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## Investigations on carotenoids in lichens.

### V. Lichens from Antarctica

ABSTRACT: Carotenoids in six species of the lichens from Antarctica (*Xanthoria elegans*, *Caloplaca regalis*, *Usnea antarctica*, *U. fasciata*, *Himantormia lugubris* and *Ramalina terebrata*) have been investigated by means of column and thin — layer chromatography. The following carotenoids were found:  $\beta$ -carotene,  $\alpha$ - and  $\beta$ -cryptoxanthin, canthaxanthin, lycophyll, lycoxanthin, lutein, lutein epoxide, zeaxanthin, antheroxanthin, adonixanthin, diatoxanthin, rhodoxanthin, rhodoxanthin derivative,  $\alpha$ -doradexanthin, astaxanthin, astaxanthin ester, mutatochrome, mutatoxanthin and cryptoflavin. Most frequently occurred  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein epoxide, zeaxanthin and mutatoxanthin.

The total carotenoid content ranged from 10.242 (*Ramalina terebrata*) to 18.700 mg/g dry weight (*Himantormia lugubris*) in October and from 4.765 (*Ramalina terebrata*) to 12.462 mg/g dry weight (*Caloplaca regalis*) in February.

Key words: Antarctica, King George Island, lichens, *Xanthoria*, *Caloplaca*, *Usnea*, *Himantormia*, *Ramalina*, carotenoid composition.

## 1. Introduction

Continental Antarctica is predominantly an ice-covered cold desert with few habitats for living organisms. In contrast, the coastal zone and off-shore islands have a milder climate, much ice-free lowland and a variety of plant and animal life. The vegetation of Antarctic regions is dominated by mosses and lichens (Lamb 1970, Lindsay 1978). On King George Island 44 species of mosses, 67 species of lichens and only two species of vascular plants were found.

King George Island (latitude 62°09'51" S, longitude 50°27'45" W) is largest among South Shetland Islands. The island has an area of 1338 km<sup>2</sup> and only 25 km<sup>2</sup> is ice-free. This island is situated in a subantarctic area of strong cyclonic activity. It has an oceanic climate characterized by a small amplitude of temperature changes and a high humidity. The average air temperature during the half a year summer period is +0.8°C, in winter it is -4.9°C. Strong direct solar radiation is typical for this island as for all Antarctic region.

Conducting research on presence of particular carotenoids in thalli of lichens (Czacuga 1979a, b; 1980a, b) we became interested in the problem of abundance of these substances in representatives of species living in specific conditions of Antarctic region (Mackenzie 1964, Lindsay 1974).

## 2. Material and methods

### 2.1. Lichens

The following 6 species of lichens were investigated: *Xanthoria elegans*, *Caloplaca regalis*, *Usnea antarctica*, *Usnea fasciata*, *Himantormia lugubris* and *Ramalina terebrata*.

### 2.2. Place of collecting the samples

The analysed material was collected in the first decade of November, December and February 1979/80 in several free of ice places on the King George Island. First of these places is an area occupied by a large colony of penguins near the H. Arctowski Station. There are several thousands nests of these birds at this colony. The area of the penguin colony is abundantly fertilized by large numbers of birds present there. In spring and summer the surface of the penguin colony is watered by the melting snow. At this place two frequently occurring species were collected: *Caloplaca regalis* and *Xanthoria elegans*. These lichens grow on sour magma rocks of volcanic origin. Near the penguin colony, at the distance of about 20 m, the third species, *Ramalina terebrata*, was collected. This lichen also grows on magma rocks of volcanic origin. The remaining species of lichens, *Usnea fasciata*, *Usnea antarctica* and *Himantormia lugubris*, were collected near Jardine Peak and Thomas Point. The lichens *Usnea fasciata* and *Himantormia lugubris* were collected at the altitude of 130 m above sea-level, near Jardine Peak. The mentioned plants grow there upon magma rocks making a rock rubble. *Usnea*

*antarctica* was collected near Point Thomas at the altitude of 50 m above sea-level. This lichen grows on magma rocks also making a rock rubble.

### 2.3. Identification of carotenoids

The material was first dried at room temperature, then immersed in 95% acetone and kept in a nitrogen atmosphere in a refrigerator (5 weeks) until the chromatographic analysis was carried out.

### 2.4. Pigments

The carotenoid pigments were extracted by means of 95% acetone in a dark room. Saponification was carried out by means of 10% KOH in ethanol at temperature of about 20°C for 24 hours in the dark in a nitrogen atmosphere.

### 2.5. Chromatography

Columnar and thin-layer chromatography, described in detail in our previous papers (Czeczuga 1979a, 1979b) were used for the separation of various carotenoids. A glass column approximately 1 cm in diameter and 15–20 in length, filled with  $\text{Al}_2\text{O}_3$ , was used in column chromatography. Silica gel was used for the thin-layer chromatography, with the appropriate solvent systems, the  $R_f$  values being determined for each spot. For identification  $\beta$ -carotene, canthaxanthin, lutein, zeaxanthin and astaxanthin co-chromatography was applied using identical carotenoids.

### 2.6. Identification of pigments

The pigments were identified by following methods: (a) behaviour on column chromatography; (b) absorption spectra of the pigments in various solvents were recorded by a Beckman spectrophotometer model 2400 DU; (c) the partition characteristics\* of the carotenoid between hexane and 95% methanol; (d) comparison of  $R_f$  on thin layer chromatography; (e) the presence of allylic hydroxyl groups was determined by the acid chloroform tests; and (f) the epoxide test.

### 2.7. Quantitative determinations

Quantitative determinations of the concentrations of carotenoid solutions were made from the quantitative absorption spectra. These determinations

were based of the extinction coefficient  $E$  1 cm at the wavelengths of maximal absorbance in petroleum ether or hexane.

### 3. Results and discussion

The received results of analysis have been presented with relation to dry weight. Therefore percentage of dry matter in the analysed material has been listed in Table I. In the thalli of the studied species of lichens 20 carotenoids were identified (Table II). In the thalli of all lichen species

Table I  
Percentage of the dry weight in the analyzed material

Species	Nov.	Dec.	Febr.
<i>Xanthoria elegans</i>	91.4	90.1	85.4
<i>Caloplaca regalis</i>	80.7	88.8	81.3
<i>Usnea antarctica</i>	86.7	91.6	84.4
<i>Usnea fasciata</i>	85.8	89.4	84.3
<i>Himantormia lugubris</i>	73.6	85.7	65.9
<i>Ramalina terebrata</i>	91.9	91.1	81.3

such carotenoids as  $\beta$ -cryptoxanthin, lutein epoxide and zeaxanthin were identified. In the thalli of the majority of the studied species also mutatoxanthin occurred, in the thalli of *Xanthoria elegans* and *Caloplaca regalis* ranging from 42.2 to 45.4% of general amount of carotenoids. It should be underlined that in the thalli of *Xanthoria elegans*, *Caloplaca regalis*, *Usnea antarctica*, the presence of rhodoxanthin, or its derivative, was found: it demonstrated the same maxima of absorption in particular solvents as rhodoxanthin, having, however, always obviously hypophasic quality. Concerning general amount of carotenoids in all studied lichen species, it was found that most of them were contained in the thalli of all species collected for analysis in November, while the least, with the exception of *Xanthoria elegans*, in February. In November the carotenoid content ranged from 10.242 (*Ramalina terebrata*) to 18.700 mg/g dry weight (*Himantormia lugubris*), while in February these amounts ranged from 4.765 (*Ramalina terebrata*) to 12.462 mg/g dry weight (*Caloplaca regalis*).

As it has already been mentioned, carotenoids such as  $\beta$ -cryptoxanthin, lutein epoxide and zeaxanthin occurred in the thalli of all studied species from Antarctica. These are carotenoids which also most frequently occur in other lichens on the European continent (Czczuga 1979a, 1979b, 1980a,



1980b). It should be underlined that the presence of diatoxanthin and rhodoxanthin in the thalli of some species was found. It is the first communication of the presence of these carotenoids in lichens. Concerning diatoxanthin, this carotenoid, as a derivative of zeaxanthin, till now has been found in algae (Cieczuga 1979c) and, concerning animals, in both marine (Matsumo et al. 1980a) and fresh-water fishes (Cieczuga 1980c). On the other hand, rhodoxanthin has been found so far in some water

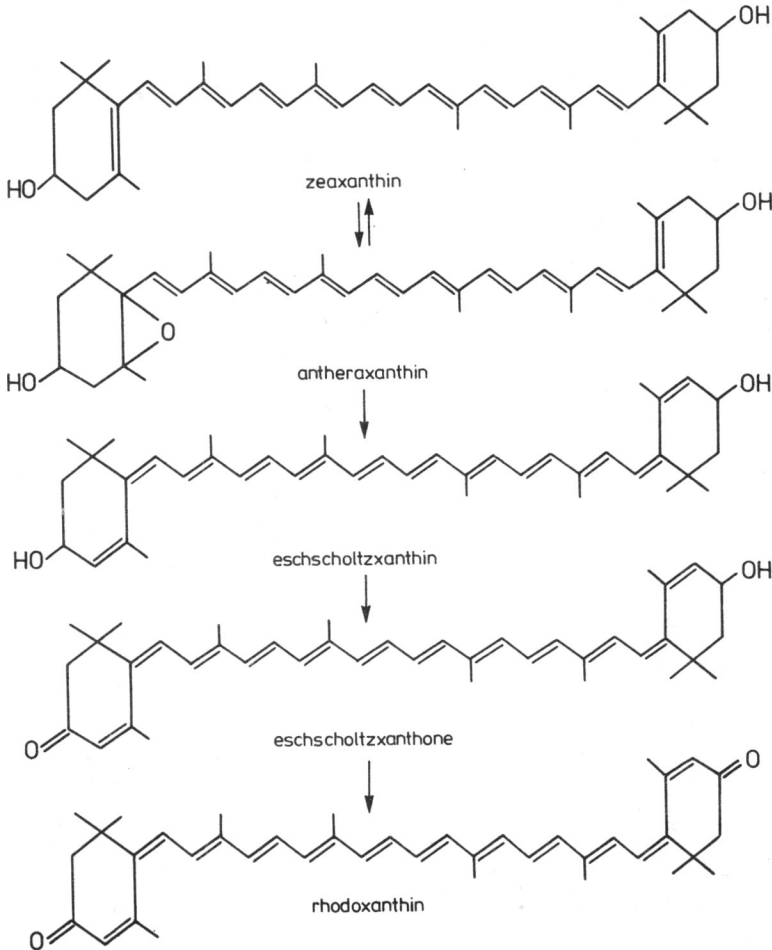


Fig. 1. Biosynthesis of rhodoxanthin in lichens

plants, first of all the *Potamogeton* genus, and in numerous conifers (Goodwin 1976, Ida 1981). Concerning animals, rhodoxanthin has been found so far only in a few species of phytophagous fishes (Matsumo et al. 1980b, Cieczuga 1981) and in several species of the tropical birds (Volker 1963) as well as in the European duck species (Cieczuga 1982). According to all probability rhodoxanthin emerges as a result of changes

of zeaxanthin (Katsuyama and Matsumo 1979) (Fig. 1), as well as diatoxanthin (Fig. 2). Perhaps the changes of zeaxanthin into rhodoxanthin is facilitated by the thermic conditions of Antarctica, particularly rapid fluctuation of temperature. It is greatly possible, since when investigating carotenoids in the mosses of this continent (Czeczuga et al. 1982) we have found the presence of certain carotenoids from the group of apo-carotenals, which emerge, as it is expected, in extreme conditions as a result of the degradation of  $\beta$ -carotene, or some of its derivatives.

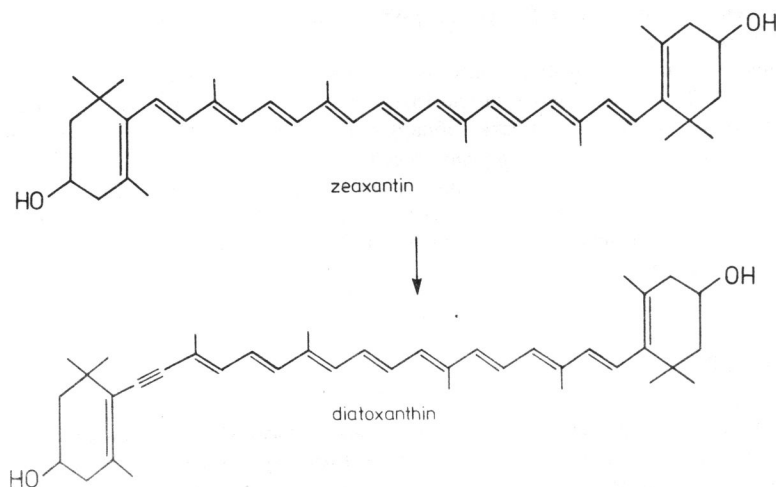


Fig. 2. Biosynthesis of diatoxanthin as derivative of zeaxanthin

Concerning general carotenoid content in the thalli of the studied lichen species, certain regularity has been noticed. Thus, thalli collected for analysis in November were the richest in carotenoids, while those collected in February were the poorest. Probably this phenomenon should also be explained as conditioned by climatic factors since it is known that in October and November a rapid increase of temperature occurs at Antarctica: it is spring and, as a result of melting snow, there is enough humidity while higher temperature at the same time facilitates the growth of these plants. On the other hand, in February, due to rapid drop in temperature, the conditions for development of plants, particularly lichens, deteriorate. This probably determines general content of carotenoids in these plants.

## 4. Резюме

Используя метод хроматографического анализа (тонкослойного и на колонке), спектрофотометрии и реакций, специфических для гидроксильных и эпоксидных групп, исследовался качественно-количественный состав каротиноидов, содержащихся в следующих видах лишайников Антарктики: *Caloplaca regalis*, *Himantormia lugubris*, *Ramalina terebrata*, *Usnea antarctica*, *U. fasciata*, *Xanthoria elegans*.

Во время анализа были определены следующие каротиноиды:  $\beta$ -каротен,  $\alpha$ - и  $\beta$ -криптоксантин, кантаксантин, ликопил, ликоксантин, лютеин, зеаксантин, антераксантин, адониксантин, диатоксантин, родоксантин,  $\alpha$ -дорадоксантин, астаксантин, мутатохром, мутатоксантин и криптофлавин. К наиболее часто выступающим каротиноидам у исследуемых лишайников относятся  $\beta$ -каротен,  $\beta$ -криптоксантин, лютеин эпоксидный, зеаксантин и мутатоксантин.

В количественном отношении общее содержание каротиноидов отчетливо доминирует в октябре — с 10,24 (*Ramalina terebrata*) до 18,70 мг/г сухого вещества (*Himantormia lugubris*), в то время как наименьшее количество обнаружено в феврале — с 4,77 (*Ramalina terebrata*) до 12,46 мг/г сухого вещества (*Caloplaca regalis*).

Полученные результаты исследований указывают на то, что анализируемые лишайники Антарктики являются очень богатыми сухим веществом, в то же время гораздо беднее каротиноидами по сравнению с лишайниками, живущими в лучших климатических и трофических условиях.

## 5. Streszczenie

Stosując metody analizy chromatograficznej (cienkowarstwowej i kolumnowej), spektrofotometrycznej oraz reakcje charakterystyczne na grupy hydroksylowe i epoksydowe badano skład jakościowo-ilościowy karotenoidów zawartych w następujących gatunkach porostów Antarktyki: *Caloplaca regalis*, *Himantormia lugubris*, *Ramalina terebrata*, *Usnea antarctica*, *U. fasciata* i *Xanthoria elegans*.

W trakcie analizy zidentyfikowano następujące karotenoidy:  $\beta$ -karoten,  $\alpha$ - i  $\beta$ -kryptoksanteryne, kantaksanteryne, likofil, likoksanteryne, luteinę, zeaksanteryne, anteraksanteryne, adoniksanteryne, rodoksanteryne, diatoksanteryne,  $\alpha$ -doradoksanteryne, astaksanteryne, mutatochrom, mutatoksanteryne i kryptoflawinę. Do najczęściej występujących karotenoidów u badanych porostów należy:  $\beta$ -karoten,  $\beta$ -kryptoksanteryna, luteina epoksydowa, zeaksanteryna i mutatoksanteryna.

Pod względem ilościowym ogólna zawartość karotenoidów wyraźnie dominuje w październiku: od 10,20 (*Ramalina terebrata*) do 18,70 mg/g suchej masy (*Himantormia lugubris*), natomiast najmniejszą ilość stwierdzono w lutym: od 4,77 (*Ramalina terebrata*) do 12,46 mg/g suchej masy (*Caloplaca regalis*).

Otrzymane wyniki badań wskazują, że analizowane porosty Antarktyki są bardzo zasobne w suchą masę, natomiast w karotenoidy są znacznie uboższe w odniesieniu do porostów żyjących w lepszych warunkach klimatycznych i troficznych.

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