

STUDIES ON CHILEAN LICHENS, VII. ¹ THE PHENOLIC CONSTITUENTS OF *PROTUSNEA MALACEA*

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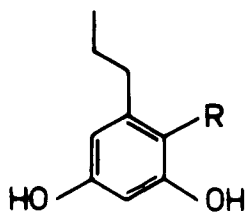
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The lichen genus *Protusnea* (Parmeliaceae) comprises approximately seven species, distributed in the *Nothofagus* forests of Chile and Patagonia and in the heathland of Tierra del Fuego and the Falkland Islands (2).

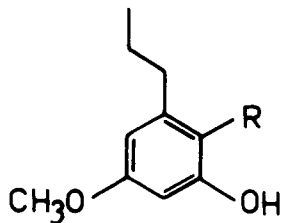
Thin-layer chromatographic studies (2) revealed the presence of usnic acid in the cortex of all species; in addition, the most common medullary substance was the orcinol depside, divaricatic acid. In *Protusnea malacea* (Stirt) Krog in particular, there has been reported by tlc, beside the aforementioned metabolites, sekikaic acid (3), previously referred to as "unknown R₁" (2).



1 R=COOH
3 R=H

The other two compounds are divaric acid (1) and ethyl divaricatinatate (2). This is the first report of the natural occurrence of 1 and 2.

A petroleum ether extract of *P. malacea* revealed a mixture of four compounds by tlc. Fractional crystallization with CHCl₃-EtOH (1:1) yielded (+)-usnic acid. The soluble fraction was concentrated and treated with *n*-hexane-CHCl₃ (1:1). The precipitate was identified as divaricatic acid. The identification of these two substances was confirmed by direct comparison with authentic samples. Preparative tlc of the soluble fraction afforded three com-



2 R=COOEt
4 R=COOH

In order to obtain usnic acid, which is a possible starting material in the synthesis of chemotherapeutic products (4), and to examine the rest of the secondary metabolites present in *P. malacea*, we analyzed specimens collected in central Chile.

This paper describes the isolation and characterization of four phenolic compounds. Two of these substances were identified as usnic and divaricatic acids.

pounds. The band with intermediate R_f was readily identified as additional divaricatic acid; the other compounds were designed as 1 and 2.

The mass spectrum of compound 1, from the band of lower R_f, exhibited a molecular ion at *m/z* 196 (C₁₀H₁₂O₄). In the ¹H-nmr spectrum, signals were exhibited corresponding to two aromatic protons, a doublet at δ 6.30 (*J*=2.5 Hz), and a doublet at δ 6.37 (*J*=2.5 Hz); a broad triplet at δ 2.97 (2H), a multiplet at δ 1.67 (2H), and a triplet at

¹For Part VI, see Quilhot *et al.* (1).

δ 0.93 (3H) suggested an *n*-propyl group linked to an aromatic ring. Additionally, the ^1H -nmr spectrum also exhibited a broadened singlet at δ 9.60 (3H) due to two phenolic and one acidic protons. The nature of these groups was also evident from the ir spectrum (3380, 1630 cm^{-1}). On the basis of these data, typical of a substituted divarinol nucleus (3), structure **1** was proposed and corresponds to divaric acid. This is the first report of the natural occurrence of divaric acid, which has been previously produced as a synthetic product (5). The structure of **1** was confirmed by partial synthesis from divaric acid.

The structure of the fourth compound, **2**, obtained from the band at higher Rf, was deduced by comparison of its spectral data with that of **1**. The ms of **2** suggested a molecular formula $\text{C}_{13}\text{H}_{18}\text{O}_4$ (M^+ , 238). The ^1H -nmr spectrum exhibited a one proton singlet at δ 11.90 assignable to one phenolic proton associated with an ester group; a doublet at δ 6.70 (1H, $J=3.0$ Hz) and a doublet at δ 6.63 (1H, $J=3.0$ Hz) were due to H-5 and H-3 of a divaric acid derivative, respectively. The other signals revealed the presence of an ethyl ester group; δ 4.43 (2H, q) and 1.43 (3H, t), and an *n*-propyl group attached to an aromatic ring: δ 2.90 (2H, t), 1.67 (2H, m), and 0.97 (3H, t). Additionally, a sharp three proton singlet at δ 3.80 indicated the presence of a methoxy group. From the above spectral data, **2** was identified as ethyl divaricatinatate. Acidic hydrolysis of **2** afforded divaricatinic acid (**4**) (5). Compound **2** was synthesized by esterification of divaric acid with EtOH. The spectral and physical data of this new compound were in complete agreement with the natural product.

The only previous study that has reported the structure of a monocyclic derivative ethyl ester related to **2** was a paper describing the structure of ethyl orsellinate (6). No evidence of artifact formation was obtained after submitting

a pure sample of divaric acid to the same treatment as described in the work-up of these compounds.

EXPERIMENTAL

GENERAL METHODS.—Mps (uncorrected) were determined on a Kofler hot plate. Optical rotations were measured in CHCl_3 with a Schmidt-Haensch polarimeter. ^1H -nmr spectra were recorded at 60 MHz (Varian T-60) and 100 MHz (Varian XL-100) in CDCl_3 with TMS as internal standard. Eims was 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 silica gel from E. Merck.

PLANT MATERIAL.—*P. malacea* collected on *Nothofagus pumilio*, Termas de Chillán, Chile, in April, was identified by Dr. H. Krog (Botanical Museum, Oslo, Norway); voucher specimens are deposited at the Herbarium of the School of Chemistry and Pharmacy, University of Valparaíso, Chile.

EXTRACTION AND ISOLATION.—The air-dried lichen thalli (500 g) were triturated and extracted with petroleum ether 40-60° in a Soxhlet for 12 h. The extract was concentrated at reduced pressure to yield 13.8 g (2.8%) of crude material. This residue was subjected to a fractional crystallization with CHCl_3 -EtOH (1:1), at room temperature. On standing, a yellow solid deposited (3.1 g, 0.62%), was removed by filtration, and was identified as (+)-usnic acid by direct comparison (mp, $[\alpha]_D$, tlc, ir, ^1H nmr, ms) with an authentic sample.

The CHCl_3 -EtOH solution was concentrated and treated with *n*-hexane- CHCl_3 (1:1). On standing, a white solid was produced (6.8 g, 1.36%), was removed by filtration and identified as divaricatinic acid by direct comparison (mp, tlc, ir, ^1H nmr, ms) with an authentic sample.

The *n*-hexane- CHCl_3 soluble fraction was also concentrated and showed a mixture of three components by tlc. This fraction, after preparative tlc on Si gel eluted with C_6H_6 -EtOAc-HCOOH (83:15:2) yielded ethyl divaricatinatate (**2**) (Rf: 0.84), divaricatinic acid (Rf: 0.56), and divaric acid (**1**) (Rf: 0.31).

ETHYL DIVARICATINATE (2).—Compound **2** (0.25 g, 0.05%) was isolated as white needles; mp 41-42° (*n*-hexane); ir ν max 3400, 2960, 2880, 1650, 1615, 1580, 1460, 1250, 1160, 710 cm^{-1} ; ^1H nmr δ 0.97 (3H, t, $J=7.0$ Hz, H-3'), 1.43 (3H, t, $J=8.0$ Hz, O- CH_2 - CH_3), 1.67 (2H, m, H-2'), 2.90 (2H, t, $J=8.0$ Hz, H-1'), 3.80 (3H, s, OCH₃-4), 4.43 (2H, q, $J=8.0$ Hz, O- CH_2 - CH_3), 6.63 (1H, d, $J=3.0$ Hz, H-3), 6.70 (1H, d, $J=3.0$ Hz, H-5), 11.90 (1H, s,

OH); eims (probe) 70 eV, m/z (rel. int.) 238 (M^+ , 40), 193 ($M-C_2H_5O$, 35), 192 ($M-CH_2H_5OH$, 100), 177 (192-Me, 9), 164 (35), 135 (41).

DIVARIC ACID (1).—Compound **1** (0.36 g, 0.07%) was isolated as white prisms; mp 157-159° (EtOAc- $CHCl_3$) [lit. (5) mp 169°]; ir ν max 3380, 2960, 2880, 1630, 1615, 1580, 1465, 1280, 1240, 800, 630 cm^{-1} ; 1H nmr δ 0.93 (3H, t, $J=7.0$ Hz, H-3'), 1.67 (2H, m, H-2'), 2.97 (2H, t, $J=8.0$ Hz, H-1'), 6.30 (1H, d, $J=2.5$ Hz, H-3), 6.37 (1H, d, $J=2.5$ Hz, H-5), 9.60 (3H, br s, 2 phenolic H and ArCOOH which disappears on D_2O exchange); eims (probe) 70 eV, m/z (rel. int.) 196 (M^+ , 37), 178 ($M-H_2O$, 100), 150 (19), 121 (34).

DIVARICATINIC ACID (4).—Compound **2** (20 mg) was dissolved in 10 ml MeOH containing 1 drop of H_2SO_4 . The solution was heated under reflux for 2 h. After cooling, the soln. was evaporated, $CHCl_3$ added and dried over anhydrous Na_2SO_4 . After evaporation of the solvent, 10 mg of pure divaricatinic acid (**4**) was obtained; mp 154-156° [lit. (5) mp 157°].

SYNTHESIS OF ETHYL DIVARICATINATE (2) AND DIVARIC ACID (1).—A solution of 200 mg divaricatinic acid in 60 ml of absolute EtOH was heated under reflux for 24 h. After evaporation of the solvent, the crude material showed three components by tlc. The substances were isolated by preparative tlc, Si gel; C_6H_6 -EtOAc- $HCOOH$

(85:13:2), and yielded **2** (80 mg, Rf: 0.90), and **1** (40 mg, Rf: 0.40). Both products were identical in every aspect (mp, tlc, 1H nmr) to the natural substances. A third compound (Rf: 0.30) was identified as divarinol (**3**) by 1H nmr and ir.

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