ANALES DE BIOLOGÍA, 16 (Biología Vegetal, 5) 1990: 131-135
SECRETARIADO DE PUBLICACIONES - UNIVERSIDAD DE MURCIA

INVESTIGATIONS ON CAROTENOIDS IN LICHENS. XXVII. CAROTENOIDS IN LICHENS FROM NORTH AFRICA AND SE SPAIN

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Recibido: 26 marzo 1990
Acetpado: 25 noviembre 1990

RESUMEN

Investigaciones en Carotenoides de Líquenes. XXVII. Carotenoides en Líquenes del N. África y SE. España.

Cromatografía en columna y capa fina revela la presencia de los siguientes carotenoides en el talo de 12 especies de líquenes recolectados en el norte de África y SE España: a-caroteno, β-caroteno, α-criptoxantina, β-criptoxantina, luteina, 3'-epiluteina, zeaxantina, diatoxantina, licopeno-5, 6-epoxido, nuevo en líquenes, β-caroteno epóxido, luteina epóxido, anteraxantina, violaxantina, mutatoxantina, neoxantina, astaxantina, β-apo-10'-carotenal y apo-12'-violaxantal.

El contenido total de carotenoides oscila entre 17,20 en *Ramalina hourgeana* y 72,52 μg g^{-1} del peso seco en *Roccella canariensis.*

Palabras clave: Líquenes, carotenoides, N. de África, SE España.

SUMMARY

Column and thin-layer chromatography revealed the presence of the following carotenoids in the thalli of 12 lichen species from the North Africa and SE Spain: a-carotene, β-carotene, a-cryptoxanthin, β-cryptoxanthin, lutein, 3'-epilutein, zeaxanthin, diatoxanthin, lycopene-5, 6-epoxide, the first record from lichens, P-carotene epoxide, lutein epoxide, antheraxanthin, violaxanthin, mutatoxanthin, neoxanthin, astaxanthin, O-apo-10'-carotenal and apo-12'violaxanthal.

The total content of carotenoids ranged from 17,20 *Ramalina hourgeana* to 72,52 μg g^{-1} dry weight *Roccella canariensis.*

Key words: Lichens, carotenoids, North Africa, SE Spain.

INTRODUCTION

As the review of literature made by EGEA and ROWE 1987 showed, studies of the lichenoflora of North Africa have a long tradition beginning with the work of WERNER, 1901 (see LLIMONA 1979). A large number of new species were described together with their ecological niches. These data considerably enriched our knowledge of the distribution of a number of lichen species.

The publication of data on the presence of

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the various carotenoids in the thalli of lichen species from this region of Africa will augment the earlier data on these lichens (CZECZUGA, 1988). Furthermore, data on carotenoids can also be of value in taxonomic research into this group of plants as was found in the case of studies of fungi (VALADON, 1976) and algae (LIAAEN-JENSEN, 1977).

MATERIALS AND METHODS

The investigations were carried out on the following species collected in North Africa and SE Spain (table 1): *Acarospora charidema* (Cl. ex Col.) Llim., *Heppia gigantea* Egea et Llim., *Heppia turgida* (Ach.) Nyl., *Peltula patellata* (Bagl.) Swinscow et Krog., *Ramalina bourgeana* Nyl., *Ramalina requienii* (De Not.) Jatta, *Roccella canariensis* Darb. em Vainio, *Roccella fuciformis* (L.) DC., *Roccella phycopsis* (Ach.) Ach., *Roccella vicentina* (Vainio) Vainio, *Solenopsora holophaea* (Mont.) Samp. and *Teloschistes lacunosus* (Rupr.) Sav., 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 until analysed for carotenoid content by column and thin-layer chromatography. Carotenoid pigments were extracted with 95% acetone in a dark room. Saponification was camed out with 10% KOH in ethanol at about 20°C for 24 h in the dark in a nitrogen atmosphere.

Column and thin-layer chromatography, described in detail in Czeczuga (1980b), 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). Pigrnents were ideniified on the basis of: (a) their behaviour on column chromatography; (b) their absorption spectra in various solvents (Beckman spectro-photometer 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 for lycopene-5, 6-epoxide (see VETTER *et al.*, 1971 for basic methodology). Concentrations of carotenoid solutions were determined from the absorption spectra, on the basis of the extinction coefficient E 1% cm^{-1} at wavelengths of maximal absorbance in petroleum ether or hexane (DAVIES, 1976).

Structure of carotenoids was given according as STRAUB (1971, 1987).

RESULTS

In the thalli of 12 lichen species from North Africa and SE Spain, the presence of 18 carotenoids was established (table 2, fig. 1). These were carotenoids previously observed in a large number of lichen species from other continents with the exception of lycopene-5, 6-epoxide which has, in these investigations, been noted for the first time in lichens. This carotenoid was determined in the *Peltula patellata, Ramalina bourgeana* and *Solenopsora holophaea* lichens. On the other hand, the carotenoids β-carotene, β-cryptoxanthin, β-carotene epoxide and lutein

TABLE 1. Investigated species of lichens from North Africa and SE Spain

Especies de líquenes estudiados de N. África y SE. España

SPECIES	LOCALITY
Acarospora charidema (Cl. ex Col.) Llim.	Spain-Almena: Tabemas; S.ª Alhamilla, altitude in 200 m.
Heppia gigantea Egea et Llim.	Spain-Almena: Tabemas; S.ª Alhamilla, altitude in 200 m.
Heppia turgida (Ach.) Nyl.	Morocco-Goulimine: Tizi Mighert, altitude in 1057 m.
Peltula patellata (Bagl.) Swinscow et Krog	Morocco-Tinerhir: Foum El Kous
Ramalina bourgeana Nyl.	Canarias-Tenerife: El Medano, altitude in 171 m.
Ramalina requienii (De Not.) Jatta	Tunisia-Tunis district
Roccella canariensis Darb. em Vainio	Morocco-Sidi Ifni
Roccella fuciformis (L.) DC.	Morocco-Bouzniza-Mohammedia: Pont Blondin, altitude in 20 m.
Roccella phycopsis (Ach.) Ach.	Algeria-Dellys: Bosque de Azazga, altitude in 40 m.
Roccella vicentina (Vainio) Vainio	Morocco-Tiznit: Tiouriza Plage
Solenopsora holophaea (Mont.) Samp.	Morocco-Anti Atlas: Subida al col. Kerdous
Teloschistes lacunosus (Rupr.) Sav.	Tunisia-Tunis distnct

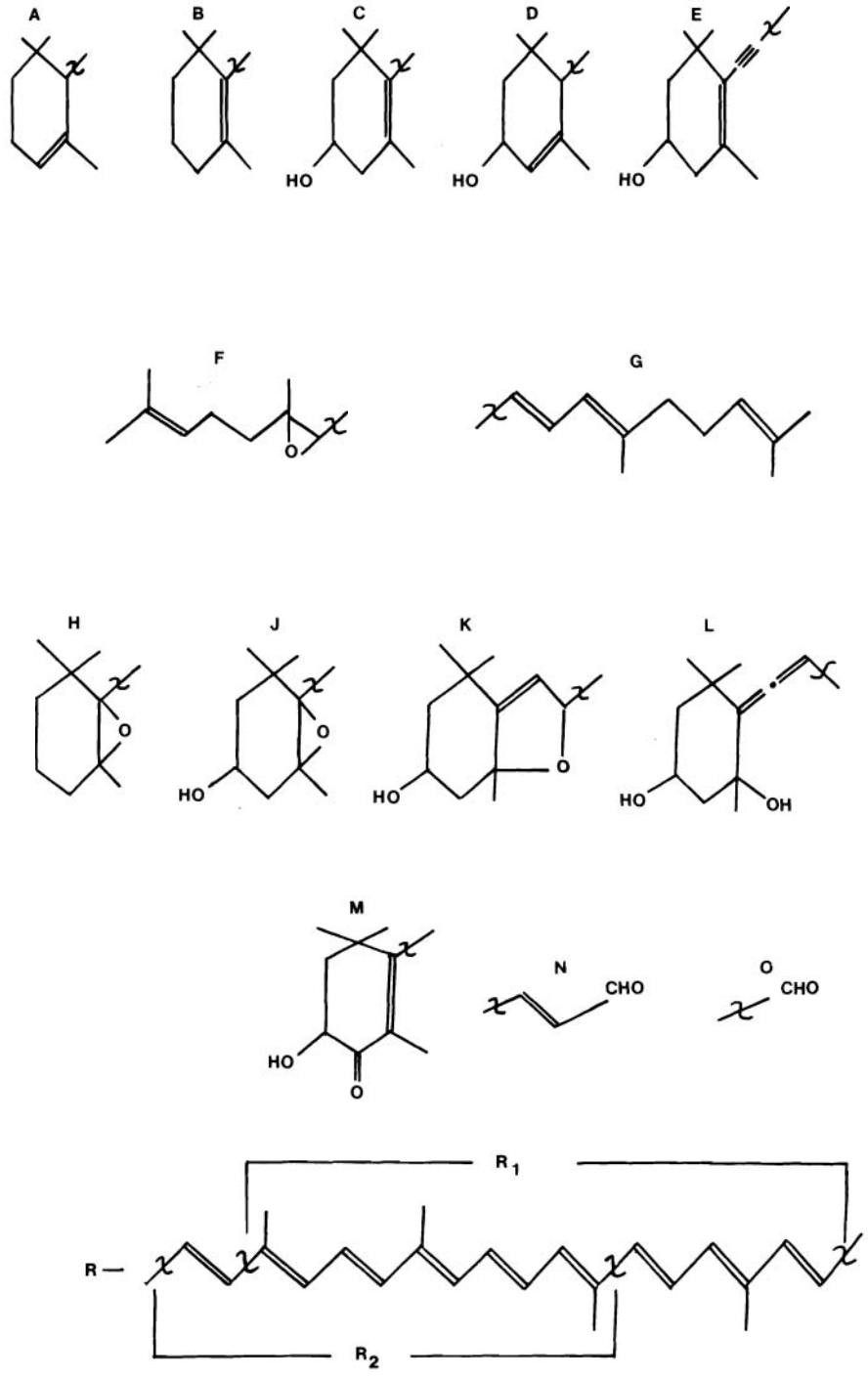

FIGURE 1. Structural features of carotenoids from investigated materials.

Características estructurales de los carotenoides de los materiales estudiados.

TABLE 2. List of the carotenoids found in lichens from North Africa and SE Spain

Lista de los carotenoides encontrados en líquenes del N. de África y SE España

CAROTENOID	STRUCTURE /SEE FIG. 1/	SEMI-SYSTEMATIC NAME
1. a-Carotene	A-R-B	β, ε-Carotene
2. β-Carotene	B-R-B	β, β-Carotene
3. a-Cryptoxanthin	A-R-C	β, ε-Caroten-3-ol
4. β-Cryptoxanthin	B-R-C	β, β-Caroten-3-ol
5. Lutein	C-R-D	β, ε-Carotene-3, 3'-diol
6. 3'-Epilutein	C-R-D	β, ε-Carotene-3, 3'-diol /stereoisomeric/
7. Zeaxanthin	C-R-C	β, β-Carotene-3-3'-diol
8. Diatoxanthin	C-R,-E	7, 8-Didehydro-β, β-carotene-3, 3'-diol
9. Lycopene-5, 6-epoxide	F-R-G	5, 6-Epoxy-5, 6-dihydro-ψ, ψ-carotene
10. β-Carotene epoxide	B-R-H	5, 6-Epoxy-5, 6-dihydro-β, β-carotene
11. Lutein epoxide	D-R-I	5, 6-Epoxy-5, 6-dihydro-β, ε-carotene-3, 3'-diol
12. Antheraxanthin	C-R-I	5, 6-Epoxy-5, 6-dihydro-β, β-carotene-3, 3'-diol
13. Violaxanthin	I-R-I	5, 6, 5', 6'-Diepoxy-5, 6, 5', 6'-tetrahydro-β, β-carotene-3, 3'-diol
14. Mutatoxanthin	C-R_1-K	5, 8-Epoxy-5, 8-dihydro-β, β-carotene-3, 3'-diol
15. Neoxanthin	I-R_1-L	5', 6'-Epoxy-6, 7-didehydro-5, 6, 5', 6'-tetrahydro-β, β-carotene-3, 5, 3'-triol
16. Astaxanthin	M-R-M	3, 3'-Dihydroxy-β, β-carotene-4, 4'-dione
17. β-Apo-10'-carotenal	B-R_2-N	10'-Apo-β-caroten-10'-al
18. Apo-12'-violaxanthal	I-R_2-O	5, 6-Epoxy-3-hydroxy-5, 6-dihydro-12'-apo-β-caroten-12'-al

TABLE 3. Carotenoid distribution in lichens from North Africa and SE Spain

Distribución de carotenoides en líquenes del N. de África y SE España

SPECIES	CAROTENOID DETECTED (SEE TABLE 2)	MAJOR CAROTENOID (%)	TOTAL CONTENT (μg g^{-1} DRY WEIGHT)
Acarospora charidema (Cl. ex Col.) Llim.	2, 4, 5, 7, 10, 11, 12, 13, 16	11 (48,7)	31,56
Heppia gigantea Egea et Llim	1, 2, 4, 8, 10, 11, 13, 14	11 (30,9)	27,20
Heppia turgida (Ach.) Nyl.	2, 4, 10, 11, 12, 13, 15, 17	11 (38,2)	32,12
Peltulapatellata (Bagl.) Swinscow et Krog	2, 4, 7, 9, 10, 11, 12, 14, 15, 18	18 (40,0)	45,01
Ramalina bourgeana Nyl.	1, 2, 4, 9, 10, 11, 14, 16, 18	11 (23,3)	17,20
Ramalina requienii (De Not.) Jatta	2, 4, 7, 10, 11, 12, 13, 15	13 (26,6)	39,50
Roccella canariensis Darb. em Vainio	2, 3, 4, 7, 10, 11, 13, 15	7 (33,8)	72,52
Roccella fuciformis (L.) DC.	2, 4, 10, 11, 12, 13, 14	11 (30,6)	33,62
Roccella phycopsis (Ach.) Ach.	2, 4, 7, 10, 11, 13, 14, 16	13 (26.1)	33,43
Roccella vicentina (Vainio) Vainio	2, 4, 10, 11, 12, 13, 15, 14	13 (26,6)	64,63
Solenopsora holophaea (Mont.) Samp.	1, 2, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15	14 (20,7)	39,05
Teloschistes lacunosus (Rupr.) Sav.	2, 4, 10, 11, 12, 13, 14, 15, 16	11 (50,4)	42,60

epoxide were found in the thalli of all the lichen species studied. The predominant carotenoids were: zeaxanthin (in 1 species), lutein epoxide (in 7 species), violaxanthin (in 2 species), mutatoxanthin (in 1 species) and apo-12'-violaxanthal (in 1 species). The total carotenoid content in the material studied ranged from 12,2 *(Ramalina bourgeana)* to 72,52 µg/g dry weight *(Roccella canariensis)* (table *3)*.

DISCUSSION

As was mentioned above, lycopene-5, 6-epoxide is a carotenoid new to lichens. This carotenoid has to date been found in ripe fruit of higher plants (GOODWIN, 1980) and in some fungus species (CZECZUGA, 1978, 1979, 1980a). Probably the presence of lycopene-5, 6-epoxide in the thalli of *Peltula patellata, Ramalina bourgeana* and *Solenopsora holophaea* is related to the presence of this carotenoid in the fungus component of these lichens.

On comparing the results of studies of such genera as *Heppia, Ramalina* and *Roccella* from North Africa. It is seen that a number of carotenoids are characteristic of species belonging to these genera. The carotenoids common to species of the *Heppia* genus were found to be p-carotene, β-cryptoxanthin, p-carotene epoxide, lutein epoxide and violaxanthin. These same carotenoids were also found in all four species of the *Roccella* genus studied. As regards the aspecies of the *Ramalina* genus, the common carotenoids were p-carotene, β-cryptoxanthin, p-carotene epoxide and lutein epoxide.

Some species of the genera studied have previously been studied from other latitudes. These are the thalli of *Roccela arboricola* from the Argentine (CZECZUGA & FERRARO DE CORONA, 1987), *Rocella hypomecha* from South Africa (CZECZUGA *et al.* 1988) and *Teloschistes exilis* from the Argentine and *Teloschistes chrysophthalmus* also from South Africa. Of the group of carotenoids common to the species of the *Roccella* genus from North Africa, the species of *Roccella arboricola* from the Argentine contained only β-carotene and lutein epoxide and the species of the *Roccella hypomecha* from South Africa contained p-carotene, lutein epoxide and violaxanthin.

The carotenoids common to all three species of the *Teloschistes* genus from the three different latitudes were found to be astaxanthin, lutein epoxide and violaxanthin. In addition, it is worthy of note that the predominant carotenoids in most of the species studied from North Africa belonged to the epoxide group, such as lutein epoxide, violaxanthin and mutatoxanthin.

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