

STUDIES ON CHILEAN LICHENS, X.¹ THE PHENOLIC CONSTITUENTS OF *PROTOUSNEA MAGELLANICA*

JUAN A. GARBARINO, MARIA C. CHAMY, VICENTE GAMBARO,

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

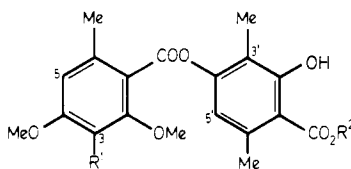
WANDA QUILHOT, OSCAR NARANJO, and EDUARDO BOLT

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

The lichen genus *Protousnea*, distributed in South America and the Falkland Islands, belongs to the Parmeliaceae family with approximately seven species (2). In Chile they are commonly known as "barbas de viejo." As a continuation of our chemical investigations of the genus *Protousnea* (3), we have studied *Protousnea magellanica* (Mont.) Krog, which has only been analyzed by tlc. In that study usnic and divaricatic acids and a substance named "unknown $\pm R^1$ " were reported to be present (2). However, neither divaricatic acid nor other orcinol depside derivatives were found in our research. In this paper the isolation and characterization of a new β -orcinol depside derivative, 2-O-methylsquamic acid [2], and the known metabolites usnic acid and diffractaic acid [1] are described from *P. magellanica*. The fact that divaricatic acid and 1 exhibited the same R_f, probably led to the erroneous conclusion by Krog (2).

The Me₂CO lichen extract was subjected to column chromatography on Si gel using mixtures of petroleum ether, EtOAc, and HCOOH to afford (+)-usnic acid (4), diffractaic acid [1] (4), and 2-O-methylsquamic acid [2].

The identification of usnic acid and 1 was confirmed by direct comparison with authentic samples. The structure of the third compound [2], was deduced by spectroscopic analysis of its dicar-



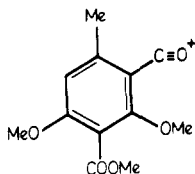
| | R ¹ | R ² |
|-----------|--------------------|----------------|
| 1 | Me | H |
| 1a | Me | Me |
| 2 | CO ₂ H | H |
| 3 | CO ₂ Me | Me |

bomethoxyl derivative, 3. In fact, the ¹H-nmr spectrum of fractions 57-65 from the chromatography suggested the presence of one major compound with two methoxy and three methyl aromatic groups. Treatment of these fractions with ethereal CH₂N₂ and further purification on a Si gel column gave 3. The dicarboxylic nature of this new lichen substance was evident from the ¹H-nmr spectrum of 3, which showed signals due to the presence of two additional methoxyl groups assigned to two ArCOOMe functions. The spectrum also showed a one-proton singlet at δ 12.00 assignable to one phenolic proton associated with an ester group, and two singlets at δ 6.57 and 6.53 were due to H-5 and H-5' of a β -orcinol depside derivative, respectively. Finally, comparison of this spectrum with that of methyl diffractate (see Experimental section) indicated that 3 lacked the Me-3 or Me-3' group. However, the base mass spectral fragment ion at *m/z* 237, obtained by rupture of the diphenyl ester linkage that occurs in most depsides (5), allowed

¹For Part IX, see Fiedler *et al.* (1).

us to postulate the partial structure **4** for this fragment.

This evidence led to the assignment of structures **2** and **3** as 2-*O*-methylsquamic acid and its dicarbomethoxyl derivative.



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EXPERIMENTAL

GENERAL METHODS.—Mps (uncorrected) were determined on a Kofler hot plate. Optical rotation was measured in CHCl_3 with a Schmidt-Haensch polarimeter. ^1H -nmr spectra were recorded at 60 MHz (Varian T-60) in CDCl_3 or CD_3COCD_3 with TMS as internal standard. Eims were recorded by direct inlet with 70 eV ionization in a Varian MAT CH-7 apparatus. Ir spectra were recorded as KBr pellets (Perkin-Elmer model 683). Tlc was performed on Si gel from E. Merck.

PLANT MATERIAL.—*P. magellanica* growing on *Nothofagus obliqua*, was collected in Laguna Icalma, IX Region, Chile, in November 1985, and identified by G. Guzman (Universidad de Playa Ancha, Valparaíso). Voucher specimens are deposited at the Herbarium of Escuela de Química y Farmacia, Universidad de Valparaíso.

EXTRACTION AND ISOLATION.—The air-dried lichen thalli (625 g) were triturated and extracted with Me_2CO at room temperature (48 h). The Me_2CO extract was concentrated in vacuum to yield 42 g of crude material. A portion of this crude material (20 g) was subjected to Si gel cc (500 g, HF_{254} for tlc) using mixtures of $\text{C}_6\text{H}_5\text{-EtOAc-HCOOH}$ (85:12.5:2.5, 2 liters; 76:21:3, 2 liters; and 65:32:3, 2 liters). Fractions of 100 ml were taken and combined based upon tlc monitoring. Fractions 8-12, containing a pure compound, were mixed and afforded (+)-usnic acid which was identified by direct comparison (mp, $[\alpha]_D$, tlc, and ^1H nmr) with an authentic sample. Fractions 29-41, containing another pure compound, were mixed and afforded diffractaic acid [**1**] which was identified by direct comparison (mp, ^1H nmr, ms) with an authentic sample. Further confirmation of the identity of **1** was ob-

tained by comparison of its ^{13}C nmr with that previously reported in the literature (6).

METHYL DIFFRACTATE [1a].—After addition of ethereal CH_2N_2 , **1** (200 mg) was transformed to **1a**. White crystals; ^1H nmr δ 11.90 (1H, s, OH-2'), 6.60 (1H, s, H-5), 6.58 (1H, s, H-5'), 3.98 (3H, s, Ar-COOMe), 3.92 (6H, s, 2 \times Ar-OMe), 2.56 (3H, s, Me-6), 2.48 (3H, s, Me-6'), 2.20 (6H, s, Me-3 and Me-3'); eims (probe) 70 eV, m/z (rel. int.) 388 (M^+ , 53), 193 (100), 179 (45), 178 (73), 164 (65), 150 (73), 136 (63), 121 (45). Its ^{13}C nmr was superimposable with that of methyl diffractate (6). Finally, fractions 57-65 gave a mixture containing **2** (60 mg).

PURIFICATION OF FRACTIONS 57-65.—The ^1H -nmr spectrum of these fractions suggested the presence of one major compound: ^1H nmr (CD_3COCD_3) δ 6.80-6.60 (*m*, Ar-H), 3.90 (3H, s, Ar-OMe), 3.83 (3H, s, Ar-OMe), 2.53 (3H, s, Ar-Me), 2.40 (3H, s, Ar-Me), 2.17 (3H, s, Ar-Me). These fractions, after treatment with ethereal CH_2N_2 , were mixed and chromatographed on a Si gel column (20 g) and eluted with $\text{C}_6\text{H}_5\text{-EtOAc-HCOOH}$ (76:21:3), yielding pure **3** (22 mg). Amorphous powder (*n*-hexane/EtOAc); ir ν max 1730, 1650, 1600, 1260, 1110, 1100, 840 cm^{-1} ; ^1H nmr δ 12.00 (1H, s, OH-2'), 6.57 (1H, s, H-5), 6.53 (1H, s, H-5'), 3.95 (3H, s, Ar-CO₂Me), 3.92 (3H, s, Ar-COOMe), 3.88 (6H, s, 2 \times Ar-OMe), 2.56 (3H, s, Me-6), 2.50 (3H, s, Me-6'), 2.20 (3H, s, Me-3'); eims (probe) 70 eV, m/z (rel. int.) 432 (M^+ , 11), 401 (71), 237 (100), 223 (55), 210 (3), 196 (6), 195 (59), 164 (78), 163 (61), 136 (55), 135 (76).

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