Methyl Psilalate: A New Antimicrobial Metabolite from *Psila boliviensis*

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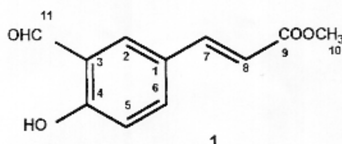
Abstract

Psila boliviensis (Wedd.) Cabr. yielded a new phenylpropanoid, named methyl psilalate. The structure was established by means of standard spectroscopic techniques. The microbiological evaluation of the compound revealed antibacterial activity against Gram-positive and Gram-negative bacteria.

Psila boliviensis (Wedd.) Cabr. (syn. *Baccharis boliviensis* (Wedd.) Cabr.), Asteraceae, known as "tola", "pesco tola" or "chijua-chijua", is a native shrub of Chile [1] which grows in the "Altiplano" region, (4000–5000 m a.s.l.). This area, which forms a unique ecological system, is sparcely inhabited by groups of Quechuan and Aymaran origin who use *P. boliviensis* to treat stomach ache [2]. Earlier works have reported the isolation of diterpenoids, sesquiterpenoids [3], a new *ent*-clerodane furanediterpenoid and two flavonoids [4]. We report the isolation and identification, from the dichloromethane extract, of a new phenylpropanoid derivative, methyl 3-formyl-*p*-coumarate, named methyl psilalate (**1**).



The antimicrobial activity of **1** was detected by bioautography of the dichloromethane extract which showed activity against Gram-positive and Gram-negative bacteria. The sensitive species were *S. aureus, M. flavus, E. coli, B. subtilis*, and *B. pumilus*. The *n*-hexane and methanol extracts showed no activity. Compound **1** was isolated using bioguided fractionation. The MS of **1** showed the parent ion peak at $m/z = 206$, consistent with a molecular formula of $C_{11}H_{10}O_4$. From the preliminary analysis of the ^{1}H- and ^{13}C-NMR spectra the base structure of a cinnamic acid derivative was deduced. In addition, these spectra exhibited signals corresponding to a methyl ester (3.80 s, 51.7 and 167.2), an aldehyde group (9.95 s and 196.2 ppm), and a phenolic group (161.6 ppm), H-bonded to the aldehyde carbonyl (11.3 ppm, in the ^{1}H-NMR spectrum). The location of the formyl and hydroxy groups at positions 3 and 4 of the aromatic ring, respectively, was established through nOe difference experiments. Thus, on irradiation of the formyl proton signal (9.95 ppm), an nOe enhancement on the doublet (J = 2 Hz) at 7.70 ppm was observed. All these results allowed us to identify this compound as the methyl ester of 3-(3-formyl-4-hydroxyphenyl)-prop-2-enoic acid (**1**), which has not been reported in the literature and for which we propose the name of psilalic acid.

Antimicrobial assay of the purified methyl 3-formyl-*p*-coumarate (**1**) showed positive activity against Gram-positive: *S. aureus, M. flavus, B. subtilis*, and *B. pumilus* and Gram-negative bacteria: *K. pneumoniae* and *S. aviatum*, but inactivity against *E. coli* and *P. aeruginosa*. Due to the small amount isolated, *S. aureus* was selected to evaluate the minimal inhibitory concentration of methyl psilalate, displaying an MIC of 75 μg/ml. As reference, the MIC of ampicillin for this strain is 5 μg/ml. Antimicrobial properties of phenol acids such as caffeic, chlorogenic, vanillic, *p*-hydroxybenzoic, ferulic and *p*-coumaric acids have been investigated [5], [6], [7] showing a remarkable activity against Gram (+) bacteria, but a weak effect against Gram (−) microorganisms. We have verified that compound **1** is active against Gram-positive bacteria and showed minor efficacy against Gram-negative bacteria in the bioautographic assay. Comparing the MIC values for methyl 3-formyl-*p*-coumarate against *S. aureus and* other phenolic compounds described in the literature such as ferulic acid (MIC higher than 200 μg/ml) [8], indicates that methyl 3-formyl-*p*-coumarate was more potent. Therefore, the replacement of the methoxy group for the aldehyde function seems to enhance the activity. These results show that this plant has antimicrobial effects due to the presence of a new phenolic compound, methyl psilalate. This activity has not been previously described for other *Psila* species, being the first report for *P. boliviensis* [8].

Materials and Methods

Melting point is uncorrected, ^{1}H-NMR (400 MHz), ^{13}C-NMR and DEPT (100 MHz) were recorded in $CDCl_3$ solution. Mass spectra (EI) were recorded under ionization energy of 70 eV. Silica gel 60G was used for column chromatography. TLC were performed on silica gel G, spots were detected under UV (254 and 366 nm) and with Liebermann-Burchard and/or *p*-anisaldehyde reagents.

The aerial parts of *Psila boliviensis* were collected in the Lauca National Park, I Region, Chile, and identified by Raúl Peña. A voucher specimen is kept at the Herbarium of the School of Chemistry and Pharmacy (SQF N° 20915), University of Chile.

Air-dried, ground material (500 g) was sequentially extracted at room temperature with *n*-hexane, dichloromethane, and methanol, yielding 13.0 g, 70.7 g, and 34.6 g of extract, respectively. The extracts were evaluated for antimicrobial activity, through a bioautographic agar overlay. The active compound was isolated from the dichloromethane extract. This extract (40.0 g) was subjected to column chromatography eluted with mixtures of *n*-hexane-CH_2Cl_2 of increasing polarity. Fractions eluted with a

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sis of TLC pattern and radical scavenging properties. Low pressure chromatography of F3 (1580–1850 ml, 0.37 g) on LiChroprep RP 18 (40–63 μm, 2.5 × 30 cm i.d.) with H_2O-MeOH (80:20, 3 ml/min, detection 280 nm) afforded compound **1** (t_R = 87,5 min, 210 mg). LiChroprep RP 18 chromatography of F5 (2090–2800 ml, 0.21 g) with H_2O (5 ml/min, detection 297 nm) gave a fraction (t_R = 61.5 min, 20 mg) which was further purified on Sephadex LH 20 (1 × 60 cm i.d.) with MeOH to afford F5–2 (14 mg). HPLC (LiChrospher Diol, 5 μm, 4 × 250 mm i.d.) with hexane-isopropanol-H_2O (50:47:3, 1 ml/min, detection at 320 nm) gave compound **2** (t_R = 4.58 min, 5.7 mg).

6-Methyl-1,2,4-trihydroxybenzene-1-O-β-D-4′-methylglucopyranoside (**1**): Cream coloured amorphous powder; R_f 0.62 (system 1); m.p. 86–89 °C; $[\alpha]_D^{25}$: −8.6° (*c* 0.746, MeOH); UV (H_2O + HCl, pH 2): λ_{max} (log ε) = 277.5 (2.95) nm; UV (H_2O + NaOH, pH 12): λ_{max} (log ε) = 294.5 (3.19) nm; ^{1}H-NMR (DMSO-d_6): δ = 6.06 (1H, d, *J* = 2.8 Hz, H-3), 6.00 (1H, d, *J* = 2.8 Hz, H-5), 4.30 (1H, d, *J* = 7.9 Hz, H-1′), 3.59 (1H, brd, *J* = 11.6 Hz, H-6a′), 3.49 (1H, m, H-6b′), 3.42 (3H, s, H-7′), 3.35 (1H, m, H-3′), 3.25 (1H, dd, *J* = 8.0, 7.9 Hz, H-2′), 3.17 (1H, ddd, *J* = 9.7, 4.8, 1.9 Hz, H-5′), 3.02 (1H, dd, *J* = 9.7, 9.5 Hz, H-4′), 2.17 (3H, s, H-7); ^{13}C-NMR (DMSO-d_6): δ = 154.2 (C-4), 149.8 (C-2), 136.9 (C-1), 132.2 (C-6), 107.4 (C-5), 106.2 (C-1′), 100.9 (C-3), 78.9 (C-4′), 75.9 (C-3′), 75.8 (C-5′), 74.0 (C-2′), 60.4 (C-6′), 59.6 (C-7′), 16.8 (C-7); ESI MS (pos. ion mode): *m/z* = 317 $[M + H]^+$, 334 $[M + NH_4]^+$, 339 $[M + Na]^+$, 650 $[2M + NH_4]^+$; ESI MS (neg. ion mode): *m/z* = 315 $[M-H]^-$, 631 $[2M-H]^-$; HR FABMS: *m/z* = 317.12420 $[M + H]^+$, 339.10708 $[M + Na]^+$ (calcd for $C_{14}H_{20}O_8$ 316,1236).

(−)-Terredionol (**2**): Slightly off-coloured glassy material; R_f 0.46 (system 1); $[\alpha]_D^{25}$: −145.2° (*c* 0.62, MeOH); ^{13}C-NMR (CD_3OD): δ = 109.8 (C-2), 67.7 (C-4 and C-6), 39.4 (C-5), 7.5 (C-7), tautomeric C-1 and C-3 not observed due to long relaxation time. ^{1}H-NMR, MS, UV, IR in agreement with published data [4]. Copies of original spectra are obtainable from the corresponding author.

The TLC-assay for radical scavengers was carried out according to [5]. A microtitre assay [6] was used for quantitative determination. The actual decrease in absorption was compared to that of positive controls (10 mM ascorbic acid and Trolox™C) and ethanol as a blank. Inhibition of lipid peroxidation was determined by quantification of thiobarbituric acid reactive substances [7]. The antioxidant activity of the samples was calculated against those of vehicle as 100% lipid peroxidation activity and expressed as IC_{50}. Cytotoxicity of crude extract and substances **1** and **2** was determined with PC12 cells and mouse L (tk-) fibroblasts. For the assay cells were plated at a density of 1 × 10^5 cells per well in a 24-well plate and test samples added to medium supplemented with 1% serum. Cytotoxicity was determined after 24 hours of exposure using LDH kit (Roche Diagnostics, Mannheim), relative to vehicle as negative control and Triton X 100-lysed cells as positive control.

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