INVESTIGATIONS ON CAROTENOIDs IN LICHENS. XXI. ASTAXANTHIN, THE DOMINANT CAROTENOID IN SOME LICHENS FROM URUGUAY

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ABSTRACT

The presence of carotenoids in four species of lichens from Uruguay (Cladia aggregata, Concamerella fistulata, Usnea amaliae, and Usnea densirostra) was studied by column and thin-layer chromatography. The investigations revealed the presence of the following carotenoids: \( \beta \)-cryptoxanthin, zeaxanthin, antheraxanthin, lutein epoxide, astaxanthin (dominant in all species), violaxanthin, mutatoxanthin, and luteoxanthin. The total carotenoid content of the material ranged from 9.2 to 12.3 \( \mu g \) g\(^{-1}\) dry weight.

The studies on carotenoids in lichens carried out by us to date have revealed that usually \( \beta \)-cryptoxanthin or some carotenoids of the epoxide group predominate in the thalli of lichens (Czeczuga, 1985a). In lichens with golden-yellow thalli, in particular, the main carotenoid of the epoxide group is mutatoxanthin (Czeczuga, 1983). In some lichens, however, auroxanthin was found to be the dominant carotenoid of this group (Czeczuga & Xavier-Filho, 1987).

On analyzing the thalli of some lichen species from Uruguay, we found that the ketocarotenoid astaxanthin is also one of the dominant carotenoids occurring in lichens.

MATERIALS AND METHODS

The investigations were carried out on the thalli of four lichen species from Uruguay: Cladia aggregata (Sw.) Nyl. (sandy soil, Pinus plantation, aerohaline zone), Concamerella fistulata (Tayl.) W. Culb. and Ch. Culb. (on rocks in Sierra de San Miguel), Usnea amaliae Mot. (on rocks in Sierra de las Animas), and Usnea densirostra Tayl. (top of boulder in Sierra Mahoma). 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

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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 Rₜ values were determined for each spot. For identification of the thallus carotenoids, 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 behavior 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 Rₜ values in TLC; (e) the presence of allylic hydroxyl groups as determined by the acid–chloroform test; (f) the epoxide test (Krinsky & Goldsmith, 1960); (g) the mass spectrum; and (h) infrared spectroscopy for astaxanthin (Vetter et al., 1971). 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).

RESULTS

The following eight carotenoids were identified in the thalli of the four lichens from Uruguay: β-cryptoxanthin, zeaxanthin, antheraxanthin, lutein epoxide, astaxanthin, violaxanthin, mutatoxanthin, and luteoxanthin (Table I and Fig. 1). β-Cryptoxanthin, lutein epoxide, and astaxanthin were found in the thalli of all the species studied. The dominant carotenoid, astaxanthin, accounted for 31.4% (Cladia aggregata) to 49.0% (Con-

![Fig. 1. Structural features of the carotenoids listed in Table I.](image)
TABLE I
Carotenoid content in some species of lichens from Uruguay

<table>
<thead>
<tr>
<th>Carotenoids</th>
<th>Structure</th>
<th>Cladia aggregata</th>
<th>Concamerella fistulata</th>
<th>Usnea amaliae</th>
<th>Usnea densirostra</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-cryptoxanthin</td>
<td>A–x–B</td>
<td>20.8</td>
<td>22.7</td>
<td>17.4</td>
<td>21.3</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>B–x–B</td>
<td></td>
<td></td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>Antheraxanthin</td>
<td>B–x–E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutein epoxide</td>
<td>C–x–E</td>
<td>21.1</td>
<td>trace</td>
<td>12.3</td>
<td>12.8</td>
</tr>
<tr>
<td>Astaxanthin</td>
<td>D–x–D</td>
<td>31.4</td>
<td>49.0</td>
<td>44.5</td>
<td>39.5</td>
</tr>
<tr>
<td>Violaxanthin</td>
<td>E–x–E</td>
<td>22.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutatoxanthin</td>
<td>B–y–F</td>
<td>4.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteoxanthin</td>
<td>E–y–F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total content</td>
<td></td>
<td>12.2</td>
<td>12.3</td>
<td>9.2</td>
<td>11.0</td>
</tr>
</tbody>
</table>

*See Figure 1. †As µg g⁻¹ dry weight.

camerella fistulata) of all the carotenoids contained in the thalli. The total carotenoid content was found to range from 9.2 (Usnea amaliae) to 12.3 µg g⁻¹ dry weight (Concamerella fistulata).

DISCUSSION

Astaxanthin was first described in aquatic crustaceans as an oxidized form of β-carotene which gives the carapace of these animals its pinkish color. It was later found that this pigment is very common in aquatic and land animals of various systematic groups. In certain species of fish and birds (Isler, 1971), it plays an important role in coloration during the mating season.

From the literature on this subject (Isler, 1971), it would seem that astaxanthin occurs only in the animal world. This made us consider advisable to give here a brief review of the occurrence of this carotenoid in plants.

Astaxanthin occurs in both higher and lower forms of plant life, but not to the same extent as in animals. So far it has not been found in bacteria, with the exception of one Brevibacterium species, where it occurs together with other ketocarotenoids (Goodwin, 1980). On the other hand, astaxanthin is present in several groups of algae. It occurs in Chlorophyta: Chlorella, Acetabularia mediterranea (Kleinig & Egger, 1967; Czeczuga, 1986a), Ankistrodesmus, and Scenedesmus (Kessler & Czygan, 1967); in Pyrrophyta: Glenodinium (Jeffrey et al., 1975); in Euglenophyta: Euglena rubida (Czeczuga, 1974) and Trachelomonas hispida; and in a number of Cyanophyta: Oscillatoria, Phormidium, Anabaena, Aphanizomen, and Synechococcus (Stransky & Hager, 1970; Czeczuga, 1979). In addition, astaxanthin has been identified in some fungus species, colonial
Ascomycetes (*Phaffia rhodozyma*) (Andrewes et al., 1976), and in a few Basidiomycetes (Arpin et al., 1966; Czeczuga, 1978). Astaxanthin is found sporadically in a few species of Bryophyta, in both the liverwort and the true musci (Czeczuga, 1980, 1985b,c). In flowering plants, astaxanthin has been noted in the petals of *Viola tricolor*, *Adonis aestivalis*, and *Adonis annua* (Neamtu & Bodea, 1969; Goodwin, 1980).

On the other hand, in lichens astaxanthin is encountered fairly often, but usually in small amounts; it has been noted in lichens from various families (Czeczuga, 1983) growing at different latitudes (Czeczuga & Cifuentes, 1986; Czeczuga & Schelkunova, 1986; Xavier-Filho et al., 1986; Czeczuga & Alstrup, 1987; Czeczuga & Ferraro de Corona, 1987). The thalli of the lichens from Uruguay contained, however, substantial amounts of astaxanthin, which was the dominant carotenoid in these plants. The fact that astaxanthin occurs much more frequently in lichens than in other groups of plants is probably due to the biology of these organisms. Lichens are perennials, and their thalli are exposed to the effect of varying environmental factors over a comparatively long period of time. Throughout this interval, under the influence of temperature, air, and light, the carotenoids are converted into more oxidized forms, the so-called xanthophylls. The effect of environmental factors has been established in studies on a wide range of plant material, e.g., the effect of light on the formation of more oxidized carotenoids, such as lutein epoxide, mutatoxanthin (Czeczuga, 1987a), and rhodoxanthin (Czeczuga, 1987b), from less oxidized precursors. Tissue senescence has a significant role in the formation and accumulation of more oxidized xanthophylls in higher plants. In deciduous species, the carotenoid content decreases suddenly in autumn, prior to leaf abscission, and the concentration of xanthophylls, including that of the highly oxidized epoxide and apocarotenal groups, increases in these leaves (Czeczuga, 1986b). The data obtained so far on the presence of various carotenoids which may be precursors of astaxanthin suggest that β-carotene may change into astaxanthin either through β-cryptoxanthin and zeaxanthin, or through echinenone and canthaxanthin (Fig. 2A). Lutein

![Fig. 2. Possible pathways of astaxanthin biosynthesis in lichens.](image-url)
was found in almost all the lichen species studied to date, though not in such large amounts as in plant leaves, where it represents the dominant carotenoid. This makes us assume that α-carotene may also participate in the biosynthesis of astaxanthin in lichens (Fig. 2B). The conversion pathway of α-carotene into astaxanthin has already been confirmed in animals (Katayama et al., 1970). Most of the carotenoids involved in the biosynthesis of astaxanthin (Fig. 2) have been found in several species of lichens from various families.

REFERENCES


