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# PHENOLICS, DEPSIDES AND TRITERPENES FROM THE CHILEAN LICHEN *PSEUDOCYPHELLARIA NUDATA* (ZAHLBR.) D.J. GALLOWAY

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## ABSTRACT

The lichen *Pseudocyphellaria nudata* is a species endemic to southern South America. From the lichen tallus, methyl orsellinate, 2-methoxy-3,6-dimethyl-4-hydroxybenzaldehyde, methyl-evernate, tenuiorin, hopan-6ß,22-diol and hopan-6α,7ß,22-triol were isolated and identified as the main lichen constituents. This is the first report of the occurrence of 2-methoxy-3,6-dimethyl-4-hydroxybenzaldehyde in lichens.

**Keywords:** *Pseudocyphellaria nudata*, lichen, monophenols, depsides, triterpenes.

## INTRODUCTION

In the cool temperate rainforests of southern South America, lichen species belonging to genus *Pseudocyphellaria* are common[1]. The genus *Pseudocyphellaria* is represented in Chile by 54 species occurring from latitude 23ºS to 56ºS. The mains constituents of the species have been identified by thin layer chromatography[1,2]. *Pseudocyphellaria nudata (*Zahlbr.) D.J. Galloway (*Lobariaceae*, lichenized Ascomycota) is endemic to southern South America. It is a robust, coriaceous lichen, with the cyanobacteria *Nosto*c as photobiont. The genus *Pseudocyphellaria* is characterized by a wide chemical diversity, including substances from the major secondary metabolism pathways. The acetate polymalonate pathway produces depsides, depsidones, and usnic acid; from the shikimic acid pathway, terphenylquinones and principally pulvinic acid derivatives are produced while the mevalonic acid pathway led to sterols and terpenoids[3]. The occurrence of triterpenoids, especially the hopane derivatives, produced also by cyanobacteria and other organisms[4], have attracted considerable interest in the organic geochemistry as indicators of sources of sedimentary organic matter and biomarkers of mature petroleum-containing layers [5,6,7]. According to Galloway[1], "the identity of the compounds appearing on thin layer chromatograms remain to be definitively characterized, and that provides an exciting opportunity for future work".

The aim of this work was the identification of the main constituents of a Chilean collection of *P. nudata* by spectroscopic means.

## EXPERIMENTAL

### *General experimental procedures*

The $^{1}H$ and $^{13}C$ NMR spectra were recorded in a Bruker AVANCE spectrometer model 400 operating at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$, using $CDCl_3$ and DMSO-$d_6$ as solvents. Chemical shifts are reported in δ ppm and coupling constants (*J*) are given in Hz. Melting points were determined on a Stuart-Scientific SMP3 apparatus. Optical rotation were measured with a sodium lamp (λ= 589 nm, D line) on a Perkin Elmer 241 digital polarimeter equipped with 1 dm cells. Column chromatography (CC) used silica gel Merck 60 G (0,032-0,063 nm), the fractions were monitored by thin-layer chromatography (TLC) used chromatoplates silica gel Merck 60 $F_{254}$. The spots were visualized under UV light (254/365 nm) and developed using $H_2SO_4$ spray reagent [1].

### *Plant material*.

*Pseudocyphellaria nudata* (Zahlbr.) D.J. Galloway (118 g) was collected from bark of *Nothofagus* spp. at 1000-1100 m altitude in Conguillio National Park (39º,39´S; 71º,43´W), Chile, in November 2005. The species was determined by W. Quilhot. Voucher specimens are deposited in the Lichen Herbarium (UV), Facultad de Farmacia, Universidad de Valparaíso

*Extraction and isolation:* The air dried lichen sample was ground, and extracted successively with chloroform and acetone (2 L), each time 72 h at room temperature. The solvents were removed under reduced pressure to give a chloroform (6.08 g) and acetone (4.50 g) extracts.

The chloroform extract was chromatographed on silica gel using a mixture of dichloromethane and ethyl-acetate with increasing polarity (49:1 and 1:49), collecting 8 ml in each fraction (260 fractions). The fractions were combined based on TLC and $^{1}H$-NMR monitoring. Fractions 5-8 were combined and reduced to dryness. Recrystallisation in $Et_2O$:MeOH (1:1) afforded an impure precipitate which was removed from the solution, and further concentration led to the isolation of a pure precipitated compound, identified as **methyl evernate** (**1**) (5.8 mg), colorless crystalline powde, mp 137-138ºC. **$^{1}H$-NMR** ($CDCl_3$): 11.57 (1H, s, OH-2'); 11.35 (1H, s, OH-2); 6.71 (1H, d, J = 2.3 Hz, H-3'); 6.60 (1H, d, J = 2.3 Hz, H-5'); 6.38 (1H, d, J = 2.7 Hz, H-5); 6.37 (1H, d, J = 2.7 Hz, H-3); 3.98 (3H, s, $CO_2Me$); 3.83 (3H, s, OMe); 2.62 (3H, s, H-8); 2.58 (3H, s, H-8'). **$^{13}C$-NMR** ($CDCl_3$): 171.7 (C-7'); 169.7 (C-7); 166.5 (C-4'); 164.8 (C-4); 164.4 (C-2); 153.9 (C-2'); 143.4 (C-6, C-6'); 116.6 (C-5'); 111.9 (C-5); 110.4 (C-1'); 108.8 (C-3'); 104.3 (C-1); 98.9 (C-3); 55.4 (OMe); 52.3 ($CO_2Me$); 24.6 (C-8); 24.2 (C-8') in accordance with literature[8].

Fractions 16-38 afforded **tenuiorin** (**2**) (1.61 g), white crystalline powder, mp 180-182 ºC. **$^{1}H$-NMR** ($CDCl_3$): 11.61 (1H, s, OH-2''); 11.34 (1H, s, OH-2); 11.15 (1H, s, OH-2'); 6.78 (1H, d, J = 1.5 Hz, H-3'); 6.73 (1H, d, J = 1.5 Hz, H-3''); 6.69 (1H, d, J = 1.5 Hz, H-5'); 6.61 (1H, d, J = 1.5 Hz, H-5''); 6.38 (2H, s, H-3 + H-5); 3.99 (3H, s, $CO_2Me$); 3.84 (3H, s, OMe); 2.70 (3H, s, H-8'); 2.64 (3H, s, H-8); 2.59 (3H, s, H-8''). **$^{13}C$-NMR** ($CDCl_3$): 171.7 (C-7''); 169.6 (C-7)*; 169.3 (C-7')*; 166.6 (C-4')$^{\#}$; 165.2 (C-4'')$^{\#}$; 164.9 (C-4); 164.4 (C-2); 154.8 (C-2')$^{\dagger}$; 153.6 (C-2'')$^{\dagger}$; 143.8 (C-6'); 143.6 (C-6''); 143.4 (C-6); 117.2 (C-5'); 116.4 (C-5''); 112.0 (C-5); 110.7 (C-1''); 109.6 (C-1'); 109.2 (C-3'); 108.7 (C-3''); 104.2 (C-1); 98.9 (C-3); 55.4 (OMe); 52.4 ($CO_2Me$); 24.6 (C-8); 24.5 (C-8'); 24.2 (C-8'').$^{\dagger}$, *, $^{\#}$ assignment may be interchanged, in accordance with literature[8].

From fractions 42-50 a residue was obtained which was recrystallized in $Et_2O$:MeOH (1:1) to afford **methyl orsellinate** (**3**) (16 mg)**,** colorless crystalline powder, mp 142-143 ºC.**$^{1}H$-NMR** ($CDCl_3$): 11.78 (1H, s, OH-2); 6.28 (1H, d, J = 2.1 Hz, H-5); 6.23 (1H, d, J = 2.1 Hz, H-3); 5.63 (1H, bs, OH-4); 3.92 (3H, s, $CO_2Me$); 2.48 (3H, s, Me-8). **$^{13}C$-NMR** ($CDCl_3$): 172.1 (C-7); 165.3 (C-4); 160.3 (C-2); 144.0 (C-6); 111.4 (C-5); 105.6 (C-1); 101.3 (C-3); 51.9 ($CO_2Me$); 24.2 (C-8) in accordance with literature[8].

Fractions 78-92 yielded the compound **hopan-7ß,22-diol (4)** (13.1 mg), colorless crystalline powder, mp 230-231 ºC, $[\alpha]_D^{25}$ = +26.3º (c 0.54 $CHCl_3$). **$^{1}H$-NMR** ($CDCl_3$): 3.89 (1H, dd, J = 5.0, 10.9 Hz, H-7); 2.23 (1H, m, H-21); 1.21 (3H, s, Me-29); 1.17 (3H, s, Me-30); 1.04 (3H, s, Me-28); 0.98 (3H, s, Me-27); 0.86 (3H, s, Me-23); 0.80 (3H, s, Me-25); 0.79 (3H, s, Me-24); 0.77 (3H, s, Me-28). **$^{13}C$-NMR** ($CDCl_3$): 73.8 (C-22); 73.5 (C-7); 53.5 (C-17); 53.2 (C-5); 51.1 (C-21); 50.5 (C-9); 50.1 (C-13); 47.7 (C-8); 44.1 (C-18); 43.4 (C-14); 41.8 (C-3), 41.4 (C-19); 40.2 (C-1); 38.4 (C-15); 37.4 (C-10); 33.2 (C-23); 33.0 (C-4); 30.8 (C-30); 29.6 (C-6); 28.8 (C-29); 26.4 (C-20); 24.1 (C-12); 22.3 (C-16); 21.5 (C-24); 20.7 (C-11); 18.6 (C-2); 17.7 (C-27); 16.1 (C-28); 15.5 (C-25); 11.2 (C-26) in accordance with literature[8].

Fractions 148-187 yielded the compound **hopan-6α,7ß,22-triol (5)** (1.46 g), colorless crystalline powder, mp 226-227 ºC, $[\alpha]_D^{25}$ = +46.9º (c 0.62 Py). **$^{1}H$-NMR** ($CDCl_3$): 3.72 (1H, dd, J = 8.6, 10.7 Hz, H-6); 3.57 (1H, d, J = 8.6 Hz, H-7); 2.23 (1H, m, H-21); 1.20 (3H, s, Me-29); 1.18 (3H, s, Me-30); 1.15 (3H, s, Me-23); 1.05 (6H, s, Me-26 + Me-27); 1.00 (3H, s, Me-24); 0.88 (3H, s, Me-25); 0.77 (3H, s, Me-28). **$^{13}C$-NMR** ($CDCl_3$): 79.4 (C-7); 73.8 (C-22); 73.3 (C-6); 56.8 (C-5); 53.5 (C-17); 51.0 (C-21); 49.9 (C-13); 49.6 (C-9); 47.3 (C-14); 44.0 (C-18); 43.6 (C-19); 43.5 (C-8); 41.4 (C-3); 40.3 (C-1, C-15); 38.8 (C-10); 36.3 (C-23); 33.6 (C-4); 30.8 (C-30); 28.8 (C-29); 26.4 (C-20); 23.9

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(C-12); 22.2 (C-16); 22.0 (C-24); 20.7 (C-11); 18.5 (C-2); 17.7 (C-27); 16.7 (C-25, C-26); 16.1(C-28) in accordance with literature[8].

The acetone extract was chromatographed on silica gel using a mixture of dichloromethane and ethyl-acetate with increasing polarity (48:2 and 2:48), collecting 8 ml in each fraction (230 fractions). Fractions 8-14 afforded **tenuiorin** (**3**) (248 mg) and the fractions 120-131 yielded compound **hopan-6α,7ß,22-triol (5)** (284 mg)**,** both compound previously found in the chloroform extract. Fractions 165-183 were combined and reduced to dryness. Recrystallisation in $Et_2O$:MeOH (1:1) afforded an impure precipitate which was removed from the solution, and further concentration led to isolation of a pure precipitated compound, identified as **2-methoxy-3,6-dimethyl-4-hydroxybenzaldehyde** (**6**) (20 mg), colorless crystalline powder, mp 155-156 ºC. **$^1$H- NMR** (DMSO-$d_6$): 10.42 (1H, s, H-7); 7.06 (1H, s, H-5); 3.89 (3H, s, OMe); 2.47 (3H, s, H-9); 2.17 (3H, s, H-8). **$^{13}$C-NMR** (DMSO-$d_6$): 186.9 (C-7); 162.6 (C-2); 151.0 (C-4); 137.6 (C-6); 114.5 (C-1); 113.2 (C-3); 112.9 (C-5); 21.7 (C-9); 9.7 (C-8) in accordance with literature[8].

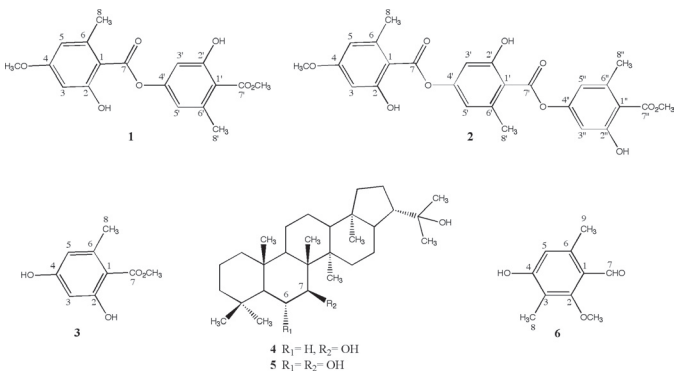


**Fig. 1.-** Metabolites isolated from *P. nudata*.

## RESULTS AND DISCUSSION

In the family Lobariaceae the most complex chemistry is found in *Pseudocyphellaria*; it has the most richly diverse chemistry of any genus, with several compounds synthesized via the shikimic acid pathway[8,9,10] in addition to contributions from other biosynthetic pathway. The genus is also especially rich in triterpenoids from several different series (hopane, stictane secostictane, lupane and fernene series), exhibiting an evolution in chemical complexity which reflect both geological age and phylogenetic relationships[11,12].

The compounds isolated from *P. nudata* were previously reported from several species of the genus. Since, with the exception of the triterpene hopane-6α,7β,22-triol (**5**), other compounds informed in *P. nudata* by means of TLC, including stictic, constictic acid, cryptostic and constictic acids, methyl gyrophorate[1], were not detected, probably because the low concentrations of the compounds in the thalli.

Tenuiorin (**2**) and methyl orsellinate (**3**) are currently found in species of *Pseudocyphellaria* and in other lichen genus[3].

The phenolic 2-methoxy-3,6-dimethyl-4-hydroxybenzaldehyde (**6**), however, is reported for the first time from a lichen. It was isolated from the fungus *Aspergillus silvaticus*[13], where additionally were reported metabolites related to 3-methylorsellinate; also methyl β-orcinolcarboxylate was isolated from *Stereocaulon alpinum*[14].

Hopanoids are pentacyclic triterpenes occurring predominantly in bacteria[15]. It has been suggested that the compounds are of great importance as cell membrane stabilizers in bacteria[16]. Hopanoids were discovered in nitrogen-fixing bacteria[17,18], and also occurs in soil bacteria as *Bradyrhizobium* root nodules in legume plants[19].

The hopanoid hopan-7ß,22-diol (**4**) has been isolated from *P. impressa* and *P crocata*[4,9], and hopan-6α,7ß,22-triol (**5**) from *P. crocata* and *Nephroma laevigatum*[10]. Hopanoids are very common in lichens with green algae and/or cyanobacteria as primary or secondary photobionts[20].

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