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CAROTENOIDS IN LICHENS OF THE *LOBARIA* AND *PELTIGERA* GENERA FROM INDIA*

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ABSTRACT

The presence of carotenoids in 10 species of the lichens form India was studied. The investigations revealed the presence of the following carotenoids ; α - carotene, β -carotene, β -cryptoxanthin, lutein, zeaxanthin, β -carotene epoxide, lutein epoxide, antheraxanthin, violaxanthin, neoxanthin, canthaxanthin, astaxanthin, mutatoxanthin, flavochrome, capsochrome and β -apo-10'-carotenal.

The total carotenoid content of the material ranged from 15.0 to 31.6 $\mu g\ g^{-1}$ dry weight.

INTRODUCTION

Apart from higher plants, cryptogamic plants to which lichens belong, have recently aroused interest as a source of biologically active substances (Galun, 1988). In addition to the well known pharmaceutical properties, lately attention has been paid to the antibiotic properties of lichen acids, particularly usnic acid (Richardson, 1988). Some lichen polysaccharides have been found to have antitumour activity (Hartwell, 1971).

Studies carried out over the last ten years (Czeczuga, 1988a) showed that lichen thalli are also rich in carotenoids, a known source of Vitamin A, which also play an important role in the normal functioning of the human organism (Isler, 1971).

In recent years, chemical methods have been increasingly used since together with the classical methods it provides better taxonomic possibilities. In taxonomic studies of fungi (Valadon, 1976) and algae (Liaaen-Jenssen, 1977, 1989; Weber and Wettern, 1980) in recent years, data on the presence or absence of the various carotenoids have been used.

In view of this we feel that the publication of data on carotenoids in lichens from India may contribute to a wider knowledge of this group of plants in the country.

MATERIALS AND METHODS

The lichen species of the genera *Lobaria* and *Peltigera* were studied and the site and habitat of these species are presented in Table 1.

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, 1980a) were used for the separation of various carotenoids. A 15-20 cm × 1 cm glass column

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(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 identification of the thallus carotenoids standards (Hoffman-La Roche and Co. Ltd., Basel, Switzerland and Sigma Chemical Co., USA), were co-chromatography 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) the infra-red spectroscopy for capsochrome (Vetter 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 wave-lengths of maximal absorbance of petroleum ether or hexane (Davies, 1976).

Structure of carotenoids is given according to Straub (1987).

RESULTS

In the thalli of 5 lichen speeies of *Lobaria* and 5 species of *Peltigera,* the presence of 16 carotenoids was determined (Table 2, Fig. 1). An interesting finding was that of the carotenoid capsochrome in the thalli of 4 species of *Peltigera* viz., *P. dolichorhiza, P. polydactyla, P. praetextata,* and *P. rufescens,* This is the first time that this carotenoid has been found in lichens. The predominant carotenoids were violaxanthin in species of the Lobaria, lutein expoxide in species of the *Peltigera* and mutatoxanthin in the thalli of two species of *L. kurokawae* and *P. canina.* The total carotenoid content in the species of the Lobaria ranged from 15.0 (*L. retigera*) to 31.6μg g^{-1} dry weight (*L. isidiosa*) and in the species of the *Peltigera* from 18.5 (*P. canina* and *P. rufescens*) to 30.2 μg g dry weight (*P. dolichorhiza*) (Table 3).

DISCUSSIONS

The carotenoid capsochrome is a derivative of capsanthin which was first isolated from the fruits of *Capsicum annuum* (Camara and Moneger, 1980). As a result of oxidation, capsanthin is converted into capsanthin mono epoxide which, in turn, is converted into capsochrome. Capsanthin, on the other hand, is formed as a result of conversion of antheraxanthin (Camara and Moneger, 1981). We now know that capsanthin does not occur only in fruit but also in the anthers, flowers, pollen and in seeds of many species of higher plants (Goodwin, 1980).

The carotenoid content of the *Lobaria retigera* thalli from South Africa was studied previously (Czeczuga *et al.*, 1988). On comparing the results of the analysis of the thalli of this species from India with those from South Africa, differences are noted, for example, β-carotene zeaxanthin, and violaxanthin occurred only in the thalli from India. On the other, the thalli of the South African species contained not only some carotenoid noted in the species from India but also lycoxanthin, astaxanthin and mutatoxanthin.

The thalli of some of the species of *Peltigera* from Poland (Czeczuga, 1980b), Bulgaria (Czeczuga, 1988c) and Ireland (Czeczuga and Richardson, 1989) have been studied for their carotenoid content. Capsochrome occurred only in the species collected from India. This observation is in agreement with those made

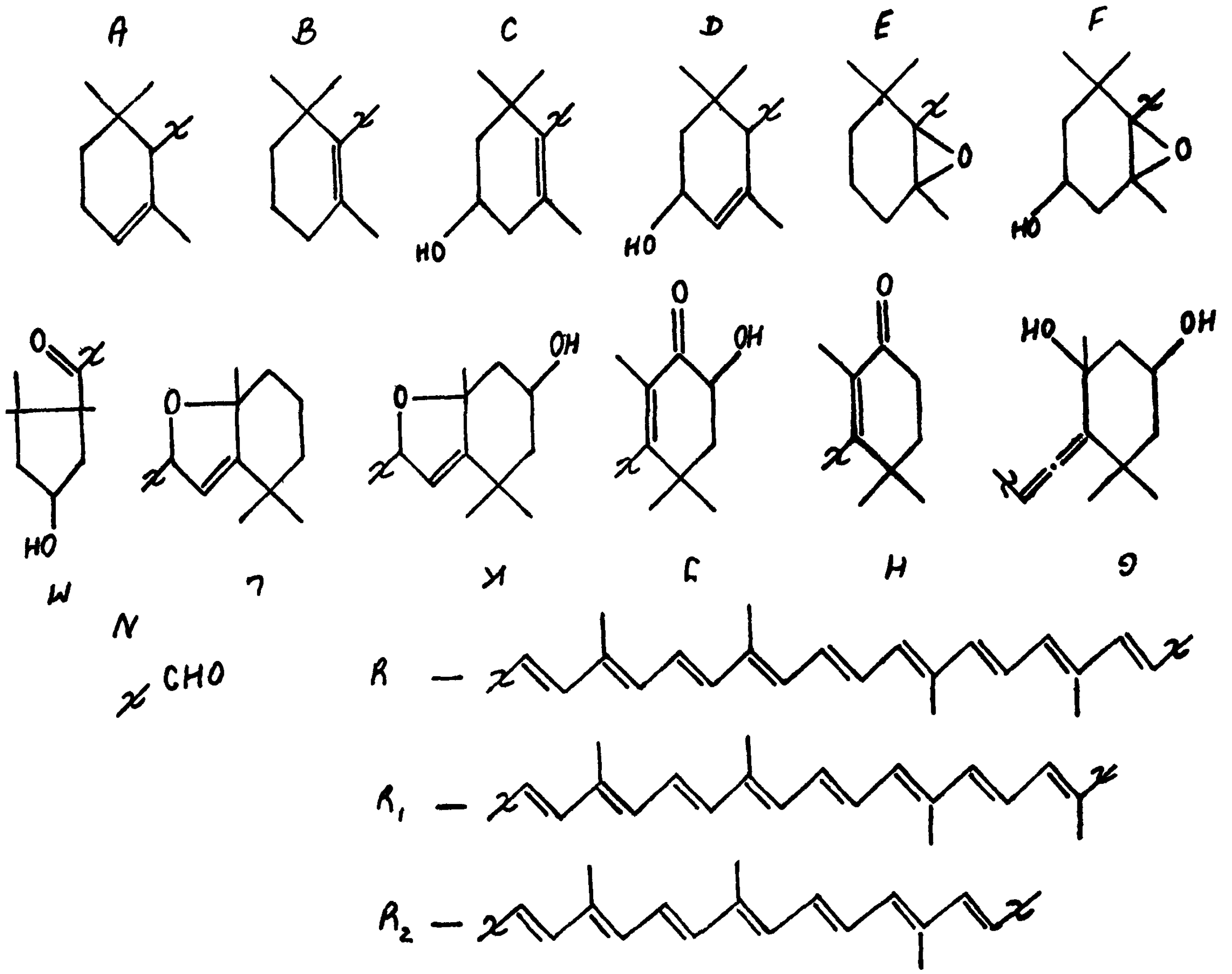

Fig. 1: Structural features of carotenoids from investigated materials.

in studying the presence of other carotenoids in *Peltigera* species from various latitudes. The data indicated the environmental factors have a marked influence on the presence of various carotenoids in lichen thalli.

As the present investigations showed, the predominant carotenoids were those of the epoxide group-lutein epoxide, violaxanthin and in two cases, mutatoxanthin. Lutein epoxide is formed from lutein by oxidation. As we know, in higher plants, the largest amounts of lutein are found to accumulate in the parts of the plants least exposed to sunlight whereas lutein epoxide is found in plants in places more insolated (Czeczuga, 1981, 1987). Other derivatives of lutein such as loroxanthin or siphonaxanthin are also formed by way of oxidation and occur in marine algae instead of lutein. These derivatives of lutein are capable of trapping green and blue rays usually penetrating the deeper parts of seas (Yokohama, 1982). Violaxanthin, on the other hand, is formed through antheraxanthin from zeaxanthin (Goodwin, 1980). As recent studies have shown (Alberte and Anderson, 1986, Owens *et al.*, 1987, Bidigare *et al.*, 1989) all three of these carotenoids (zeaxanthin, antheraxanthin, violaxanthin) in algae are capable of trapping rays of a shorter wave-length, that is usually the green and blue, than those trapped by chlorophylls. This property particularly applies to violaxanthin. In some algae, especially Cyanophyceae and Rhodophyta, apart from chlorophylls and carotenoids which act as antennae trapping light rays, phycobiliprotein pigments also perform this function (Czeczuga, 1988b). As our studies of several species of *Peltigera* among others, *P. polydactyla* and *P. rufescens,* the phycobiliprotein pigments particularly the phycoerythrin and phycocyanin groups, play an important role in chromatic adaptation (Czeczuga, 1986). Most of the lichen species of *Peltigera* and a considerable number of species of *Lobaria* the phycobionts are Cyanophyceae, usually of *Nostoc.* Where the lighting is poor, the total content of phycobilin and C-phycocyanin increases in species of *Peltigera,* whereas with intense light, the content of C-phycoerythrin increases. Furthermore, the content of the various phycobiliprotein pigments change with the seasons ; in periods when the amount of long rays in the spectrum is greater (late autumn, winter and early spring) the C-phycocyanin content rises whereas in summer when short rays are predominant, the content of C-phycoerythrin increases in the thalli of these lichens (Czeczuga, 1988b). Furthermore, where the lighting is poor, the amount of bilisomes, carriers of phycobiliprotein pigments, increases in Cyanophyceae (Vierling and Alberte, 1980). Poor lighting conditions favour an increase in phycobiliprotein pigments more than in chlorophyll pigments (Foy and Gibson, 1982).

In all the species of *Lobaria,* the carotenoids β-carotene, lutein, lutein epoxide, zeaxanthin and violaxanthin were found in the thalli. On the other hand, in the thalli of all the other species of *Peltigera,* α-carotene, β-carotene and lutein epoxide were present. In conclusion, it should be noted that each genus has its own characteristic carotenoids, a fact that may be of value in taxonomic research.

Table 1 : Species of lichens from India that were investigated

Species	Locality	Habitat	Altitude (in m)
1. *Lobaria isidiosa* (Mull.-Arg.) Vainio	West Bengal-Darjeeling, Tongu	rock	3225
2. *L. kurokawae* Yoshim.	Uttar Pradesh-Chamoli district	rock	2100
3. *L, pindarensis* Ras.	Almora district-Pindari	rock	3000
4. *L. pseudopulmonaria* Gyel.	Uttar Pradesh-Chamoli district	rock	4050
5. *L. retigera* (Bory) Trev.	Uttar Pradesh-Tehri district	bark	2250
6. *Peltigera canina* (L.) Willd.	Uttar Pradesh-Chamoli district	rock	3075
7. *P. dolichorhiza* (Nyl.). Nyl.	Himachal Pradesh-Lakarmandi	rock	2700
8. *P. polydactyla* (Neck) Hoffm.	Uttar Pradesh-Pithoragarh district	rock	1500
9. *P. praetextata* (Flk.) Vainio	Uttar Pradesh-Naini Tal district	soil	1800
10. *P. rufescens* (Weis.) Humb.	Uttar Pradesh-Mussorie	soil	2000

Table 2 : List of the carotenoids found in lichens from India

Carotenoid	Structure (See Figure 1)	Semi-systematic name
1. α-Carotene	A R B	β, ϵ -carotene
2. β-Carotene	B R B	β, β -carotene
3. β-Cryptoxanthin	B R C	β, β-carotene-3-ol
4. Lutein	C R D	β, ϵ-carotene-3, 3'-diol
5. Zeaxanthin	C R C	β, β-carotene-3, 3'-diol
6. β-Carotene epoxide	B R E	5, 6-Epoxy-5, 6-dihydro-β, β-carotene
7. Lutein epoxide	D R F	5, 6-Epoxy-5, 6-dihydro-β, ϵ-carotene-3, 3'-diol
8. Antheraxanthin	C R F	5, 6-Epoxy-5, 6-dihydro-β, β-carotene-3, 3'-diol
9. Violaxanthin	F R F	5, 6, 5', 6' -Diepoxy-5, 6, 5', 6', tetrahydro-β, β-carotene-3, 3'-diol
10. Neoxanthin	F R_1 G	5'-6-Epoxy-6, 7,-didehydro-5, 6, 5', 6', tetrahydro-β, β-carotene-3, 5, 3'-triol
11. Canthaxanthin	H R - H	β, β -carotene-4, 4'-dione
12. Astaxanthin	I - R I	3, 3' -Dihydroxy-β, β-carotene-4, 4'-dione
13. Mutatoxanthin	C R_1 K	5, 8-Epoxy-5, 8-dihydro-β, β-carotene-3, 3'-diol
14. Flavochrome	A R_1 L	5, 8-Epoxy-5, 8-dihydro-β, ϵ -carotene
15. Capsochrome	K R_1 M	5, 8-Epoxy-3, 3'-dihyroxy-5, 8-dihydro-β, K -carotene-6'-one
16. β-Apo-10'-carotenal	B R_2 - N	10'-Apo-β-carotene-10'-al

Table 3 : Carotenoid distribution in lichens from India

Species	Carotenoid detected (see Table 1)	Major carotenoids	Total content ($\mu g\ g^{-1}$ dry weight)
1. *Lobaria isidiosa* (Mull.-Arg.) Vainio	2, 3, 4, 7, 9, 10	9/24.1/	31.6
2. *L. kurokawae* Yoshim.	2, 3, 4, 6, 7, 9, 13	13/42.5/	25.0
3. *L. pindarensis* Ras.	2, 3, 4, 7, 9	9/62.6/	28.1
4. *L. pseudopulmonaria* Gyel.	2, 3, 4, 7, 8, 9	9/57.4/	22.1
5. *L. retigera* (Bory) Trev.	2, 3, 4, 5, 7, 9, 10	9/30.6/	15.0
6. *Peltigera canina* (L.) Willd.	1, 2, 3, 4, 7, 9, 13	13/30.6/	18.3
7. *P. dolichorhiza* (Nyl.) Nyl.	1, 2, 7, 9, 10, 15	7/44.6/	30.2
8. *P. polydactyla* (Neek) Hoffm.	1, 2, 6, 7, 10, 11, 14, 15	7/42.1/	19.5
9. *P. praetextata* (Flk.) Vainio	1, 2, 4, 5, 7, 8, 15, 16	7/41.1/	21.4
10. *P. rufescens* (Weis.) Humb.	1, 2, 5, 6, 7, 8, 12, 13, 15	7/44.5/	18.3

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