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Ascochital, a new metabolite from the marine ascomycete *Kirschsteiniothelia maritima*

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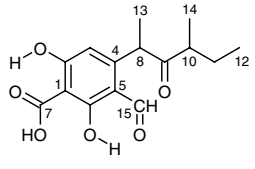
The organic extracts of the culture filtrate of the marine ascomycete *Kirschsteiniothelia maritima* (Linder) D. Hawksw. (Pleomassariaceae) displayed antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Micrococcus flavus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Bioactivity guided separation of these extracts led to ascochital (**1**), a new aromatic aldehyde, besides the closely related ascochitine (**2**). Here we report on the isolation, structure elucidation and antibacterial activity of these compounds.

The fungal strain of *K. maritima* was taken from submerged wood and cultivated in a liquid shake culture on Hagem broth for 15 days. Chromatography of an EtOAc–Me_2CO extract of the culture broth on Sephadex LH-20 afforded two main products **1** and **2**. The major compound ascochitine (**2**), a cristalline yellow solid, was identified from its UV and NMR spectral data [1, 2]. Previously, **2** has been isolated as phytotoxic metabolite from cultures of *Ascochyta fabae* [3] and *A. pisi* [4]. The new metabolite, ascochital (**1**), was obtained as brown oil. Negative-mode DCI-MS and high-resolution DCI-MS of **1** established the elemental composition $C_{15}H_{18}O_6$ for the molecular ion at *m/z* 294. The ^{1}H- and ^{13}C NMR spectra of **1** in DMSO-d_6 were complicated by partial doubling of signals indicating the presence of two inseparable diastereomers in a nearly equivalent ratio (A and B in the Table). Because the doubled ^{13}C NMR signals appear as well separated pairs, all 25 observed signals could be assigned to the 15 carbon atoms. A doubled 2-butyl and CH_3–CH< residue were identified in the ^{1}H,^{1}H-COSY spectrum. The analysis of the HMQC spectrum correlated all signals of carbon-bound protons with their respective ^{13}C NMR signals and further showed the presence of an aldehyde group ($\delta_{H/C} = 10.3/189$; C-15) and of an unsaturated methine group ($\delta_{H/C} = 5.8/107$; C-3). Thus, the signals of one keto group, a further five quarternary sp^2 carbons, and of two signals of H,D-exchangeable OH protons remained to be specified in the NMR spectra. A third exchangeable acidic proton, which was not visible in the ^{1}H NMR spectrum due to fast exchange with the residual water, was assigned to the carboxylic acid group ($\delta_C = 174$; C-7). The direct ^{13}C, ^{1}H NMR couplings ($^1J_{C,H}$) visible in the long-range ^{1}H, ^{13}C-correlation NMR spectrum (HMBC) revealed the aromatic character of methine C-3 by $^1J_{C,H} = 159$ Hz and confirmed the aldehyde group ($^1J_{C,H} = 174$ Hz). An obvious $^2J_{C,H}$ coupling of 21 Hz and a long-range correlation signal with the H-15 aldehyde proton identified C-5 as point of attachment for the aldehyde group. The HMBQ correlation between



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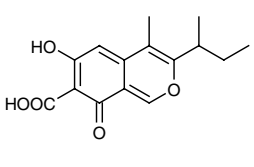

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Table: ^{1}H and ^{13}C NMR Data[a] of ascochital (1) in DMSO-d_6

Proton	δ_H (ppm)	m	J (Hz)	carbon	δ_C (ppm)	m	correlated H in HMBQ
–	–	–	–	1A + B	102.0	s	OH-2, OH-6, 3
					102.0	s	
OH-2	15.68	s	–	2	167.9	s	OH-2
	15.66	s	–				
H-3A	5.83	s	–	3	106.9	d	OH-2 ($^1J_{CH} = 159$ Hz)
H-3B	5.81	s	–				
–	–	–	–	4A + B	147.3	s	15, 13
					147.1	s	
–	–	s	–	5	111.7	s	6-OH 15, 3 ($^2J_{CH} = 21$ Hz)
OH-6	16.47	s	–	6	170.2	s	6-OH
OH-7	n.o.	–	–	7	174.9	s	–
H-8A	5.30	m	–	8A	46.1	d	14A
H-8B	5.22	m	–	8B	44.7	d	14B
–	–	–	–	9B	213.1	s	10B, 11Ba, 13B, 14B
				9A	212.6	s	10A, 11Aa, 13A, 14A
H-10A	2.37	m	–	10A	45.4	d	14A, 12A, 11Aa + b
H-10B	2.44	m	–	10B	44.9	d	14B, 12B, 11Ba + b
Ha-11A	1.49	m	–	11A	24.9	t	14A, 12A, 10A
Hb-11A	1.14	m	–				
Ha-11B	1.49	m	–	11B	26.3	t	14B, 12B, 10B
Hb-11B	1.25	m	–				
H-12A	0.60	t	7.6	12A	11.5	q	10A, 11Aa + b
H-12B	0.77	t	7.6	12B	11.3	q	10B, 11Ba + b
H-13A	0.94	d	6.9	14A	17.0	q	10A, 11Aa + b
H-13B	0.82	d	6.9	14B	15.8	q	10B, 11Ba + b
H-14A	1.17	d	6.3	13A	17.2	q	–
H-14B	1.15	d	6.9	13B	17.0	q	–
H-15	10.30	s	–	15A + B	189.2	d	– ($^1J_{CH} = 174$ Hz)
					189.1	d	

[a] A/B specifies signals of the isomers; ^{1}H at 300 MHz; ^{13}C at 75 MHz; Multiplicity was determined by a DEPT experiment

the signals of C-2 and C-6 ($\delta_C = 167.9$ and 170.2) and those of the exchangeable protons at $\delta_H = 15.7$ and 16.5, respectively, identified two phenolic OH groups.

Further analysis of the HMBC data provided the complete structure **1** (Table, isomers A and B). The doubling of the NMR signals of **1** can either be explained by an equilibrium of inseparable diastereomers generated by enolization of the ketone or by a hindered rotation at the C-7-C-8 bond. Heating of the sample to 100 °C gave sharper signals, but no indication of a possible coalescence of the doubled signals was observed. Although no enol intermediate was directly visible in the NMR spectra, the enolization was observed in the ^{1}H NMR spectrum of **1** in methanol-d_4 by H/D exchange of H-8.

Compounds **1** and **2** exhibit antibacterial activity against the Gram-positive bacterium *Bacillus subtilis* with a minimal inhibitory concentration of 0.5 μg/ml and 0.1 μg/ml, respectively.

Experimental

1. Apparatus

NMR spectra were recorded on a Bruker AM-300 NMR spectrometer. EI and FAB MS were carried out on a Finnigan MAT 95 spectrometer.

2. Fungal material

K. maritima (Linder) D. Hawksw. was originally isolated from submerged wood, collected in December 1992 at the coast of the Greifswalder Bodden, Baltic Sea, Germany. The material was identified by Prof. Dr. H. Kreisel, Institute of Biology, University Greifswald. The culture has been deposited in the culture collection of the Department of Pharmaceutical Biology, University of Greifswald (No. M9). *K. maritima* was grown in liquid shake culture on a rotary shaker (220–240 rpm) in 500 ml Erlenmeyer flasks containing 150 ml Hagem broth (ammoniumsuccinate 0.5 g; KH_2PO_4 0.5 g; $MgSO_4 \times 7\ H_2O$ 0.5 g; $FeCl_3$ (1%) 0.5 ml; glucose 5.0 g; malt extract 5.0 g; aqua purificata 1000 ml) at 21 °C for 15 days.

3. Extraction and isolation

After separation of the mycelium by filtration the culture broth was extracted with EtOAc–Me_2CO (9 : 1). The organic layer was dried over sodium sulfate and evaporated to give a brown residue (140.7 mg from 10 l of culture broth). The extract was separated by CC on Sephadex LH-20 with CH_2Cl_2 : hexane (4 : 1). The major compound **2**, a crystalline yellow solid, was identified as ascochitine by comparison of spectral data [1, 2]. Ascochital (**1**) was eluted from a column of Sephadex LH-20 with MeOH : H_2O (2 : 1): m.p. 122–124 °C; UV (MeOH): $\lambda_{max} = 235$, 251, 281, 334 nm; IR (KBr): $\nu_{max} = 3429$, 2967, 2990, 2875, 1706, 1589, 1433, 1402, 1279, 1227, 1191, 1113, 1028, 929, 856 cm^{-1}; (–) DCI-MS (i-butane): m/z (%) = 294 (100) $[M]^-$, 276 (59), 265 (15), 252 (10), 251 (89), 250 (80), 249 (31), 232 (55), 153, (46); HR-DCI-MS (i-butane, resolution 9000): 294.1103 (calcd. for $C_{15}H_{18}O_6$, 294.1104).

4. Bioassay

The minimal inhibitory concentration against *B. subtilis* SBUG 14 was established by convential serial broth dilution method.

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