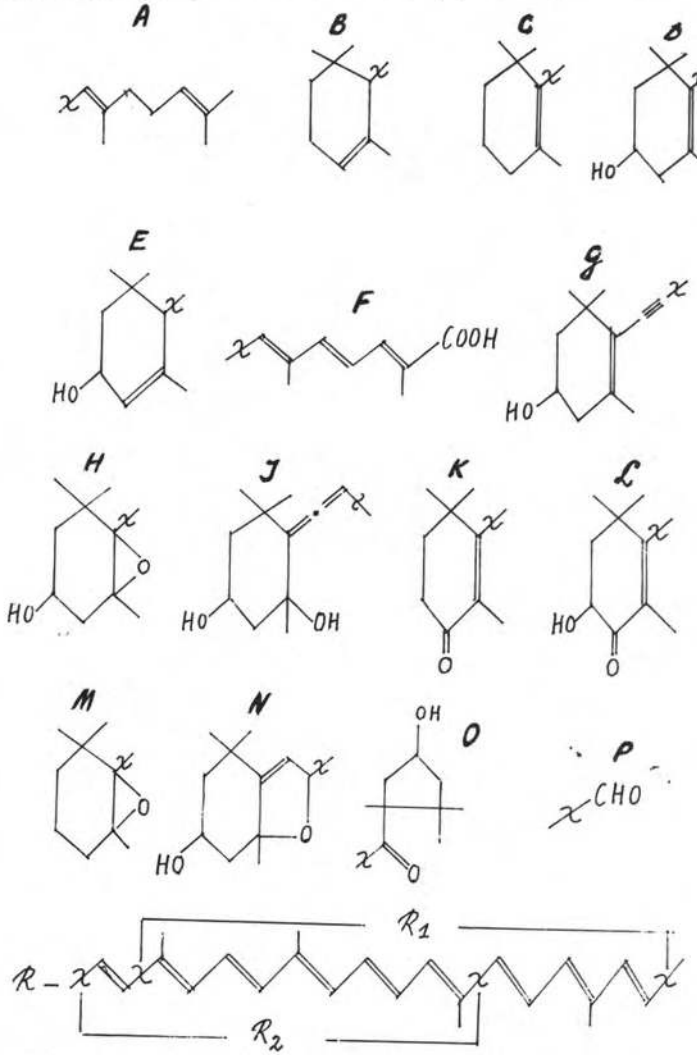

Fig. 1. Structural features of carotenoids from investigated materials

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-Apol-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 (μg g^{-1} dry weight)
Collema rugosum Krempelh.	2, 8, 9, 12, 13, 14, 17, 20, 21	2 (43.4)	77.57
Heterodermia leucomelos (L.) Poelt subsp. *boryi* (Fee) et Krog	2, 7, 8, 16, 19, 20, 21	20 (30.3)	31.08
Heterodermia speciosa (Wulf.) Trevis.	2, 3, 5, 7, 18, 19, 20, 22	22 (56.6)	18.92
Hypogymnia bitteri (Lynge) Ahti	2, 5, 6, 7, 12, 18, 20, 21, 26	20 (16.2)	21.24
Lasallia pustulata (Ach.) Llano	2, 3, 7, 12, 19, 20, 21, 22	22 (35.6)	25.86
Lobaria pulmonaria (L.) Hoffm.	2, 3, 7, 8, 19, 20, 21, 24	24 (22.5)	16.18
Parmotrema abessinicum (Krempelh.) Hale	2, 5, 8, 17, 19, 20, 21	17 (30.2)	31.54
Phaeophyscia adiastola (Essl.) Essl.	2, 3, 6, 8, 12, 20, 21, 22	22 (40.2)	30.14
Physcia albata (Wils.) Hale	2, 3, 8, 11, 12, 19, 20, 21	21 (26.6)	16.52
Physcia albo-plumbea (Tayl.) Nyl.	2, 3, 5, 6, 19, 20, 21, 24	20 (49.4)	26.16
Pseudocyphellaria clathrata (de Not) Malme	2, 4, 16, 17, 20, 25, 26	17 (29.2)	24.50
Pseudocyphellaria cracata (L.) Vainio	2, 10, 15, 16, 17, 18, 20	17 (37.3)	28.60
Pyxine cocoes (Swinscow) Nyl.	2, 3, 5, 8, 20, 21, 24	21 (47.3)	15.89
Pyxine reticulata (Vainio) Vainio	2, 3, 8, 9, 12, 19, 20, 21, 23	20 (28.4)	34.02
Ramalina celastri (Sprengel) Krog et Swinsow	2, 7, 8, 17, 20, 21	20 (43.4)	44.08
Teloschistes chrysophthalmus (L.) Th.Fr.	2, 3, 5, 8, 9, 19, 20, 21, 22	22 (79.3)	135.44
Teloschistes perrugosus Müll. Arg.	2, 3, 5, 8, 9, 19, 20, 22	22 (51.0)	98.13
Umbilicaria haumaniana (L.) Ach. em. Frey	2, 3, 6, 7, 8, 9, 11, 20, 21, 27	21 (21.3)	17.14
Usnea exasperata (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 cocoes*) to 133.44 μg 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 neurosporence. 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 Bubrick 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 (Straub 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 there 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 Xaver-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 (Czeczuga 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.

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

Streszczenie

Stosując chromatografię kolumnową i cienkowarstwową 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, mutatoksantyna, flawoksantyna, kapsochrom, β-apo-8'-karotenal, β-apo-10'-karotenal i apo-12'-wiolaksantal. Ogólna zawartość karotenoidów wahała się od 15,88 (*Pyxine cocoes*) do 135,44 μg g^{-1} suchej masy (*Teloschistes chrysophthalmus*).