

## Preliminary data on the accumulation of usnic acid related to ozone depletion in two Antarctic lichens.

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### ABSTRACT

The absorbing properties of UV radiation of lichen phenols agree with their photoprotector capacity determined by *in vivo* and *in vitro* methods. The photostability that characterizes these compounds makes it possible to study the relations between the UV absorbers and ozone levels in different time scales. Usnic acid is the most frequent UV-B absorber in antarctic lichens. In *Neuropogon aurantiaco-ater* (Jacq.) I. M. Lamb. and *Ramalina terebrata* Hook. et Tayl., collected in Antarctica over a period of 30 years, the highest concentrations of usnic acid were observed when ozone diminished to critical values.

**Key words:** usnic acid, UV-B absorbers, photochemical stability, ozone.

## Acumulación de ácido úsnico en dos especies de líquenes antárticos relacionada con la disminución del ozono estratosférico.

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### RESUMEN

Fenoles de origen líquénico absorben radiación ultravioleta (UV), propiedad que ha sido comprobada por métodos *in vivo* e *in vitro*. Debido a su fotoestabilidad, es posible evaluar las relaciones entre estos metabolitos y los niveles de ozono en diferentes escalas de tiempo. El ácido úsnico, que absorbe radiación UV-B, es frecuente en líquenes antárticos. En *Neuropogon aurantiaco-ater* (Jacq.) I. M. Lamb. y *Ramalina terebrata* Hook. et Tayl., recolectados en la Antártica en un período de 30 años, las concentraciones más elevadas de ácido úsnico se observaron cuando el ozono disminuyó a valores críticos.

**Palabras clave:** metabolitos fotoprotectores, ácido úsnico, estabilidad fotoquímica, ozono.

### INTRODUCTION

One of the most significant environmental changes is the increase in ultraviolet radiation (UV) due to the depletion of the stratospheric ozone layer. The most affected continent is Antarctica. However, the overall impact of the UV radiation levels on Antarctic ecosystems is not yet known. Declines in the primary productivity, changes in community structure and subsequent alterations in

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trophic dynamics, and shifts in the balance of the natural ecosystems are among the expected effects of this global change (Karentz *et al.*, 1992).

Living organisms have developed strategies to minimise damage induced by UV radiation. Some of these strategies are: synthesis of screening compounds which absorb and dissipate the UV radiation as fluorescence or heat, synthesis of accessory compounds which can use the UV radiation in photosynthesis or in other metabolic processes, synthesis of antioxidant enzymes or accessory compounds to protect cells from active forms of oxygen (Holm-Hansen *et al.*, 1993).

Phenolic compounds produced by lichens, containing a carbonyl-ortho-hydroxyl chromophore unit strongly absorb in both UV-A and UV-B and exhibit chemical and photochemical stability (Hidalgo *et al.*, 1992; Quilhot *et al.*, 1994a). Phenols, with the exception of usnic acid, fluoresce between 410 and 490 nm at basic pH and between 435 and 485 nm in solid state where chlorophylls absorb (Quilhot *et al.*, 1994b). The absorption capacity of these metabolites has been determined by *in vitro* and *in vivo* methods (Fernández *et al.*, 1996). Some of these compounds also show antioxidant capacity (Hidalgo *et al.*, 1994). Others biological roles proposed for these metabolites are that they increase the light available for photosynthesis (Rao and Le Blanc, 1965) and that they can supply energetic deficits due to the winter depression of photosynthesis (Quilhot *et al.*, 1991a).

Usnic acid is the more frequent UV-B absorber in Antarctic lichens (Huneck *et al.*, 1984; Quilhot *et al.*, 1989; Piovano *et al.*, 1992, 1994). The compound presents chemical and photochemical stability with a  $\epsilon = 2.8 \times 10^6$  (Quilhot *et al.*, 1994b). These properties permit the analysis of herbarium specimens, of any age, in order to know the evolution of some environmental change, in the foremost increase of UV radiation because of ozone depletion. This paper reports the accumulation levels of usnic acid in two Antarctic lichens, *Neuropogon aurantiaco-ater* (Jacq.) I. M. Lamb. and *Ramalina terebrata* Hook. et Tayl., and its relation with the ozone observed, over a 30 years time scale.

## Experimental

Herbarium specimens of *N. aurantiaco-ater* and *R. terebrata* were collected during spring and summer in South Shetland Islands, Antarctica (Table 1). Voucher specimens are deposited in the herbarium of the Escuela de Química y Farmacia, Universidad de Valparaíso. The lichen material was extracted with chloroform during 48 h and 24 h respectively. The extracts were filtered and mixed and evaporated to dryness; the residue was dissolved in acetone. The acetone solutions were spotted over HF<sub>254</sub> Merck plates using a Camag Linomatt III applicator and were eluted in toluene-ethyl acetate-formic acid (35:5:1 v/v). The plates were scanned in a Camag HPTLC photodensitometer equipped with a Chromatopac Shimadzu C-R6A Model data processor, at  $\lambda = 313$  nm.

The concentrations of usnic acid were calculated using formulae from standard curves with an authentic sample. Variability within thalli from different years was determined by ANOVA and the differences among means were tested using the Bonferroni test (CRAPHPAD INSTAT 1.15).

Table 1

Origin of lichen specimens investigated.

Species	N°	Origin (South Shetland Islands)
<i>N. aurantiaco-ater</i>		
	6	King George Island, January 1966
	11	King George Island, January 1977
	5	Greenwich Island, November 1983
	10	Greenwich Island, January 1987
	10	Greenwich Island, January 1988
	10	Greenwich Island, January 1989
	10	Greenwich Island, January 1990
	7	Robert Island, January 1996
<i>R. terebrata</i>		
	6	Robert Island, January 1966
	11	King George Island, December 1975
	8	Livingston Island, November 1977
	5	Robert Island, January 1986
	7	Robert Island, January 1987
	8	Robert Island, January 1996

N° : number of thalli analyzed.

## RESULTS

The comparative results of variation in usnic acid concentration in individuals of *N. aurantiaco-ater* and *R. terebrata*, collected during spring and summer in several years, related to ozone values, are shown in Fig. 1.

Usnic acid levels in *N. aurantiaco-ater* were highest from 1987 to 1996 ( $p < 0.01$ ), with values ranging between 1,28% and 1,50% dry weight. In *R. terebrata* significant differences in usnic acid concentration ( $p < 0.001$ ) were observed from 1987 to 1996, 0,30% and 0,34% dry weight respectively. Correlation coefficients observed were  $r = 0.8503$  in *N. aurantiaco-ater* and  $r = 0.8987$  in *R. terebrata*.

There is a correlation between the higher concentrations of usnic acid in both lichen species and the lower values of ozone observed from 1987 to 1995 in the Antarctica (S. Diaz, Personal communication)

### Usnic acid related to ozone depletion

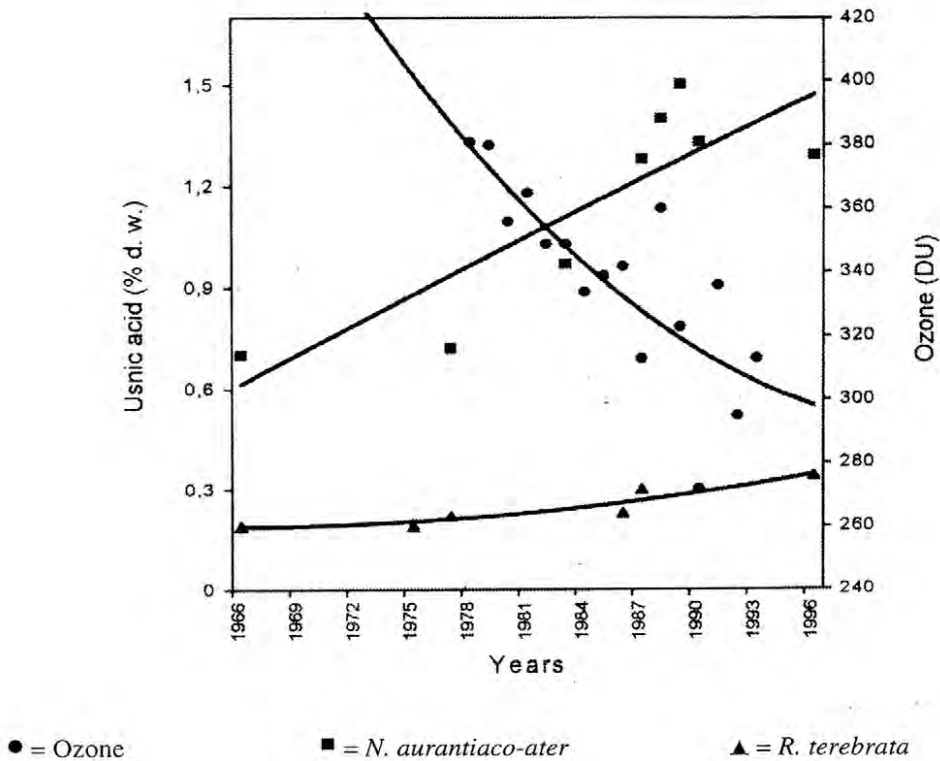


Fig. 1.-Relationships between usnic acid (mean concentrations) and ozone levels in *N. aurantiaco-ater* and *R. terebrata*, collected in South Shetland Islands. Ozone data were provided by Dra. Díaz, CADIC-CONICET.

### DISCUSSION

UV radiation induces the synthesis of flavonoids in several plants (Caldwell *et al*, 1989; Khare & Gurprasad, 1993). It has been shown (Markham *et al*, 1990) that absolute levels of photoprotective flavonoids in mosses from the Ross Sea, correlate directly with measured levels of ozone, and it is suggested that flavonoids concentrations in herbarium specimens have the potential to provide otherwise inaccessible data on historical trends in ozone levels in the Antarctic.

Lichen phenols which present very low consumption quantum yield, less than  $10^{-2}$  (Quilhot *et al.*, 1994) can be used to determine the evolution of UV levels worldwide. The decomposition rates could be negligible in herbarium specimens because of the chemical and photochemical stability of the phenols and the natural protection against bacteria or fungi due to their antibiotic activity (Cavieres *et al.*, 1995).

Lichens in alpine and high latitude environments worldwide are subject to high levels of UV radiation, currently increased by ozone thinning; the production of the screening compounds would protect the sensitive photosystems of the photobiont from the damaging effect of radiation (Galloway, 1993). A direct relationship has been observed between the usnic acid concentration and the available light in some lichen species from natural habitats (Rundel, 1979). The higher concentrations of usnic acid in *N. aurantiaco-ater* and *R. terebrata* were determined when ozone diminished to critical values. These results are in accordance with the hypothesis that UV light enhances the synthesis of usnic acid more than visible light (Fahselt, 1994).

The total content of lichen metabolites is generally very similar in different thalli of the same species (Egan, 1986), even when there are intrathalline variations related to the age of the tissues. It is known that usnic acid is more concentrated in young tissues (Fahselt, 1984; Quilhot *et. al.*, 1987, 1991b). In our experiments we used the whole thallus in every determination.

It has often been difficult to demonstrate consistently significant plant responses to UV-B radiation in greenhouse studies or field studies or to reproduce results from greenhouse studies under field conditions (Middleton and Teramura, 1994). Numerous plant studies evaluating the effectiveness UV-B radiation indicate that the responses vary qualitatively and quantitatively and are dependent on many factors, including the UV-B radiation exposure, the sensitivity of the species and other environmental stresses (Teramura, 1986). Moreover, UV-B is generally considered to be a damaging radiation.

In spite of those qualifications, our preliminary results support the hypothesis that lichens can be used to monitor levels of UV radiation elsewhere in nature. We agree with the idea (Galloway, 1993) that the use of lichens as indicators of climatic and environmental changes in the Southern Hemisphere, although in its infancy, is a field of enormous and exciting potential.

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#### REFERENCES

- CADWELL, M.M., A.H. TERAMURA and M. TEVINI, 1989. The changing solar ultraviolet climate and the ecological consequences for higher plants. *TREE* 4:363-366.
- CAVIERES, F., E. FERNÁNDEZ, C. RUBIO y W. QUILHOT, 1995. Importancia de las colecciones de líquenes en el estudio de problemas químico-ambientales. *Bol. Antártico Chileno* 14: 2-4.
- CULBERSON, CH. F. and J.A. ELIX, 1989. Lichen substances. In: *Methods in Plant Biochemistry* Vol. 1, Academic Press, pp. 509-535.
- EGAN, R.S., 1986. Correlations and non correlations of chemical variation patterns with lichen morphology and geography. *Bryologist* 89: 99-110.
- FAHSELT, D., 1984. Interthalline variability in levels of lichen products within stands of *Cladonia stellaris*. *Bryologist* 87:50-56.
- FAHSELT, D., 1994. Secondary biochemistry of lichens. *Symbiosis* 16: 117-165.
- FERNANDEZ, E., W. QUILHOT, I. GONZALEZ, M.E. HIDALGO, J. MOLINA and I. MENESES, 1996. Photoprotector capacity of lichen metabolites against UV-B radiation. *Cosmetic and Toiletries* 111 : 69-74.

- GALLOWAY, D.J., 1993. Global environmental change: lichens and chemistry. *Biblioth. Lichenol.* 53: 87-95.
- HIDALGO, M.E., E. FERNANDEZ, W. QUILHOT and E.A. LISSI, 1992. Solubilization and photophysical and photochemical behaviour of depsides and depsidones in water and Brij-35 solutions at different pH values. *J. Photochem. Photobiol. A: Chem.* 67: 245-254.
- HIDALGO, M.E., E. FERNANDEZ, W. QUILHOT and E.A. LISSI, 1994. Antioxydant capacity of depsides and depsidones. *Phytochemistry* 37: 1585-1587.
- HOLM-HANSEN, O., D. LUBIN and W. HEBLING, 1993. Ultraviolet radiation and its effects on organisms in aquatic environments. In: *Environmental UV photobiology* (A.P. Young, L.E. Bjorn, J. Moon and W. Nultsch, ed.), Plenum Press; pp. 379-314.
- HUNECK, S., M. SAINSBURY, T.M.A. RICKARD and R.I. LEWIS SMITH, 1984. Ecological and chemical investigations of lichens from South Georgia and the Maritime Antarctica. *Journ. Hattori Bot. Lab.* 56: 461-480.
- KARENTS, D., I. BOSH and W. DUNLAP, 1992. Distribution of UV-absorbing compounds in the antarctic limpet, *Nacella concinna*. *Antarctic J.* 27: 121-122.
- KHARE, M. and K.M. GURPRASAD, 1993. UV-B Induced anthocyanin synthesis in maize regulated by FMN and inhibitors of FMN photoreactions. *Plant Science* 91: 1-5.
- LAWREY, J.D., 1986. Biological role of lichen substances. *Bryologist* 89: 111-122.
- MARKHAM, R., A. FRANKE, D.R. GIVEN and P. BRONWENSEY, P., 1990. Antarctic ozone level trends from herbarium specimens flavonoids. *Bull. Lias. Gr. Polyphenols* 15: 230-235.
- MIDDLETON, E.M and A.H. TERAMURA, 1994. Understanding photosynthesis pigment and growth responses induced by UV-B and UV-A irradiances. *Photochemistry and Photobiology* 60: 38-45.
- PIOVANO, M., J.A.GARBARINO, M.C. CHAMY, V. ZUÑIGA, E. MIRANDA, E. CESPEDES, P. FIEDLER, W. QUILHOT and G. ARAYA, 1992. Studies on Chilean lichens. XVI. Advances in the chemistry of Antarctic lichens. *Ser. Cient. INACH* 41: 91-97.
- QUILHOT, W., G. LEIGHTON, E. FLORES, E. FERNANDEZ, W. PEÑA and G. GUZMAN, 1987. Factores exógenos y endógenos determinantes de la acumulación de ácido úsnico en líquenes. *Acta Farm. Bonaerense* 6: 15-22.
- QUILHOT, W., J.A. GARBARINO, M. PIOVANO, M.C. CHAMY, V. GAMBARO, M.L. OYARZUN, C. VINET, V. HORMAECHEA and P. FIEDLER, 1989. Studies on Chilean lichens. XI. Secondary metabolites from Antarctic lichens. *Ser. Cient. INACH* 39: 75-89.
- QUILHOT, W., W. PEÑA, E. FLORES, M.E. HIDALGO, E. FERNANDEZ and G. LEIGHTON, 1991a. Temporal variation of usnic acid concentration in *Usnea aurantiaco-atra*. *Ser. Cient. INACH* 41: 99-106.
- QUILHOT, W., M.G. SAGREDO, E. CAMPALANS, M.E. HIDALGO, W. PEÑA, E. FERNANDEZ and M. PIOVANO. 1991b. Quantitative variation of phenolic compounds related to thallus age in *Umbilicaria antarctica*. *Ser. Cient. INACH* 41: 91-97.

- QUILHOT, W., E. FERNANDEZ and M.E. HIDALGO, 1994a. Photoprotection mechanisms in lichens against UV radiation. *British Lichen Soc. Bull.* 75: 1-5.
- QUILHOT, W., E. FERNANDEZ and M.E. HIDALGO, 1994b. Bases experimentales relacionadas al potencial adaptativo de fenoles liquénicos. In: *Actas II Simposio Internacional Química de Productos Naturales y sus Aplicaciones* (M. Silva & M. Bittner, eds.). Concepción, Chile, pp: 148-154.
- RAO, D.N. and F. LE BLANC, 1965. A possible role of atranorin in the lichen thallus. *Bryologist* 68: 284-289.
- RUNDEL, P.W., 1979. Clinal variation in the production of usnic acid in *Cladonia subtenuis*. *Bryologist* 72: 40-44.
- TERAMURA, A.H., 1986. Interaction between UV-B radiation and other stresses in plants. In: *Stratospheric ozone Reduction, Solar Ultraviolet Radiation and Plant Life*, NATO ASI Series G: Ecological Sciences, (R.C. Worrest & M.M. Cadwell, eds.), Vol 8: 327-343. Springer-Verlag, Berlin.
- TEZUKA, T., T. HOTTA and I. WATANABE, 1993. Growth promotion of tomato and radish plants by solar UV radiation reaching the Earth's surface. *J. Photochem. Photobiol. B: Biol.* 19: 61-66.