—報告—
Report

Carotenoids in Lichens from the Antarctic

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南極地域産地衣類から得られたカロテノイド

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要旨: 東南極の宗谷海岸, Schirmacher Oasis, 南シェトランド諸島キング・ジョージ島産地衣類 33 種について, カラム及び薄層クロマトグラフィー法によりカロテノイドを分析した. 結果として, α-carotene をはじめとして 21 種のカロテノイドの存在を明らかにした.

Abstract: Column-, and thin-layer chromatography revealed the presence of the following carotenoids in the thalli of 33 lichen species from the Antarctic: α-carotene, β-carotene, δ-carotene, ε-carotene, β-cryptoxanthin, lutein, zeaxanthin, echinenone, hydroxyechinenone, canthaxanthin, α-doradexanthin, β-doradexanthin, astaxanthin, lycopene-5,6-epoxide, lutein epoxide, antheraxanthin, neoxanthin, violaxanthin, auroxanthin, mutatoxanthin, and capsochrome.

The total content of carotenoids ranged from 23.25 (*Leptogium puberulum*) to 123.50 $\mu g\,g^{-1}$ dry wt (*Polycauliona regalis*).

1. Introduction

Among Antarctic flora, the lichen shows extensive species diversity (DODGE, 1973). Thus, it has around taxonomical or ecological interest of many lichenologists from various countries (DARBISHIRE, 1910, 1912; LAMB, 1964, 1968; KASHIWADANI, 1970, 1979; LINDSAY, 1973, 1974; GUZMAN and REDON, 1981; ANDREEV, 1988; JACOBSEN and KAPPEN, 1988; INOUE, 1989, 1991a, b, 1993). The studies have detected new species and widended the known occurrence areas of many species (LINDSAY, 1969, 1977). The following investigations have focused on the chemical composition of thalli of species from various ecological niches (HUNECK *et al.*, 1984) and on the carotenoid content (CZECZUGA *et al.*, 1986b; CZECZUGA and XAVIER-FILHO, 1987; CZECZUGA and OLECH, 1989). A significant effect has been found of seasonal changes on the total carotenoid content, and new carotenoids have been detected in a number of lichen species.

The present study is a continuation of investigations of carotenoids in thalli of various lichen species of the Antarctic.

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2. Materials and Methods

The thalli of thirty three lichen species collected in the Antarctic were used for this study (Table 1, Fig. 1).

The thalli were cleaned of all organic debris, placed in dark glass bottles and macerated with acetone. The air above the fluid in the bottle was replaced with nitrogen to ensure an anaerobic atmosphere. Samples were kept in a refrigerator

Table 1. Investigated species of lichens from the Antarctic.

No. Species	Locality
1. *Acarospora gwynnii* DODGE and RUDOLPH	Schirmacher Oasis, around lake near Russian Station
2. *Buellia frigida* DARB.	Sôya Coast, Langhovde
3. *Buellia pallida* DODGE and BAKER	Schirmacher Oasis, Lake behind Russian Station
4. *Carbonea capsulata* (DODGE and BAKER) HALE	Schirmacher Oasis, on Stream extreme south of Maitri
5. *Evernia* sp.	Schirmacher Oasis, Lake behind Russian Station
6. *Himantormia lugubris* (HUE) LAMB	South Shetland Islands, King George
7. *Hypogymnia lugubris* (PERS.) KROG	South Shetland Islands, King George
8. *Lecanora fuscobrunnea* DODGE and BAKER	Schirmacher Oasis, Maitri, near Shivalinga
9. *Lecidea cancriformis* DODGE and BAKER	Schirmacher Oasis, Maitri, North side of Zub lake
10. *Lecidella siplei* (DODGE and BAKER) INOUE	Schirmacher Oasis, at the base of Trishul Hill
11. *Lepraria membranacea* (DICKS) LETT.	Schirmacher Oasis, North side of Maitri near ice shelf
12. *Leptogium puberulum* HUE	South Shetland Islands, King George
13. *Pannaria hookerii* (BORRY) NYL.	South Shetland Islands, King George
14. *Ochrolechia antarctica* (MULLER. ARG.) DARB.	South Shetland Islands, King George
15. *Parmelia saxatilis* (L.) ACH.	South Shetland Islands, King George
16. *Pertusaria* sp.	Schirmacher Oasis, North side of Maitri
17. *Physcia caesia* (HOFFM.) HAMPE	South Shetland Islands, King George
18. *Physcia caesia* (HOFFM.) HAMPE	Sôya Coast, Cape Tama
19. *Physcia caesia* (HOFFM.) HAMPE	Schirmacher Oasis, East side of Maitri, behind Flat top
20. *Physconia muscigena* (ACH.) POELT	South Shetland Islands, Nelson Island
21. *Polycauliona regalis* (VAIN.) HUE	South Shetland Islands, King George
22. *Psoroma hypnorum* (VAHL.) GRAY	South Shetland Islands, Nelson Island
23. *Ramalina terebrata* HOOKER f. and TAYLOR	South Shetland Islands, King George
24. *Rhizocarpon flavum* DODGE and BAKER	Schirmacher Oasis, around lake near Trishul Hill
25. *Rhizocarpon geographicum* (L.) DC	Schirmacher Oasis, Maitri, North side of the Zub lake
26. *Rinodina olivaceobrumea* DODGE and BAKER	Schirmacher Oasis, on way from Maitri to Russian Station
27. *Rinodina peternanii* (HUE) DARBISHIRE	Schirmacher Oasis, Maitri, Zub lake
28. *Rinodina endophragmia* (HUE) DARBISHIRE	Schirmacher Oasis, at the base of Trishul Hill
29. *Umbilicaria antarctica* FREY and LAMB	South Shetland Islands, King George
30. *Umbilicaria aprina* NYL.	Sôya Coast, Langhovde
31. *Umbilicaria aprina* NYL.	Schirmacher Oasis, Maitri, Zub lake
32. *Umbilicaria decussata* (VILL.) ZAHLBR.	Sôya Coast, Padda
33. *Umbilicaria decussata* (VILL.) ZAHLBR.	Schirmacher Oasis, at the base of Trishul Hill
34. *Usnea antarctica* DR.	South Shetland Islands, King George
35. *Usnea aurantiacoatra* (JACQ.) BORRY	South Shetland Islands, Nelson Island
36. *Usnea sphacerata* R. BR.	Sôya Coast, Langhovde
37. *Usnea subcapillaris* WALKER	South Walthat
38. *Xanthoria elegans* (LINK.) TH. FR.	Sôya Coast, Skarvsnes
39. *Xanthoria elegans* (LINK.) TH. FR.	Schirmacher Oasis, East side of Maitri, behind Flat top

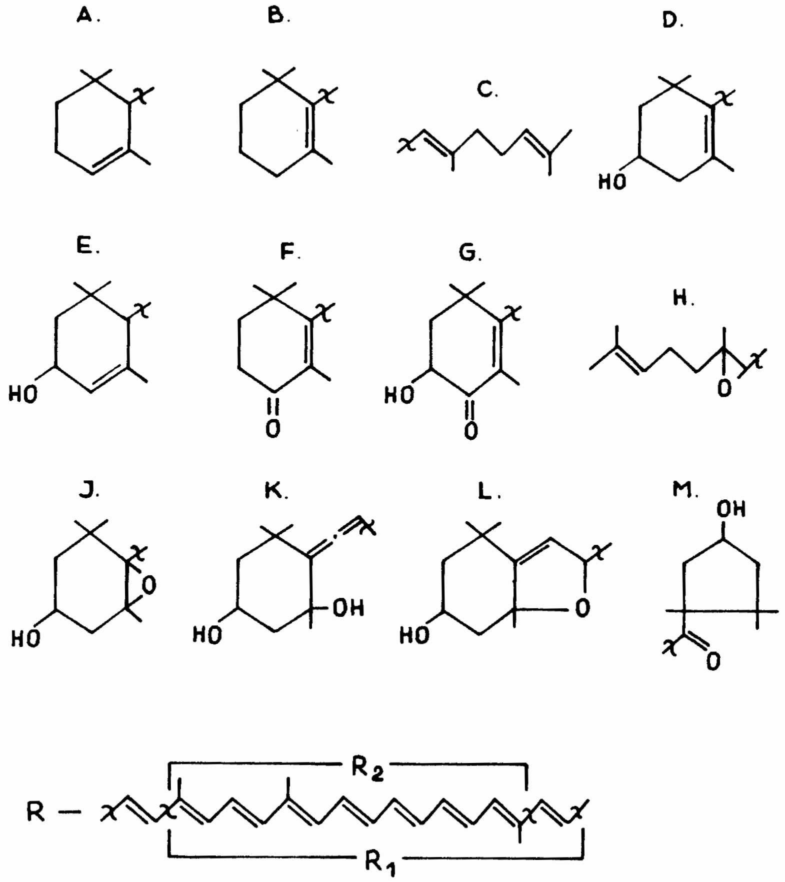

Fig. 1. Structural features of carotenoids from investigated materials (see Table 1).

until analysed for their carotenoid content by column and thin-layer chromatography. 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 ha in dark condition in a nitrogen atmosphere.

Column and thin-layer chromatographic methods shown in detail in by CZECZUGA (1980a) were used to separate various carotenoids. These were identified by performing replicate chromatography with standard carotenoids (Hoffman-La Roche and Co. Ltd., Basel, Switzerland, and Sigma Company, USA). Pigments were identified on the basis of: (a) their behaviour in column chromatography; (b) their absorption spectra in various solvents (Beckman spectrophotometer model 2400); (c) their partition between hexane and 95% methanol; (d) their Rf values (TLC); (e) the presence of allylic hydroxyl groups, determined by the acid-chloroform test; (f) the epoxide test, and (g) the mass spectrum of end groups (see VETTER *et al.*, 1971 for basic methodology). Concentrations of carotenoid solutions were determined by the absorption spectra, on the basis of the extinction coefficients E 1% cm^{-1} at wavelengths absorbed in petroleum ether or hexane (DAVIES, 1976). The structures of the carotenoids have been reported previously by STRAUB (1987).

3. Results

Twenty-one carotenoids were detected in the material studied (Table 2). Most of the carotenoids found have previously been encountered in lichen thalli from various latitudes. However, δ-carotene, ε-carotene, β-doradexanthin and lycopene-5,6-epoxide have rarely been reported. The predominant carotenoid was astaxanthin (Table 3). The total carotenoid content in the material examined ranged from 23.25 (*Leptogium puberum*) to 123.50 $\mu g\, g^{-1}$ (*Polycauliona regalis*) of dry mass.

Table 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. δ-carotene	A-R-C	ε,ψ-Carotene
4. ε-carotene	A-R-A	ε,ε-Carotene
5. β-cryptoxanthin	B-R-D	β,β-Caroten-3-ol
6. lutein	D-R-E	β,ε-Carotene-3,3′-diol
7. zeaxanthin	D-R-D	β,β-Carotene-3,3′-diol
8. echinenone	B-R-F	β,β-Caroten-4-one
9. hydroxyechinenone	B-R-G	3-Hydroxy-β,β-caroten-4-one
10. canthaxanthin	F-R-F	β,β-Carotene-4,4′-dione
11. α-doradexanthin	E-R-G	3,3′-Dihydroxy-β,ε-caroten-4-one
12. β-doradexanthin	D-R-G	3,3′-Dihydroxy-β,β-caroten-4-one
13. astaxanthin	G-R-G	3,3′-Dihydroxy-β,β-carotene-4,4′-dione
14. lycopene-5,6-epoxide	C-R-H	5,6-Epoxy-5,6-dihydro-ψ,ψ-carotene
15. lutein epoxide	E-R-I	5,6-Epoxy-5,6-dihydro-β,ε-carotene-3,3′-diol
16. antheraxanthin	D-R-I	5,6-Epoxy-5,6-dihydro-β,β-carotene-3,3′-diol
17. neoxanthin	I-R_1-K	5,6-Epoxy-6,7-didehydro-5,6,5′,6′-β,β-carotene-3,5,3′-triol
18. violaxanthin	I-R-I	5,6,5′,6′-Diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol
19. auroxanthin	L-R_2-L	5,8,5′,8′-Diepoxy-5,8,5′,8′-tetrahydro-β,β-carotene-3,3′-diol
20. mutatoxanthin	D-R_1-L	5,8-Epoxy-5,8-dihydro-β,β-carotene-3,3′-diol
21. capsochrome	L-R_1-M	5,8-Epoxy-3,3′-dihydroxy-5,8-dihydro-β,χ-caroten-6′-one

Table 3. Carotenoid distribution in lichens from the Antarctic.

No. Species	Carotenoid (see Table 2)	Major carote-noids (%)	Total content ($\mu g\, g^{-1}$ dry wt)
1. *Acarospora gwynnii* DODGE and RUDOLPH	2, 6, 9, 13, 15, 16, 18	18 (41.0)	62.05
2. *Buellia frigida* DARB.	1, 2, 5, 6, 9, 11, 12, 13, 15, 17, 18, 21	13 (30.5)	40.73
3. *Buellia pallida* DODGE and BAKER	2, 6, 7, 9, 10, 11, 15, 17, 18	18 (50.7)	46.75
4. *Carbonea capsulata* (DODGE and BAKER) HALE	2, 6, 7, 9, 12, 13, 15, 17	13 (29.4)	69.18
5. *Evernia* sp.	2, 5, 6, 7, 11, 15, 16, 18	16 (25.8)	68.62
6. *Himantormia lugubris* (HUE) LAMB	2, 8, 9, 10, 13, 15, 17, 18, 21	10 (34.6)	57.46
7. *Hypogymnia lugubris* (PERS.) KROG	1, 6, 7, 9, 15, 18, 21	7 (39.2)	44.48
8. *Lecanora fuscobrunnea* DODGE and BAKER	1, 2, 7, 10, 13, 15, 17, 18, 20	7 (23.6)	41.80
9. *Lecidea cancriformis* DODGE and BAKER	4, 5, 6, 13, 15, 17, 18, 21	13 (32.9)	46.44
10. *Lecidella siplei* (DODGE and BAKER) INOUE	2, 6, 12, 13, 15, 17, 18	13 (32.3)	37.79

Table 3. (Continued)

No. Species	Carotenoid (see Table 2)	Major carote-noids (%)	Total content ($\mu g\ g^{-1}$ dry wt)
11. *Lepraria membranacea* (DICKS) LETT.	6, 13, 15, 17, 18, 21	13 (39.9)	31.01
12. *Leptogium puberulum* HUE	2, 5, 6, 10, 15, 17, 18, 21	21 (30.6)	23.25
13. *Pannaria hookerii* (BORRY) NYL.	2, 7, 10, 12, 13, 15, 17, 21	12 (33.2)	26.86
14. *Ochrolechia antarctica* (MULLER. ARG.) DARB.	5, 6, 9, 10, 12, 13, 18	13 (25.4)	54.45
15. *Parmelia saxatilis* (L.) ACH.	2, 5, 6, 12, 13, 15, 18	12 (25.0)	60.12
16. *Pertusaria* sp.	2, 4, 5, 7, 9, 13, 14, 15, 21	13 (40.4)	50.21
17. *Physcia caesia* (HOFFM.) HAMPE	2, 5, 6, 10, 13, 15, 17, 18, 21	13 (35.8)	44.77
18. *Physcia caesia* (HOFFM.) HAMPE	2, 6, 7, 10, 13, 15, 17, 18	13 (22.9)	24.66
19. *Physcia caesia* (HOFFM.) HAMPE	1, 2, 6, 10, 12, 13, 15	10 (29.4)	74.93
20. *Physconia muscigena* (ACH.) POELT	2, 5, 7, 9, 10, 12, 13, 15	13 (49.5)	45.91
21. *Polycauliona regalis* (VAIN.) HUE	2, 10, 15, 17, 19	19 (62.7)	123.50
22. *Psoroma hypnorum* (VAHL.) GRAY	2, 10, 12, 13, 15, 16, 17, 18	12 (25.1)	31.70
23. *Ramalina terebreta* HOOKER f. and TAYLOR	6, 7, 9, 12, 13, 15, 17, 18	18 (27.0)	38.87
24. *Rhizocarpon flavum* DODGE and BAKER	4, 5, 6, 10, 12, 15, 16, 18	10 (32.7)	85.03
25. *Rhizocarpon geographicum* (L.) DC	2, 4, 5, 6, 9, 10, 13, 15, 18, 21	13 (42.1)	67.17
26. *Rinodina olivaceobrumea* DODGE and BAKER	2, 6, 7, 10, 13, 15, 17, 18, 21	7 (27.6)	53.42
27. *Rinodina peternanii* (HUE) DARBISHIRE	1, 2, 5, 6, 7, 10, 13, 15, 17, 18, 21	7 (21.7)	54.45
28. *Rinodina endophragmia* (HUE) DARBISHIRE	2, 7, 10, 13, 15, 17, 18, 21	17 (25.1)	36.19
29. *Umbilicaria antarctica* FREY and LAMB	2, 5, 6, 13, 15, 16, 18, 21	13 (32.8)	35.47
30. *Umbilicaria aprina* NYL.	5, 6, 12, 13, 15, 17, 18	13 (26.3)	35.14
31. *Umbilicaria aprina* NYL.	2, 3, 6, 10, 13, 14, 15, 16, 18	10 (27.2)	65.55
32. *Umbilicaria decussata* (VILL.) ZAHLBR.	2, 6, 10, 13, 15, 16, 18, 21	10 (31.2)	49.45
33. *Umbilicaria decussata* (VILL.) ZAHLBR.	2, 5, 6, 13, 15, 17, 18, 21	13 (46.3)	34.04
34. *Usnea antarctica* DR.	2, 5, 6, 9, 10, 15, 17, 18, 21	18 (47.2)	68.69
35. *Usnea aurantiacoatra* (JACQ.) BORRY	2, 4, 6, 10, 13, 15, 17, 21	10 (41.9)	44.16
36. *Usnea sphacerata* R. BR.	2, 5, 6, 10, 15, 17, 21	21 (42.8)	49.71
37. *Usnea subcapillaris* WALKER	1, 2, 6, 8, 15, 16, 17, 18, 21	18 (27.3)	61.60
38. *Xanthoria elegans* (LINK.) TH. FR.	2, 5, 6, 9, 13, 15, 17, 20	20 (62.4)	107.81
39. *Xanthoria elegans* (LINK.) TH. FR.	2, 5, 7, 8, 15, 16, 18, 20	20 (59.6)	112.57

4. Discussion

Worth noting is the finding of several very rare carotenoids in the material studied. δ-Carotene has been commonly found in higher plants (GOODWIN, 1980). It has only been found in *Cetraria islandica* (L.) ACH. from Białystok, Poland (CZECZUGA, 1980b). In the present study, however, it was detected in the thalli of *Umbilicaria aprina* (No. 31). ε-Carotene, another rare carotene, was detected in several species such as *Lecidea cancriformis*, *Pertusaria* sp., *Rhizocarpon flavum* and *Usnea aurantiacoatra.* Perhaps the conditions in this part of the Antarctic stimulate biosynthesis of this carotene. In previous studies of lichen, ε-carotene was observed in *Rhizoplaca peltata* from the Tien-Shan mountains (CZECZUGA *et al.*, 1993) and in the following four species: *Pseudocyphellaria coriacea*, *Pseudocyphellaria faevolata*, *Stereocaulon corticatum* and *Sticta fuliginosa* from New Zealand (CZECZUGA and TAYLOR, 1991), and three species (*Cetraria nivalis* (L.) ACH., *Pseudephebe pubescens* (L.) CHOISY and *Umbilicaria artica* (ACH.) NYL.) from West Greenland

(CZECZUGA and JACOBSEN, 1993). ε-Carotene has also been found in algae (GOODWIN, 1980).

β-Doradexanthin, or adonixanthin, was observed in *Evernia* sp. This is a ketocarotenoid, derived during astaxanthin biosynthesis from β-carotene. It has previously been found in the phylum Bryophyta from the Antarctic (CZECZUGA *et al.*, 1982) and in several lichen species from northern Siberia (CZECZUGA and SHCHELKUNOVA, 1986), in *Stereocaulon alpinum* from Lapland (CZECZUGA, 1986) and in *Parmelia saxatilis* (L.) ACH. from the Far East (CZECZUGA *et al.*, 1986a). Adonixanthin was previously found in several lichen species from the Antarctic. It was detected from *Xanthoria elegans*, *Usnea fasciata* and *Sphaerophorus globosus* collected on King George Island (CZECZUGA *et al.*, 1986b; CZECZUGA and XAVIER-FILHO, 1987).

Lycopene-5,6-epoxide was detected in *Pertusaria* sp. and *Umbilicaria aprina*. It has previously been found in *Ramalina bourgeana* NYL. from the Canaries (CZECZUGA *et al.*, 1988), *Peltula patellata*, *Ramalina bourgeana* and *Solenospora holophaea* from the northern part of Africa (CZECZUGA and EGEA, 1990), and also in *Phaeophyscia rubropulchra*, *Physcia stellaris* and *Punctelia perreticulata* from the State of Illinois, USA (CZECZUGA and WILHELM, 1992). In higher plants, lycopene-5,6-epoxide is frequently encountered in ripe fruit (GOODWIN, 1980).

Our previous studies have demonstrated that the carotenoid composition of thalli of a respective lichen species is environmental factor-dependent, and that in thalli collected from various environmental sites only constant carotenoids, typical of a respective species, are always present (CZECZUGA *et al.*, 1991). Thus, for the chemical characteristics of a given species, these constant carotenoids are taxonomically significant. In the present materials which were collected from various sites, the thalli of *Physcia caesia* had β-carotene, lutein, canthaxanthin, astaxanthin and lutein epoxide in common, *Umbilicaria aprina* collected from two sites shared lutein, astaxanthin, lutein epoxide and violaxanthin. Common carotenoids from *Umbilicaria decussata* were those found in *Umbilicaria aprina* and capsochrome. *Xanthoria elegans* had β-carotene, β-cryptoxanthin, lutein epoxide and mutatoxanthin in common.

It should be mentioned that five species of *Umbilicaria* from central Japan have produced different carotenoids such as neoxanthin, mutatoxanthin and luteoxanthin in addition to lutein, lutein epoxide and astaxanthin (CZECZUGA and YOSHIDA, 1991). Four species of *Usnea* from the East Antarctic also differ from the tropical (Indonesian and Malaysian) taxa of the same genus in sharing β-carotene, lutein, lutein epoxide, neoxanthin and capsochrome; the latter taxa only contained β-carotene and lutein epoxide as carotenoids (CZECZUGA *et al.*, 1994).

The same carotenoids have been detected in other species of this genus, and from other continents.

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