Montadial A, a Cytotoxic Metabolite from *Bondarzewia montana*†

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A new meroterpenoid, montadial A (**1**), has been isolated from the polypore *Bondarzewia montana*. Its structure was elucidated by spectroscopic techniques. Montadial A exhibits cytotoxic effects and develops a striking yellow color when treated with KOH.

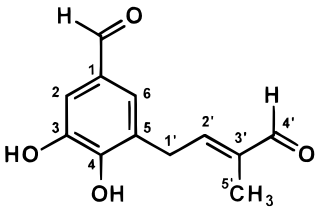
The rare white-rot fungus *Bondarzewia montana* (Quel.) Sing. (German: *Bergporling*) grows at the base of *Abies* trees and other conifers. It forms yellow to brownish fruitbodies that originate from a gnarled, pale yellow rooting base. Treatment of these mycelial roots with aqueous KOH causes an intense yellow color. Taxonomically the genus *Bondarzewia* has been placed in the order Russulales, which is supported by the occurrence of stearoyl−velutinal, the chemotaxonomic marker compound for this order.[1]

To identify the chromogens, two big roots from *B. montana* were extracted with ethyl acetate. Purification of the extract by preparative HPLC on reversed phase yielded the main chromogen, named montadial A, as a yellow, oily film. The compound develops an intense yellow color reaction with bases, and the UV maximum (MeOH) is shifted from 312 to 351 nm. The HREIMS of montadial A shows a $[M]^+$ peak at *m/z* 220.0734, corresponding to $C_{12}H_{12}O_4$. The IR spectrum (KBr) reveals the presence of OH (3421, 1304 cm^{-1}) and α,*β*-unsaturated carbonyl groups [1677 cm^{-1} (s), 1593 (m)]. In the ^1H NMR spectrum (CD_3OD) montadial A exhibits two aldehyde signals at *δ* 9.38 and 9.67 and doublets at *δ* 7.22 and 7.26 (*J* = 1.8 Hz), which can be assigned to two aromatic *meta*-protons. A triplet of quartets at *δ* 6.75 (*J* = 7.5, 1.2 Hz) indicates an olefinic proton directly coupled to a methylene group with additional allylic coupling to a methyl group.

In the ^{13}C NMR spectrum, signals for the aldehyde functions and the aromatic CH groups were observed at *δ* 193.1/197.2 and *δ* 113.7/126.8, respectively. In addition, five quaternary carbons, an olefinic CH group at *δ* 154.0, a methylene group at 30.6, and a methyl group at 9.20 were visible. From these data, montadial A was identified as 3,4-dihydroxy-5-(3′-methyl-4′-oxobut-2′-enyl)benzaldehyde (**1**). The compound has not been reported before.

Formula **1** is supported by 2D NMR experiments. Thus, the NOESY spectrum revealed a correlation between the aromatic aldehyde group at *δ* 9.67 and the aromatic proton at *δ* 7.26 (6-CH). Structure **1** was confirmed by comparison of the ^1H NMR data of montadial A with those of compounds **2**[2,3] and **3**,[4] which are described in the literature (Table 1), and also with the ^{13}C NMR data of other related compounds.[5,6]

Montadial A (**1**) shows weak phytotoxic effects against *Setaria italica* at concentrations of 50 μg/disk. A strong cytotoxic activity against L1210 tumor cells (lymphocytic leukemia of mice) is observed at concentrations of 10 μg/mL and higher, as well as against HL60 tumor cells (promyelocytic human leukemia) at 5 μg/mL and higher. Analytical HPLC examination revealed the presence of several other compounds with the same UV spectra as montadial A, but their presence in very small quantities prevented a structure investigation.



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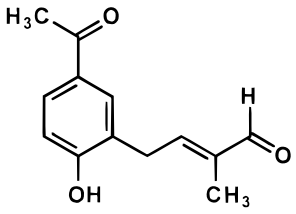

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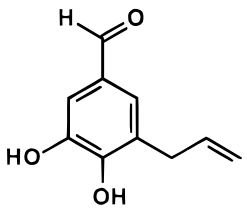

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Experimental Section

General Experimental Procedures. UV−vis spectra were measured on a Perkin−Elmer Lambda 16 spectrophotometer in CH_3OH. IR spectra were obtained with a Perkin−Elmer FT-IR 1000. NMR spectra were obtained on Bruker ARX-300 and AMX-600 instruments in CD_3OD or $(CD_3)_2CO$ with the solvent as internal standard. EIMS were recorded with a Finnigan MAT 90. GC−MS was carried out with a Varian GC 3200 coupled with a Finnigan MAT Magnum mass spectrometer (ion trap); column: J & W DB-5ms, length 30 m, diameter 0.25 mm; temperature gradient: 50 °C for 2 min,

Table 1. ^1H NMR Data of **1** [300 MHz, CD_3OD], **2**[2,3] [270 MHz, $CDCl_3$], and **3**[4] [220 MHz, $(CD_3)_2CO$]

	1	*J* (Hz)	**2**	*J* (Hz)	**3**	*J* (Hz)
2-H	7.22 d	1.8	7.79 dd	8, 2	7.28 s	
3-H			6.92 dd	8		
6-H	7.26 d	1.8	7.80 d	2	7.30 s	
1′-H	3.74 d	7.5	3.73 d	7.2	3.46 d	7.5
2′-H	6.75 tq	7.5, 1.2	6.68 tq	7.2, 1	6.02 m	
4′-H	9.38 s		9.41 s		5.10 m	
5′-H	1.85 d	1.2	1.89 d	1		
1-CHO	9.67 s				9.76 s	

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then heating at 5 °C/min up to 300 °C. TLC was carried out on commercial Si gel plates (Merck Kieselgel 60 F_{254}, thickness 0.2 mm); solvent system: EtOAc−hexane (2:1); the pale yellow spots were visualized by spraying with a mixture of 4-methoxybenzaldehyde−AcOH−H_2SO_4−EtOH (10:12:12:210) (ANIS) and heating. Before HPLC, solid-phase extraction was performed with Chromabond C_{18} cartridges (Macherey & Nagel). Analytical HPLC was carried out with a Waters 600 E pump and system controller coupled with a diode-array detector 990+; a Knauer Vertex column 4 × 250 mm was used, packed with Nucleosil 100 C_{18}, 5 μm; eluent A: H_2O−CH_3CN 9:1; eluent B: CH_3CN; linear gradient: 0 min, A 100%; 30 min, B 100%; flow rate 1 mL/min; detection range 200−400 nm. Preparative HPLC was performed with a Merck Hitachi L 6200 intelligent pump coupled with a Merck 655A variable wavelength UV monitor; Knauer Vertex columns 16 × 250 mm were used, packed with Nucleosil 100 C_{18}, 7 μm; eluents as for analytical HPLC; linear gradient: 0 min, A 100%; 60 min, B 100%; flow rate 7 mL/min; detection at 290 nm.

Biological Material. *Bondarzewia montana* was found in September 1997, at the base of *Abies alba* near Furth im Wald, Bavaria, and stored at −10 °C. A voucher specimen is kept in the herbarium of the Ludwig-Maximilians-Universität München, Institut für Organische Chemie.

Extraction and Isolation of Montadial A (1). The pale yellow skin from 350 g mycelial roots of *B. montana* was exhaustively extracted with EtOAc (4 × 200 mL) until the resulting solution was colorless. After evaporation of the solvent, the brown, oily residue (ca. 0.3 g) was dissolved in MeOH, and the mixture separated by reversed-phase preparative HPLC with a H_2O−MeCN gradient. Montadial A (t_R = 25.10 min) was obtained as a yellow, oily film, yield 8.6 mg (0.002%).

Montadial A (1): R_f 0.44, violet spot with ANIS followed by heating; UV (MeOH) λ_{max} (log ϵ) 230 (4.12), 285 (3.74), 312 (3.63) nm; IR (KBr) ν_{max} 3421, 2928, 1677, 1593, 1447, 1304, 1210, 1138, 869 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz), see Table 1; ^1H NMR (Me_2CO-d_6, 600 MHz) δ 1.85 (3H, d, J = 1.2 Hz, H-5′), 3.79 (2H, d, J = 7.2 Hz, H-1′), 6.76 (1H, tq, J = 7.2, 1.2 Hz, H-2′), 7.31 (1H, d, J = 1.8 Hz, H-2), 7.35 (1H, d, J = 1.8 Hz, H-6), 9.46 (1H, s, H-4′), 9.76 (1H, s, 1-CHO); ^{13}C NMR (CD_3-OD, 600 MHz) δ 9.2 (q, C-5′), 30.6 (t, C-1′), 113.1 (s, C-5), 113.7 (d, C-2), 120.2 (s, C-1), 126.8 (d, C-6), 130.2 (s, C-3′), 146.9 (s, C-3), 154.0 (d, C-2′), 155.2 (s, C-4), 193.1 (d, C-4′), 197.2 (d, 1-CHO); EIMS *m/z* 220 $[M]^+$ (100), 202 (13), 189 (17), 163 (22), 145 (38), 117 (20), 115 (13), 91 (9), 77 (10); HREIMS *m/z* 220.0734 (calcd for $C_{12}H_{12}O_4$, 220.0736).

Trimethylsilylation of 1. To montadial A (1 mg) was added 3 drops of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, and the mixture was kept for 5 min at 25 °C. GC−MS analysis of the mixture revealed the formation of a tris-(trimethylsilyl) derivative of montadial A (*m/z* 436) with a retention time of 26.02 min. The introduction of the third trimethylsilyl residue can be explained by dienol formation and subsequent silylation. A similar observation was made by Bohlmann et al.[2] on acetylation of aldehyde **2**.

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