Supporting Information

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Sesquiterpene Lactones of *Amphoricarpos autariatus* ssp. *autariatus* from Montenegro - Antifungal Leaf - Surface Constituents

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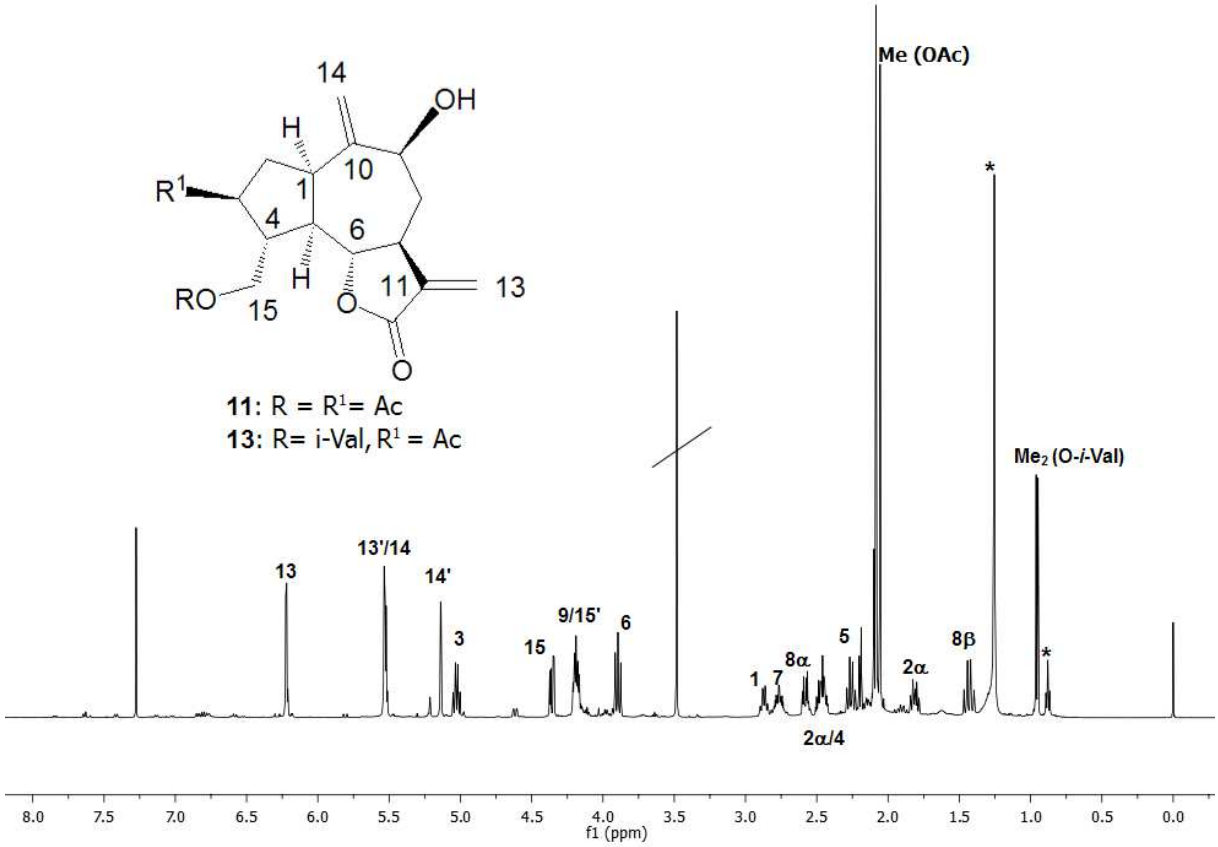
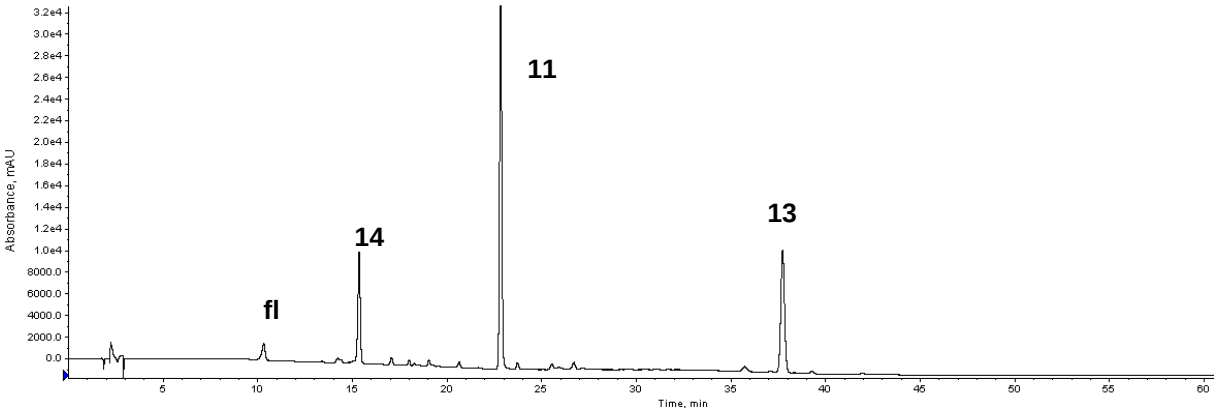
S1: *GC-MS analysis* of *n*-alkanes. The analysis of the *n*-alkane fraction was carried out on an Agilent Technologies 7890A GC System with 5975 inert XL EI/CI MSD, split/splitless injector (250 ^{o}C), column HP-5 MS (30 m x 0.25 mm, 0.25 μm), equipped with autosampler 7638B Series Injector, GC - conditions: injection volume, 1 μL, carrier gas helium (2.2 ml/min), column temperature 50 - 285 ^{o}C, 15 ^{o}C/min, EI MS range *m/z* 30 - 550.

S2:



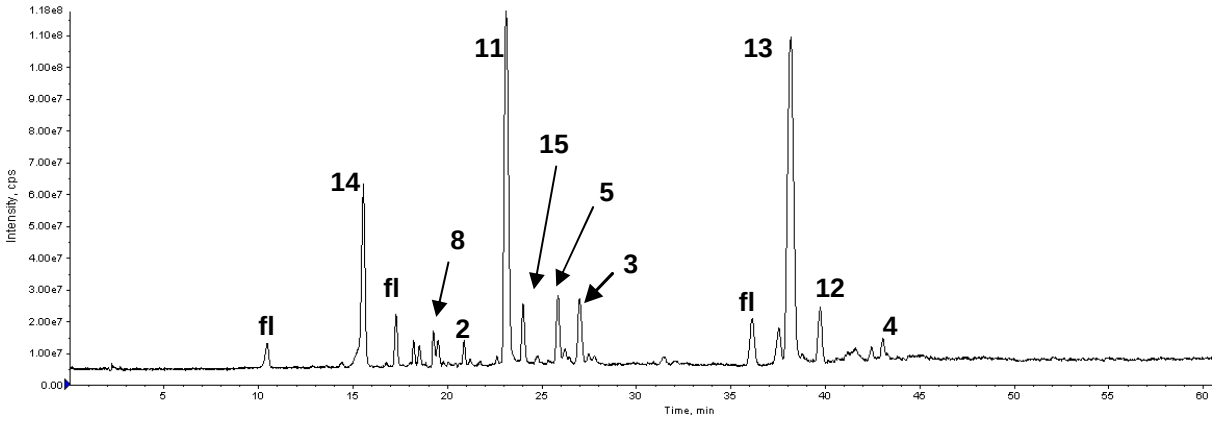
^{1}H 500 MHz ($CDCl_3$) NMR spectrum of the surface lactone fraction from the sample originating from Tara canyon; **11** and **13**, major constituents (≥85% of the lactone mixture according to LC-ESI TOF MS), the assignment of resonances are based on 2D NMR [ref. 3 in the manuscript]; **n*-alkane signals

S3: *LC-DAD analysis of the lactone fraction.*The analysis of the lactone mixture was carried out on an Agilent Technologies 1100 Series liquid chromatograph equipped with a diode array detector (λ = 210 nm) and autosampler; LC conditions: injection volume 20 μL (c ~ 7 mg/ml, MeOH), LiChrospher 100 RP-18 (250 x 4 mm; 5μm), column temp. 20^{o}C, mobile phase (1 mL/min): 5 mM ammonium formate in water (A) + ACN (B), combined isocratic and gradient elution, 10% B (0 - 5 min), 10 - 35% B (5 - 20 min), 35% B (20 - 30 min), 35 - 50% B (30 - 40 min), 50% B (40 - 60 min).



LC/DAD chromatogram (λ=210 nm) of the surface lactone fraction; **fl**: flavone

S4: *LC-ESI HR MS TOF analysis of the lactone fraction.* The analysis of the lactone mixture was performed on LC Agilent Technologies 1100 Series chromatograph (see above) coupled with Agilent Technologies 6210 Time-of-flight mass spectrometer; LC - conditions same as above. ESI MS TOF conditions: injecton volume 10 μL (c ~ 7 mg/mL, MeOH); drying gas (N_2) flow 12 l/min, nubulizer pressure 45 psig, drying gas temp. 350^o; capillary voltage, 4000 V; fragmentor voltage, 140 V; skimmer, 60 V; Oct RF voltage 250 V; positive mode, mass range *m/z* 100 - 1500; 10,000 transients/scan. The compounds were identified, using coinjection technique, by identity of HR MS and LC (t_R) data with those of the lactones isolated on the preparative scale from the same plant material.



LC/ESI TOF MS (+ mode) total ion current chromatogram (TIC) of the surface lactone fraction; **fl**: flavone

S5: *Antifungal-activity assay.* Eight fungi were used: *Aspergillus ochraceus* (ATCC 12066), *A. niger* (ATCC 6275), *A. versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *P. ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061), *Fusarium verticillioides* (plant isolate) and *Fulvia fulvum* (TK 5318). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. The micromycetes were maintained on malt agar and the cultures stored at 4° C and sub-cultured once a month [1]. In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used [2, 3]. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10^5 in a final volume of 100 μL per well. The inocula were stored at 4° C for further use. Dilutions of the inocula were cultured on solid malt agar

to verify the absence of contamination and to check the validity of the inoculum. Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (1 mg/mL) and added in broth Malt medium with inoculum. The microplates were incubated at Rotary shaker (160 rpm) for 72 h at 28° C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 μL of tested compounds dissolved in medium and inoculated for 72 h, into microtiter plates containing 100 μL of broth per well and further incubation 72 h at 28° C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. Solution of 5% DMSO was used as a negative control, commercial fungicides, bifonazole (Srbolek, Belgrade, Serbia) and ketoconazole (Zorkapharma, Šabac, Serbia), were used as positive controls (1–3500 μg/mL). All experiments were performed in duplicate and repeated three times.

[1] C. Booth (1971). Fungal Culture Media, In: Methods in Microbiology IV, *eds*: J. R. Norris and D. W. Ribbons, Academic Press, London and New York. p.p. 49-94.

[2] A. Espinel-Ingroff (2001). Comparison of the E-test with the NCCLS M38-P Method for Antifungal Susceptibility Testing of Common and Emerging Pathogenic Filamentous Fungi. *J. Clin. Microbiol.* **39**, 1360-1367.

[3] H. Hanel and W. Raether (1988). A More Sophisticated Method of Determining the Fungicidal Effect of Water-Insoluble Preparations with a Cell Harvester, Using Miconazole as an Example. *Mycoses*. **31**, 148-154.