SYNTHESIS OF SECONDARY COMPOUNDS AS A UV-SCREENING STRATEGY OF LICHENIZED FUNGI FROM THE TROPICAL ANDES AND ITS POSSIBLE ROLE ON THE EARLY EARTH

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ABSTRACT

Photochemical analysis of secondary compounds in lichens from the Venezuelan Andean snow and glacier zones (4800-5000 m) was carried out in order to determine the absorbance capacity of UV radiation in the UVA, UVB and UVC ranges, and to characterize the probable UV-protective function. Spectrotometric (UV-VIS, NIR, FTIR, MS, NMR) and chromatographic (HPTLC) standardized techniques were utilized to identify the lichen compounds. UVB radiation in the glacier zone (5000 m) revealed a value of ~ 3 W m^{-2} which is sufficient to produce important biochemical and cell alterations. Of a total of 25 lichen species distributed in the glacier and snow zones, 68% showed the presence of phenolic compounds having strong absorption for UVC radiation, 96% had strong absorption for UVB radiation and 100% had strong absorption for UVA radiation. The substance groups that had the highest resistance to UVA and UVB radiation were characterized by ester bonds among both phenolic units (depsides). They were the most abundant products to be found among the lichens, whereas substances having ester and ether bonds in both phenolic units (depsidones) had a higher capacity to absorb UVC radiation. Microorganisms having adaptive UV-screening responses similar to the lichens investigated are expected to occur on the early Earth in O_2 levels $\leq 10^{-2}$.

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KEY WORDS

Venezuelan Andes, Snow and Glacier Zones, Cryoextremophile Lichens, UV radiation, UV-Screening Compounds.

SÍNTESIS DE COMPUESTOS SECUNDARIOS COMO UNA ESTRATEGIA DE PANTALLA CONTRA RADIACIÓN ULTRAVIOLETA EN HONGOS LIQUENIZADOS DE LOS ANDES TROPICALES Y SU POSIBLE ROL EN LA EDAD TEMPRANA DE LA TIERRA

COMPENDIO

Se realizó un análisis fotoquímico de compuestos secundarios presentes en líquenes procedentes de las zonas nival y glaciar de los Andes Venezolanos con la finalidad de determinar la capacidad de absorbancia de radiación UV en los rangos UVA, UVB y UVC y caracterizar de esta manera la probable función UV-protectora. Técnicas normalizadas espectrométricas (UV-VIS, NIR, FTIR, MS, NMR) y cromatográficas (HPTLC) se utilizaron para identificar los compuesto liquénicos. La UVB radiación en la zona glaciar (5000 m) mostró un valor de ~ 3 W m^{-2} lo cual es suficiente para producir importantes alteraciones celulares y bioquímicas. De un total de 25 especies de líquenes distribuidas en las zonas nival y glaciar, el 68% reveló la presencia de compuestos fenólicos presentando una fuerte absorción para la radiación UVC; un 96% presentó una fuerte absorción para la radiación UVB, mientras el 100% mostró una fuerte absorción para la radiación UVA. Los grupos de sustancias que presentaron la más alta resistencia para las radiaciones UVA y UVB estuvieron caracterizados por enlaces ester entre ambas unidades fenólicas (dépsidos). Estos productos fueron los más abundantes hallados en los líquenes, mientras sustancias presentando enlaces ester y éter entre ambas unidades fenólicas (depsidonas) mostraron la más alta capacidad para absorber la radiación UVC. Microorganismos presentando respuestas adaptativas para protegerse de la radiación UV, de manera similar a los líquenes investigados, pudieron haber estado presentes en la tierra primitiva cuando los niveles de oxígeno fueron $\leq 10^{-2}$.

PALABRAS CLAVE

Andes Venezolanos, Zonas Nival y Glaciar, Líquenes Crioextremofilicos, Radiación UV, Compuestos UV-protector.

INTRODUCTION

In the early earth, organisms exposed to intense UV radiation at surface habitats could have synthesized UV screening and energy quenching compounds in order to avoid damages in the biochemical machinery (Vishniac 1996, Garcia-Pichel 1998, Garcia-Pichel and Castenholz 1991, Quesada and Vincent 1997, Cockell and Knowland 1999, Wynn-Williams and Edwards 2000, Cockell and Horneck 2001, Wynn-Williams *et al*. 2002, Onofri *et al*. 2003, De Vera *et al*. 2003, Marcano *et al*. 2001, 2002a, 2002b, 2006).

The development of UV-screening strategies in heterotrophic-photosynthetic organisms similar to lichens could have allowed their occurrence in surface habitats before 1 Gyr ago when the ozone shield was not fully formed. Although the oldest certain fossil lichen is Early Devonian (Taylor *et al*. 1995), there is strong evidence for the occurrence of lichen-like associations from Witwatersrand, South Africa, dated between 2.2 and 2.7 billion years old (Hallbauer and van Warmelo 1974). Lichens are able to synthesize secondary compounds by mevalonic acid, acetate-polymalonate, and shikimic pathways. These compounds are generally extracellular and concentrate in the external tissues of lichens (e.g. cortical layer). Likewise, these compounds are found in the internal tissues (e.g. medulla). The most common fate of acetate-polymalonate-derived phenolic acids is intermolecular esterification of two or three similar units. For instance, the carboxylic acid of one unit is joined to the hydroxyl *para* to the carboxylic acid of the second unit. Such esterifications lead to the *para*-depsides. If an ester linkage joins the first unit to a position *meta* to the carboxylic acid of the second ring, a *meta*-depside results (Culberson 1969). The compounds synthesized by the mevalonic acid and acetate-polymalonate pathways, such as phenolic carboxylic acid derivatives (e.g. *para*- and *meta*-depsides, and depsidones), xanthones, dibenzofurane derivatives (e.g. usnic acid) (Marcano *et al*. 1999), and anthraquinones, show absorbances ranging between 200 and 400 nm that offer protection to the lichen before the UV radiation could cause a lethal effect at the molecular level (Harborne 1968, Rundel 1978, Solhaug and Gauslaa 1996, Bacherau and Asta 1997, Wynn-Williams and Edwards 2000, Wynn-Williams *et al*. 1999, 2002, Bjerke *et al*.

2002). For instance, the high absorbance between 280-320 nm exhibited by these compounds suggests a protective effect for aromatic amino acids, proteins, purines, pyrimidines, or nucleic acids, of the UVB (280-315 nm) radiation fluxes. UVA (315-400 nm) radiation is less damaging than UVC (200-280 nm), but it can mediate photooxidative damage through reactive oxygen species such as H_2O_2 within cells (Jagger 1985, Mancinelli and White 2000). Although these pigments can also absorb UVC radiation (Harborne 1968, Towers 1968), such function is irrelevant today because the atmosphere of modern Earth screens out all UVC before it reaches the biosphere. However, this capability would have been vital on early Earth.

Our interest with this paper is to determine the capacity of the secondary compounds synthesized by lichens occurring in the Tropical Andean glacier and snow zones to absorb UV radiation in the UVA, UVB, and UVC wavelength ranges, and therefore, to infer its possible UV-protective function to the lethal effects of this radiation on the cell components and functions of the lichen. Thus, it is expected that the results obtained in this work may contribute to a better understanding of the characteristics and limits of the adaptations exhibited by the organisms in the tropical high mountain regions, and thereby may increase the knowledge about adaptative responses developed by microorganisms in ages when the ozone shield would have not fully screened UV radiation (Cockell 2000a, 2000b 2002). Likewise, the study of the UV-screening role of the lichen compounds may constitute an important contribution in the biology of terrestrial environments exposed to intense UV radiation.

MATERIALES Y MÉTODOS

STUDY AREA

The study area was located at the Venezuelan Andean glacier and snow zones, forming part of the Sierra Nevada de Mérida National Park (8°32.5' N, 71°035' W), and comprises the Pico Espejo ($\geq$ 4765 m, periglacier desert) and Pico Bolívar (~ 5000 m, glacier desert) sectors (Monasterio and Reyes 1980, Diaz *et al*. 1997). Both glacier and snow zones show mean annual temperatures $\leq$ 2 °C, wide daily surface thermal oscillations ($\geq$ 20 ºC), daily cycles of freezing and thawing, intense UVB, UVA, and PAR (400-700 nm) radiation, and low O_2 (120 ~ mbars), N_2 (410 ~ mbars), and CO_2 (~ 16 x 10^{-2} mbars) partial pressures. Mean values of atmospheric pressure in the glacier and snow zones were near 50% sea level (~ 561 mbar; sea level, 1014 mbar) (Marcano *et al*. 2003). An increment of the mean soil temperature (> 3 ºC) with the increment of depth

(10, 20 and 40 cm) may allow microbian activity concerning nitrogen fixation (Azócar and Monasterio 1980, Diaz *et al*. 1997, Marcano *et al*. 2003). Because of the extreme climatic conditions occurring in the glacier and snow zones, the dominant species were some grasses having anthocyaninic pigments (***Agrostis***, ***Calamagrostis***), mosses, epilithic and endolithic microlichens, cyanobacteria (***Oscillatoria***, ***Nostoc)***, chromobacteria, and diazotroph and heterotrophic soil microorganisms (Marcano and Morales 1994a, 1995, Marcano *et al.* 1996, 1997).

High UV and PAR radiation, and low temperatures in the glacier and snow zones determine the existence of lichens having specialized adaptations in order to avoid enzymatic inactivation and possible damages in membranes and biomolecules (Jagger 1985, Mancinelli and White 2000, Rothschild and Mancinelli 2002). Although such adaptations are not well known in the glacier and snow zones, it is expected that microorganisms exhibit the following features:

1. Production of UV-screening secondary metabolites such as polyketides, xanthones, anthraquinones, and usnic acids (Quesada and Vincent 1997, Garcia-Pichel 1998, Wynn-Williams *et al*. 1999, 2002).
2. Capacity to colonize cracks in rock formations as a permanent habitat, and in subsoil in order to resist freezing (Friedmann 1982).
3. Synthesis of antifreeze substances such as proteins, sugars and phenolic acids (Kappen 1973, Morita 1975, Rothschild and Mancinelli 2002).
4. Production of higher amounts of carotenoids, in comparison to the amounts of chlorophyll (chl *a*) in photobionts, in order to compensate the oxidizing effects of UV radiation (Cockell and Knowland 1999, George *et al*. 2001).

CLIMATIC MEASUREMENTS

Climatic data were obtained from several climatic stations placed within the Pico Espejo and Pico Bolívar sectors. These climatic stations belong to the Bioclimatic Scientific Program of the Sierra Nevada de Mérida National Park, which is supported by the University of the Andes, NASA Ames Research Center, USA, and Institute of Nuclear Sciences of the UNAM, México. Thermal sensors (mod. HOBO H8 4-channel logger, Onset Computer Corporation) provided with 4 thermocouples (TMC6-HA), were placed beneath the soil surface at 10, 20 and 40 cm. One thermocouple was placed on the soil surface. Humidity and precipitation data were registered at 2 m above the surface utilizing HOBO loggers. The error in the temperature measurement is ± 0.5 ºC, whereas in the relative humidity measurement the error is ± 3%. Recorded data were

retrieved by a field computer using BOXCAR 3.6. The stations were emplaced on 15 March 2001 and they are operating continuously. The complete dataset as well as climate data from other years are available at the website: http://www.ing.ula.ve/~cme/red. UVB and UVA radiation records were obtained utilizing Thies UV sensors (data loggers). On the other hand, values about the altitudinal distribution of the UVB and UVA radiation at the Andean tropical high mountain were also modeled for average conditions of total ozone O_3 (D)= 260 (7º N), solar zenith angle ϭ = 30º, and cloudiness (F = 0.6), according to Feister (1994), Piazena (1996) and Dvorkin and Steinberger (1999).

Statistical analyses

Plot analysis of the climatic and chemical data was carried out using MATLAB 5.3. Parametric statistical tests were conducted using Graphpad Instat 2.4a and SPSS 7.5.

CHEMICAL ANALYSIS OF PIGMENTS

Separation of lichen compounds was carried out utilizing HPTLC or nano-TLC standardized methods (Culberson 1972, Culberson and Johnson 1976; Culberson *et al*. 1981, White and James 1985, Arup *et al*. 1993). In a first HPTLC analysis, acetone extracts of the lichen thalli were separated on silica gel Merck 60 F_{254} (10 x 10 cm) plates that had been cleaned prior to use with repetitive solvent elutions, and were activated at 120°C for 2 hours. After application of the extract and elution standards, the HPTLC plates were developed with a mixture of toluene/dioxane/acetic acid (180:45:5), and toluene/acetic acid (170:30). The dried plates were sprayed with H_2SO_4 10 % v/v and then exposed to UV (ë 354 nm) light to display the bands. Rf values were analyzed using blanks and the Wintabolites 3.2 software (Mietzch *et al*. 1994). On the other hand, a second HPTLC analysis was carried out on plates not sprayed with H_2SO_4. This analysis was made by running large samples as bands that were detected with the help of a short wave UV-lamp, cut from the chromatogram, scraped off, eluted with methanol, and analyzed by UV spectrophotometry at 322 and 212 nm against blanks. Lichen compounds utilized as blanks were characterized previously by Fourier transform infrared (FTIR), mass (MS), and nuclear magnetic resonance (RMN) spectroscopy (Morales and Marcano 1992, Marcano and Morales 1994b, Marcano *et al*. 1999).

Additionally, a collection of spectra at the 200-400 nm region corresponding to lichen methanolic and ethanolic extracts were obtained in order to infer the

absorbance capacity in the UV-spectrum region of the lichen compounds. Likewise, molar extinction coefficients (ε), of the extracted and separated lichen compounds, were determined for each wavelength of the full UV spectrum. Ultraviolet spectra of the lichen compounds were obtained utilizing a Shimadzu spectrophotometer.

MICROSCOPICAL ANALYSIS

The cell morphology was evaluated by fluorescence microscopy (FM). Particularly, this technique allowed the location in the lichen tissues (e.g. epicortex, cortex or medulla) of the synthesized metabolites, due to the color of the fluorescence response emitted by such substances (Kauppi and Verseghy-Patay 1990). Specimens were examined in a Zeiss fluorescence microscope.

RESULTS

Altitudinal distribution of effective UVB radiation (W m^{-2}) at the Andean tropical high mountain revealed an increase estimated at ~ 18 mW m^{-2} per 100 m, corresponding to ~ 3 W m^{-2} in the region located in the glacier and snow zones (Fig. 1). On the other hand, the altitudinal distribution of the UV effective global radiation (J m^{-2})$_{plr}$, producing responses in plants from the Andean tropical high mountain, showed an increment of ~ 150 J m^{-2} per 100 m, and a value of ~ 2.25 kJ m^{-2} for the region located in the glacier and snow zones (Fig. 2).

A total of 25 lichen species were registered in the glacier and nival zones (Table I). The more abundant and frequent species were ***Candelariella vitellina*** Hoffm. Müll. Arg., ***Rhizocarpon geographicum*** (L.) DC., ***Stereocaulon strictum*** Th. Fr., ***Tephromela atra*** (Huds.) Hafelln., ***Umbilicaria polyrrhiza*** (L.) Fr., ***U. polyphylla*** (L.) Baumg, ***Xanthoparmelia conspersa*** (Ach.) Hale**,** and ***Xanthoria elegans*** (Link) Th. Fr. Particularly, ***Xanthoria elegans*** has been reported previously in the Antarctic revealing the presence of the anthraquinone parietin as an UV-screening compound (Solhaug and Gauslaa 1996, Wynn-Williams and Edwards 2000, Wynn-Williams *et al*. 1999, 2002, Edwards 2004). Likewise, ***Rhizocarpon geographicum***, having rhizocarpic (product from the shikimic acid pathway) and barbatic acids (β-orcinol *para*-depside), and ***Tephromela atra***, containing atranorin (β-orcinol *para*-depside), have been reported for the Antarctic continent (Olech 2001, Østedal and Lewis-Smith 2001). However, several other lichen genera and species occurring in the Andean glacier and nival zones are also known from the Antarctic (Friedmann

1982, Kappen 1993, Wynn-Williams *et al*. 1999, Østedal and Lewis-Smith 2001). That continent exhibits large ozone changes during the year and, thereby, increases of UVB radiation (Madronich *et al*. 1996). Spectrophotometric analysis of the total extracts revealed that 32% of the species found in the glacier and snow zones showed vulnerability to biochemical damages produced by UVC radiation, 4% were vulnerable to such damages at the UVB region, and no species showed evidences of vulnerability at the UVA region (Table II). On the other hand, 68% of the species showed a potential resistance to effects of biochemical damages of UVC radiation, 96% to UVB, and 100% to UVA radiation damage effects (Table III).

Particularly, analyses of the absorption spectra corresponding to total extracts revealed that ***Lecanora*** sp. shows a total vulnerability to the damaginge effects of UVC radiation, indicated by the low absorbance in the 220 and 280 nm wavelengths. Likewise, ***Candellariela*** sp., ***Umbilicaria polyphylla*** and ***U. polyrrhiza*** showed a remarkable vulnerability at the UVC region (Fig. 3). On the other hand, ***Hypotrachyna*** sp., ***Candelariella vitellina*** and ***Dictyonema zahlbrucknerii*** (Schiffn.) V. Marcano exhibited higher potential resistance to the damage effects of the UVB and UVA radiation (Fig. 4). ***Pertusaria pertusa*** Dibb., ***Rhizocarpon geographicum***, ***Stereocaulon strictum*** and ***Tephromela atra*** also showed a high absorbance at the UVB and UVA spectrum range. In ***D. zahlbrucknerii*** lichen compounds were not detected but, due to the occurrence of cyanobacterial symbionts, it is thought that their high absorbance at the UVC region could be a consequence of the presence of cyanobacterial pigments, such as scytonemin and mycosporine-like amino acid derivatives (Garcia-Pichel and Castenholz 1991, Budel *et al*. 1997, Wynn-Williams *et al*. 1999). Analysis by fluorescence microscopy and HPTLC revealed the frequent occurrence of β-orcinol depsidones, and orcinol and β-orcinol depsides, in the medullar tissue of lichen, whereas dibenzofurane derivatives and atranorin (β-orcinol *para*-depside) were located in the cortical tissue.

Analyses of the lichen compounds separated by HPTLC, utilizing blanks identified by FTIR, MS and NMR spectrometry, revealed the presence of 11 major compounds. Comparisons of the molar extinction coefficients (ε) of lichen compounds showed that the β-orcinol depsidones have higher absorbance at the UVC region, whereas the orcinol and β-orcinol depsides (*para*- or *meta*) exhibited lower absorbance (Fig. 5). At the UVB region the depsides exhibited higher absorbance (Fig. 6), whereas at the UVA region thamnolic acid (β-orcinol *meta*-depside) and usnic acid (dibenzofurane derivative) showed higher absorbance in comparison to the depsidones and other depsides that exhibited lower absorbance (Fig. 7). At the biochemically important wavelengths (220,

260 and 280 nm), usnic, didimic (dibenzofurane derivatives), sequicaic (orcinol *meta*-depside), thamnolic (β-orcinol *meta*-depside), and lecanoric acids (orcinol *para*-depside) showed lower e values, whereas salazinic, protocetraric, and stictic acids (β-orcinol depsidones) exhibited higher e values at the 220 and 260 nm wavelengths, and barbatic acid (β-orcinol *para*-depside) at 280 nm (Table IV). At the full UV spectrum, the lichen compounds showed higher absorbance only at 211, 306 and 324 nm (Table V).

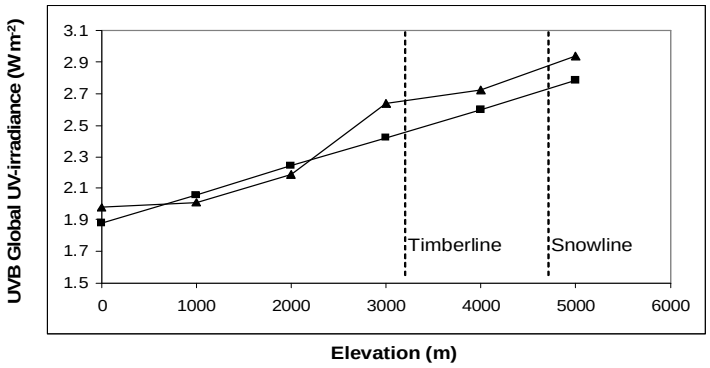

Fig. 1. Altitudinal distribution of effective UVB (280-320 nm) radiation reaching the ground at the Sierra Nevada de Mérida, Venezuelan Andes. A. Mean values obtained *in situ* using UV sensors (▲). B. Modeled for average conditions of total ozone O_3 (D) = 260 (7º N), solar zenith angle è = 30º, and cloudiness (■), according to Feister (1994), Piazena (1996) and Dvorkin and Steinberger (1999).

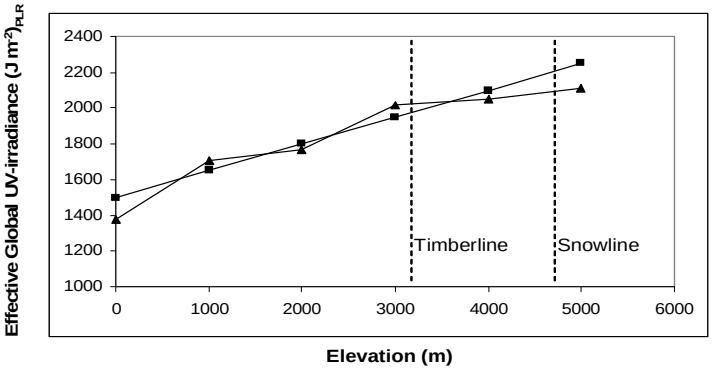

Fig. 2. Altitudinal distribution of effective global UV-radiation producing responses in plants from the Sierra Nevada de Mérida, Venezuelan Andes. A. Mean values obtained *in situ* using UV sensors (▲). B. Modeled for average conditions of total ozone O_3 (D) = 260 (7º N), solar zenith angle è = 30º, and cloudiness (■), according to Feister (1994), Piazena (1996) and Dvorkin and Steinberger (1999).

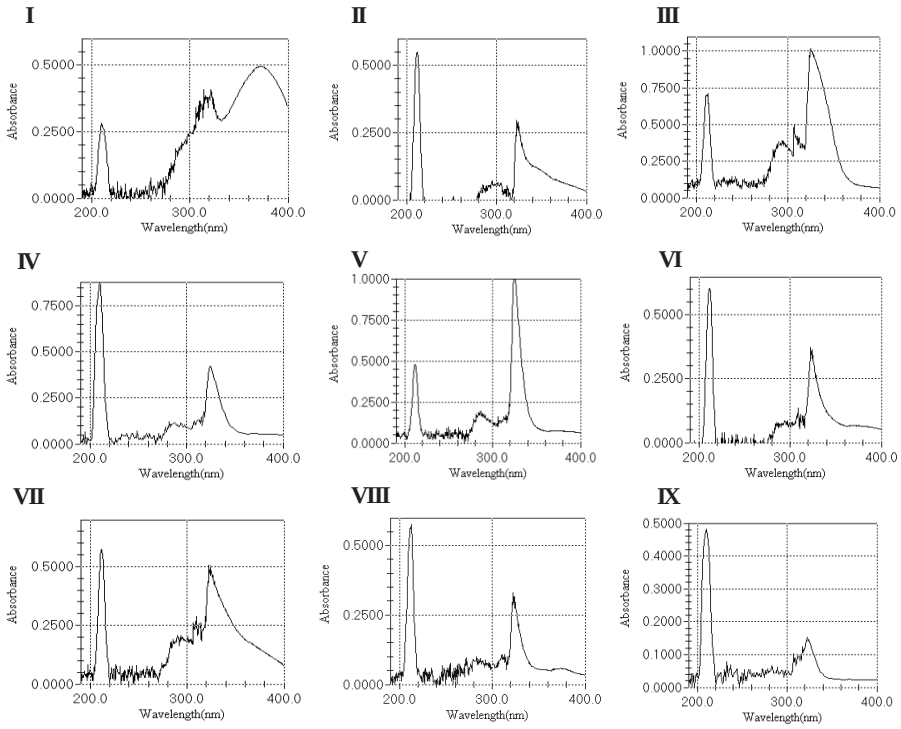

Fig. 3. Absorption spectra of total extracts corresponding to lichens, vulnerable potentially to UVC and UVB radiation from the glacier and snow zones of the Sierra Nevada de Mérida National Park, Venezuelan Andes. I, ***Candelariella*** sp. II, ***Lecanora*** sp. III, ***Leprocaulon congestum***. IV, ***Physcia*** sp. V, ***Umbilicaria polyphylla***. VI, ***Verrucaria*** sp. VII, ***Xanthoparmelia conspersa***. VIII, ***Umbilicaria polyrrhiza*** and IX, ***Protoblastemia*** sp.

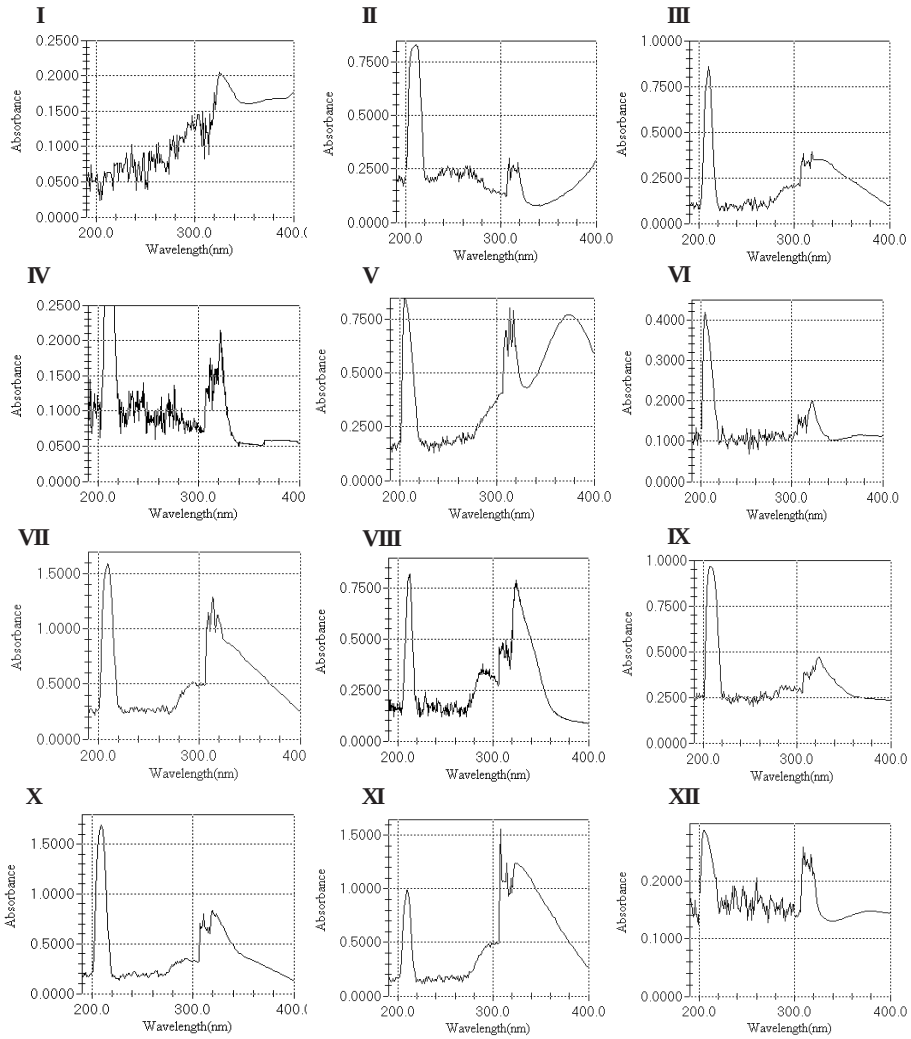

Fig. 4. Absorption spectra of total extracts corresponding to lichens, resistant potentially to UVC, UVB and UVA radiation from Pico Espejo and Pico Bolívar, Venezuelan Andes. I, ***Hypotrachyna*** sp. II, ***Xanthoria elegans***. III, Lecidea sp. IV, ***Lecidella*** sp. V, ***Candelariella vitellina***. VI, ***Peltigera*** sp. VII, ***Siphula fastigiata***. VIII, ***Stereocaulon strictum***. IX, ***Tephromela atra***. X, ***Alectoria ochroleuca***. XI, ***Cladonia coccifera*** and XII, ***Dictyonema zahlbrucknerii***.

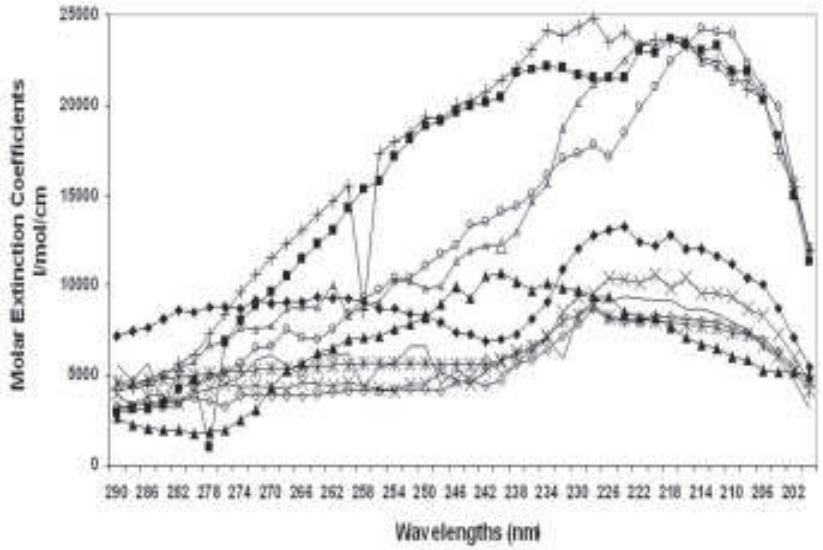

Fig. 5. Molar extinction coefficients (ε) at the UVC region estimated for secondary metabolites separated from lichen species from the glacier and snow zones of the Sierra Nevada de Mérida, Venezuelan Andes. ◇ usnic acid, △ stictic acid, - sequicaic acid, + salazinic acid, ■ protocetraric acid, ∗ lecanoric acid, O fumarprotocetraric acid, ▲didimic acid, ◆ barbatic acid, and ×atranorin.

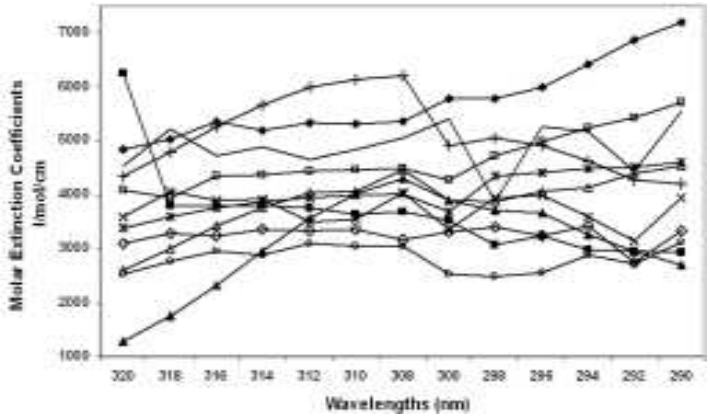

Fig. 6. Molar extinction coefficients (ε) at the UVB region estimated for secondary metabolites separated from lichen species of the glacier and snow zones of the Sierra Nevada de Mérida, Venezuelan Andes. ◇ usnic acid, □ thamnolic acid, △ stictic acid, – sequicaic acid, + salazinic acid, ■ protocetraric acid, ∗ lecanoric acid, O fumarprotocetraric acid, ▲ didimic acid, ◆ barbatic acid, and × atranorin.

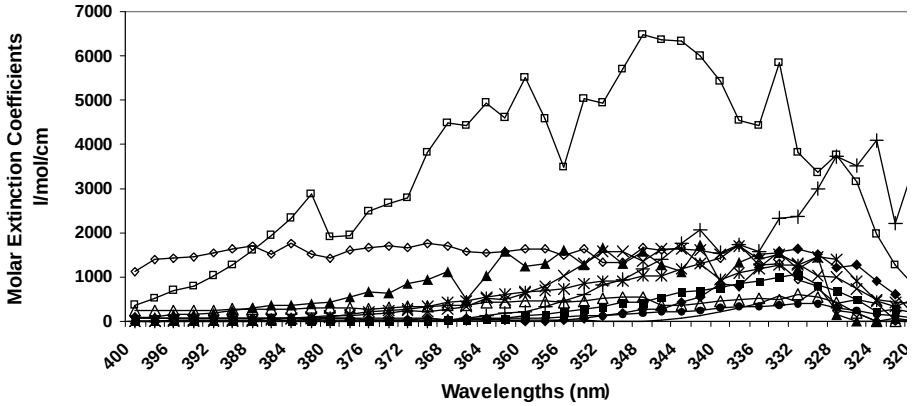

Fig. 7. Molar extinction coefficients (ε) at the UVA region estimated for secondary metabolites separated from lichen species of the glacier and snow zones of the Sierra Nevada de Mérida, Venezuelan Andes. ◇ usnic acid, □ thamnolic acid, △ stictic acid, – sequicaic acid, + salazinic acid, ■ protocetraric acid, ∗ lecanoric acid, O fumarprotocetraric acid, ▲ didimic acid, ◆ barbatic acid, and × atranorin.

Table I. Lichen species collected in the glacier and snow zones of the Sierra Nevada de Mérida National Park, Pico Bolívar and Pico Espejo sectors (4800-5000 msnm), Venezuelan Andes.

Alectoria ochroleuca	***Physcia*** sp.
Arthrorhapis citrinella	***Protoblastemia*** sp.
Candellariella vitellina	***Rhizocarpon geographicum***
Candellariella sp.	***Siphula fastigiata***
Cladonia coccifera	***Stereocaulon strictum***
Dictyonema zahlbrucknerii	***Tephromela aglaea***
Hypotrachyna sp.	***Tephromela atra***
Lecanora sp.	***Umbilicaria polyphylla***
Lecidea sp.	***Umbilicaria polyrrhiza***
Lecidella sp.	***Verrucaria*** sp.
Leprocaulon congestum	***Xanthoparmelia conspersa***
Peltigera sp.	***Xanthoria elegans***
Pertusaria pertusa	

Table II. Lichen species, potentially* vulnerable to damaginge effects of UV radiation, from the glacier and snow zones of the Sierra Nevada de Mérida National Park, Pico Bolívar and Pico Espejo sectors (4800-5000 msnm), Venezuelan Andes.

Lichen Species	Ultraviolet radiation		
	UVC	UVB	UVA
Candellariella sp.	++		
Lecanora sp.	+++	+	
Leprocaulon congestum	+		
Physcia sp.	++		
Umbilicaria polyphylla	+		
Umbilicaria polyrrhiza	+		
Verrucaria sp.	+++		
Xanthoparmelia conspersa	+		
Number of species	8 (32%)	1 (4%)	0 (0%)

* Qualitative estimation of the vulnerability to the damage effects of UV radiation based on molar extinction coefficients and absorbance values at the UV spectrum region for total extracts of lichen compounds (+ low vulnerability, ++ median vulnerability and +++ high vulnerability).

Table III. Lichen species potentially* resistant to damaginge effects of UV radiation, from the glacier and snow zones of the Sierra Nevada de Mérida National Park, Pico Bolívar and Pico Espejo sectors (4800-5000 msnm), Venezuelan Andes.

Lichen Species	Ultraviolet radiation		
	UVC	UVB	UVA
Alectoria ochroleuca	* *	* * *	* * *
Arthrorhapis citrinella	*	* * *	* * *
Candellariella sp.		* * *	**
Candellariella vitellina		* * *	* * *
Cladonia coccifera	*	* * *	* * *
Dictyonema zahlbrucknerii	* * *	* * *	* * *

Table III. Continuación.

Lichen Species	Ultraviolet radiation		
	UVC	UVB	UVA
Hypotrachyna sp.	* * *	* * *	* * *
Lecanora sp.			* *
Lecidea sp.	*	* *	* * *
Lecidella sp.	* * *	* * *	* * *
Leprocaulon congestum		* * *	* * *
Peltigera sp.	* * *	* * *	* * *
Pertusaria pertusa	*	* * *	* * *
Physcia sp.		* * *	* *
Protoblastemia sp.	*	* * *	*
Rhizocarpon geographicum	*	* * *	* * *
Siphula fastigiata	* *	* * *	* * *
Stereocaulon strictum	* * *	* * *	* * *
Tephromela aglaea	* * *	* * *	* * *
Tephromela atra	* *	* * *	* *
Umbillicaria polyphylla		* *	* * *
Umbillicaria polyrrhiza		* *	* *
Verrucaria sp.		* * *	* * *
Xanthoparmelia conspersa		* * *	* * *
Xanthoria elegans	* * *	* * *	* * *
Number of species	17 (68%)	24 (96%)	25 (100%)

* Qualitative estimation of the vulnerability to the damaginge effects of UV radiation based on molar extinction coefficients and absorbance values at the UV spectrum region for total extracts of lichen compounds (+ low resistance, ++ median resistance and +++ high resistance).

Table IV. Molar extinction coefficients (ε)* of lichen substances (depsides, depsidones and dibenzofurane derivatives) occurring in lichens from the glacier and snow zones of Pico Espejo and Pico Bolívar, Venezuelan Andes, for biochemically important wavelengths.

Lichen compounds	ε 220	ε 260	ε 280
Depsides			
Atranorin	10569.6	4624.6	4032.7
Barbatic acid	12252.7	9271.3	8512.8
Lecanoric acid	8026.3	5625.7	5026.1
Sequicaic acid	9203.8	6267.1	4356.1
Thamnolic acid	8746.5	6696.4	5998.1
Depsidones			
Fumarprotocetraric acid	20986.2	8382.7	4354.1
Protocetraric acid	22935.3	14299.7	4758.9
Salazinic acid	23659.4	15549.1	6133.6
Stictic acid	23326.1	8332.1	5816.3
Dibenzofurane derivatives			
Didimic acid	8254.4	7009.9	776.0
Usnic acid	8300.3	4140.3	3744.7

* $l\ mol^{-1}\ cm^{-1}$

Table V. Maximum absorbance peaks of the UV spectrum region corresponding to secondary metabolites (depsides, depsidones, and dibenzofurane derivatives) occurring in lichens from the glacier and snow zones of Pico Espejo and Pico Bolívar, Sierra Nevada de Mérida National Park, Venezuelan Andes.

Lichen compounds	UV λ max	UVC λ max (200-280 nm)	UVB λ max (290-315 nm)	UVA λ max (320-415 nm)
Depsides				
Atranorin	324.5	212	308	324.5
Barbatic acid	325.5	212	296	325.5
Lecanoric acid	325	210	308	325
Sequicaic acid	322.5	212	307.6	322.5
Thamnolic acid	324.5	211	306.6	324.5
Depsidones				
Fumarpro-tocetraric acid	324.5	212.5	300	324.5
Protocetraric acid	212	212	307.5	322.5
Salazinic acid	211.5	211.5	315	324.5
Stictic acid	211.5	211.5	307	322.5
Dibenzofurane derivatives				
Didimic acid	324.5	280	309.3	324.5
Usnic acid	321	212	312	321

DISCUSSION

Both effective UVB radiation and effective global UV radiation, producing responses in plants, revealed values ~ 1.6 times higher in the glacier and snow zones in comparison to that registered at sea level. This intensity would be sufficient to produce important biochemical and cell alterations (Jagger 1985, Feister 1994). Because the Sierra Nevada de Mérida is located in the tropical region (8° 32' N), it is expected that the effective UV radiation reaching the ground would be two and four times higher during all the year than at latitudes from subtropical regions (Caldwell *et al*. 1980, Feister 1994). UVB fluxes reaching the ground in the glacier and snow zones were similar to those UVB fluxes reaching the ground estimated for the Mid-Proterozoic atmosphere containing ~ 10^{-2} PAL of O_2 (Segura *et al*. 2003).

On the Earth's surface, only 4% of the UVB and 96% of the UVA radiation would penetrate. Although the DNA absorption spectrum shows major absorbance located at 260 nm, an important absorption is also observed at the UVB region. UVB irradiance at the Andean high mountain could be sufficient to generate damages in the lipoproteins of cell membranes and organelles, and deleterious mutations in DNA, viz. thymine dimers (Jagger 1985, Cockell and Knowland 1999), if organisms have no efficient UV-screening strategies. Amino acids, such as tryptophan, tyrosine, and cystine, also have an important absorbance at the UVC and UVB regions (Jagger 1985). Thus, proteins constituted by aromatic residues or having disulphur bonds could be susceptible to structural and functional changes when exposed to intense UV radiation in tropical mountain environments above 4000 m (Caldwell *et al*. 1980).

However, lichen species exposed to high UV radiation at this tropical high mountain have UV-screening strategies based on the synthesis of phenolic secondary metabolites, generated probably during millions years of evolution. According to Cockell (1998) and Cockell and Knowland (1999), all the lichen-substance groups studied would have showed absorption maxima at the UV region depending upon the presence of conjugate structures having π–electron systems, and causing energetic transitions of π-electrons to anti-bonding π^*-electron orbitals, when they are exposed to UV radiation. The remarkable capacity of absorbance at the UVB and UVA regions that the lichen compounds from the Andean glacier and snow zones have, and further, the high frequency of occurrence of these substances in the lichens studied (96%), suggest an adequate adaptation to such an environment. On the other hand, the fact that only 68% of the species are resistant to the UVC radiation would be expected, because the penetration of this radiation is avoided by the ozone shield before reaching the ground.

Molar extinction coefficients (ε) of the analyzed compounds revealed that the β-orcinol depsidones have higher absorbance at the UVC region whereas the orcinol and β-orcinol, depsides and dibenzofurane derivatives, showed lower absorbance in that UV spectrum region. Depsidones are constituted of two acetate-polymalonate-derived phenolic acid units linked by an ester bond between the 4' and 1 positions of both phenolic units, and generally by an ether bond between the 2 and 5' positions, whereas the depsides have the same ester bond only between the 4' and 1 positions of both phenolic units (Culberson 1969). Both depsides and depsidones are considered products of orsellinic acid-type cyclization unique to lichens (Marcano 1994, Huneck and Yoshimura 1996). On the other hand, dibenzofurane derivatives are constituted of two such acetate-

polymalonate-derived phenolic acid units linked by an ether bond and a carbon-carbon bond, and are considered products either of orsellinic acid-type cyclization (didimic acid) or phloroglucinol-type cyclization (usnic acid). These compounds are unknown in non-lichen-forming fungi and appear to be extremely rare in all living systems (Culberson 1969).

According to Sala and Sargent (1981), and Rogers (1989), there is strong evidence that β-orcinol depsidones were derived not from *para*-depsides by oxidation, but by acylation of one orsellinic acid with another, followed by intramolecular rearrangements. Thus, this substance group could be considered as old or older than depsides. The presence of ether bonds in esterified compounds, such as β-orcinol depsidones, is related to the capacity to absorb significantly UVC radiation. These substances could have been originated ~ 2 billions years ago before the formation of the ozone shield (Kasting 1987, Cockell 2000a). On the other hand, in carbonaceous meteorites (e.g. Murchison) there is evidence for the existence of phenolic carboxylic acid and dibenzofurane derivatives similar to those occurring in lichens (Hayatsu *et al*. 1980). These substances would be synthesized abiogenically by Fischer-Tropsch-type reactions during the solar nebula formation, or by other processes (Pizzarello 2004). However, both observations could suggest the old age of these compounds and the possibility of the occurrence of life forms based on similar UV-screening strategies in terrestrial environments exposed to intense UV radiation.

CONCLUSIONS

In this work, we have attempted to demonstrate that phenolic carboxylic acid derivatives have the capacity to screen out UV radiation, and thereby, to avoid the lethal effects that such radiation could cause in organisms exposed at high elevations. Among the species studied, 96-100% showed a high absorbance of UVB and UVA radiation, whereas 68% showed a high absorbance of UVC radiation. Lichen capacity to synthesize phenolic compounds having electronic transitions π to π^* could be considered a success for colonizing the high-UV, tropical high mountain glacier and snow zones.

Phenolic substances presenting a high resistance to UVA radiation are characterized by ester bonds among both phenolic units (depsides). These substances constitute the more abundant products found in lichens. Compounds presenting both ester and ether bonds among both phenolic units (depsidones), and absorbing UVC radiation, could have been originated probably before the

Proterozoic and Phanerozoic, when the ozone shield was not fully formed. The relative low frequency of depsidones in lichens could suggest a reduced UV-screening role nowadays due to the existence of the ozone shield. Finally, it is important to point out that the data obtained in this study are from a region of the Earth having «very extreme» bioclimatic conditions due to the effects of the latitude on the daily surface thermal oscillations, and to the altitudinal effects on the O_2, CO_2, and N_2 partial pressures, and on surface temperatures.

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