

Structure Elucidation of Auxofuran, a Metabolite Involved in Stimulating Growth of Fly Agaric, Produced by the Mycorrhiza Helper Bacterium *Streptomyces* AcH 505

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Received: August 21, 2006 / Accepted: November 16, 2006

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Abstract Mycorrhiza helper bacterium *Streptomyces* strain AcH 505 stimulates ectomycorrhiza formation between spruce and fly agaric by supporting fungal growth whereas growth of pathogenic fungi is suppressed. A fungal growth promoting substance was isolated and the chemical structure elucidated by mass spectrometry and NMR spectroscopy. The absolute configuration of the novel fungal growth promoting compound auxofuran (**1**) was deduced from NMR data with the help of Mosher esters.

Keywords mycorrhiza helper bacterium, growth promoting substance, structure elucidation

Intact terrestrial ecosystems depend on the ubiquitous symbiosis between plants and fungi called mycorrhiza. Mycorrhizal associations provide the plant with better access to water as well as to nutrients such as phosphorus, nitrogen and iron. Another positive effect for the plant is protection against pathogens and parasites. In boreal, alpine and temperate regions, the predominant form of association between plants and fungi is the ectomycorrhizal symbiosis. Apart from plants and fungi, mycorrhiza often involves bacteria. The so-called mycorrhiza helper bacteria [1] stimulate fungal growth as well as mycorrhiza formation. Isolation and characterization of the substances responsible for these effects will provide a basis for better understanding them. In the present work, a compound produced by the

Streptomyces strain AcH 505 that showed a stimulating effect on the growth of fly agaric (*Amanita muscaria*) [2–4], thus influencing ectomycorrhiza formation with spruce (*Picea abies*), was isolated and the structure elucidated.

The taxonomy of the producing *Streptomyces* strain AcH 505, fermentation, isolation and biological activities are reported in preceding papers [2, 3]. For structure elucidation, auxofuran (**1**) was analyzed by mass spectrometry and NMR spectroscopy. The high-resolution ESI-FTICR mass spectra of **1** showed a mass of m/z 195.10197 [(M+H)⁺, C₁₁H₁₄O₃, (M+H)⁺_{theor} = 195.10212, Δm = 0.77 ppm]. Database searches of the molecular formula did not match with any known compound from the DNP database [5] or the CAS-online database [6]. The physico-chemical properties of **1** are shown in Table 1.

The ¹H and ¹³C NMR spectral data of compound **1** are shown in Table 2. The ¹H NMR spectrum showed a methyl group, four methylene groups, a methine group, a hydroxy proton, and an aromatic proton. The ¹³C NMR data

Table 1 Physico-chemical properties of **1**

Appearance	Yellow oil
Molecular formula	C ₁₁ H ₁₄ O ₃
ESI-FTICR (m/z)	
Found [M+H] ⁺	195.10197
Calcd [M+H] ⁺	195.10212
UV λ _{max} ^{MeOH} [nm] (ε)	272 (7992)
IR ν _{max} (cm ⁻¹)	3388, 2958, 2926, 2872, 2855, 1744, 1714, 1669, 1464, 1379, 1173, 1045, 1026

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supported the presence of these groups and revealed four quaternary carbon atoms. From 2D NMR spectra, we identified compound **1** to be 5,6-dihydro-4-hydroxy-3-propyl-4*H*-benzofuran-7-one (Fig. 1). Connectivities from OH-4 to H-4, from H-4 to H₂-6, and H₂-8 to H₃-10 were established based on analyses of ¹H-¹H COSY NMR data (Fig. 2) as well as HMBC NMR data. The positions of the hydroxy and the keto groups were assigned according to HMBC spectra (Fig. 2). HMBC correlation for the proton of the hydroxy group to C-4, C-5 and C-3a suggested that the OH group is attached to C-4. The absolute configuration was determined by the modified Mosher's method [7] by preparing (*S*)- and (*R*)- α -methoxy- α -trifluoromethylphenylacetic acid (MTPA) esters of auxofuran.

(*R*)- and (*S*)-MTPA esters were prepared as follows: To a solution of auxofuran (2.9 mg, 15 μ mol), (dimethylamino)-pyridine (7.3 mg, 60 μ mol), and triethylamine (3 μ l, 21 μ mol) in dry dichloromethane (*R*)- or (*S*)-MTPA

Table 2 ¹H and ¹³C NMR shifts of auxofuran

Position	¹ H (J in Hz)	¹³ C
2	7.72 (s)	144.9
3	—	125.8
3a	—	140.9
4	4.85 (ddd, <i>J</i> =4.3, 6.5, 6.9)	62.0
4-OH	5.37 (d, <i>J</i> =6.9)	
5	2.20 (m) 1.96 (m)	33.8
6	2.60 (ddd, <i>J</i> =4.5, 8.0, 16.8) 2.39 (ddd, <i>J</i> =4.5, 8.4, 16.8)	34.7
7	—	185.5
7a	—	146.3
8	2.46 (t, <i>J</i> =7.3)	24.8
191	1.57 (tq, <i>J</i> =7.3, <i>J</i> =7.3)	22.1
10	0.98, t (7.3)	14.1

DMSO-*d*₆, 400 MHz.

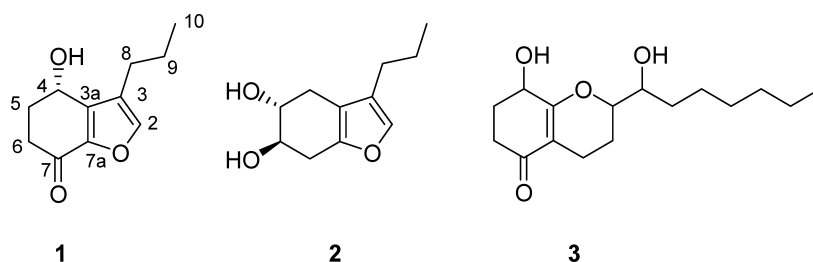


Fig. 1 Structures of auxofuran (**1**), ulufuranol (**2**), and koniginin B (**3**).

chloride (5.6 μ l, 30 μ mol) were added and stirred at 27°C for 12 hours. Further purification of the (*R*)- and (*S*)-MTPA esters of auxofuran was accomplished by preparative HPLC using RP-18 with a gradient of H₂O+0.1% formic acid and MeCN+0.1% formic acid from 20% B to 100% B, with the MTPA esters eluting at 80% B. (*R*)-MTPA ester of auxofuran: ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.80 (1H, H-2), 6.31 (1H, H-4), 2.56 (1H, H-5a), 2.32 (1H, H-5b), 2.55 (2H, H-6), 2.08 (2H, H-8), 1.34 (2H, H-9), 0.75 (3H, H-10), 3.52 (3H, O-CH₃), 7.47 (5H, phenyl). (*S*)-MTPA ester of auxofuran: ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.89 (1H, H-2), 6.35 (1H, H-4), 2.49 (1H, H-5a), 2.25 (1H, H-5b), 2.48 (1H, H-6), 2.36 (2H, H-8), 1.52 (2H, H-9), 0.88 (3H, H-10), 3.41 (3H, O-CH₃), 7.46 (5H, phenyl).

For the (*S*)-MTPA ester, compared to the (*R*)-MTPA ester, H-5 and H-6 exhibit an upfield shift, whereas H-2, H-8, and H-9 exhibit a downfield shift, suggesting an *S*-configuration at C-4.

Due to the absence of the hydroxy proton in MeOH-*d*₆, the hydroxy and the keto group were inadvertently assigned wrongly at first [3]. 5,6-dihydro-4-hydroxy-3-propyl-4*H*-benzofuran-7-one is a previously unknown natural product. Due to its similarity to indole-3-acetic acid, the most important native auxin, it was named auxofuran. Auxofuran also bears structural resemblance to ulufuranol (**2**) [8] and the herbicidal active koniginin B (**3**) [9] (Fig. 1).

Auxofuran is the first fungal growth promoting substance from a mycorrhiza helper bacterium belonging to the genus *Streptomyces* that has been characterized. As described in [3], auxofuran improves fungal growth and ectomycorrhiza

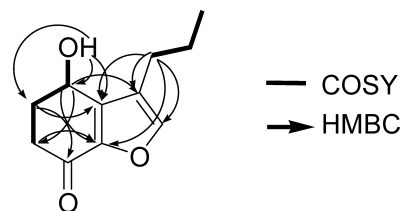


Fig. 2 ¹H-¹H COSY and selected HMBC correlations of auxofuran (**1**).

formation between *A. muscaria* and spruce. On the other hand, co-cultivation of auxofuran producing strain AcH 505 and *A. muscaria* stimulated auxofuran production.

Acknowledgments The compounds were kindly provided by Prof. Hans-Peter Fiedler, University of Tübingen/Germany. His support for the project is appreciated very much. We thank Mr. Graeme Nicholson for obtaining the FT-ICR spectra. The work was supported by a research grant of the Emmy-Noether program (SU-239/2) of the DFG and the Schering AG, Germany.

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