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Investigations on Carotenoids in Lichens. XXXIII. Carotenoids in lichens from heathland in Iceland.

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Abstract: Column and thin-layer chromatography were used to study the occurrence of various carotenoids in 12 lichen species collected in Iceland. The following carotenoids were found: β-carotene, β-cryptoxanthin, lutein, zeaxanthin, diatoxanthin, α-doradexanthin, canthaxanthin, astaxanthin, lutein epoxide, antheraxanthin, violaxanthin, auroxanthin, mutatoxanthin, luteoxanthin, neoxanthin and apo-12'-violaxanthal. The total carotenoid contents ranged from 14.0 (*Cladina rangiferina*) to 57.3 μg g^{-1} dry weight (*Cladonia furcata*).

While carrying out studies of carotenoids in lichens from various latitudes (CZECZUGA 1988) we noticed, that in many cases in the Atlantic lichens from Ireland, the thalli contained far more lutein than the epoxide form of this carotenoid (CZECZUGA and RICHARDSON 1989). Our investigations of lichens from other latitudes indicated, that the epoxide form of lutein usually predominated and if lutein was present, it occurred in much smaller amounts than lutein epoxide.

As numerous reports have shown (GALLØE 1932, LYNGE 1940, DEGELIUS 1957, KRISTINSSON 1972, 1974, 1981, ORANGE 1990) lichens constitute a considerable part of the flora of Iceland. In view of this, it was felt that it would be of interest to study the occurrence of carotenoids in lichens from a continent of a typical Atlantic climate, Iceland, with particular attention being paid to their lutein content. The results obtained may prove to be of assistance in chemotaxonomic studies of this group of plants (CULBERSON and KRISTINSSON 1970, KRISTINSSON 1971) since carotenoids have been used in chemotaxonomic investigations of fungi (VALADON 1976) and algae (LIAAEN-JENSEN 1977, 1989) which are, as we know, components of lichens.

MATERIALS AND METHODS

The investigations were carried out on the following species: *Nephroma expallidum* (Nyl.) Nyl., *Peltigera aphthosa* (L.) Willd., *Cladina arbuscula* (Wallr.) Hale et W. Culb., *Cladina rangiferina* (L.) Nyl., *Cladonia ecmocyna*

Leight., *Cladonia furcata* (Huds.) Schrad., *Cladonia gracilis* (L.) Willd., *Cladonia stricta* (Nyl.) Nyl., *Stereocaulon alpinum* Laur., *Cetraria islandica* (L.) Ach., *Alectoria nigricans* (Ach.) Nyl. and *Alectoria ochroleuca* (Hoffm.) Mass. All the species were collected on November 8th 1987 in heathland in Northern Iceland in the same locality: Province Suður-Þingeyjarsýsla, Vaðlaheiði, Hrossadalur.

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 hours 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 glas column (Quickfit, England) packed 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

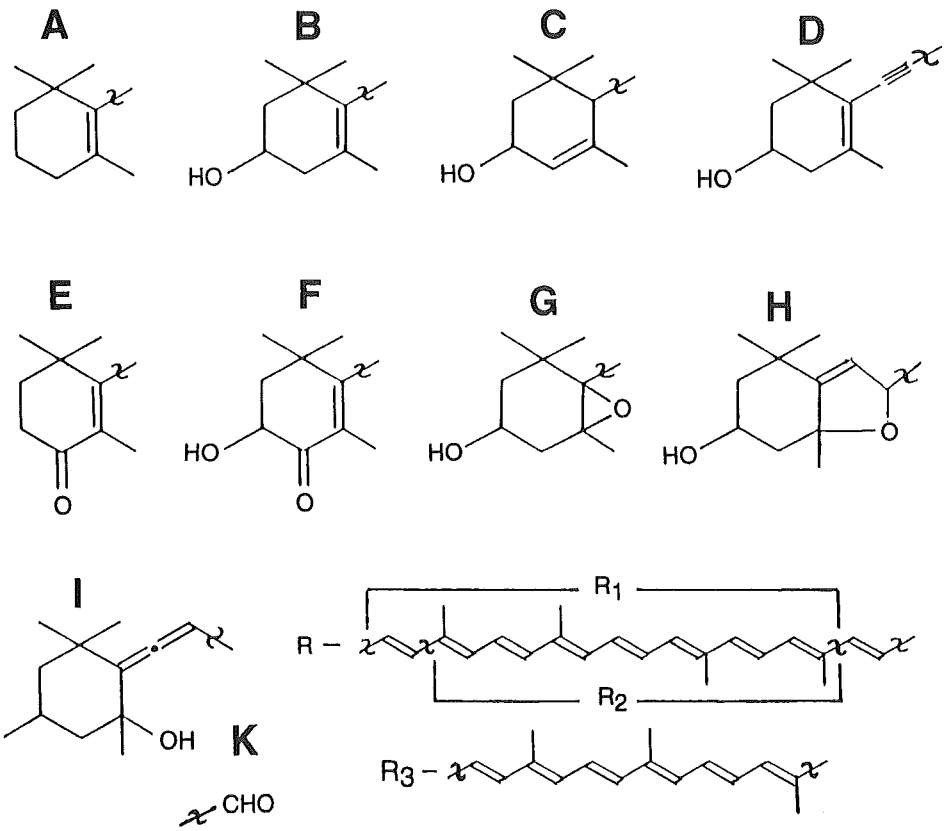

FIGURE 1. Structural features of carotenoids.

TABLE 1. List of the carotenoids from the investigated lichen species

Carotenoid	Structure (see Fig.1)	Semisystematic name
1. ß-carotene	A-R-A	ß, ß-carotene
2. ß-cryptoxanthin	A-R-B	ß, ß-carotene-3-ol
3. lutein	B-R-C	ß, ε-carotene-3,3'-diol
4. zeaxanthin	B-R-B	ß, ß-carotene-3,3'-diol
5. diatoxanthin	B-R_1-D	7,8-didehydro-ß, ß-carotene-3,3'-diol
6. α-doradexanthin	C-R-F	3,3'-dihydroxy-ß, ε-carotene-4-one
7. canthaxanthin	E-R-E	ß, ß-carotene-4,4'-dione
8. astaxanthin	F-R-F	3,3'-dihydroxy- ß, ß-carotene-4,4'-dione
9. lutein epoxide	C-R-G	5,6-epoxy-5,6-dihydro-ß, ε-carotene-3,3'-diol
10. antheraxanthin	B-R-G	5,6-epoxy-5,6-dihydro-ß,-ß-carotene-3,3'-diol
11. violaxanthin	G-R-G	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro-ß, ß-carotene-3,3'-diol
12. auroxanthin	H-R_9-H	5,8,5',8',-diepoxy-5,8',5',8'-tetrahydro-ß, ß-carotene-3,3'-diol
13. mutatoxanthin	B-R_1-H	5,8-epoxy-5,8-dihydro-ß, ß-carotene-3,3'-diol
14. luteoxanthin	G-R_1-H	5,6,5',8'-diepoxy-5,6,5',8'-tetrahydro-ß, ß-carotene-3,3'-diol
15 neoxanthin	G-R_1-I	5',6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro-ß, ß-carotene-5,5,3'-triol
16. apo-12'-violaxanthal	G-R_9-K	5,6-epoxy-3-hydroxy-5,6-dihydro-12'-apo-ß-carotene-12'-al

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 (LIAAEN-JENSEN 1971).

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; g) the mass spectrum; and h) the infrared spectroscopy (VETTER et al. 1971 for basic methodology) were recorded by a Specord M-80 Carl Zeiss, Jena. 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 to STRAUB (1987).

RESULTS

In the thalli of 12 common species from Iceland, 16 carotenoids were identified (table 1), which had previously been noted in the thalli of many

other lichen species from various latitudes. The predominant carotenoid in the thalli of all the species except *Alectoria nigricans* was lutein (table 2). In the *Alectoria nigricans* thalli, violaxanthin constituted the greatest percentage of all the carotenoids (29.8%) whereas lutein constituted 24.7%. The total carotenoid content in the material studied ranged from 14.0 (*Cladina rangiferina*) to 57.3 µg dry weight (*Cladonia furcata*).

TABLE 2. Carotenoid distribution in lichens from Iceland

Families and species	Carotenoid (see Table 1)	Major carotenoid (%)	Total content (µg g^{-1} dry wt)
Peltigeraceae			
Nephroma expallidum	1,3,7,8,9,10,11	3 (33.6)	42.2
Peltigera aphthosa	1,3,6,7,8,9,11	3 (38.3)	39.3
Cladoniaceae			
Cladina arbuscula	1,3,4,6,8,9	3 (33.3)	18.8
Cladina rangiferina	1,3,4,6,9,11,15	3 (26.7)	14.0
Cladonia ecmocyna	1,2,3,6,9,10,11	3 (29.6)	24.2
Cladonia furcata	1,2,3,4,6,7,8,9,11,12,16	3 (32.7)	57.3
Cladonia gracilis	1,3,5,6,8,9,11,13	3 (18.6)	22.3
Cladonia stricta	1,2,3,4,6,8,9,11	3 (18.8)	18.5
Stereocaulaceae			
Stereocaulon alpinum	3,4,8,9,11,13	3 (19.0)	21.1
Parmeliaceae			
Cetraria islandica	1,2,3,4,7,8,9,14	3 (25.9)	29.7
Usneaceae			
Alectoria nigricans	1,3,8,9,11,13	11(29.8)-3(24.7)	57.2
Alectoria ochroleuca	1,3,4,8,9,10	3 (28.9)	28.2

DISCUSSION

Of the results obtained the presence of β-carotene in nearly all the lichen species (with the exception of *Stereocaulon alpinum*) and that of lutein in all the species, is worthy of note. As was mentioned above, with the exception of the *Alectoria nigricans* thalli, lutein was the predominant carotenoid. Our previous studies to date have clearly shown that in both lichens and in higher plants (CZECZUGA 1981, 1983, 1987a) the total carotenoid content and the presence of the various carotenoids are markedly influenced by the intensity of insolation. In sites well insolated, the total carotenoid content is considerably lower than in shady places. Furthermore, in insolated places the leaves of higher plants contain fewer non-oxidized carotenoids including β -carotene, and in leaves exposed to sunlight far more lutein epoxide accumulates (CZECZUGA 1987a). This also applies to a number of other carotenoids, among others, rhodoxanthin (CZECZUGA 1987b). As was mentioned in the introduction to this report, the thalli of most of the lichen species from Ireland contained substantially more lutein than lutein epoxide

(CZECZUGA and RICHARDSON 1989). The climatic conditions prevalent in Ireland are similar in general to those of Iceland. It is also interesting to note that there was a comparatively high total content of carotenoids, particularly in the representatives of the Cladoniaceae. In our previous investigations of carotenoids in many representatives of the Cladoniaceae from north-eastern Poland (CZECZUGA 1985) we found a far lower total carotenoid content. The total carotenoid contents of thalli of *Cladonia* species collected from the far north in Siberia from the region of Taimyr (CZECZUGA and SHCHELKUNOVA 1986) were of similar values to those noted in the lichens from Iceland.

The thalli of five species of lichens of the genus *Cladonia* from a heathland in Iceland were found to have following carotenoids in common: β-carotene, lutein, α-doradexanthin and lutein epoxide. On the other hand, the carotenoids common to two species of the genus *Alectoria* were β-carotene, lutein, astaxanthin and lutein epoxide. It was also of interest to find β-carotene, lutein, and lutein epoxide in the thalli of species of *Cladonia* and *Alectoria*. The only differences were observed in carotenoids of the keta-carotenoid group; α-doradexanthin was found in the *Cladonia* species, instead of astaxanthin in the representatives of *Alectoria*.

The results of this analysis of the carotenoid content of the lichens from Iceland once again confirm our observations of the significant effect of environmental factors on the carotenoid content of lichens.

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