

Studies on Chilean lichens. XI Secondary metabolites from Antarctic lichens.

WANDA QUILHOT¹, JUAN A. GARBARINO², MARISA PIOVANO², MARÍA CRISTINA CHAMY², VICENTE GAMBARO²,
MARÍA LUISA OYARZÚN², CINDY VINET¹, VALERIA HORMAECHEA¹ AND PETER FIEDLER².

ABSTRACT

The major secondary metabolites of 17 Antarctic lichen species were studied via macroextraction. The most frequently found compounds were usnic acid and atranorin. Two polysubstituted depsides were isolated from Lecania brialmontii, brialmontins 1 and 2; from Catillaria corymbosa a derivative of hiassic acid was isolated, 2'-O-methylhiassic acid. Peroxygosterol, a sterol infrequent in lichens, was found in four species.

Key words: Antarctic lichens, secondary metabolites, depsides, depsidones, dibenzofuran derivatives, usnic acid, terpenes.

Estudios sobre líquenes chilenos. XI. Metabolitos secundarios de líquenes antárticos

WANDA QUILHOT¹, JUAN A. GARBARINO², MARISA PIOVANO², MARÍA CRISTINA CHAMY², VICENTE GAMBARO²,
MARÍA LUISA OYARZÚN², CINDY VINET¹, VALERIA HORMAECHEA¹ Y PETER FIEDLER².

RESUMEN

Se estudiaron los metabolitos secundarios mayoritarios de 17 especies de líquenes antárticos mediante macroextracción. Los compuestos más frecuentes fueron el ácido úsnico y la atranorina. De Lecania brialmontii se aislaron dos interesantes dépsidos polisustituídos, la brialmontina 1 y la brialmontina 2, y de Catillaria corymbosa el ácido 2'-O-metilhiásico, un derivado del ácido hiásico. El peroxigosterol, un esteroles poco frecuente en líquenes, fue aislado de cuatro especies.

Palabras claves: Líquenes antárticos, metabolitos secundarios, dépsidos, depsidonas, dibenzofuranos, ácido úsnico, terpenos.

INTRODUCTION

Lichens accumulate products of their secondary metabolism, most of them being specific; they are formed from phenolic units which have a common origin in polyketonic tricarboxylic acids derived from acetic acid. These constitute the structural base of depsides, depsidones, diaryl ethers, dibenzofurans and usnic acids, depending on whether the cyclization follows the orsellinic acid model or the phloroglucinol model (Vicente, 1976; Elix *et al.*, 1984). Less frequently,

¹ Escuela de Química y Farmacia, Facultad de Medicina, Universidad de Valparaíso, Casilla 92-V, Valparaíso, Chile.

² Departamento de Química, Facultad de Ciencia, Universidad Federico Santa María, Casilla 110-V Valparaíso, Chile.

they also synthesize triterpenes with a hopane skeleton, xanthenes, anthraquinones, and compounds related to shikimic acid (Asahina and Shibata, 1954; Culberson, 1969).

The lichens are known by the differentiation of chemical races with little or no change in morphology. According to Culberson (1970), chemical races are established through a change in the constituent natural products by the replacement of one substance by another or the addition (or deletion) of one or more accessory substances.

Lichens are the most conspicuous macroscopic plants in Antarctica, in terms of species, biomass and distribution (Lamb, 1970; Lindsay, 1978). In spite of the large number of species—more than 300—only a few chemical studies have been undertaken so far (Hertel and Leuckert, 1969; Huneck *et al.*, 1984).

The purpose of this work is to appraise the chemical variability of macrolichens collected on Robert and King George Islands, South Shetland Islands, during January and February, 1987.

EXPERIMENTAL

General procedures

Melting points are uncorrected and were determined on a Kofler hot plate. Optical rotation was measured in CHCl_3 with a Schmidt-Haensch polarimeter. $^1\text{H-NMR}$ spectra were recorded at 60 MHz (Varian T-60) in CDCl_3 , CD_3COCD_3 and DMSO-d_6 , with TMS as internal standard. MS were obtained on a Varian MAT (CH-7) spectrometer by direct probe insertion at 70 eV. IR spectra were recorded in KBr pellets (Perkin Elmer, model 683). Thin layer chromatography and column chromatography were performed on Silica gel from Merck.

The lichen material was dried and ground, and was extracted, successively, with naphtha (60-80°), chloroform, and acetone, each time for a period of 24 hours, at room temperature.

The new compounds were identified by physical and spectroscopic methods, and the known ones were additionally compared with authentic samples.

Voucher specimens were deposited in the herbarium of the School of Pharmacy, Universidad de Valparaiso.

Buellia cladocarpiza Lamb. 116 g., collected in Copper Mine Cove, Robert Island. Atranorin (1) (1.14 g; 0.98%), identical to an authentic sample, was isolated from the chloroform and acetone extracts, after fractionating on a silica gel column by elution with benzene.

Catillaria corymbosa (Hue) Lamb. 730 g, collected on Robert Island. Atranorin (1) (1.16 g; 0.16%), chloroatranorin (2) (0.22 g; 0.03%) and 2'-O-methylhiassic acid (9) (1.09 g; 0.15%), were isolated from the chloroform and acetone extracts, after fractionating on a silica gel column, using toluene-ethyl acetate-formic acid (35: 23: 1) as eluent.

Cornicularia aculeata (Schreb.) Ach. - 665 g, collected in Ardley Cove, King George Island. Usnic acid (15) (0.01g; 0.002%) and (+)-protolichesterinic acid (19) (5.70 g; 0.87%) were isolated from the chloroform and acetone extracts, after fractionating on a silica gel column, using benzene-ethyl acetate-formic acid (20:2:0.5) as eluent.

Lecanora atra (Huds.) Ach. - 871 g. collected on Robert Island. From the naphtha extract traces of atranorin were obtained. Atranorin (1) (0.19 g; 0.02%), α -collatolic acid (11) (0.16 g; 0.02%), and alectoronic acid (12) (0.19 g; 0.02%), were isolated from the chloroform and acetone extracts, after fractionating on a silica gel column, using benzene-ethyl acetate-formic acid (40:2:1) as eluent.

Lecania brialmontii Vain.- 750 g of lichen material, collected in Copper Mine Cove, Robert Island. Brialmontin 1 (3) (0.38 g; 0.05%) was isolated from the naphtha, chloroform and acetone extracts by elution from a silica gel column with naphtha-ethyl acetate (30:1); and brialmontin 2 (4) (0.15 g; 0.02%), using naphtha-ethyl acetate (30:2) as eluent.

Ochrolechia antarctica (Müll. Arg.) Darb.- 1000 g of lichen material, collected on Robert Island. Usnic acid (15) (0.20 g; 0.002%) was isolated from the naphtha extract. Variolaric acid (14) (2.20 g; 0.22%) and lecanoric acid (7) (0.05 g; 0.005%) were obtained from the chloroform and acetone extracts.

Ochrolechia frigida (Sw.) Lynge.- 330 g of lichen material, collected on Robert Island. Usnic acid (15) (0.10 g; 0.03%), and traces of atranorin (1) and zeorin (17) were isolated from the naphtha extract. The chloroform and acetone extracts gave gyrophoric acid (10) (2.50 g; 0.76%).

Parmelia saxatilis (L.) Ach.- 730 g of lichen material, collected on Robert Island. Usnic acid (15) (0.04 g; 0.005%), atranorin (1) (0.51 g; 0.70%), and zeorin (17) (0.013 g; 0.002%), were isolated from the naphtha extract. From the chloroform and acetone extracts salazinic acid (5) (2.02 g; 0.28%) was isolated.

Pannaria hookeri (Borr. ex Sm.) Nyl.- 280 g of material collected on Robert Island. Traces of atranorin (1) and ergosterol peroxide (18) (0.09 g; 0.012%) were isolated from the acetone extract.

Psoroma tenue Henssen var.- *tenue*.- 300 g of lichen material, collected on Robert Island, Copper Mine Cove. Porphyrilic acid methyl ester (16) (0.39 g; 0.13%) and atranorin (1) (0.01 g; 0.003%) were isolated from the acetone extract after fractionating on a silica gel column by elution with naphtha-ethyl acetate (20:1).

Psoroma hypnorum (Vahl.) Gray.- 230 g of lichen material, collected in Copper Mine Cove, Robert Island. Ergosterol peroxide (18) (0.02 g; 0.009%) was isolated from the acetone extract, by precipitation and recrystallization from acetone.

Placopsis contortuplicata Lamb.- 690 g of lichen material, collected on Robert Island. Gyrophoric acid (10) (4.70 g; 0.68%) was obtained from the acetone extract, by precipitation and recrystallization from acetone. Gyrophoric acid (10) was detected by thin layer chromatography of separated cephalodia, extracted with acetone.

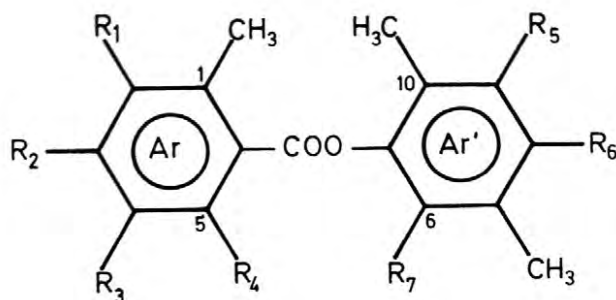
Ramalina terebrata Hook et Tayl.- 1275 g of material, collected in Copper Mine Cove, Robert Island. The naphtha extract gave usnic acid (15) (2.20 g; 0.17%).

Rhizoplaca aspidophora (Vain.) Redon.- 239 g of material, collected on Robert Island. Usnic acid (15) (2.39 g; 1.00%) and zeorin (17) (2.70 g; 1.13%) were isolated from the naphtha and chloroform extracts. The acetone extract gave stictic acid (6) (0.02 g; 0.008%).

Sphaerophorus globosus (Huds.) Vain.- 647 g of lichen material, collected on Robert Island. Usnic acid (15) (0.01 g; 0.002%) was isolated from the naphtha extract. Sphaerophorin (8) (1.07 g; 0.16%) was obtained from the chloroform and acetone extracts after fractionating on a silica gel column by elution with naphtha-ethyl acetate-formic acid (36:4:1).

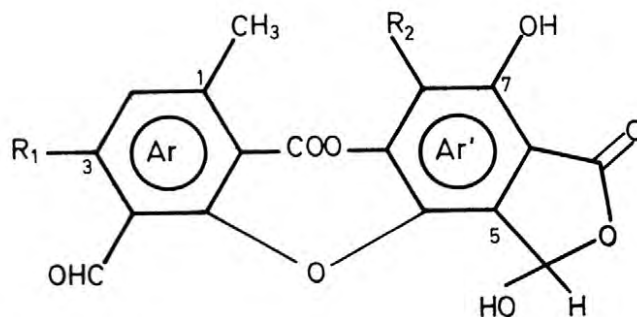
Stereocaulon alpinum Laur.- 1300 g of lichen material, collected on Robert Island, and in Ardley Cove, King George Island. Usnic acid (15) (0.01 g; 0.001%) and atranorin (1) (0.02 g; 0.002%) were isolated from the naphtha extract. From the chloroform extract atranorin (1) (4.00 g; 0.30%), lobaric acid (13) (6.00 g; 0.46%) and traces of ergosterol peroxide (18) were isolated by fractionation on a silica gel column, eluting with benzene.

DEPSIDES



COMPOUND		R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
ATRANORIN	(1)	H	OH	CHO	OH	OH	COOCH ₃	H
CHLOROATRANORIN	(2)	CL	OH	CHO	OH	OH	COOCH ₃	H
BRIALMONTIN 1	(3)	CH ₃	OCH ₃	CH ₃	OCH ₃	OCH ₃	H	CH ₃
BRIALMONTIN 2	(4)	CH ₃	OCH ₃	CH ₃	OH	OCH ₃	H	CH ₃

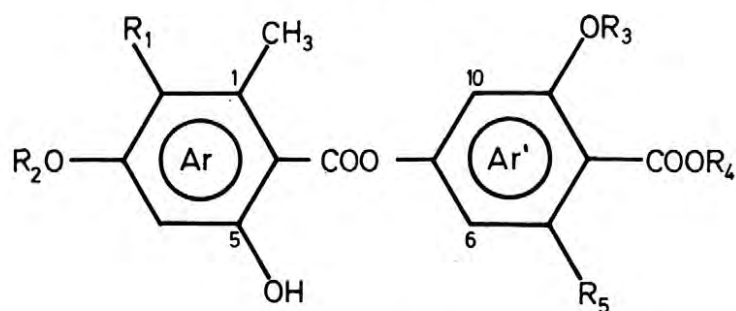
DEPSIDONES

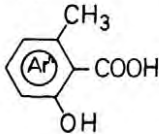
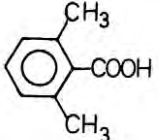


COMPOUND		R ₁	R ₂
SALAZINIC ACID	(5)	OH	CH ₂ OH
STICTIC ACID	(6)	OCH ₃	CH ₃

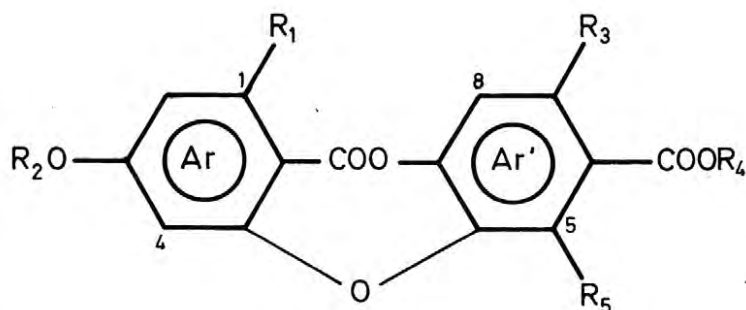
Scheme 1: β -orcinol derivatives

DEPSIDES



COMPOUND		R ₁	R ₂	R ₃	R ₄	R ₅
LECANORIC ACID	(7)	H	H	H	H	CH ₃
SPHAEROPHORIN	(8)	H	CH ₃	H	H	C ₇ H ₁₅
2-O-METHYLHIASCIC ACID	(9)	OH	H	CH ₃		CH ₃
GYROPHORIC ACID	(10)	H	H	H		CH ₃

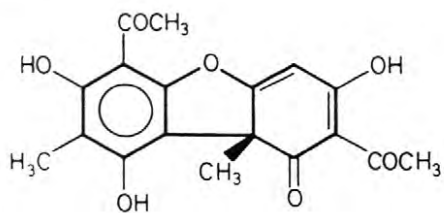
DEPSIDONES



COMPOUND		R ₁	R ₂	R ₃	R ₄	R ₅
α-COLLATOLIC ACID	(11)	CH ₂ COC ₅ H ₁₁	CH ₃	OH	H	CH ₂ COC ₅ H ₁₁
ALECTORONIC ACID	(12)	CH ₂ COC ₅ H ₁₁	H	OH	H	CH ₂ COC ₅ H ₁₁
LOBARIC ACID	(13)	COC ₄ H ₉	CH ₃	OH	H	C ₅ H ₁₁
VARIOLARIC ACID	(14)	CH ₃	H	-CH ₂ -		

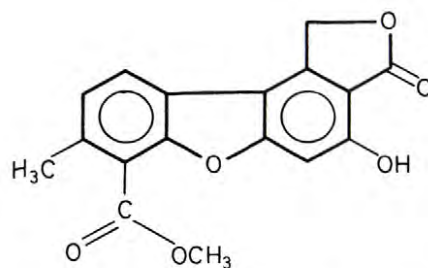
Scheme 2: Orcinol derivatives

a) PHLOROGLUCINOL DERIVATIVE



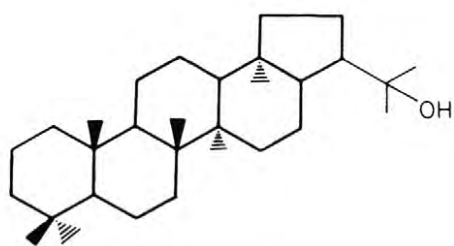
(+)-USNIC ACID (15)

b) DIBENZOFURAN DERIVATIVE



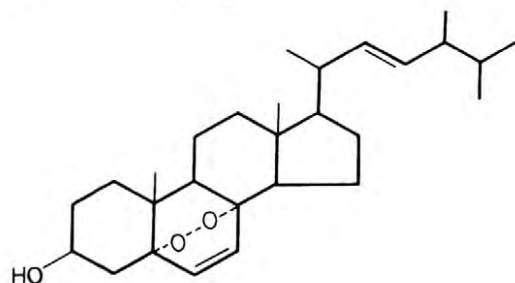
PORPHYRILIC ACID METHYL ESTER (16)

c) TERPENE



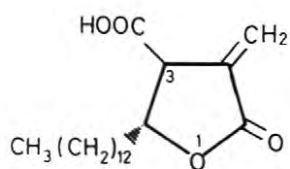
ZEORIN (17)

d) STEROL



ERGOSTEROL PEROXIDE (18)

e) ALIPHATIC ACID



(-)-PROTOLICHESTERINIC ACID (19)

Scheme 3. Further structure of Antarctic lichen metabolites.

Umbilicaria antarctica Frey et Lamb. - 1300 g of material, collected on Robert Island. Usnic acid (15) (0.04 g; 0.03%) and atranorin (1) (0.04 g; 0.03%) were isolated from the naphtha extract. After fractionating the acetone extract eluting with naphtha-ethyl acetate (3:1), gyrophoric acid (10) (12.00 g; 0.92%) and ergosterol peroxide (18) (0.09 g; 0.007%) were obtained.

Characterization by means of physical and spectroscopic data of the compounds isolated

Atranorin (1) (Wojciechowski *et al.*, 1973): mp = 194-196° (lit 195-197°).

IR_{max}^{KBr} cm⁻¹: 2920 (OH); 1650 (C=O); 1575 (arom.); 1250 (ArCOOCH₃); 1120 (C-O-C); 810 (arom.).

¹H-NMR (CDCl₃) δ: 2.06 (3H, s, CH₃-1); 2.53 (3H, s, CH₃-10); 2.70 (3H, s, CH₃-7); 4.00 (3H, s, COOCH₃); 6.65 (1H, s, H-2); 10.46 (1H, s, CHO); 12.03 (1H, s, OH-9); 12.60 (1H, s, OH-3); 12.70 (1H, s, OH-5).

Chloroatranorin (2) (Vinet, 1987) mp = 208-210° (lit. 208-208.5°; Culberson, 1969).

IR_{max}^{KBr} cm⁻¹: 2910 (OH); 1655 (C=O); 1580 (arom.); 1120 (C-O-C); 810 (arom.).

¹H-NMR (CDCl₃) δ: 2.10 (3H, s, CH₃-1); 2.56 (3H, s, CH₃-10); 2.90 (3H, s, CH₃-7); 4.03 (3H, s, COOCH₃); 6.40 (1H, s, H-5); 10.46 (1H, s, CHO); 12.03 (1H, s, OH-9); 12.43 (1H, s, OH-3); 12.70 (1H, s, OH-5).

Ms m/z: 410 (M⁺ + 2; 6.3%); 408 (M⁺; 8.2); 213 (56.0); 212 (19.9); 196 (75.5); 164 (100); 136 (50.5).

Brialmontin 1 (3) (Vinet, 1987): mp = 102-104°.

IR_{max}^{KBr} cm⁻¹: 2900 (C-H); 1740 (C=O); 1615 (arom.); 1450 (Ar-CH₃); 1280 (C-O-C); 850 (arom.).

¹H-NMR (CDCl₃) δ: 2.19 (9H, s, CH₃-1,2,7); 2.27 (3H, s, CH₃-6); 2.30 (3H, s, CH₃-4); 2.36 (3H, s, CH₃-10); 3.70 (3H, s, OCH₃-9); 3.81 (6H, s, OCH₃-3,5); 6.63 (1H, s, H-8).

Ms m/z: 372 (M⁺; 3.5%); 207 (100); 193 (1.1); 192 (1.3); 166 (1.7); 165 (2.4); 164 (5.8); 149 (3.4).

Brialmontin 2 (4) (Vinet, 1987): mp = 106-109°.

IR_{max}^{KBr} cm⁻¹: 3400 (OH); 2925 (C-H); 1650 (C=O); 1630 (arom.); 1470 (ArCH₃); 1275 (C-O-C); 810 (arom.).

¹H-NMR (CDCl₃) δ: 2.05 (6H, s, CH₃-1,7); 2.22 (3H, s, CH₃-5); 2.25 (3H, s, CH₃-10); 2.32 (3H, s, CH₃-4); 2.67 (3H, s, CH₃-6); 3.75 (3H, s, OCH₃-9); 3.84 (3H, s, OCH₃-3); 6.66 (1H, s, H-8); 11.42 (1H, s, OH-5).

Ms m/z: 358 (M⁺; 4%); 193 (100); 192 (3.8); 166 (9.5); 165 (2.9); 164 (2.9); 149 (9.5).

α-Collatolic acid (11) (Elix *et al.*, 1974): mp = 122-123° (lit. 122-123°).

IR_{max}^{KBr} cm⁻¹: 3350 (br OH); 1730, 1680, 1620 (C=O); 1570 (arom.); 1055 (Ar-OH); 890, 860, 800 (arom.).

¹H-NMR (CD₃COCD₃) δ: 0.93 (6H, m, (CH₂)₄CH₃); 1.20-1.80 (12 H, m, (CH₂)₃), 2.14 (2H, m, ArCH₂COCH₂); 2.50 (2H, t, J = 8Hz, Ar'CH₂COCH₂); 3.69 (2H, brs, Ar'CH₂CO); 3.83 (3H, s, OCH₃); 4.02 (2H, s, ArCH₂CO); 6.80 (2H, dd, J = 8, 4Hz, ArH-2,4).

Alectoronic acid (12) (Elix *et al.*, 1974): mp = 185-186° (lit. 190-191°).

IR_{max}^{KBr} cm⁻¹: 3300 (br OH); 1740, 1705, 1675 (C=O); 1605 (arom.); 1090 (Ar-OH); 890, 800 (arom.).

¹H-NMR (CD₃COCD₃) δ: 0.67-1.10 (6H, m, (CH₂)₄CH₃); 1.10-1.80 (12H, m, (CH₂)₃); 2.06 (2H, m, ArCH₂COCH₂); 2.53 (2H, s, Ar.CH₂COCH₂); 3.20-4.00 (2H, brs, Ar'CH₂CO); 4.03 (2H, s, ArCH₂CO) 6.60-6.90 (2H, m, ArH-2,4); 6.60-6.90 (1H m, Ar'-H-8).

- Gyrophoric acid (10)* (Bryan *et al.*, 1976): mp = 220-225° dec. (lit. 223° dec.).
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3300, 3000 (br OH); 1720, 1600 (C = O); 1250 (C-O-C); 1050 (C-OH).
 $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.40 (9H, s, Ar-CH₃); 6.22 (2H, s, H-2, 4); 6.59-6.68 (4H, m, H-6, 9, 10, 14); 9.97 (1H, bs, OH or COOH); 10.34, 10, 40 (each 1H, s, bonded OH).
 Ms m/z: 318 (0, 4%); 168 (50); 151 (50); 150 (90); 124 (100); 123 (54); 122 (55).
- 2'-O-methylhiassic acid (9)* (Vinet, 1987): mp = 207-209°.
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3300 (OH); 2880 (CH); 1660 (arom.); 1250 (C-O-C); 840 (arom.).
 $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.27 (3H, s, Ar'CH₃); 2.37 (3H, s, Ar''CH₃); 2.43 (3H, s, ArCH₃); 3.63 (3H, s, OCH₃); 6.38 (1H, s, Ar-H); 6.60 (2H, dd, J = 1.5, 0.5 Hz, Ar'H); 6.70 (2H, dd, J = 1.5, 0.5 Hz, Ar''H); 9.94 (2H, s, OH-2,3); 10.54 (1H, s, OH-5).
 Ms m/z: 168 (11%); 166 (11); 165 (3); 151 (11); 150 (25); 138 (25); 124 (100).
- Lecanoric acid (7)* (Bryan *et al.*, 1976): mp = 175-185° dec. (lit. 185° dec.).
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3480-3400 (OH); 1730, 1650 (C = O); 1250 (C-O-C); 1050 (C-OH).
 $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.60 (6H, s, ArCH₃); 6.32 (2H, s, H-2, 4); 6.60, 6.68 (each 1H, d, J = 3.0 Hz, H-6, 10); 10.00 (2H, s, OH or COOH); 11.19 (1H, s, bonded OH).
 Ms m/z: 318 (M + ; 16%); 317 (20); 300 (5); 273 (8); 167 (100); 150 (58).
- Lobaric acid (13)* (Huneck *et al.*, 1968): mp = 196-198° (lit. 196-197°; Culberson, 1969).
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3140 (OH); 1720, 1685, 1600 (C = O); 1270 (C-O-ester); 1100 (C-O-ether).
 Ms m/z: 456 (M + ; 22%); 438 (18); 412 (100); 397 (72); 355 (20); 194 (100).
- Porphyritic acid methyl ester (16)* (Renner *et al.*, 1981): mp = 265-266°.
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3300 (OH); 1735, 1660 (C = O); 1600 (C = C); 1275 (C-O-ester); 1065 (C-O-ether).
 Ms m/z: 328 (M + ; 39%) 299 (15); 297 (13); 271 (19); 270 (100); 269 (16); 242 (16); 241 (95); 213 (12); 212 (8); 185 (12); 183 (7).
- (+)-Protolichesterinic acid (19)* (Boll, 1968): mp = 102-104° [α]_D²² + 18.2°, CHCl₃ (lit.: [α]_D^{19.5} = 12.1°, CHCl₃; Culberson, 1969).
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3200 (OH); 2780 (CH); 1740 (C = O) lactone; 1720 (C = O acid).
 $^1\text{H-NMR}$ (CDCl₃) δ 0.86 (3H, m, CH₃); 1.30 (br, s (CH₂)₁₂); 3.62 (1H, m, H-2); 4.82 (1H, m, H-3); 6.10 (1H, d, J = 4.0 Hz, = CH₂); 6.55 (1H, d, J = 4.0 Hz, = CH₂).
- Salazinic acid (5)* (O'Donovan *et al.*, 1980): mp = 260-268° (lit. 260-280°; Culberson, 1969).
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3500, 3240 (OH). 1760 (C = O lactone), 1730 (C = O aldehyde), 1650 (C = O ester), 1250 (C-O-C), 1070 (C-O-H).
 $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.45 (3H, s, CH₃-1); 4.70 (2H, s, CH₂OH); 6.80 (1H, s, H-2); 6.83 (1H, s, H-lactol); 10.83 (1H, s, CHO).
- Stictic acid (6)* (Hirayama *et al.*, 1976; Elix *et al.*, 1987): mp = 270-272° dec. (lit. 268-269° dec.).
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3350, 3200 (OH); 1750, 1720, 1680 (C = O); 1100 (C-OH).
 $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.23 (3H, s, CH₃-1); 2.48 (3H, s, CH₃-8); 3.97 (3H, s, OCH₃-3); 10.55 (1H, s, CHO).
- (+)-Usnic acid (15)* (Talvitic, 1983): mp = 201-203° (lit. 203-204°). [α]_D²² = 505°, CHCl₃ (lit. [α]_D²⁰ = 495°; Culberson, 1969).
 $\text{IR}_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 1685 (C = O); 1190 (C-O-C); 840, 820 (arom.).

$^1\text{H-NMR}$ (CDCl_3) δ : 1.90 (3H, s, Ar- CH_3); 2.12 (3H, s, $\text{CH}_3\text{-C-CO}$); 2.67 (6H, s, COCH_3); 6.00 (1H, s, Ar-H).

Variolaric acid (14) (Rana and Sargent, 1975): mp = 296-298°, dec. (lit. 294-296° dec.).

$\text{IR}_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3340 (OH); 1740 (C = O lactone); 1720 (C = O ester); 1280 (COO); 1080 (C-OH).

$^1\text{H-NMR}$ ($\text{CDCl}_3\text{-DMSO-d}_6$) δ : 2.41 (3H, s, CH_3); 5.18 (2H, s, CH_2); 6.59, 6.98 (2H, s, ABq, J = 2.3 Hz, H-2, 4); 6.79 (1H, s, H-8).

Ms m/z: 315 (19%); 314 (M+; 100); 201 (24); 164 (26); 151 (59); 135 (24); 134 (36); 115 (20); 107 (20); 105 (20).

Sphaerophorin (8) (Culberson, 1969): mp = 137-139° (lit. 137°).

$\text{IR}_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450 (OH); 1650 (C = O); 1600 (Arom.); 1240 (C-O); 1040 (C-OH); 850, 790 (Arom.).

Ms m/z: 416 (M+; 8%); 415 (15); 398 (3); 372 (34); 251 (15); 225 (3); 206 (52); 181 (64); 165 (100); 137 (64); 122 (3).

Zeorin (17) (Yosioka *et al.*, 1966): mp = 217-219° (lit. 221-223°).

$[\alpha]_{\text{D}}^{22} + 54.5^\circ$, CHCl_3 (lit. $[\alpha]_{\text{D}}^{25} = 54^\circ$; Culberson, 1969). $\text{IR}_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3250 (br, OH); 2920 (CH).

$^1\text{H-NMR}$ ($\text{CDCl}_3 + \text{CD}_3\text{OD}$) δ : 1.19; 1.19; 1.15; 1.06; 1.01; 0.98; 0.88; 0.77 (3H, s, 8 CH_3).

Ms m/z: 444 (M+, 16%); 207 (70); 191 (47); 189 (100); 151 (17); 149 (61).

Ergosterol peroxide (18) (González *et al.*, 1973; Wojciechowski *et al.*, 1973): mp = 175-178° (lit, 175-177°).

$[\alpha]_{\text{D}}^{20} - 30.0^\circ$, CHCl_3 (lit. $[\alpha]_{\text{D}}^{20} - 27.4^\circ$).

$\text{IR}_{\text{max}}^{\text{KBr}}$: 3525 (OH); 1465 (C = C); 1375 (C-H); 1045 (C-OH); 970 (C-OO-C).

$^1\text{H-NMR}$ (CDCl_3) δ : 6.50, 6.24 (each 1H, d, J = 8Hz, AB-system-C-CH = CH-C); 5.2 (2H, m, -CH = CH-); 4.0 (1H, W 1/2 = 14Hz, H-C-OH); 0.90, 0.83 (6H, s, CH_3 tert.); 1.0, 0.92, 0.84, 0.83 (12H, s, CH_3 sec.).

RESULTS AND DISCUSSION

The analytical results corresponding to 17 macrolichen species from the vicinity of the Antarctic peninsula are collected in Table 1. Usnic acid (15), a phloroglucinol derivative, and the depside atranorin (1) of the orcinol series, are present in the majority of the species. The concentrations of both metabolites are extremely variable: from traces to about 1%.

The presence of atranorin (1) in *Buellia cladocarpiza*, a species considered endemic to Antarctica, is in accordance with previous reports (Culberson, 1969; Culberson *et al.*, 1977). Atranorin (1) is the most frequently found compound in species of *Buellia*, as shown in a phytochemical and chemotaxonomic study of 73 taxa (Huneck and Follmann, 1970).

Atranorin (1) and α -collatolic acid (11) had already been reported for *Lecanora atra* (Culberson *et al.*, 1977). The Robert Island sample presents, in addition, alectoronic acid (12). α -collatolic (11) and alectoronic (12) acids—depsidones of the orcinol series—are very similar structurally: they only differ by the methylation of a phenol group at position 4 of the former.

Stressful conditions prevailing in Antarctica would make the hypothetical methylation difficult, which should cause the coexistence of both compounds in the plant.

Parmelia saxatilis accumulates atranorin (1), usnic acid (15), the depsidone salazinic acid (5)

and the triterpene zeorin (17). Atranorin (1), chloroatranorin (2), and usnic acid (15) had been identified in an Antarctic sample (Lindsay, 1973). Except for chloroatranorin (2), the same metabolites had been reported for material collected in other geographic areas (Culberson, 1969; Culberson, 1970; Culberson *et al.*, 1977).

Rhizoplaca aspidophora (syn. *Lecanora aspidophora*) accumulates stictic (6) and usnic (15) acids, as well as ergosterol peroxide (18). For this species zeorin (17) and usnic acid (15) had been reported previously (Culberson *et al.*, 1977). Two dibenzofuran derivatives, (-)-placodiolic and (-)-pseudoplacodiolic acids, as well as (-)-usnic acid had been isolated from a related species, *R. chrysoleuca* (Connolly *et al.*, 1984).

The presence of atranorin (1), laboric acid (13) (Culberson *et al.*, 1977), stictic acid (6) (Culberson, 1970), and methyl orsellinate (Ingolfsdottir *et al.*, 1986), had been reported for *Stereocaulon alpinum*. Our material gave usnic (15) and lobaric (13) acids, atranorin (1), and ergosterol peroxide (18). Ergosterol peroxide, isolated for the first time from the fungus *Aspergillus fumigatus* (Wieland and Prelog, 1947), was synthesized in 1954 (Barton and Robinson, 1954); its presence in lichens is infrequent (González *et al.*, 1973); Hirayama *et al.*, 1973; Wojciechowski *et al.*, 1973).

The chemistry of the genus *Ramalina* is well known. 80% of more than 100 taxa analyzed accumulate usnic acid (15) (Culberson, 1969; Culberson, 1970; Culberson *et al.*, 1977). This compound was reisolated from the Antarctic sample in agreement with previously reported data (Huneck *et al.*, 1984).

Sphaerophorus globosus is a species characterized by chemical variation (Culberson, 1970). Two chemical races have been reported for Antarctic samples: one produces thamnolic acid, and the other, squamatic acid (Lindsay, 1972). A third chemical race, from South Georgia, produces squamatic acid and sphaerophorin (8) (Huneck *et al.*, 1984). According to our results, *S. globosus* from Robert Island corresponds to a new chemical race which accumulates sphaerophorin (8).

Cornicularia aculeata accumulates a major compound, (+)-protolichesterinic acid (19), and usnic acid (15). A previous study of Antarctic material (Lindsay, 1973) reports the presence of (+)-protolichesterinic acid (19); for samples collected in other geographic areas the same compound have been reported, as well as rangiformic acid and cornicularin (Culberson, 1969).

Gyrophoric (10), lecanoric (7), and variolaric (14) acids are frequent in the genera *Ochrolechia* and *Placopsis*, belonging to the family Pertusariaceae (Culberson, 1969; Culberson, 1970; Culberson *et al.*, 1977). Lecanoric acid (7) is the depside corresponding to the tridepside gyrophoric acid (10) which derives from three orsellinic acids units, related to orcinol. Both compounds often coexist; in *Parmelia pulla* chemosyndromes lecanoric acid (7) is not produced if gyrophoric acid (10) is absent (Culberson *et al.*, 1977). Nevertheless, a very small amount of lecanoric acid (7) (0.005%) was isolated from *Ochrolechia antarctica*; this species gave variolaric acid (14) as the major product, as well as usnic acid (15); gyrophoric acid (10) was not detected. These metabolites had been reported for samples of *O. antarctica* and *O. frigida* (Culberson *et al.*, 1977). In our *O. frigida* material gyrophoric acid (10) is present, as well as atranorin (1), usnic acid (15) and zeorin (17). Usnic acid (15) is reported here for the first time as a constituent of an *Ochrolechia* species.

Gyrophoric acid (10) was isolated from *Placopsis contortuplicata*; this is also the major compound found in isolated cephalodia. Gyrophoric (10) and lecanoric (7) acids had been reported for this species (Culberson *et al.*, 1977).

The number of lichen compounds reported for taxa of the genus *Umbilicaria* is small. Most of

Table 1
COMPOSITION AND CONTENTS (% DRY WEIGHT) OF THE SECONDARY METABOLITES
IN 17 SPECIES OF ANTARCTIC LICHENS.

Species	Compounds																			
	Atranorin	Chloroatranorin	Brialmontin 1	Brialmontin 2	Salazinic acid	Stictic acid	Lecanoric acid	Sphaerophorin	2'-O-methylhiascic acid	Gyrophoric acid	α -collatolic acid	Alectoronic acid	Lobaric acid	Variolaric acid	(+)-Usnic acid	Porphyritic acid methyl ester	Zeorin	Ergos terolperoxide	(+)-Protolichesterinic acid	
<i>Buellia cladocarpiza</i>	0.980																			
<i>Catillaria carymbosa</i>	0.160	0.030						0.150												
<i>Cornicularia aculeata</i>															0.002				0.860	
<i>Lecanora atra</i>	0.020									0.020	0.020									
<i>Lecania brialmontii</i>			0.050	0.020																
<i>Ochrolechia antarctica</i>							0.005							0.220	0.002					
<i>O. frigida</i>	tr									0.760					0.030		tr			
<i>Parmelia saxatilis</i>	0.070				0.280										0.005		0.002			
<i>Pannaria hookeri</i>	tr																		0.012	
<i>Placopsis contortuplicata</i>										0.680										
<i>Psoroma hypnorum</i>																				0.009
<i>P. tenue var tenue</i>	0.003															0.130				
<i>Ramalina terebrata</i>															0.170					
<i>Rhizoplaca aspidophora</i>						0.008									1.000		1.130			
<i>Sphaerophorus globosus</i>								0.160							0.002					
<i>Stereocaulon alpinum</i>	0.302											0.460			tr			tr		
<i>Umbilicaria antarctica</i>	0.030									0.920					0.030				0.007	

tr = traces

the species studied accumulate gyrophoric acid (10); less frequent substances are lecanoric (7), umbilicic, stictic (6) and norstictic acids (Culberson, 1969; Culberson 1970; Culberson *et al.*, 1977). Gyrophoric acid (10) and triglycerides had been reported for species collected on the South Orkneys (Huneck *et al.*, 1984). Our material gave gyrophoric acid (10) as the major product, as well as small amounts of atranorin (1) and usnic acid (15).

Catillaria corymbosa, *Lecania brialmontii* and *Pannaria hookeri* had not been analyzed before. *C. corymbosa* yielded 2'-O-methylhiassic acid (9), an orcinol tridepside related to hiassic acid reported (Vinet, 1987); atranorin (1) and chloroatranorin (2) were also isolated from this species. In two members of the genus *Catillaria*—*C. griffithii* and *C. reichertiana*— atranorin (1) and usnic acid (15) had been detected (Culberson *et al.*, 1977).

The chemistry of the genus *Lecania* is almost unknown. The only existing reference (Follmann and Huneck, 1970) reports a neutral compound, mp = 163-164°, the structure of which was not established, isolated from *L. aispopila*. From *L. brialmontii* two compounds, brialmontin 1 (3) and brialmontin 2 (4), were isolated. These belong to a small group of polysubstituted depsides (Bruun, 1971; Huneck, 1984).

Pannaria hookeri accumulates atranorin (1) and ergosterol peroxide (18). *Pannaria*, as well as *Psoroma*, belong to the family Pannariaceae, whose members characteristically synthesize biogenetically related chlorinated depsidones derived from β -orcinol (Elix *et al.*, 1982; Piovano *et al.*, 1985). One of the members of this biogenetic series is pannarin which has been reported for *P. leucostictoides* and, together with atranorin (1), for *P. pityrea* (Culberson, 1969; Culberson, 1970). Neither in *P. hookeri* nor in both the *Psoroma* taxa studied—*P. hypnorum* and *P. tenue* var-*tenue*— have pannarin or other chlorinated depsidones been detected. From *P. hypnorum* only ergosterol peroxide (18) has been isolated, and in a very low concentration (0.009%); and from *P. tenue*, atranorin (1) and porphyritic acid methyl ester (16), a dibenzofuran derivative. This last compound has been reported for *P. hypnorum/palaceum* aggr., and for *P. tenue* (Renner, *et al.*, 1981).

CONCLUSIONS

The specific secondary metabolite spectrum in lichens is broad in the Antarctic species studied: depsides, depsidones, dibenzofurans and (+)-usnic acid (15) are present in these species. *Psoroma hypnorum* is exceptional in that it accumulates just one extractable compound, namely ergosterol peroxide (18), and only in very low concentration.

Yields, in general, are lower than those obtained from lichens of other geographic areas (Culberson, 1969; Culberson, 1970; Culberson *et al.*, 1977). Usnic acid (15) and zeorin (17), a compound not considered specific for lichens, reach their highest concentrations in *Rhizoplaca aspidophora*, with 1% and 1.13%, respectively, of the dry weight of the lichen. The highest yield of atranorin (1), 0.98%, was obtained from *Buellia cladocarpiza*.

The stressful conditions of Antarctica, as regards temperature and luminosity, might determine the low concentration of these compounds due to limited synthesis, or to the use of the metabolites as energy sources it has been reported that the lichen phenols are degraded in the interior of thalli so as to be able to enter the energy cycle when lichens are subjected to nutrient stress (Vicente *et al.*, 1980).

This study adds to the knowledge of the diversity of lichen metabolites, with the isolation and structure determination of brialmontins 1 (3) and 2 (4) which are accumulated by *Lecania brialmontii*, and of 2'-O-methylhiassic acid (9) from *Catillaria corymbosa*.

Chemical races are frequent in lichens. Even considering importance of the testing of individual thalli for the verification of bulk extractions (Imshaug and Harris, 1971), undoubtedly *Sphaerophorus globosus* from Robert Island, which only accumulates sphaerophorin (8), corresponds to a new chemical race of the species, since no other related products were isolated, or unrelated substances which are present in specimens from other geographic areas.

ACKNOWLEDGEMENTS

The authors thank the Instituto Antártico Chileno for its valuable contribution to the funding of this research through Project INACH 1986/1987.

We are also grateful to Mss. C. Miranda, V. Zúñiga and H. Arancibia, Natural Products Chemistry Lab., U. F. Santa María, for technical assistance; to Dr. E. Valdés, U. F. Santa María, Dr. B. Didyk, RPC, Concón, Chile; Dr. E. Gros, U. Buenos Aires, Argentina; and Dr. M. Nicoletti, U. Rome, Italy, for recording spectra; finally, to Prof. G. Guzmán, U. Playa Ancha, Chile, for determining some of the lichen species.

REFERENCES

- ASAHINA, I. and S. SHIBATA, 1954. Chemistry of Lichen Substances. Japan Society for the Promotion of Science; Ueno, Tokyo, Japan. 1-224. pags.
- BARTON, D. H. R., and C. H. ROBINSON, 1954. The stereo-specificity of carbanion reduction processes. J. Chem. Soc.: 3045-3051.
- BOLL, P. M., 1968. Naturally occurring lactones and lactams. Acta Chem. Scand. 22: 3245-3250.
- BRUNN, T., 1971. Phenarctin, a fully substituted depside from *Nephroma arcticum*. Acta Chem. Scand. 25: 2831-2835.
- BRYAN, A. J., J. A. ELIX and S. NORFOLK, 1976. Synthesis of orcinol tridepsides and aphtosin, an orcinol tetradepside. Aust. J. Chem. 29: 1079-1086.
- CONNOLY, J. D., A. A. FREER and S. HUNECK, 1984. Crystal structure: of (-)-placodiolic acid, a dibenzofuran derivative from the lichen *Rhizoplaca chrysoleuca*. Phytochemistry 23,3: 702.
- CULBERSON, Ch. F., 1969. Chemical and Botanical Guide to Lichen Products. Univ. of North Carolina Press-Chapel Hill, 1-628 pags.
- CULBERSON, Ch. F., 1970. Supplement to Chemical and Botanical Guide to Lichen Products. Bryologist 73: 177-377.
- CULBERSON, Ch. F., W. L. CULBERSON and A. JOHNSON, 1977. Second Supplement to Chemical and Botanical Guide to Lichen Products. Amer. Bryol. Lichenol. Soc. Missouri Bot. Garden, St. Louis, 1-400. pags.
- CULBERSON, Ch. F., W. L. CULBERSON and T. L. ESSLINGER, 1977. Chemosyndromic variation in the *Parmelia pulla* group. Bryologist 80: 125-135.
- CULBERSON, W. L., 1970. Chemosystematics and ecology of lichen forming-fungi. Annual Review of Ecology and Systematics 1: 153-170.
- ELIX, J. A., B. A. FERGUSON and M. V. SARGENT, 1974. The structure of alectoronic acid and related lichen metabolites. Aust. J. Chem. 27: 2403-2408.
- ELIX, J. A. 1975. Synthesis of para- β -orcinol. Aust. J. Chem. 28: 1113-1115.
- ELIX, J. A., L. LAJIDE and D. J. GALLOWAY, 1982. Metabolites from the lichen genus *Psoroma*. Aust. J. Chem. 35: 2325-2329.
- ELIX, J. A., M. V. SARGENT, Y. SHIMIZU, D. A. TAYLOR and A. A. WHITTON, 1984. Progress in the chemistry of Organic Natural Products. Ed. Springer Verlag, Wien, 45: 103-219.
- ELIX, J. A., K. L. GAUL, P. W. JAMES and O. W. PURVIS, 1987. Three new lichen depsidones. Aust. J. Chem. 40: 417-423.

- FOLLMANN, G. und S. HUNECK, 1970. Mitteilungen über Flechteninhaltsstoffe LXXVII. Ergänzende Flechtenanalysen. *Willdenowia* 6:3-12.
- GONZALEZ, A. G., J. D. MARTIN y M. MELIAN MARTIN. 1973. Componentes químicos en Líquenes. II. Estudio de la *Ramalina tingitana*. *Anales de Química* 69: 807-809.
- HERTEL, H. und C. LEUCKERT, 1969. Über Flechtenstoffe und Systematik einiger Arten der Gattungen *Lecidea*, *Placopsis*, und *Trapelia* mit c⁺ rot. reagierenden Thallus. *Willdenowia* 5: 369-383.
- HIRAYAMA, T., F. FUJIKAWA, I. YOSIOKA and I. KITAGAWA, 1975. Vittatolic acid, a new depsidone isolated from the lichen *Hypogymnia vittata* (Ach.) Gas. Chem. Pharm. Bull. 23, 3: 693-695.
- HIRAYAMA, T., F. FUJIKAWA, I. YOSIOKA and I. KITAGAWA, 1976. On the constituents of the lichen in the Genera *Menegazzia*. Menegazzaic acid, a new depsidone from *Menegazzia asahine* (Yas, ex Zahlbr.) Sant. and *Menegazzia terebrata* (Hoffm.) Mass. Chem. Pharm. Bull. 24, 10: 2340-2344.
- HUNECK, S., 1984. Pseudocypbellarins A and B., two fully substituted depsides from the lichen *Pseudocypbellaria endochrysea*. *Phytochemistry* 23: 431-434.
- HUNECK, S. und G. FOLLMANN, 1970. Mitteilungen über Flechteninhaltsstoffe LXXV. Zur Phytochemie und Chemotaxonomie der Buelliaceae. *Biochem. Physiol. Pflanzen (BPP)*, Bd. 161: 191-214.
- HUNECK, S., C. DIERASSI, B. BECHER, M. BARBER, M. VON ANDENNE, K. STEINFELDER und R. TUMBER, 1968. Flechteninhaltsstoffe XXXI. Massenspektrometrie und ihre Anwendung auf strukturelle und stereochemische Probleme. CXXIII. Massenspektrometrie von Depsonen, Dibenzofuranen und Diphenylbutadienen mit positiven und negativen Ionen. *Tetrahedron* 24: 2707-2755.
- 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 Antarctic. *Journ. Hattori Bot. Lab.* 56: 461-480.
- IMSCHAUG, H. A. and R. C. HARRIS, 1971. Notes on the taxonomic relationships and chemistry of *Ramalina subdecipiens*. *J. Stein. Bryologist* 74: 369-371.
- INGOLFSDOTTIR, K., P. J. HYLANDS and Y. SOLBERG, 1986. Structure of vesuvianic acid from *Stereocaulon* species. *Phytochemistry* 25, 2: 550-553.
- LAMB, M. I., 1964. Antarctic Lichens. I. The Genera *Usnea*, *Ramalina*, *Himantormia*, *Alectoria*, *Cornicularia*. *Br. Antarct. Surv. Bull.*, 38: 1-35.
- LAMB, M. I., 1970. Antarctic terrestrial plants and their ecology. In: M. W. Hogate (ed.). *Antarctic, Ecology* 2: 733-751. New York.
- LINDSAY, D. C. 1972. Notes on Antarctic Lichens. VII. The genera *Sphaerophorus* Pers. *Br. Antarct. Surv. Bull.* 28: 43-48.
- LINDSAY, D. C., 1973. Notes on Antarctic Lichens. VII. The Genera *Cetraria* Hoffm., *Hypogymnia* (Nyl.) Nyl., *Menegazzia* Massal., *Parmelia* Ach. and *Plastimatia* Culb. et C. S. *Br. Antarct. Surv. Bull.* 36: 105-114.
- LINDSAY, D. C., 1978. The role of lichens in Antarctic Ecosystems. *Bryologist* 8,2: 268-276.
- O'DONOVAN, D. G., G. ROBERTS and M.F. KEOGH, 1980. Structure of the β -orcinol depsidones connorstictic and consalazinic acids. *Phytochemistry* 19: 2497-2499.
- PIOVANO, M., M. I. GARRIDO, V. GAMBARO, J. A. GARBARINO and W. QUILHOT, 1985. Studies on Chilean Lichens. VIII. Depsidones from *Psoroma* species. *J. Nat. Prod.* 48: 854-855.
- RANA, N. M. and M. V. SARGENT, 1975. Structure of the lichen depsidone variolaric acid. *J. C. S. Perkin I*: 1992-1995
- RENNER, B., A. HENSSEN und E. GERTSNER, 1981. Pannarsäure und Porphyrilsäuremethylester - Sekundärstoffe der Flechten- gattung *Psoroma*. *Z. Naturforsch.* 36c: 893-895.
- TALVITÄ, A., 1983. Reassignment of the ¹H-NMR signals of the methyl groups. *Finn. Chem. Lett.* 8: 225-230.
- VARENTE, C., 1976. Fisiología de las sustancias líquénicas. Alhambra, Madrid, 1-162. págs.

- VICENTE, C., J. L. RUIZ and M. P. ESTÉVEZ, 1980. Mobilization of usnic acid in *Evernia prunastri* under critical conditions of nutrient availability. *Phyton* 39: 15-20.
- VINET, C., 1987. Chemical Study of Antarctic Lichens. Thesis, Universidad de Valparaíso, Chile 1-85.
- WIELAND, P. und V. PRELOG, 1947. Über die Isolierung von Ergosterin, Ergosterin-palmitat und Ergosterin-peroxyd aus dem Mycel von *Aspergillus fumigatus* mut. *helvola*, Yuill. *Helv. Chim. Acta* 30: 1028-1030.
- WOJCIECHOWSKI, Z. A., L. J. GOAD and T. W. GOODWIN, 1973. Sterols of the Lichen *Pseudoevernia furfuracea*. *Phytochemistry* 12: 1433-1436.
- YOSIOKA, I., M. YAMAKI and I. KITAGAWA, 1966. On the triterpene constituents of a Lichen, *Parmelia entotheiochroa* Hue: zeorin, leucotylin, leucotylic acid and five new related triterpenoids. *Chem. Pharm. Bull.* 7: 804-807.

Recibido: 16.05.88. Aprobado: 24.06.88